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To: Commissioner for Patents
P.O. Box 1450
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NEW APPLICATION TRANSMITTAL - UTILITY

Sir:

Transmitted herewith for filing is a utility patent application:

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Masoud MOKHTARANI

Title: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

I. PAPERS ENCLOSED HEREWITH FOR FILING UNDER 37 CFR § 1.53(b):

41 Page(s) of Written Description
3 Page(s) Claims
1 Page(s) Abstract
7 Sheets of Drawings
Sheets of Sequence Listing

II. ADDITIONAL PAPERS ENCLOSED IN CONNECTION WITH THIS FILING:

- ☐ Declaration
- ☐ Power of Attorney ☐ Separate ☐ Combined with Declaration
- ☐ Assignment to and assignment cover sheet
- ☐ Certified Copy of Priority Document No(s): _____
- ☐ Information Disclosure Statement w/PTO 1449 ☐ Copy of Citations
- ☐ Preliminary Amendment
- ☐ Sequence Listing Diskette and Declaration
- ☐ Request and Certification under 35 U.S.C. § 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35
- ☐ Return Postcard

III. U.S. PRIORITY:

The present application claims priority to U.S. Provisional Application No. 61/636,256, filed April 20, 2012, the disclosure of which is incorporated by reference herein in its entirety, including drawings.

IV. FEES:

- ☒ Applicant claims small entity status pursuant to 37 CFR § 1.27
- ☒ This application is being filed without fee or Declaration under 37 CFR § 1.53.

V. CORRESPONDENCE ADDRESS

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METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 61/636,256, filed April 20, 2012, the disclosure of which is incorporated by reference herein in its entirety, including drawings.

BACKGROUND

[0002] Nitrogen retention disorders associated with elevated ammonia levels include urea cycle disorders (UCDs), hepatic encephalopathy (HE), and advanced kidney disease or kidney failure, often referred to as end-stage renal disease (ESRD).

[0003] UCDs include several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia, including enzymes involved in the urea cycle. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. With reference to Figure 1, N-acetyl glutamine synthetase (NAGS)-derived *N*-acetylglutamate binds to carbamyl phosphate synthetase (CPS), which activates CPS and results in the conversion of ammonia and bicarbonate to carbamyl phosphate. In turn, carbamyl phosphate reacts with ornithine to produce citrulline in a reaction mediated by ornithine transcarbamylase (OTC). A second molecule of waste nitrogen is incorporated into the urea cycle in the next reaction, mediated by arginosuccinate synthetase (ASS), in which citrulline is condensed with aspartic acid to form argininosuccinic acid. Argininosuccinic acid is cleaved by argininosuccinic lyase (ASL) to produce arginine and fumarate. In the final reaction of the urea cycle, arginase (ARG) cleaves arginine to produce ornithine and urea. Of the two atoms of nitrogen incorporated into urea, one originates from free ammonia (NH_4^+) and the other from aspartate. UCD individuals born with no meaningful residual urea synthetic capacity typically present in the first few days of life (neonatal presentation). Individuals with residual function typically present later in childhood or even in adulthood, and symptoms may be precipitated by increased dietary protein or physiological stress (*e.g.*, intercurrent illness). For UCD patients, lowering blood ammonia is the cornerstone of treatment.

[0004] HE refers to a spectrum of neurologic signs and symptoms believed to result from hyperammonemia, which frequently occur in subjects with cirrhosis or certain other types of liver

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disease. HE is a common manifestation of clinically decompensated liver disease and most commonly results from liver cirrhosis with diverse etiologies that include excessive alcohol use, hepatitis B or C virus infection, autoimmune liver disease, or chronic cholestatic disorders such as primary biliary cirrhosis. Patients with HE typically show altered mental status ranging from subtle changes to coma, features similar to patients with UCDs. It is believed that an increase in blood ammonia due to dysfunctional liver in detoxifying dietary protein is the main pathophysiology associated with HE (Ong 2003).

[0005] ESRD results from a variety of causes including diabetes, hypertension, and hereditary disorders. ESRD is manifested by accumulation in the bloodstream of substances normally excreted in the urine, including but not limited to urea and creatinine. This accumulation in the bloodstream of substances, including toxins, normally excreted in the urine is generally believed to result in the clinical manifestations of ESRD, sometimes referred to also as uremia or uremic syndrome. ESRD is ordinarily treated by dialysis or kidney transplantation. To the extent that urea, per se, contributes to these manifestations and that administration of a phenylacetic (PAA) prodrug may decrease synthesis of urea (see, e.g., Brusilow 1993) and hence lower blood urea concentration, PAA prodrug administration may be beneficial for patients with ESRD.

[0006] Subjects with nitrogen retention disorders whose ammonia levels and/or symptoms are not adequately controlled by dietary restriction of protein and/or dietary supplements are generally treated with nitrogen scavenging agents such as sodium phenylbutyrate (NaPBA, approved in the United States as BUPHENYL® and in Europe as AMMONAPS®), sodium benzoate, or a combination of sodium phenylacetate and sodium benzoate (AMMONUL®). These are often referred to as alternate pathway drugs because they provide the body with an alternate pathway to urea for excretion of waste nitrogen (Brusilow 1980; Brusilow 1991). NaPBA is a PAA prodrug. Another nitrogen scavenging drug currently in development for the treatment of nitrogen retention disorders is glyceryl tri-[4-phenylbutyrate] (HPN-100), which is described in U.S. Patent No. 5,968,979. HPN-100, which is commonly referred to as GT4P or glycerol PBA, is a prodrug of PBA and a pre-prodrug of PAA. The difference between HPN-100 and NaPBA with respect to metabolism is that HPN-100 is a triglyceride and requires digestion, presumably by pancreatic

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lipases, to release PBA (McGuire 2010), while NaPBA is a salt and is readily hydrolyzed after absorption to release PBA.

[0007] HPN-100 and NaPBA share the same general mechanism of action: PBA is converted to PAA via beta oxidation, and PAA is conjugated enzymatically with glutamine to form phenylacetylglutamine (PAGN), which is excreted in the urine. The structures of PBA, PAA, and PAGN are set forth below:

$CO_2^-Na^+$

phenylbutyrate

OH

O

Phenylacetic acid

O NH$_2$

O

HO

N

H

O

Phenylacetylglutamine

[0008] The clinical benefit of NaPBA and HPN-100 with regard to nitrogen retention disorders derives from the ability of PAGN to effectively replace urea as a vehicle for waste nitrogen excretion and/or to reduce the need for urea synthesis (Brusilow 1991; Brusilow 1993). Because each glutamine contains two molecules of nitrogen, the body rids itself of two waste nitrogen atoms for every molecule of PAGN excreted in the urine. Therefore, two equivalents of nitrogen are removed for each mole of PAA converted to PAGN. PAGN represents the predominant terminal metabolite, and one that is stoichiometrically related to waste nitrogen removal, a measure of efficacy in the case of nitrogen retention states.

[0009] In addition to nitrogen retention states, PAA prodrugs may be beneficial in a variety of other disorders for which PBA and/or PAA are believed to modify gene expression and/or exert post-translational effects on protein function. In the case of maple syrup urine disease (MSUD, also

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known as branched-chain ketoaciduria), for example, the apparently beneficial effect of NaPBA in lowering plasma levels of branched chain amino acids is reported to be mediated by PBA-induced inhibition of the kinase that regulates activity of branched chain alpha-keto acid dehydrogenase complex or BCKDC. BCKDC is the enzyme that normally breaks down branched-chain amino acids and is genetically defective in MSUD patients (Bruneti-Pieri 2011). Similarly, the putative beneficial effects of PAA prodrugs for the treatment of cancer (Chung 2000), neurodegenerative diseases (Ryu 2005), and sickle cell disease (Perrine 2008) all involve alteration of gene expression and/or post-translational effects on protein function via PBA and/or PAA.

[0010] Numerous publications reports adverse events following administration of PBA and/or PAA (Mokhtarani 2012), and PAA is reported to cause reversible toxicity when present in high levels in circulation. While many of these publications have not recorded PAA blood levels and/or temporally correlated adverse events with PAA levels, toxicities such as nausea, headache, emesis, fatigue, weakness, lethargy, somnolence, dizziness, slurred speech, memory loss, confusion, and disorientation have been shown to be temporally associated with PAA levels ranging from 499–1285 μg/mL in cancer patients receiving PAA intravenously, and these toxicities have been shown to resolve with discontinuation of PAA administration (Thiebault 1994; Thiebault 1995). Therefore, when administering PAA prodrugs for treatment of nitrogen retention disorders and other conditions, it is important to optimize dosing so as to achieve the desired therapeutic effect while minimizing the risk of PAA associated toxicity.

SUMMARY

[0011] Provided herein is a clinically practical approach for utilizing and interpreting blood levels of PAA and PAGN to adjust the dose of a PAA prodrug in order to minimize the risk of toxicities and maximize drug effectiveness.

[0012] Provided herein in certain embodiments are methods of treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject comprising the steps of administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In

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certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, the nitrogen retention disorder is UCD, HE, or ESRD. In certain embodiments, the condition for which PAA prodrug administration is expected to be beneficial is cancer, a neurodegenerative diseases, a metabolic disorder, or sickle cell disease. In certain embodiments, the PAA prodrug is HPN-100 or NaPBA. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0013] Provided herein in certain embodiments are methods of treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject who has previously received a first dosage of PAA prodrug comprising the steps of measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining

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whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, the nitrogen retention disorder is UCD, HE, or ESRD. In certain embodiments, the condition for which PAA prodrug administration is expected to be beneficial is cancer, a neurodegenerative diseases, a metabolic disorder, or sickle cell disease. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0014] Provided herein in certain embodiments are methods of adjusting the dosage of a PAA prodrug to be administered to a subject comprising the steps of administering a first dosage of a

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PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0015] Provided herein in certain embodiments are methods of determining whether a first dosage of a PAA prodrug can be safely administered to a subject comprising the steps of administering the first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the first dosage can be safely

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administered based on whether the PAA:PAGN ratio falls above a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the first dosage is unsafe and needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the first dosage is potentially unsafe and may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 2 to 2.5 where the target range is 1 to 2.5) indicates that the first dosage is likely safe, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0016] Provided herein in certain embodiments are methods of determining whether a first dosage of a PAA prodrug is likely to be effective for treating a nitrogen retention disorder or another disorder for which PAA prodrug administration is expected to be beneficial comprising the steps of administering the first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the first dosage is likely to be effective based on whether the PAA:PAGN ratio falls below a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the first dosage is unlikely to be effective needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the first dosage is potentially ineffective and may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain

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embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 where the target range is 1 to 2.5) indicates that the first dosage is likely effective, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0017] In certain embodiments, methods are provided for optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been adminsitered a first dosage of PAA prodrug comprising the steps of measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased. In other embodiments, a PAA:PAGN ratio above the target range indicates that the dosage may need to be decreased, with the final determination of whether to decrease the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In other embodiments, a PAA:PAGN ratio below the target range indicates that the dosage may need to be increased, with the final determination of whether to increase the dosage taking into account other characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. In certain embodiments, a PAA:PAGN ratio that is within the target range but within a particular subrange (e.g., 1 to 1.5 or 2 to 2.5 where the target range is 1 to 2.5) indicates that the dosage of the PAA prodrug does not need to be adjusted, but that the subject needs to be subjected to more frequent monitoring. In certain embodiments, the methods further comprise a step of administering an adjusted second dosage if

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such an adjustment is determined to be necessary based on the PAA:PAGN ratio and, optionally, other characteristics of the subject. In other embodiments, the methods further comprise a step of administering a second dosage that is the same as or nearly the same as the first dosage if no adjustment in dosage is deemed to be necessary. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

[0018] In certain embodiments, methods are provided for obtaining a plasma PAA:PAGN ratio within a target range in a subject comprising the steps of administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating a plasma PAA:PAGN ratio, and determining whether the PAA:PAGN ratio falls within the target range. If the PAA:PAGN ratio does not fall within the target range, an adjusted second dosage is administered, and these steps are repeated until a plasma PAA:PAGN ratio falling within the target range is achieved. In certain embodiments, the target range is 1 to 2.5, 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain embodiments, a PAA:PAGN ratio above the target range indicates that the dosage of the PAA prodrug needs to be decreased and a PAA:PAGN ratio below the target range indicates that the dosage of the PAA prodrug needs to be increased. In certain embodiments, measurement of plasma PAA and PAGN levels takes place after the first dosage of the PAA prodrug has had sufficient time to reach steady state, such as at 48 hours to 1 week after administration.

BRIEF DESCRIPTION OF DRAWINGS

[0019] Figure 1: Urea cycle.

[0020] Figure 2: Plasma PAA levels versus plasma PAA:PAGN ratio in (A) all subjects combined (healthy adults, patients age 2 months and above with UCDs, and patients with cirrhosis), (B) patients age 2 months and above with UCDs, and (C) patients with cirrhosis.

[0021] Figure 3: Estimated probability (95% confidence interval (c.i.)) of correctly detecting elevated plasma PAA:PAGN ratio ($\geq$2.0) with a single blood sample at a designated time.

[0022] Figure 4:Distribution of plasma PAA:PAGN ratio (log scale) by time since dosing (hours) and category of maximum PAA:PAGN ratio in all subjects combined.

[0023] Figure 5: Distribution of plasma PAA concentrations (μg/mL) by PAA:PAGN ratio for (A) all subjects and (B) UCD and HE subjects.

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DETAILED DESCRIPTION

[0024] The following description of the invention is merely intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

[0025] The enzymes responsible for beta oxidation of PBA to PAA are present in most cell types capable of utilizing fatty acids as energy substrates, and the widespread distribution of these enzymes presumably accounts for the rapid and essentially complete conversion of PBA to PAA. However, the enzymes that conjugate PAA with glutamine to form PAGN are found primarily in the liver and to a lesser extend in kidneys (Moldave 1957). Therefore, the conversion of PAA to PAGN may be affected under several circumstances, including the following: a) if conjugation capacity is saturated (e.g., by high doses of PAA prodrug); b) if conjugation capacity is compromised (e.g., by severe hepatic and/or renal dysfunction); c) if the substrate (glutamine) for PAA to PAGN conjugation is rate limiting; d) genetically determined variability (i.e., polymorphisms) in the enzymes responsible for PAA to PAGN conversion, or e) in young children, since the capacity to convert PAA to PAGN varies with body size measured as body surface area (Monteleone 2012). The presence of any one of these conditions may lead to accumulation of PAA in the body, which causes reversible toxicity.

[0026] The goal of PAA prodrug administration in subjects with nitrogen retention disorders is to provide a sufficient dosage to obtain a desired level of nitrogen removal while avoiding excess build-up of PAA. The goal of PAA prodrug administration in patients without a nitrogen retention disorder (e.g., a neurodegenerative disease) is to achieve circulating metabolite levels necessary to produce a clinical benefit by alteration of gene expression and/or protein folding or function. However, there are several difficulties associated with determining the proper dosage in patients with nitrogen retention disorders.

[0027] Plasma PAA and PAGN levels are affected by various factors, including timing of the blood draw in relation to drug administration, hepatic function, availability of metabolizing enzymes, and availability of substrates required for metabolism. A random PAA level drawn during

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an outpatient visit to determine if levels are in the toxicity range without considering concomitant PAGN level is insufficient to inform dosing. First, PAA levels vary many-fold over the course of the day, fluctuating a great deal between peak and trough levels. For example, in the Hyperion pivotal study evaluating HPN-100 for use in treating adult UCD (Study ID HPN-100-006, Clinical Trials ID NCT00992459), serial blood samples were obtained for PK studies over a 24 hour period during which subjects were receiving HPN-100 or NaPBA. The fluctuation index for PAA over a 24 hour period, which represents the fluctuation between maximum concentration (typically observed after the last daily dose or at approximately 12 hours) and minimum concentration (typically observed in the morning after overnight fasting or at 0 hours), indicated a very high degree of variability (2150% for NaPBA and 1368% for HPN-100). Therefore, a single plasma PAA level may not be representative of the highest PAA level a patient may experience during the day. Second, a high plasma PAA level may only be indicative of the high doses a subject is receiving rather than a point of concern if the subject is effectively conjugating PAA with glutamine to form PAGN. Therefore, basing dose adjustment on only on a high PAA level without considering concomitant plasma PAGN level may result in unnecessary dose reduction and under-treatment of the patient. Conversely, a PAA level seemingly below the levels associated with toxicity might be taken as an indication of satisfactory dosing without appreciating the fact that the concomitant PAGN level may not be proportional to PAA, indicating that PAA is not being efficiently utilized and may be accumulating.

[0028] Previous studies have shown that conversion of PAA to PAGN is a saturable process that varies considerably among individuals (see, e.g., Monteleone 2012), and that patients with hepatic impairment have higher PAA levels than patients without hepatic impairment (Ghabril et al., "Glycerol phenylbutyrate (GPD) administration in patients with cirrhosis and episodic hepatic encephalopathy (HE)," submitted to Digestive Disease Week, 2012). If PAGN formation is affected by any of the above factors, PAA will be accumulated and waste nitrogen may not be removed from the body. Previous studies have also shown that a small proportion of individuals, including both healthy adults ad patients with UCDs or HE, have higher PAA levels than the remainder of the population, presumably due to individual differences in conjugating PAA to PAGN, and that PAA levels fluctuate many-fold during the day depending on the dose and the timing of blood sample

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relative to the last dose so that a single plasma level may not be informative (Lee 2010; Lichter 2011).

[0029] Although the goal of PAA prodrug therapy for nitrogen retention disorders is to achieve ammonia levels within a normal limit, there is no correlation between plasma PAA levels and blood ammonia. Nitrogen retention disorder subjects are normally "dosed to effect," meaning that subjects with absent or severely deficient urea synthetic capacity require higher doses of PAA prodrugs than do mildly deficient UCD patients. These higher dosages are generally associated with higher PAA levels, such that the conventional PK/PD response (higher active moiety, i.e., PAA, correlates with lower harmful substance, i.e., ammonia) does not apply. Therefore, there is no single target plasma PAA level that can be applied to patients with UCDs or other nitrogen retention disorders based on their blood ammonia.

[0030] Patients with severe hepatic impairment are at increased risk of PAA accumulation due to inadequate levels of PAA conjugating enzymes if treated with PAA-prodrugs. UCD patients without hepatic impairment whose PAA conjugating enzymes are readily saturated are also at increased risk of PAA accumulation if treated with PAA-producing compounds. Other patients without nitrogen retention are at increased risk of PAA accumulation due to limited availability of glutamine as the substrate to form PAGN if treated with PAA-producing compounds, which accumulates in patients with nitrogen retention states.

[0031] WO09/134460 and WO10/025303 disclose methods for determining an effective dosage of a PAA prodrug based on urinary PAGN levels, which was found to be a more reliable indictor of effective dosage than plasma levels of PAA or other metabolites. Although such measurements are highly useful for evaluating waste nitrogen removal, they do not provide complete information regarding a subject's ability to utilize the prodrug.

[0032] Since PAA, PAGN, and ammonia levels do not provide the information necessary to determine whether a subject is effectively converting PBA to PAGN (i.e., effectively utilizing the PAA prodrug), there is a need for improved methods of adjusting PAA prodrug dosage and incorporating such adjustments into methods of treating nitrogen retention disorders.

[0033] As disclosed herein, plasma PAA:PAGN ratio has been found to provide an unexpectedly accurate measure of PAA prodrug metabolism in subjects with nitrogen retention

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disorders and/or hepatic impairment. It was found that subjects who can readily convert PAA to PAGN and have not reached the saturation point with respect to PAA to PAGN conversion will have a plasma PAA:PAGN ratio of 2.5 or below (when both are measured in µg/mL), and that subjects with PAA:PAGN ratios above 2.5 have a significantly higher chance of experience a PAA level above 400 µg/mL or 500 µg/mL over a 24 hour period. A PAA/PAGN ratio of less than 2.5 was associated primarily with healthy adult or adolescent subjects and normal liver function, with subjects having a ratio below 2.5 exhibiting a 1% probability of experiencing a PAA level greater than 400 µg/mL and almost no chance of exhibiting a PAA level greater than 500 µg/mL at any point during a 24 hour period. A ratio greater than 2.5, on the other hand, was generally seen in subjects with moderate hepatic impairment, a subset of healthy subjects or UCD patients with relatively lower saturation point and difficulty conjugating PAA to form PAGN, and patients with a low body surface area. Subjects with a ratio greater than 2.5, on the other hand, exhibited a 20-36% likelihood of experiencing a PAA level greater than 400 µg/mL during the day, and an approximately 10% likelihood of experiencing a PAA level of 500 µg/L or greater. In subjects with a ratio greater than 3, the likelihood of experiencing a PAA level higher than 500 µg/mL increased to as high as 25%. These results show that a plasma PAA:PAGN ratio exceeding 2.5 in a patient with unexplained neurological adverse events and normal ammonia indicates that dosage adjustment should be considered. Thus, plasma PAA:PAGN ratio provides a clinically useful surrogate for evaluating the efficiency of PAA to PAGN conversion.

[0034] Plasma PAA:PAGN ratio indicates whether a PAA prodrug is being effectively utilized and scavenging nitrogen, and therefore provides an indirect and simple measure of saturation of conjugating enzymes, availability of substrate, and possible effect of hepatic or renal impairment on this process. Calculating this ratio will allow effective treatment and dose adjustment in subjects with known hepatic impairment, subjects presenting with signs and symptoms overlapping between hyperammonemia and PAA toxicities, and subjects who are not clinically controlled despite increasing the dosage of drugs.

[0035] One of ordinary skill in the art would generally not consider the ratio of an active metabolite such as PAA to a terminal metabolite such as PAGN when making therapeutic decisions because they would expect that higher levels of the active metabolite would result in a

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proportionately higher response (as measured by PAGN production) and increased efficacy (i.e., waste nitrogen removal). However, the results provided herein show that the use of plasma PAA:PAGN ratios to evaluate and adjust PAA prodrug dosage is unexpectedly superior to the use of PAA or PAGN levels alone. Once a subject exceeds a specific PAA:PAGN ratio, there is a high likelihood that they are not effectively utilizing the active moiety and that further increasing PAA prodrug dosage may not increase efficacy and may actually result in PAA accumulation and toxicity.

[0036] Based on these findings, methods are provided herein for treating nitrogen retention disorders and evaluating and adjusting the dosage of a PAA prodrug based on plasma PAA:PAGN ratio. Generally, these methods comprise steps of measuring plasma PAA and PAGN levels, calculating the PAA:PAGN ratio, and determining whether the ratio falls within a target range, with this determination being used at least in part to decide whether to adjust PAA prodrug dosage. In these methods, PAA:PAGN ratio can be used to ensure that urinary PAGN output, plasma ammonia concentration, and/or PAA levels fall within a predefined target range. Such methods represent an improvement over previously developed methods for evaluating PAA prodrug dosage and efficacy in that they allow for more accurate dosing, greater efficacy, and decreased risk of toxicity associated with PAA accumulation.

[0037] Disclosed herein are target ranges for the ratio of plasma PAA to PAGN in subjects who are receiving PAA prodrug therapy. In certain embodiments, a subject exhibiting a PAA:PAGN ratio falling within a target range is classified as properly dosed, meaning that they do not require a PAA prodrug dosage adjustment, while a subject exhibiting a PAA:PAGN ratio falling outside the target range is classified as improperly dosed, meaning that they require an adjustment in PAA prodrug dosage. In certain of these embodiments, a subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as requiring an increased dosage of PAA prodrug. In other embodiments, a subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as potentially requiring an increase in PAA prodrug dosage. In still other embodiments, a

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subject exhibiting a plasma PAA:PAGN ratio falling above a target range is classified as potentially requiring a decreased dosage of PAA prodrug, while a subject exhibiting a plasma PAA:PAGN ratio falling below a target range is classified as potentially requiring an increase in PAA prodrug dosage. In those embodiments where a subject is classified as potentially requiring an increase or decrease in PAA prodrug dosage based on their PAA:PAGN ratio, a decision as to whether to increase or decrease dosage may be based on one or more additional characteristics of the subject such as biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health.

[0038] In certain embodiments, the target range for plasma PAA:PAGN ratio is 1 to 2.5, meaning that a subject exhibiting a PAA:PAGN falling within this range is classified as properly dosed. In other embodiments, the target range for plasma PAA:PAGN ratio is 1 to 2, 1 to 1.5, 1.5 to 2, or 1.5 to 2.5. In certain of those embodiments where the target range is 1 to 2.5, a subject with a PAA:PAGN ratio above 2.5 is classified as requiring a decrease in PAA prodrug dosage, while a subject with a PAA:PAGN ratio falling below 1 is classified as potentially requiring an increase in PAA prodrug dosage. In certain of these embodiments, a subject is necessarily classified as requiring an increase in PAA prodrug dosage if their ratio is below 1. In other embodiments, a subject with a PAA:PAGN ratio of less than 1 is only classified as requiring an increase in PAA prodrug dosage if one or more additional clinical or biochemical characteristics are satisfied (e.g., the subject is exhibiting severe symptoms of a nitrogen retention disorder).

[0039] In certain embodiments, the target range for plasma PAA:PAGN ratio may comprise one or more subranges, with subjects falling within different subranges being treated differently despite falling within the target range. For example, where a target range is 1 to 2.5, a subject exhibiting a PAA:PAGN ratio below 1 or above 2.5 may be classified as requiring an adjustment in PAA prodrug dosage. Within the target range, subjects with a PAA:PAGN ratio falling within a particular subrange may be treated as properly dosed, improperly dosed (i.e., requiring a dosage adjustment), or properly dosed but requiring more frequent monitoring. For example, subjects having a PAA:PAGN ratio greater than 2 but not greater than 2.5 may be classified as properly dosed but requiring more frequent monitoring.

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[0040] In certain embodiments, subrange boundaries or the treatment of subjects falling within a particular subrange will depend in part on a subject's specific characteristics, including for example biochemical profile or clinical characteristics such as target nitrogen excretion, actual nitrogen excretion, symptom severity, disorder duration, age, or overall health. For example, in certain embodiments a first subject with a PAA:PAGN ratio falling within the subrange of 2 to 2.5 may be classified as properly dosed but requiring frequent monitoring, while a second subject falling within the same subrange may be classified as requiring a decreased dosage of PAA prodrug. Similarly, a first subject with a PAA:PAGN ratio falling within the subrange of 1 to 1.5 may be classified as properly dosed but requiring frequent monitoring, while a second subject falling within the same subrange may be classified as requiring an increased dosage of PAA prodrug. For example, a subject who has recently exhibited particularly acute symptoms associated with a particular disorder may be classified as requiring an increased dosage of PAA prodrug when exhibiting a PAA:PAGN ratio of 1 to 1.5, while a subject who is clinically controlled may be classified as properly dosed despite a ratio falling within the same subrange.

[0041] In certain embodiments, methods are provided herein for treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject that has previously received a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more other characteristics of the subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the

subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0042] In certain embodiments, methods are provided for treating a nitrogen retention disorder or a condition for which PAA prodrug administration is expected to be beneficial in a subject that has not previously been administered a PAA prodrug. These methods comprise administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the

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second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more additional characteristics of the subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific clinical or biochemical characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0043] A method of administering a PAA prodrug to a subject with a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. These methods comprise administering a first dosage of the PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug

dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the second dosage is greater than the first dosage if the PAA:PAGN ratio is less than 1 (i.e., the dosage is increased) and less than the first dosage if the PAA:PAGN ratio is greater than 2.5 (i.e., the dosage is decreased). In other embodiments, the second dosage may or may not be greater than the first dosage if the PAA:PAGN ratio is less than 1, depending on one or more additional characteristics of the subject. In certain embodiments, the second dosage is equal to the first dosage when the PAA:PAGN ratio is 1 to 2.5, i.e., falling within the target range. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the second dosage may be equal to the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the second dosage may be greater than the first dosage if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, the second dosage may be less than the first dosage if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, determining whether the PAA

prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within the target range, and administering a third dosage of the PAA prodrug.

[0044] In certain embodiments, methods are provided herein for achieving a target plasma PAA:PAGN ratio in a subject with a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. These methods comprise administering a first dosage of a PAA prodrug, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, and administering a second dosage of the PAA prodrug based on the PAA:PAGN ratio. If the PAA:PAGN ratio is above the target range, the second dosage is less than the first dosage. If the PAA:PAGN ratio is below the target range, the second dosage is greater than the first dosage. These steps are repeated until a target plasma PAA:PAGN ratio is achieved. In certain embodiments, the target ratio falls within a target range of 1 to 2.5 or 1 to 2. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration).

[0045] In certain embodiments, methods are provided for evaluating the dosage of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the first dosage of the PAA prodrug is effective based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments, the first dosage is considered too low if the PAA:PAGN ratio is less than 1, and too high if the PAA:PAGN ratio is greater than 2.5. In other embodiments, the first dosage is considered potentially too low if

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PAA:PAGN ratio is less than 1, with a final decision depending on one or more additional characteristics of the subject. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, the first dosage is considered potentially effective if the PAA:PAGN ratio is 1 to 1.5 or 2 to 2.5, but the subject may be subjected to more frequent monitoring. In certain other embodiments, the first dosage may be considered too low if the PAA:PAGN ratio is 1 to 1.5 or 1 to 2 and the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments the first dosage may be considered too high if the PAA:PAGN ratio is greater than 1.5 or 2 but not greater than 2.5, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the methods further comprise a step of administering a second dosage that differs from the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that differs from the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug is effective based on whether the PAA:PAGN ratio falls within a target range.

[0046] In certain embodiments, methods are provided for adjusting the dosage of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug. These methods comprise measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether to adjust the dosage of the PAA prodrug based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio of less than 1 indicates the PAA prodrug dosage needs to be adjusted upwards, while a PAA:PAGN ratio above 2.5 indicates the PAA prodrug dosage needs to be adjusted downwards. In other embodiments, a PAA:PAGN ratio of less than 1 indicates that the

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PAA prodrug dosage potentially needs to be adjusted upwards, with a final decision depending on one or more additional characteristics of the subject. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 or 2 to 2.5 indicates that the dosage need not be adjusted, but that the subject should be subjected to more frequent monitoring. In certain other embodiments, a PAA:PAGN ratio of 1 to 1.5 or 1 to 2 indicates that the dosage needs to be increased when the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments a PAA:PAGN ratio greater than 1.5 or 2 but not greater than 2.5 may indicate that the dosage needs to be decreased, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the dosage needs to be adjusted, the methods further comprise a step of administering a second dosage that differs from the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that differs from the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range. In certain embodiments, the increase or decrease in the second dosage versus the first dosage depends on the precise plasma PAA:PAGN ratio. For example, where the plasma PAA:PAGN ratio is only slightly less than 1, the dosage may be increased only slightly, but where the PAA:PAGN ratio is significantly less than 1, the dosage may be increased more. Similarly, the decrease in dosage for subjects exhibiting a ratio above 2.5 may vary depending on how far above 2.5 the ratio extends.

[0047] In certain embodiments, methods are provided for optimizing the therapeutic efficacy of a PAA prodrug for use in treating a nitrogen retention disorder in a subject. These methods comprise measuring plasma PAA and PAGN levels in a subject who has previously been

administered a PAA prodrug, calculating the plasma PAA:PAGN ratio, determining whether to adjust the dosage of the PAA prodrug based on whether the PAA:PAGN ratio falls within a target range, and administering an adjusted dosage of the PAA prodrug as necessary. These steps are repeated until the subject exhibits a plasma PAA:PAGN ratio falling within the target range (e.g., 1 to 2.5 or 1 to 2). In certain embodiments where the target range is 1 to 2.5, a plasma PAA:PAGN ratio of less than 1 indicates that the dosage needs to be adjusted upwards, while a ratio greater than 2.5 indicates that the dosage needs to be decreased. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 or 2 to 2.5 indicates that the dosage does not need to be adjusted, but that the subject should be subjected to more frequent monitoring. In certain other embodiments, a PAA:PAGN ratio of 1 to 1.5 or 1 to 2 indicates that the dosage needs to be increased when the subject has recently exhibited particularly acute symptoms of a nitrogen retention disorder or another condition for which PAA prodrug administration is expected to be beneficial. Similarly, in certain embodiments a PAA:PAGN ratio greater than 1.5 or 2 but not greater than 2.5 may indicate that the dosage needs to be decreased, depending on the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments, the magnitude of the increase or decrease in dosage may be based on the precise PAA:PAGN ratio. For example, a PAA:PAGN ratio that is slightly less than 1 may indicate that the dosage needs to be increased slightly, while a ratio significantly less than 1 may indicate the dosage needs to be increased to a greater degree. In certain embodiments, the above steps are repeated until the subject exhibits a PAA:PAGN ratio falling within the target range.

[0048] In certain embodiments, methods are provided for determining whether a prescribed first dosage of a PAA prodrug can be safely administered to a subject. These methods comprise administering the prescribed first dosage to the subject, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the prescribed first dosage is safe for the subject based on whether the PAA:PAGN ratio falls above a target range, wherein a PAA:PAGN ratio falling above the target range indicates that the first dosage cannot be or

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potentially cannot be safely administered to the subject. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio above 2.5 indicates the PAA prodrug dosage is unsafe and needs to be adjusted downwards. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 2 to 2.5 indicates that the first dosage is safe, but that the subject should be subjected to more frequent monitoring. In other embodiments, a PAA:PAGN ratio of 2 to 2.5 indicates that the first dosage is potentially unsafe, with a final determination of safety taking into account the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the first dosage is unsafe and needs to be decreased, the methods further comprise a step of administering a second dosage that is lower than the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that is lower than the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug can be safely administered to the subject based on whether the PAA:PAGN ratio falls above a target range.

[0049] In certain embodiments, methods are provided for determining whether a prescribed first dosage of a PAA prodrug will be effective for treating a nitrogen retention disorder or another disorder for which PAA prodrug administration is expected to be beneficial. These methods comprise administering the prescribed first dosage to the subject, measuring plasma PAA and PAGN levels, calculating the plasma PAA:PAGN ratio, and determining whether the prescribed first dosage will be effective for the subject based on whether the PAA:PAGN ratio falls below a target range, wherein a PAA:PAGN ratio falling below the target range indicates that the first dosage will not be or potentially will not be effective for treating a disorder. In certain embodiments, the target range for PAA:PAGN ratio is 1 to 2.5 or 1 to 2. In certain of these embodiments where the target range is 1 to 2.5, a PAA:PAGN ratio below 1 indicates the PAA

prodrug dosage is unlikely to be effective and needs to be adjusted upwards. In other embodiments, a PAA:PAGN ratio below 1 indicates that the first dosage is potentially ineffective, with a final determination of whether the dosage is likely to be ineffective based on the subject's specific biochemical or clinical characteristics. In certain embodiments, the target range is divided into one or more subranges. In certain of these embodiments, a PAA:PAGN ratio of 1 to 1.5 indicates that the first dosage is likely to be effective, but that the subject should be subjected to more frequent monitoring. In other embodiments, a PAA:PAGN ratio of 1 to 1.5 indicates that the first dosage is potentially ineffective, with a final determination of whether the dosage is likely to be ineffective taking into account the subject's specific biochemical or clinical characteristics. In certain embodiments, measurement of plasma PAA and PAGN ratio takes place after the PAA prodrug has had sufficient time to reach steady state (e.g., 48 hours, 48 to 72 hours, 72 hours to 1 week, 1 week to 2 weeks, or greater than 2 weeks after PAA prodrug administration). In certain embodiments where a determination is made that the first dosage is likely to be ineffective and needs to be increased, the methods further comprise a step of administering a second dosage that is higher than the first dosage, and in certain of these embodiments the above steps may be repeated until a desired plasma PAA:PAGN ratio (e.g., 1 to 2.5 or 1 to 2) is achieved. For example, the methods may comprise administering a second dosage that is higher than the first dosage, measuring plasma PAA and PAGN levels after administration of the second dosage, calculating the plasma PAA:PAGN ratio, and determining whether the second dosage of the PAA prodrug is likely to be ineffective for treating a disorder based on whether the PAA:PAGN ratio falls above a target range.

[0050] Provided herein in certain embodiments are methods for monitoring therapy with a PAA prodrug in patients with a nitrogen retention disorder. These methods comprise administering a PAA prodrug to the subject, measuring plasma PAA and PAGN levels, and calculating the plasma PAA:PAGN ratio. In these methods, a PAA:PAGN ratio falling within a target range (e.g., 1 to 2.5 or 1 to 2) indicates that the therapy is effective, while a ratio falling outside this range indicates that the therapy may need to be adjusted. In certain embodiments, the plasma PAA:PAGN ratio is compared to a previously obtained PAA:PAGN ratio from the same subject to evaluate the effectiveness of PAA prodrug administration.

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[0051] In certain embodiments, the methods provided herein may be used in conjunction with the methods described in WO09/134460 and WO10/025303. In these embodiments, urinary PAGN levels may be determined in addition to plasma PAA:PAGN ratio, with both measurements being used to evaluate or adjust PAA prodrug dosage.

[0052] A "PAA prodrug" as used herein refers to any drug that contains or is converted to PAA following administration to a subject, or to any pharmaceutically acceptable salt, ester, acid, or derivative thereof. A PAA prodrug may be administered via any route, including oral or parenteral administration. A PAA prodrug may be converted directly to PAA (e.g., a salt or ester of PAA; PBA or a salt or ester thereof such as NaPBA), or it may be converted to PAA via an intermediate (e.g., a pre-prodrug such as HPN-100). Other examples of PAA prodrugs include butyroyloxymethyl-4-phenylbutyrate.

[0053] An adjustment to the dosage of a PAA prodrug as discussed herein may refer to a change in the amount of drug per administration (e.g., an increase from a first dosage of 3 mL to a second dosage of 6 mL), a change in the number of administration within a particular time period (e.g., an increase from once a day to twice a day), or any combination thereof.

[0054] A "subject in need thereof" as used herein refers to any individual having a condition or suspected of having a condition for which administration of a PAA prodrug is expected to be beneficial. For example, a subject may be an individual with a nitrogen retention disorder or suspected of having a nitrogen retention disorder, including for example UCD, HE, and/or kidney failure/ESRD (Lee 2010; McGuire 2010; Lichter 2011). Likewise, a subject may have or be suspected of having another condition for which PAA prodrug administration is expected to be beneficial, including for example cancer (Thiebault 1994; Thiebault 1995), neurodegenerative disorders such as Huntington's Disease (Hogarth 2007), amyotrophic lateral sclerosis (ALS) (Cudkowicz 2009), and spinal muscular atrophy (SMA) (Mercuri 2004; Brahe 2005), metabolic disorders (e.g., maple syrup urine disease (MSUD) (Bruneti-Pieri 2011), or sickle cell disease (Hines 2008).

[0055] A subject that has previously been administered a PAA prodrug may have been administered the drug for any duration of time sufficient to reach steady state. For example, the

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subject may have been administered the drug over a period of 2 to 7 days, 1 week to 2 weeks, 2 weeks to 4 weeks, 4 weeks to 8 weeks, 8 weeks to 16 weeks, or longer than 16 weeks.

[0056] A "PAA prodrug" as used herein refers to any drug that contains or is converted to PAA following administration to a subject, or to any pharmaceutically acceptable salt, ester, acid, or derivative thereof. A PAA prodrug may be administered via any route, including oral or parenteral administration. A PAA prodrug may be converted directly to PAA (e.g., PBA or a salt thereof such as NaPBA), or it may be converted to PAA via an intermediate (e.g., a pre-prodrug such as HPN-100). Other examples of PAA prodrugs include butyroyloxymethyl-4-phenylbutyrate.

[0057] An adjustment to the dosage of a PAA prodrug as discussed herein may refer to a change in the amount of drug per administration (e.g., an increase from a first dosage of 3 mL to a second dosage of 6 mL), a change in the number of administration within a particular time period (e.g., an increase from once a day to twice a day), or any combination thereof.

[0058] The terms "treat," "treating," or "treatment" as used herein may refer to preventing a disorder, slowing the onset or rate of development of a disorder, reducing the risk of developing a disorder, preventing or delaying the development of symptoms associated with a disorder, reducing or ending symptoms associated with a disorder, generating a complete or partial regression of a disorder, or some combination thereof. For example, where the disorder being treated is a nitrogen retention disorder, "treating" may refer to lowering waste nitrogen levels below a threshold level, preventing waste nitrogen levels from reaching a threshold level, decreasing the likelihood of waste nitrogen levels exceeding a threshold level, reducing or ending symptoms associated with elevated waste nitrogen levels, or a combination thereof.

[0059] With regard to the methods of treatment disclosed herein, interpretation of the PAA:PAGN ratio must be performed in the context of the therapeutic objective. For example, in subjects being treated for a nitrogen retention disorder, the therapeutic objective is elimination of waste nitrogen in the form of PAGN. In subjects being treated for other disorders for which PAA prodrug administration is expected to be beneficial (e.g., neurodegenerative disorders, MSUD), the therapeutic objective is safely achieving target plasma levels of PAA and/or PBA.

[0060] Any methods known in the art may be used to obtain a plasma blood sample. For example, blood from a subject may be drawn into a tube containing heparin or

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ethylenediaminetetraacetic acid (EDTA). In certain embodiments, the sample can be placed on ice and centrifuged to obtain plasma within 15 minutes of collection, stored at 2-8°C (36-46°F) and analyzed within 3 hours of collection. In other embodiments, the blood plasma sample is snap frozen, stored at ≤-18°C (≤0°F) and analyzed at a later time. For example, the sample may be analyzed at 0-12 hours, 12-24 hours, 24-48, 48-96 hours after freezing, or within any other timeframe over which the sample has demonstrated stability. In certain of these embodiments, the blood sample is stored at a temperature between 0-15°C, such as 2-8°C. In other embodiments, the blood sample is stored below 0°C or below -18°C.

[0061] Measurement of PAA and PAGN levels in a plasma sample is carried out using techniques known in the art. For example, PAA and PAGN levels may be measured using liquid chromatography/mass spec analyses.

[0062] Any combination of embodiments described herein can be envisioned. Although individual features may be included in different claims, these may be advantageously combined.

[0063] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the invention. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention. It will be understood that many variations can be made in the procedures herein described while still remaining within the bounds of the present invention. It is the intention of the inventors that such variations are included within the scope of the invention.

EXAMPLES

Example 1: Analysis of PAA:PAGN ratio in UCD and HE subjects:

[0064] Plasma PAA and PAGN levels and PAA:PAGN ratio were analyzed in more than 4000 plasma samples obtained from various clinical trials of healthy adults, severely hepatic impaired adults with clinically decompensated Child-Pugh B or C cirrhosis, and UCD patients ages 29 days or older. Healthy and hepatically impaired adults received HPN-100, while UCD subjects received both HPN-100 and NaPBA. Clinical trial populations are summarized in Tables 1 and 2.

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Table 1: Clinical studies and analysis populations

Study Group	Description	Demographics	Protocols Included	Analysis Populations
1	Short-term (<= 2-4 weeks) exposure in UCD subjects	Adults and children ages 29 days or greater (N=81)	UP 1204-003 HPN-100-005SO HPN-100-006 HPN-100-012	A, B
2	Long-term exposure in UCD and HE subjects	Adults and children ages 6 years or greater (N=180)	HPN-100-005SE HPN-100-007 HPN-100-008 Part B	A
3	Short-term (<= 4 weeks) exposure in hepatic impaired subjects	Adults (N=15)	HPN-100-008 Part A	A, B
4	Short-term exposure (<= 4 weeks) in healthy subjects	Adults (N=98)	HPN-100-010	A, B

Table 2: Demographics and number of samples used

	Attribute	**No. of subjects**		**No. of sample points (Population A)**		**No. of time-specific PK sample points (Population B)**	
		Count	Percent	Count	Percent	Count	Percent
Population	Healthy	86	17.0	2126	34.4	2126	38.5
	Hepatic Encephalopathy (HE)	103	20.4	830	13.4	830	15.0
	UCD	158	31.3	1616	26.1	1281	23.2
	Total	**347**	**100.0**	**4572**	**100.0**	**4237**	**100.0**
Age	29 days -< 6 yrs	15	4.3	110	2.4	110	2.6
	6 -< 18 yrs	47	13.5	373	8.2	213	5.0
	18+ yrs	285	82.1	4089	89.4	3914	92.4
Sex	F	199	57.3	2394	52.4	2152	50.8
	M	148	42.7	2178	47.6	2085	49.2

[0065] Analysis Population A consisted of quantifiable levels of PAA and PAGN metabolites derived from all studies described above. All PAA and PAGN levels used for analysis came from blood samples drawn once dosing with NaPBA or HPN-100 had reached steady state. Analysis Population B consisted of quantifiable levels of PAA and PAGN metabolites during studies in which pharmacokinetics were analyzed and for which blood draws were performed over 12 or 24 hours at steady state and for which the timing of the blood sample in relation to dosing was known. Subjects in study groups 1, 3 and 4 above contributed to these points. Analysis Population B was

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the source of analyses that examined how PAA levels changed with time relative to dosing, where dosing could have been with either NaPBA or HPN-100. To be eligible for Analysis Population B, the time of the blood draw relative to the time of initiation of dosing during the dosing period had to have been recorded.

[0066] Data on metabolite levels were pooled across a wide range of age levels- infants, toddlers, children, adolescents, and adults. All children, defined as ages under 18, were UCD patients. The majority of the blood sampling points came from adults (89.4%). Newborn infants (< 29 days old) were not studied in any of the clinical trials for the investigational agent HPN-100. The population of blood sampling points were roughly equally divided between female and male (57.3% female, 42.7% male).

[0067] To examine the predictive ability of PAA:PAGN ratios, a subject was considered to have achieved a high value of PAA if any PAA value up to 24 hours since initiation of dosing equaled or exceeded 400 μg/mL or equaled or exceeded 500 μg/mL. PAA:PAGN ratios were grouped into one of three categorization schemes: a.) [0-<= 2.0] , [> 2.0] , b.) [0- <= 2.5, > 2.5], c.) [0- <= 3.0, > 3.0]. The repeated measures categorical outcome was modeled using GEE with a logit link function, ratio category as the independent variable, and SUBJECTID as the repeated measures factor. Confidence intervals for the predicted probabilities were computed by bootstrap estimation of 1000 resamplings of the original data, as detailed in Davison & Hinkley, "Bootstrap Methods and Their Application," Cambridge Univ. Press (1997), pp. 358-362.

[0068] Results are summarized in Figures 2-5. A striking curvilinear relationship was observed between plasma PAA levels and PAA:PAGN ratio at any given timepoint. Figure 2A shows the relationship between the ratio of PAA:PAGN concentrations and absolute PAA levels in micrograms per milliliter among blood samples that had quantifiable values for both PAA and PAGN. The ratio axis (i.e. 'X' axis) is plotted on a logarithmic (base e) scale. For ratios less than 1.0, increases in ratio are not associated with correspondingly elevated or increased levels of PAA. Above ratios of 1.0, there is a gradual increase in PAA levels, and a noticeable upswing in PAA levels that begins in the vicinity of a ratio of 2.0. This finding suggests that when the ratio of PAA precursor to PAGN product approaches higher values, the values of PAA are also correspondingly

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high. This increase in the ratio of precursor (PAA) to product (PAGN) implies ineffective PAA to PAGN conversion, regardless of whether the PAA is derived from HPN-100 or NaPBA.

[0069] To determine whether excessive PAA build-up is a function of dosing, the plots mentioned above were repeated, but this time adjusting for assigned dose level of NaPBA or HPN-100 at the time of the blood draw. Since the UCD population consisted of a mixture of children and adults undergoing both short-term therapy and long-term therapy, total assigned daily dose for UCD patients was standardized to body surface area and reported in PBA-equivalent grams meter^2. Healthy and HE subjects were all adults and their assigned dose was not adjusted by body surface area. Dose levels for healthy and HE subjects were reported in HPN-100 equivalent mL. Dose levels for UCD subjects were reported in NaPBA-equivalent grams.

[0070] The excess of PAA over PAGN, indicated by larger ratios as PAA increases, was evident across all dosage groups, disease populations, and types of treatment in UCD patients (i.e., applies to both NaPBA and HPN-100). This finding suggests that analysis of the precursor (PAA) to product (PAGN) ratio may be predictive of the efficiency of conversion among patients with or without liver dysfunction (UCD patients have normal liver function apart from their urea cycle dysfunction) and independently of dose. As a corollary, the presence of liver dysfunction (e.g. cirrhosis) by itself, is not necessarily a reliable determinant of whether a particular patient is at risk for high PAA levels.

[0071] The ability of PAA:PAGN ratios to predict extremely high plasma PAA concentrations was determined by modeling the probability that a subject would exceed a PAA value of 400 or 500 μg/mL anytime during a 24 hour dosing period, based on the ratio of PAA to PAGN computed at pre-dose (presumably trough), 12 hours after dosing (presumably peak), and the maximum ratio encountered anytime between pre-dose and 12 hours post-dose. This interval of 0-12 hours was chosen for practical reasons, as it would encompass the entire interval corresponding to the usual outpatient visit.

[0072] Since subjects could have multiple dosing periods within a given clinical study, the probability was modeled using Generalized Estimating Equations. Three categorizations of ratios were modeled: a.) [0-<= 2.0] [> 2.0] , b.) [0- <= 2.5, > 2.5], c.) [0- <= 3.0, > 3.0]. The models were

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repeated with PAA values greater than or equal to 500 μg/mL considered extreme. Results are summarized in Table 3.

Table 3: Probabilities of extreme PAA values encountered during 24 hour PK sampling with PAA:PAGN ratios (all subjects combined)

PAA Value Considered High		Time of Blood Draw Used For Ratio Classification	Observed Ratio of PAA/PAGN	Probability that a Subject With This Ratio Will Exceed High Value* (%)	Bootstrapped 95% Confidence Interval**
[<=2.0, >2.0]	>=400 μg/mL	t=0 (fasting)	<= 2.0	0.005 (0.5%)	0.004, 0.020
[<=2.0, >2.0]	>=400 μg/mL	t=0 (fasting)	> 2.0	0.164 (16.4%)	0.041, 0.281
[<=2.0, >2.0]	>=400 μg/mL	t = 12 hours	<= 2.0	0.003 (0.3%)	0.004, 0.021
[<=2.0, >2.0]	>=400 μg/mL	t = 12 hours	> 2.0	0.227 (22.7%)	0.048, 0.412
[<=2.0, >2.0]	>=400 μg/mL	MAX(0-12)	<= 2.0	0.002 (0.2%)	0.004, 0.010
[<=2.0, >2.0]	>=400 μg/mL	MAX(0-12)	> 2.0	0.143 (14.3%)	0.036, 0.263
[<=2.0, >2.0]	>=500 μg/mL	t=0 (fasting)	<= 2.0 > 2.0	did not converge	
[<=2.0, >2.0]	>=500 μg/mL	t = 12 hours	<= 2.0 > 2.0	did not converge	
[<=2.0, >2.0]	>=500 μg/mL	MAX(0-12)	<= 2.0 > 2.0	did not converge	
[<=2.5, >2.5]	>=400 μg/mL	t=0 (fasting)	<= 2.5	0.008 (0.8%)	0.004, 0.023
[<=2.5, >2.5]	>=400 μg/mL	t=0 (fasting)	> 2.5	0.191 (19.1%)	0.053, 0.366
[<=2.5, >2.5]	>=400 μg/mL	t = 12 hours	<= 2.5	0.007 (0.7%)	0.004, 0.016
[<=2.5, >2.5]	>=400 μg/mL	t = 12 hours	> 2.5	0.364 (36.4%)	0.125, 0.752
[<=2.5, >2.5]	>=400 μg/mL	MAX(0-12)	<= 2.5	0.003 (0.3%)	0.004, 0.013
[<=2.5, >2.5]	>=400 μg/mL	MAX(0-12)	> 2.5	0.200 (20.0%)	0.050, 0.381
[<=2.5, >2.5]	>=500 μg/mL	t=0 (fasting)	<= 2.5	0.003 (0.3%)	0.004, 0.011
[<=2.5, >2.5]	>=500 μg/mL	t=0 (fasting)	> 2.5	0.084 (8.4%)	0.029, 0.214
[<=2.5, >2.5]	>=500 μg/mL	t = 12 hours	<= 2.5 > 2.5	did not converge	
[<=2.5, >2.5]	>=500 μg/mL	MAX(0-12)	<= 2.5 > 2.5	did not converge	
[<=3, >3]	>=400 μg/mL	t=0 (fasting)	<= 3.0	0.010 (1.0%)	0.004, 0.025
[<=3, >3]	>=400 μg/mL	t=0 (fasting)	> 3.0	0.205 (20.5%)	0.059, 0.398
[<=3, >3]	>=400 μg/mL	t = 12 hours	<= 3.0	0.013 (1.3%)	0.004, 0.028
[<=3, >3]	>=400 μg/mL	t = 12 hours	> 3.0	0.250 (25.0%)	0.113, 0.576
[<=3, >3]	>=400 μg/mL	MAX(0-12)	<= 3.0	0.003 (0.3%)	0.004, 0.014
[<=3, >3]	>=400 μg/mL	MAX(0-12)	> 3.0	0.229 (22.9%)	0.059, 0.438
[<=3, >3]	>=500 μg/mL	t=0 (fasting)	<= 3.0	0.003 (0.3%)	0.004, 0.010
[<=3, >3]	>=500 μg/mL	t=0 (fasting)	> 3.0	0.102 (10.2%)	0.032, 0.255
[<=3, >3]	>=500 μg/mL	t = 12 hours	<= 3.0 > 3.0	did not converge	
[<=3, >3]	>=500 μg/mL	MAX(0-12)	<= 3.0 > 3.0	did not converge	

Analysis repeated for each ratio cut off category independently.
* Probability derived from Generalized Estimating Equations model with logit link function.

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** Confidence interval derived from method disclosed in Davison & Hinkley, "Bootstrap Methods and Their Application," Cambridge Univ. Press (1997), pp. 358-362, using 1000 re-samplings of original data.

[0073] Because of the sparseness of samples in which PAA equaled or exceeded 500 μg/mL, 400 μg/mL proved to be a more stable and predictable target (i.e. high) value. Of the three categorizations of ratio considered, the cutpoint of 2.5 was the best discriminator and predictor of the risk of experiencing an high value. For example, referring to Table 3, a subject with a PAA:PAGN ratio > 2.5 at t=12 hours after dosing has a 36.4% chance (95% c. i.= 0.125, 0.752) of exceeding 400 μg/mL in PAA sometime during the 24-hour PK sampling period.

[0074] Results were similar whether the ratio was computed from plasma drawn at pre-dose, 12 hours after initiation of dosing, or the maximum ratio encountered anytime between pre-dose and 12 hours after initiation of dosing.

[0075] Due to the very high intra-day variability of plasma PAA levels, a PAA:PAGN ratio observed as exceeding 2.0 at a certain time following dosing may not remain greater than 2.0 in subsequent times. To evaluate the optimal time for obtaining a PAA:PAGN ratio measurement (i.e., the time that gives the greatest probability of correctly detecting a subject whose PAA:PAGN ratio ever equals or exceeds 2.0 during the dosing period), ratios were evaluated at 0 (pre-dose) and 2, 4, 6, 8, 10, and 12 hours post-dosing and modeled using GEE methodology. Pairwise differences in sensitivity between time points were evaluated using LS means and confidence intervals were computed.

[0076] Figure 3 plots the estimated probabilities of correctly detecting a ratio profile that ever equals of exceeds 2.0. With the exception of time= 2 hours and time=10 hours, time points of 0, 4, 6, 8, and 12 hours post-dosing were equally effective in detecting subjects who equal or exceed a PAA:PAGN ratio of 2.0 at some point during the dosing period. Sensitivities were in the range of 75-90 percent. There were too few blood samples collected at t=10 hours to analyze inter-time differences. Differences in predictive value were observed. For example, blood samples collected at t= 2 hours post-dosing had a significantly lower probability of detecting subjects who equal or exceed a PAA:PAGN ratio of 2.0 than samples collected at t=0 (p = 0.036), 4 (p = 0.032), or 6 hours (p = 0.017) post-dosing (p values are comparisons of t=2 hour probability with other time points). Similarly, a sample collected at t=12 hours following initiation of dosing had the highest probability (87%) of detecting a subject whose ratio ever equals or exceeds 2.0. However, for

practical clinical purposes, the differences in predictive value among time points was trivial relative to the dramatically greater variability in PAA values themselves, meaning that random blood draws can be used for measurement of PAA:PAGN ratio.

[0077] Further exploration of the fluctuation of PAA:PAGN ratios over time was conducted by dividing the subject population into cohorts according to the maximum PAA:PAGN ratio achieved during the 24-hour PK sampling time during the dosing period. Cohorts were divided into "low" (maximum ratio <= 2.0), "medium" (maximum ratio: 2.01-2.50), and "high" (maximum ratio > 2.50). Each cohort was then followed over time during the dosing period at t= 0 hours(pre-dose), 4, 6, and 8 hours post-dosing and the distribution of PAA:PAGN ratios within the cohort summarized using a box-and-whisker plot at each time point. This analysis was conducted for the PK-timepoint-specific population as a whole (analysis population B) as well as for each disease subpopulation separately.

[0078] Figure 4 plots the progression of ratios for all subjects combined. Each "panel" of the plot that divides the graphing space into thirds represents one cohort. Subjects in the high cohort had high ratios throughout the day and not only at a particular time point. Therefore, subjects in this cohort (n=73 subject/dosing periods) started with high ratios (median ratio > 2.5) and remained high throughout the first 12 hours. This finding is consistent with the findings plotted in Figure 3 which revealed the consistency of sensitivity in ratios.

[0079] The relationship between PAA levels and PAA:PAGN ratios was further analyzed by categorizing ratios into "low" (maximum ratio <= 2.0), "medium" (maximum ratio: 2.01-2.50), and "high" (maximum ratio > 2.50). Unlike the previous analysis, this analysis did not associate subject/dosing periods with particular cohorts (i.e., all samples and all time points are combined with regard to the subject or dosing period).

[0080] Figure 5A shows the box-and-whisker plots of PAA levels grouped by the above categories of PAA:PAGN ratio for all subjects, while Figure 5B shows the same for UCD and HE subjects only. The results were very similar in both analysis sets. Following a statistically significant overall Kruskal-Wallis test ($p < 0.0001$), pairwise comparisons of PAA levels were conducted using Wilcoxon-Mann-Whitney with a Bonferroni alpha correction of (0.0167). In both analysis sets, ratios greater than 2.5 had significantly higher PAA levels ($p < 0.001$) than either

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ratios between 2.0 – 2.5 or ratios less than 2.0. Furthermore, ratios between 2.0 – 2.5 were associated with significantly higher PAA levels than ratios less than 2.0 ($p < 0.001$).

Example 2: Analysis of PAA:PAGN ratio as a guide to dose adjustment and monitoring in a UCD patient:

[0081] Patient 1 was a 15 year old partial OTC female receiving HPN-100 as maintenance therapy for her UCD at a dose of 9 mL/day. The patient's ammonia had been controlled since her last routine visit around 6 months ago, but she was complaining of headache and lack of appetite for the past 3 days. Ammonia and metabolite levels were tested after overnight fasting and showed the following results: ammonia 55 μmol/L, PAA and PAGN below levels of quantification. The physician suspected non-compliance with drug and repeated the tests in midday several hours after lunch and found the following results: ammonia: 117 μmol/L; PAA 55 μg/L, PAGN 121 μg/L, and PAA:PAGN ratio approximately 0.5. The patient indicated that she had been fully compliant with her medication. Based on the PAA to PAGN ratio of 0.5 and ammonia of 117, the physician decided to increase the dosage of HPN-100 to 12 mL/day. After one week of treatment with the new dose of HPN-100, all symptoms resolved and the laboratory tests after overnight fasting showed the following: ammonia 9 μmol/L; PAA 12.9 μg/L, PAGN of 9 μg/L, and PAA:PAGN ratio of 1.3. Midday tests showed the following: ammonia 35 μmol/L, PAA 165 μg/L, PAGN 130 μg/L, and PAA:PAGN ratio of ~1.2. The patient was considered controlled and the dose remained at 12 mL/day.

Example 3: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a UCD patient:

[0082] Patient 2 was a 1 year old male OTC receiving 600 mg/kg of NaPBA per day. The patient presented with poor feeding and somnolence. Laboratory tests showed ammonia levels of <9 μmol/L, PAA levels of 530 μg/L, PAGN levels of 178 μg/L, and a PAA:PAGN ratio of >2.5, suggesting that the dose of NaPBA was greater than the patient could effectively convert to PAGN. The treating physician decided to decrease the dose of NaPBA to 450 mg/Kg/day. After one week of treatment with the new dosage, the patient's mother reported that he was eating well and was no longer somnolent. Laboratory tests showed the following: ammonia 20 μmol/L, PAA 280 μg/L, and PAGN 150 μg/L.

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Example 4: Analysis of PAA:PAGN ratio as a guide to assessment of importance of a high PAA level in a UCD patient:

[0083] Patient 3 is a 25 year old OTC female who is being treated with HPN-100. The physician had to increase the dose of HPN-100 several times in order to achieve clinical and blood ammonia within normal limits. Patient 3 was treated at a dose of 18 mL/day for her UCD for the past month. In her next office visit, she did not have any complaints and the following lab results were reported: ammonia 22 μmol/L, PAA 409 μg/L, PAGN 259 μg/L, and PAA:PAGN ratio of 1.5. Despite the patient's relatively high PAA levels, the PAA:PAGN ratio indicated that the subject was being adequately treated and that the patient was able to effectively metabolize the high dose of HPN-100 that she was receiving. The physician decided to continue the treatment as planned.

Example 5: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with spinal muscular atrophy and concomitant liver disease:

[0084] Patient 4 was a 2 year old female being treated with a liquid form of NaPBA for her type II SMA. The patient also suffered from chronic hepatitis C virus infection acquired perinatally from her infected mother. The patient had been having mild to moderate elevation of transaminases since birth, with episodes of icterus and a recent liver biopsy has confirmed presence of chronic hepatitis and cirrhosis. The patient was receiving 4 g of NaPBA per day, and the physician wanted to increase the dosage due to the patient's growth but was concerned about the effects of liver dysfunction on drug metabolism. The physician ordered plasma PAA and PAGN levels and the results were as follows: PAA 110 μg/L, PAGN 85 μg/L, PAA:PAGN ratio of 1.2. The physician decided to increase the dosage of NaPBA to 6 g/day, and repeated the plasma metabolite level measurements after one week of treatment with the new regimen. The results were as follows: PAA 155 μg/L, PAGN 110 μg/L, and PAA:PAGN ratio of 1.4. The physician decided to leave the patient on 6 g/day of NaPBA since his liver seems to have adequate capacity to metabolize 6 g of NaPBA.

Example 6: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with Huntington's Disease and concomitant liver disease:

[0085] Patient 5 was a 56 year old male diagnosed with Huntington's disease several years ago. He also had a history of alcohol abuse and was diagnosed with alcoholic cirrhosis last year. His

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wife enrolled him in clinical trials that involved an experimental drug delivering PBA at a slow rate, thereby enabling once-a-day dosing of the drug. The study had an option for dose escalation after 2 weeks of treatment if clinically safe. Although the protocol did not exclude patients with liver dysfunction, the investigator was concerned about PBA metabolism and possible accumulation of PAA in higher doses due to the patient's liver dysfunction. The investigator enrolled the patient in the low dose group and performed plasma PBA, PAA and PAGN measurements after 6 weeks of treatment with experimental drug. The patient reported improvement in his HD symptoms with no specific complains. Plasma metabolite levels after six weeks of treatment were as follows: PBA 45 μg/L; PAA 159 μg/L, and PAGN 134 μg/L. The dosage of the drug was increased by 50%. After four days of treatment at the new dosage, the patient started to complain about short episodes of somnolence. The investigator performed a blood test and observed the following: PBA 44 μg/L; PAA 550 μg/L, PAGN 180 μg/L, and PAA:PAGN ratio of >3. The PAA:PAGN ratio of greater than 2.5 indicated that the patient's liver could not effectively metabolize the higher dose of the drug, and the investigator therefore decided to reduce the dosage of the experimental drug and not continue dose escalation.

Example 7: Analysis of PAA:PAGN ratio as a guide to dose adjustment in a patient with MSUD:

[0086] Patient 6 was a 4 year old female being treated with HPN-100 for MSUD. The patient was receiving 6 mL of HPN-100 once a day, and the physician wanted to increase the dosage due to the patient's growth. Midday plasma PAA and PAGN measurements after the dose of medication were as follows: PAA 550 μg/L, PAGN 180 μg/L, and PAA:PAGN ratio of >2.5. The physician believed a lower dosage of HPN-100 would not be as effective for the patient, and decided to change the dosing regimen to 3 mL BID instead of 6 mL QD based on the high PAA:PAGN ratio. The tests were repeated after one week of treatment with the new BID regimen, with the following results: PAA 350 μg/L, PAGN 190 μg/L, and PAA:PAGN ratio of 1.8. Based on the ratio of 1.8, the physician decided to leave the patient on 3 mL BID since she can efficiently use a total dose of 6 mL/day given in divided doses but not as a bolus.

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Example 8: Analysis of PAA:PAGN ratio as a guide to monitor a patient with HE and hepatic impairment:

[0087] Patient 7 was a 55 year old Caucasian male diagnosed with alcoholic cirrhosis 3 years ago. His transaminase levels had been mildly elevated and he had recently experienced mild episodes of HE. In the last assessment at the time of hospital admission for a grade 2 HE episode, the patient had a blood ammonia of 85 μmol/L, ALT of 55 U/L, and AST of 47 U/L, and a calculated MELD score of 11. The physician decided to start an ammonia scavenging therapy for the patient and treated him with HPN-100 6 mL BID. The patient returned for a follow up visit after 3 months, during which time he had experienced no episodes of HE. His laboratory assessments showed the following: ammonia of 30 μmol/L, plasma PAA level of 285 μg/mL, PAGN level of 120 μg/L, ALT of 66 U/L, AST of 50 U/L, and calculated MELD score of 13. The physician suspected that the patient's hepatic function may be deteriorating and was concerned about possible accumulation of PAA. She calculated the ratio of PAA to PAGN as 2.4, and confirmed that the patient had not experienced any unusual symptoms such as dizziness, headache, or nausea. Considering patient's ammonia control, lack of specific side effects, and clinical remission, the physician decided not to change the dose and to see the patient in two weeks to repeat the laboratory tests. The physician also warned the patient to call her immediately if he experienced any of these symptoms. In two weeks, the patient's laboratory assessments were essentially unchanged from the previous visit, with a PAA to PAGN ratio of 2.3, and the patient did not report any unusual symptoms. Based on the PAA:PAGN ratio of less than 2.5, the physician decided to continue dosing with 6 mL BID of HPN-100 until the next routine visit.

Example 9: Analysis of PAA:PAGN ratio as a guide to monitoring treatment in a patient with Parkinson's Disease:

[0088] HPN-100 treatment was initiated at a dose of 4mL twice a day in a patient with Parkinson's Disease to produce target circulating levels of PAA expected to produce clinical benefit. After one week of treatment, the patient's circulating PAA level of 50 μg/mL was below the target range, and the PAA:PAGN ratio was determined to be 0.9. The physician concluded that the HPN-100 dose could be safely adjusted upward, and the dose was increased by 50% to 6 mL BID. The PAA level and PAA/PAGN ratio one week later were found to be 75 μg/mL and 1.4,

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respectively. Since 75 μg/mL was still below the therapeutic PAA target level and the PAA:PAGN ratio of 1.4 indicated that conversion of PAA to PAGN had not been saturated, the patient's dosage was increased again by 50% to 9 mL BID. One week later, the patient's PAA and PAA:PAGN ratio were found to be 159 μg/mL and 2.6, respectively. Since the target PAA level was now approximately therapeutic but the PAA:PAGN ratio indicated that PAA to PAGN conversion was approaching saturation, HPN-100 dosage was decreased to 8 mL BID, at which time the patient's circulating PAA level was determined to be close to the target range and his PAA:PAGN ratio was determined to be 2. The patient's dose was not further adjusted and he continued to be monitored.

[0089] As stated above, the foregoing is merely intended to illustrate various embodiments of the present invention. The specific modifications discussed above are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein. All references cited herein are incorporated by reference as if fully set forth herein.

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What is claimed is:

1. A method of treating a nitrogen retention disorder in a subject comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

2. A method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

3. A method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

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4. A method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

5. A method of adjusting the dosage of a PAA prodrug comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

6. A method of optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug as necessary based on the determination in (c).

7. The method of claim 1 or 2, wherein the nitrogen retention disorder is selected from the group consisting of UCD, HE, and ESRD.

8. The method of claim 3 or 4, wherein the disorder is selected from the group consisting of cancer, a neurodegenerative diseases, a metabolic disorder, and sickle cell disease.

9. The method of any of claims 1-6, wherein the target range is 1 to 2.5.

10. The method of any of claims 1-6, wherein the target range is 1 to 2.

11. The method of any of claims 1-6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state.

12. The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of PAA prodrug is administered.

13. The method of any of claims 1-6, wherein the PAA prodrug is selected from the group consisting of NaPBA and HPN-100.

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ABSTRACT

The present disclosure provides methods for adjusting the dosage of PAA prodrugs (*e.g.*, HPN-100, PBA) based on measurement of PAA and PAGN in plasma and calculating the PAA:PAGN ratio so as to determine whether PAA to PAGN conversion is saturated.

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Figure 1

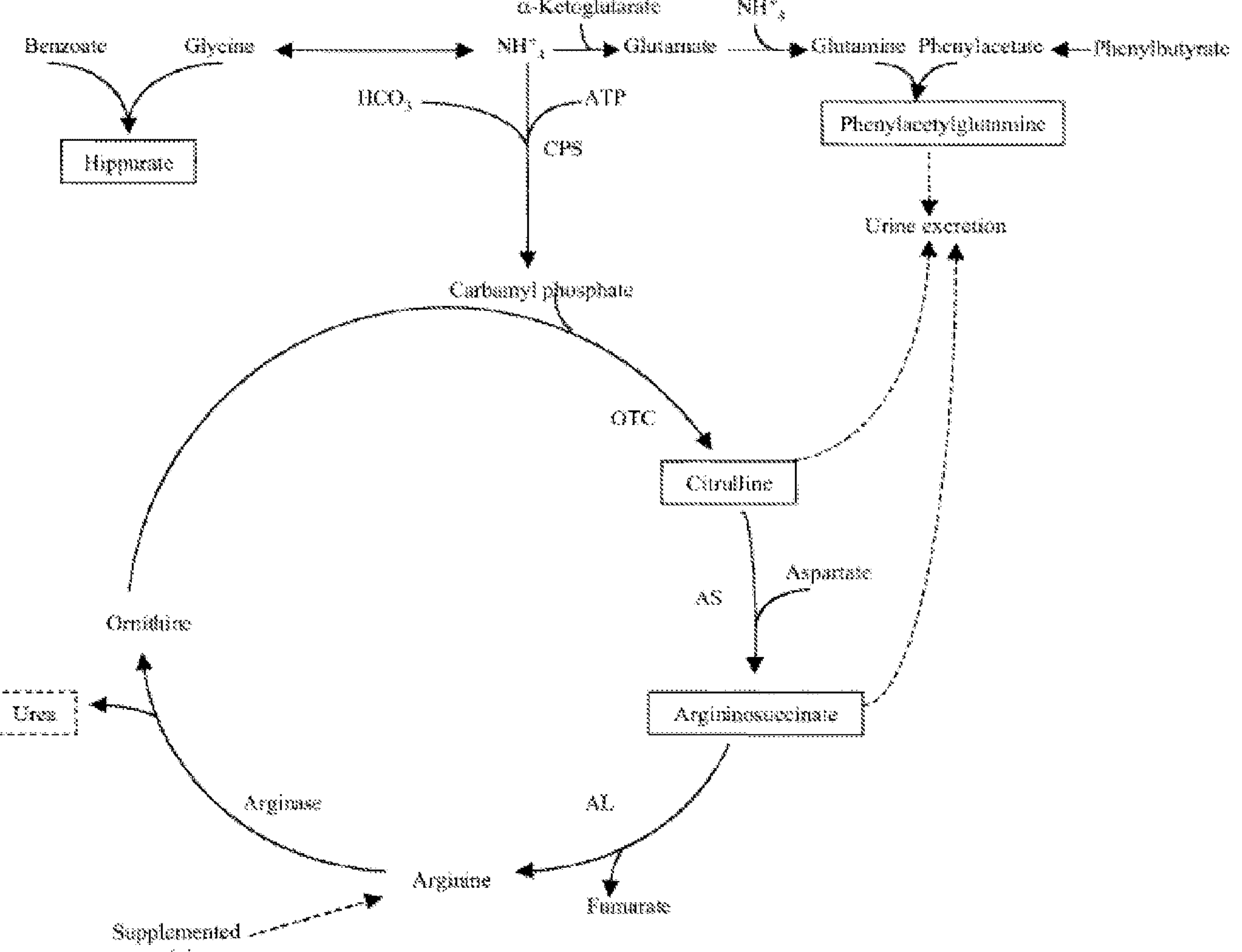

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Figure 2A

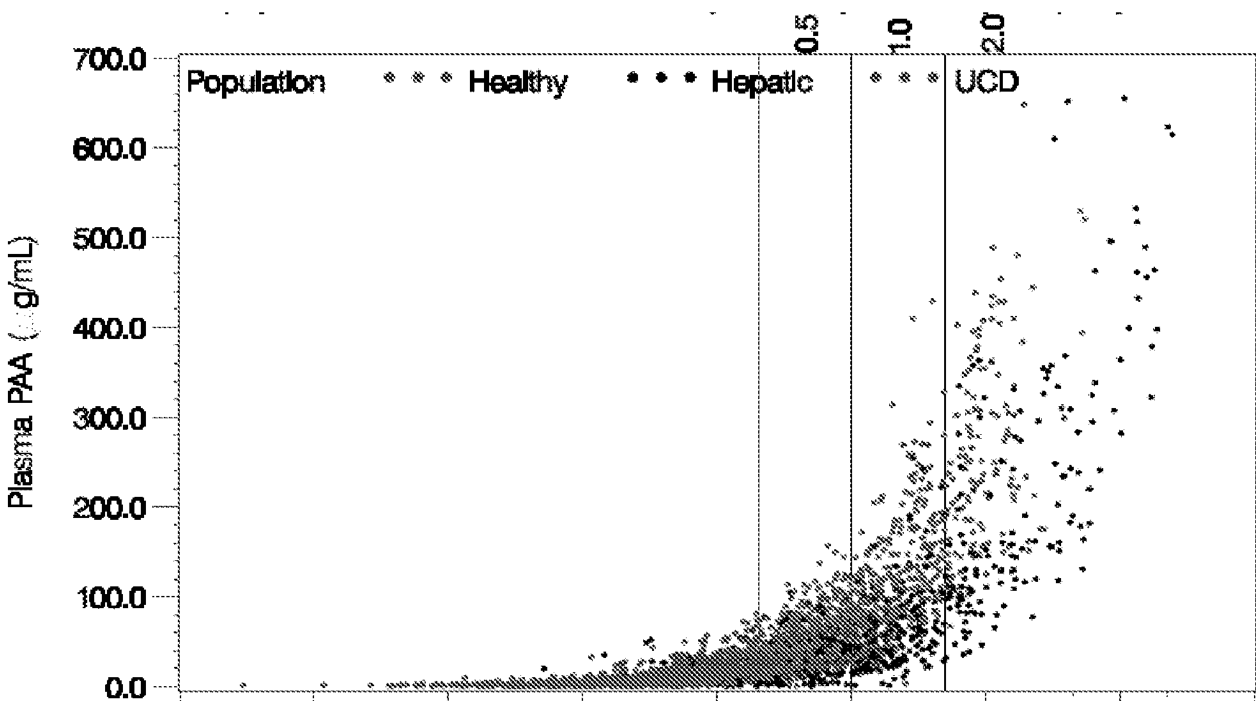

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Figure 2B

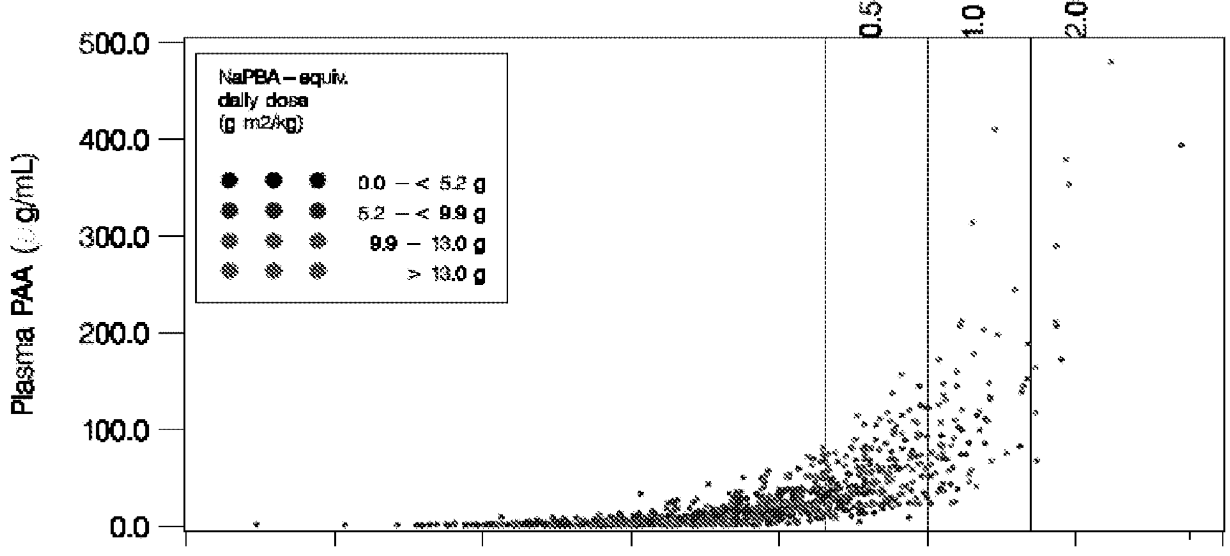

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Figure 2C

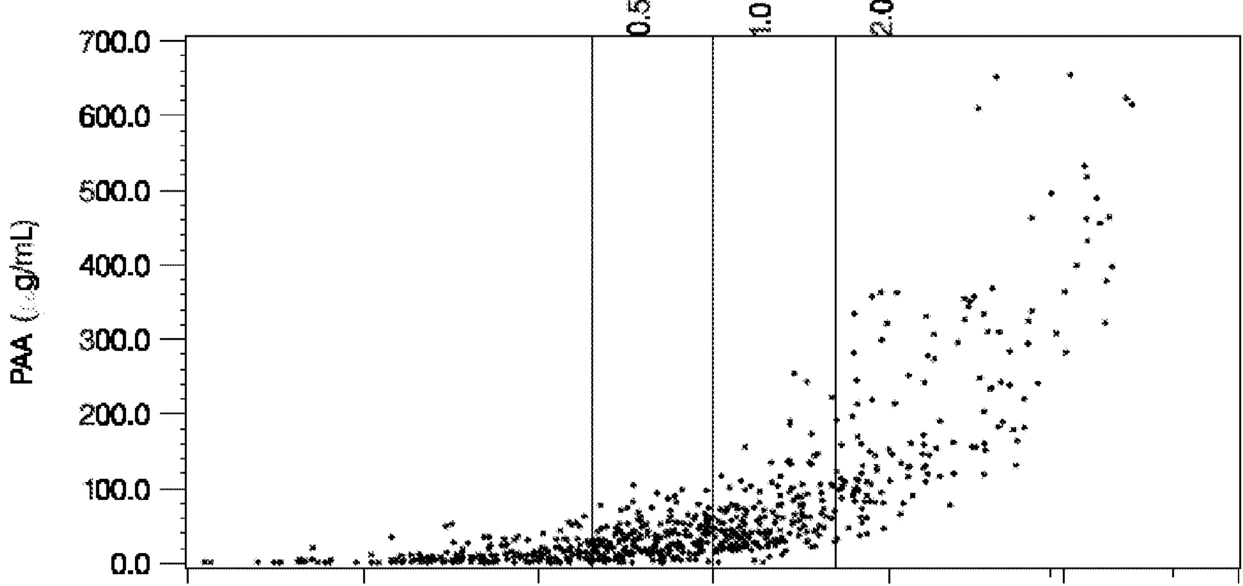

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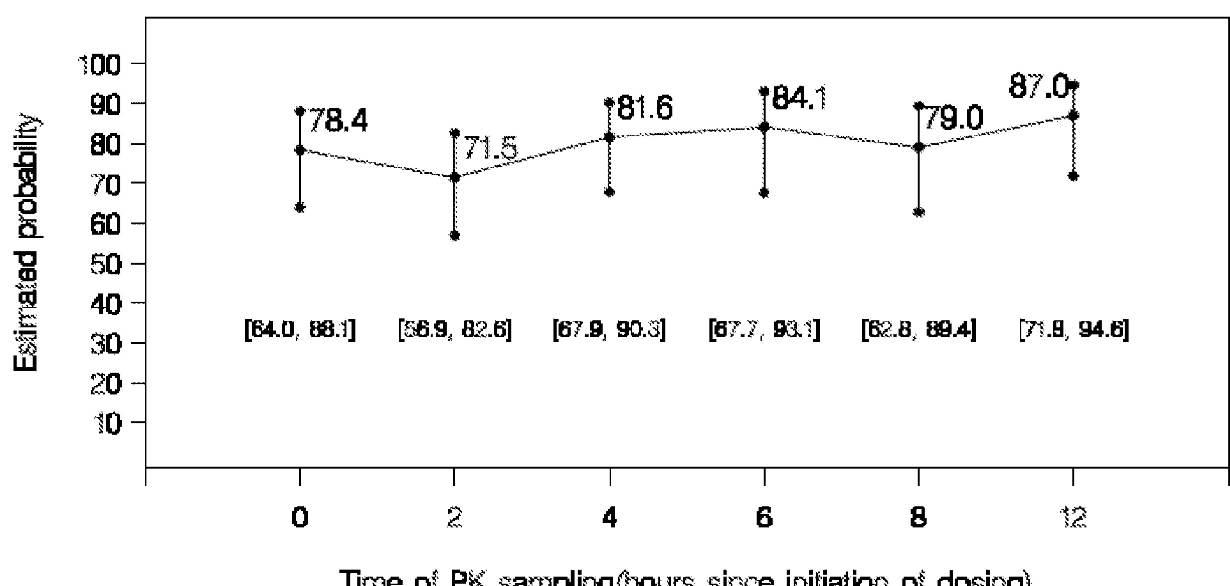
Figure 3



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No other time differences statistically significant. Time=10 omitted due to too few observations

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Figure 4

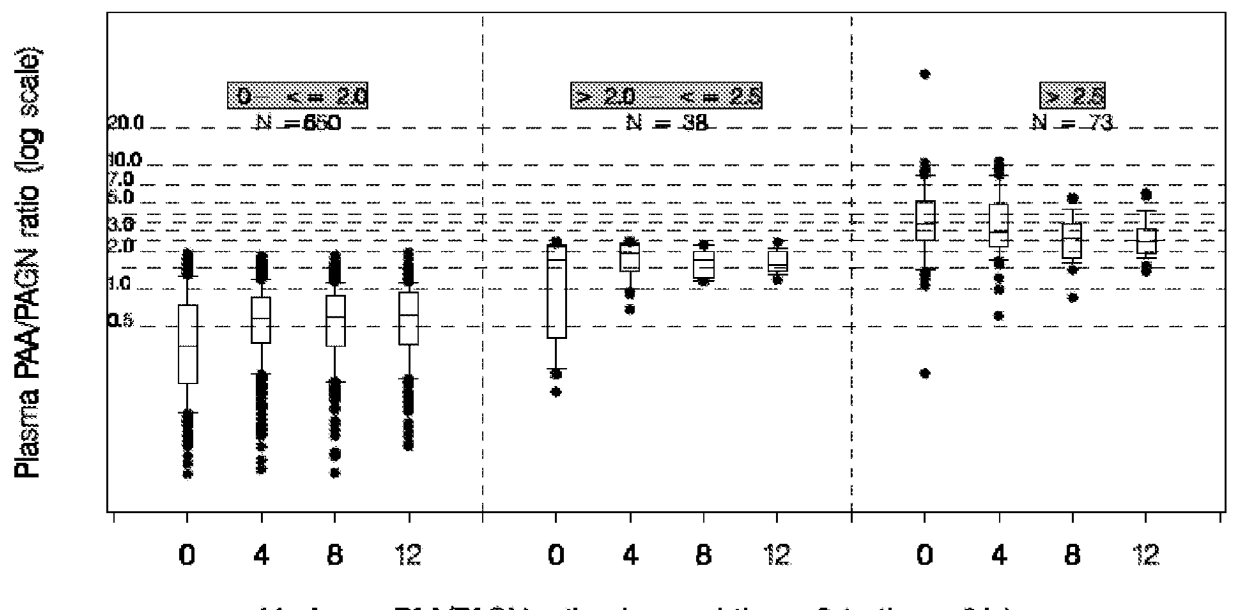

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Figure 5A

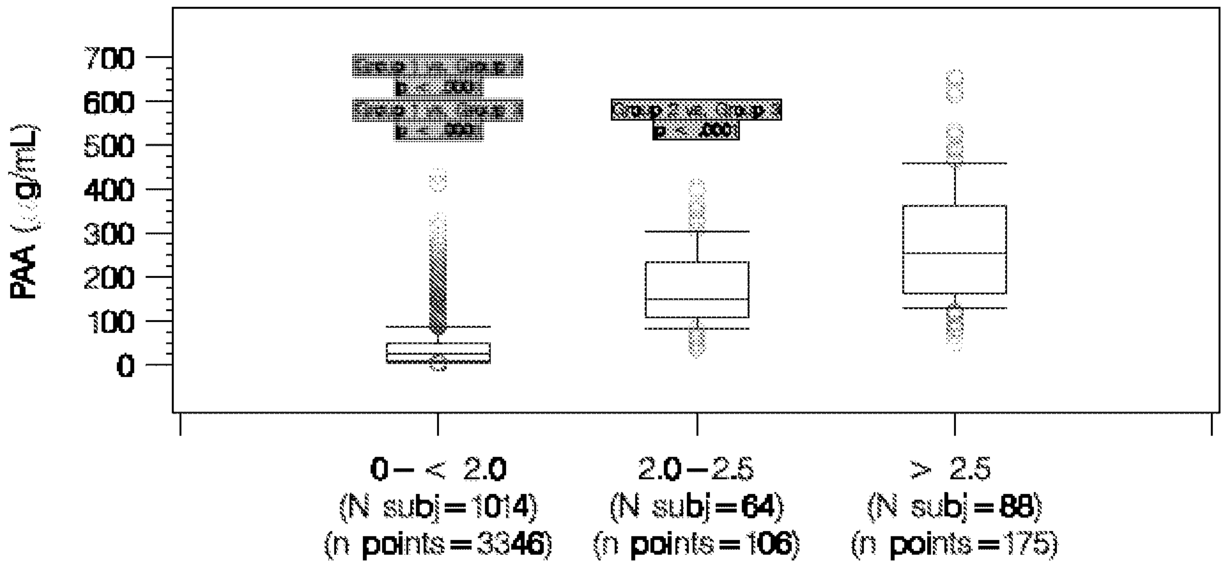
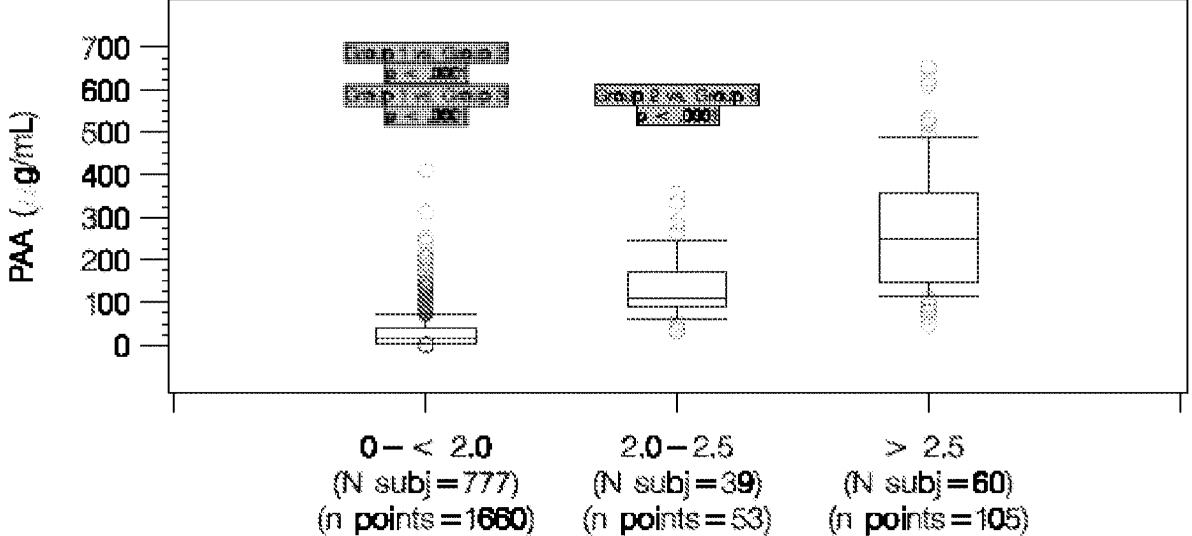


Figure 5B



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Electronic Acknowledgement Receipt

EFS ID:	13716069
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce SCHARSCHMIDT
Customer Number:	34055
Filer:	Patrick D. Morris/Colleen Kirchner
Filer Authorized By:	Patrick D. Morris
Attorney Docket Number:	79532.8004.US01
Receipt Date:	11-SEP-2012
Filing Date:	
Time Stamp:	18:32:27
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	US_transmittal.pdf	70868 bd58f1fcf889ecda569722dbae623d8be9d1e274	no	2

Warnings:

Information:

2		US_Specification.pdf	421452 23dcf88e635702ec2320a497198d2dbec21cbf92	yes	52

	Multipart Description/PDF files in .zip description		
	Document Description	**Start**	**End**
	Specification	1	41
	Claims	42	44
	Abstract	45	45
	Drawings-only black and white line drawings	46	52

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Application Number: 13610580 Document Date: 09/11/2012

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/610,580

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED		(Column 2) NUMBER EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A		N/A	N/A	95		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A		N/A	N/A	310		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A		N/A	N/A	125		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	40	minus 20=	* 20	x 30 =	600	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	6	minus 3 =	* 3	x 125 =	375			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).				0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))					225			
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL	1730		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	SMALL ENTITY RATE($)	SMALL ENTITY ADDITIONAL FEE($)	OR	OTHER THAN SMALL ENTITY RATE($)	OTHER THAN SMALL ENTITY ADDITIONAL FEE($)
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	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)	OR	RATE($)	ADDITIONAL FEE($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
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13/610,580	09/11/2012	1765	0.00	79532.8004.US01	13	6

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OC000000056731558

Date Mailed: 09/26/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Bruce Scharschmidt, Residence Not Provided;
Masoud Mokhtarani, Residence Not Provided;

Applicant(s)

Bruce Scharschmidt, Residence Not Provided;
Masoud Mokhtarani, Residence Not Provided;

Power of Attorney: None

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/636,256 04/20/2012

Foreign Applications (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.)

If Required, Foreign Filing License Granted: 09/24/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/610,580**

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 59 of 1354

Title

METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Preliminary Class

528

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

SelectUSA

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage, facilitate, and accelerate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/610,580	09/11/2012	Bruce Scharschmidt	79532.8004.US01

CONFIRMATION NO. 1957

34055
PERKINS COIE LLP
POST OFFICE BOX 1208
SEATTLE, WA 98111-1208

FORMALITIES LETTER

OC000000056731559

Date Mailed: 09/26/2012

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
  *Applicant must submit $**95** to complete the basic filing fee for a small entity.*
- The oath or declaration is missing.

A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.

Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Additional claim fees of $ **1200** as a small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- A surcharge (for late submission of the basic filing fee, search fee, examination fee or inventor's oath or declaration) as set forth in 37 CFR 1.16(f) of $ **65** for a small entity in compliance with 37 CFR 1.27, must be submitted.

SUMMARY OF FEES DUE:

Total fee(s) required within **TWO MONTHS** from the date of this Notice is $ **1795** for a small entity

- $ **95** Statutory basic filing fee.
- $ **65** Surcharge.
- The application search fee has not been paid. Applicant must submit $ **310** to complete the search fee.

- The application examination fee has not been paid. Applicant must submit $ **125** to complete the examination fee for a small entity in compliance with 37 CFR 1.27.
- Total additional claim fee(s) for this application is $ **1200**
  - $ **375** for **3** independent claims over 3.
  - $ **600** for **20** total claims over 20.
  - $ **225** for multiple dependent claim surcharge.

Replies should be mailed to:

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Registered users of EFS-Web may alternatively submit their reply to this notice via EFS-Web.
https://sportal.uspto.gov/authenticate/AuthenticateUserLocalEPF.html

For more information about EFS-Web please call the USPTO Electronic Business Center at **1-866-217-9197** or visit our website at http://www.uspto.gov/ebc.

If you are not using EFS-Web to submit your reply, you must include a copy of this notice.

/rerry/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)	Application Number 13610580	Filing Date
	Applicant(s) Bruce Scharschmidt	

* May be used for additional claims or amendments

CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT	
	Indep	Depend	Indep	Depend	Indep	Depend
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPL. OF:	BRUCE SCHARSCHMIDT ET AL.	ART UNIT:	1765
APPLICATION NO.:	13/610,580	CONF. NO:	1957
FILED:	SEPTEMBER 11, 2012		
FOR: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS			

RESPONSE TO NOTICE TO FILE MISSING PARTS OF APPLICATION

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Notice to File Missing Parts of Nonprovisional Application mailed on September 26, 2012, applicants submit the following:

☒ an executed Declaration of Inventorship;

☒ an executed Power of Attorney by Assignee; and

☒ a Preliminary Amendment.

1. Authorization for Extensions of Time Under 37 C.F.R. § 1.136 (a)(3)

Applicants petition for an Extension of Time if necessary for timely filing of this Response. The Commissioner is authorized to treat this or any future reply requiring a Petition for Extension of Time under 37 C.F.R. § 1.136 (a)(3) for its timely submission as incorporating a petition herefore for the appropriate length of time. Please charge all required extension of time fees in this application to Deposit Account No. 50-2586.

2. Fee Calculation and Payment

For:	(Col. 1) No. Filed	(Col. 2) No. Extra	Small Entity			Other Than a Small Entity	
			Rate	Fee		Rate	Fee
Filing Fee			$95	$95.00	or	$380	$
Search Fee			$310	$310.00	or	$620	$
Examination Fee			$125	$125.00	or	$250	$
Total Claims	23 – 20	3	X $31=	$93.00	or	X $60=	$
Independent Claims	4 – 3	1	X $125=	$125.00	or	X $250=	$
☒ Multiple Dependent Claim Presented			+ $230=	$230.00	or	+ $450=	$
Application Size Fee – for each additional 50 sheets that exceeds 100 sheets			X $160=	$	or	X $310=	$
Missing Parts Surcharge			$65.00	$65.00		$130	$
Extension of Time Fee				$			$
*If the difference in Col. 1 is less than zero, enter "0" in Col. 2.			TOTAL	$1043.00	or	TOTAL	**$**

☒ Please charge Deposit Account No. 50-2586 in the amount of $1,043.00 for the requisite fees.

☒ Please charge any deficiency or credit to Deposit Account No. 50-2586.

Dated: November 21, 2012

Respectfully submitted,

PERKINS COIE LLP

By: /Patrick D. Morris/
Patrick D. Morris, Ph.D.
Reg. No. 53,351

Correspondence Address:
Customer No. 34055
Perkins Coie LLP
Patent - LA
P.O. Box 1208
Seattle, WA 98111-1208
Phone: (310) 788-9900
Fax: (206) 332-7198

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPL. OF:	BRUCE SCHARSCHMIDT ET AL.	ART UNIT:	1765
APPLICATION NO.:	13/610,580	CONF. NO:	1957
FILED:	SEPTEMBER 11, 2012		
FOR:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS		

PRELIMINARY AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Prior to examination on the merits, please amend the above-identified application as follows:

Amendments to the Claims are reflected in the listing of claims beginning on page 2.

Conclusion begins on page 5.

[Continued on next page.]

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 67 of 1354

AMENDMENTS TO THE CLAIMS

The following is a complete listing of the claims pending in the application, as amended:

1. (original) A method of treating a nitrogen retention disorder in a subject comprising:

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

2. (original) A method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

3. (canceled)

4. (canceled)

5. (original) A method of adjusting the dosage of a PAA prodrug comprising:

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 68 of 1354

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

6. (original) A method of optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug comprising:

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug as necessary based on the determination in (c).

7. (original) The method of claim 1 or 2, wherein the nitrogen retention disorder is selected from the group consisting of UCD, HE, and ESRD.

8. (canceled)

9. (currently amended)The method of any of claims 1[[-]], 2, 5, or 6, wherein the target range is 1 to 2.5.

10. (currently amended)The method of any of claims 1[[-]], 2, 5, or 6, wherein the target range is 1 to 2.

11. (currently amended)The method of any of claims 1[[-]], 2, 5, or 6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state.

12. (original) The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of PAA prodrug is administered.

13. (currently amended)The method of any of claims 1[[-]], 2, 5, or 6, wherein the PAA prodrug is selected from the group consisting of NaPBA and HPN-100.

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
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CONCLUSION

Applicant respectfully requests consideration of the application in view of this preliminary amendment. If the Examiner has any questions or matters that can be expediently handled by telephone, he or she is encouraged to contact the undersigned at (310) 788-9900.

Respectfully submitted,
Perkins Coie LLP

Date: November 21, 2012

/Patrick D. Morris/
Patrick D. Morris, Ph.D.
Reg. No. 53,351

Correspondence Address:
Customer No. 34055
Perkins Coie LLP
Patent – LA
P.O. Box 1208
Seattle, WA 98111-1208
Phone: (310) 788-9900
Fax: (206) 332-7198

UTILITY DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS**, the specification of which

(Check One) ☐ is attached hereto OR
☒ was deposited on September 11, 2012 and accorded United States Application No. 13/610,580.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Date of Filing	Priority Claimed	
			Yes	No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date
61/636,256	April 20, 2012

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code § 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 72 of 1354

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date	Status-Patented, Pending or Abandoned

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, United States Code, § 1001 and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

201					
	FULL NAME OF INVENTOR	FIRST Name Bruce	MIDDLE Initial	LAST Name SCHARSCHMIDT	
	RESIDENCE & CITIZENSHIP	City San Francisco	State or Foreign Country CA	Country of Citizenship USA	
	POST OFFICE ADDRESS	45 St. Francis Boulevard	City San Francisco	State or Country CA	Zip Code 94127

INVENTOR'S SIGNATURE [signature] DATE November 9 2012

201					
	FULL NAME OF INVENTOR	FIRST Name Masoud	MIDDLE Initial	LAST Name MOKHTARANI	
	RESIDENCE & CITIZENSHIP	City Walnut Creek	State or Foreign Country CA	Country of Citizenship USA	
	POST OFFICE ADDRESS	725 Castle Rock Road	City Walnut Creek	State or Country CA	Zip Code 94598

INVENTOR'S SIGNATURE [signature] DATE 11/9/2012

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: BRUCE SCHARSCHMIDT ET AL.
APPLICATION NO.: 13/610,580
FILING DATE: SEPTEMBER 11, 2012
FOR: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

CONFIRMATION NO.: 1957
ART UNIT: 1765

Power of Attorney by Assignee and Certification Under 37 C.F.R. § 3.73(b)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned, acting on behalf of the Assignee of the entire right, title and interest in the above-identified patent application, by virtue of an Assignment attached hereto appoint the attorneys and agents listed below to prosecute this patent and transact all business with the U.S. Patent and Trademark Office in connection therewith. This appointment is to the exclusion of the inventor(s) and their attorney(s) and agent(s) in accordance with the provisions of 37 C.F.R. § 3.71.

All prior powers of attorney for this application are hereby revoked. The Assignee hereby appoints all of the registered practitioners identified by Customer Number 34055:

Customer Number 34055
Perkins Coie LLP
Patent – LA
P.O. Box 1208
Seattle, WA 98111-1208
Phone: (310) 788-9900
Fax: (206) 332-7198

Please direct all inquires to Patrick D. Morris at the above Customer Number.

In accordance with 37 C.F.R. § 3.73(b), I hereby certify that I am empowered to act on behalf of the Assignee. To the best of my knowledge and belief, title is in the Assignee, as evidenced by the Assignment noted above.

I further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Title 18, USC § 1001 and that such willful false statements may jeopardize the validity of this patent.

ASSIGNEE: HYPERION THERAPEUTICS, INC.

Signature:

Typed Name: Jeff Farrow

Title: CFO

Date: 11/9/12

Address: 601 Gateway Blvd., Suite 200, South San Francisco, CA 94080

ASSIGNMENT

THIS ASSIGNMENT is by Bruce SCHARSCHMIDT and Masoud MOKHTARANI (hereinafter collectively referred to as "Assignors"). Assignors have invented one or more certain inventions described in a United States Utility Patent Application entitled **METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS** (the "Application"), which was filed on September 11, 2012, as Application No. 13/610,580 (the "Invention(s)").

HYPERION THERAPEUTICS, INC., a corporation of the State of Delaware having a principal place of business at 601 Gateway Blvd., Suite 200, South San Francisco, CA 94080 ("Assignee"), desires to acquire the entire right, title, and interest in and to the Invention(s) and the Application, and in and to any patents (collectively, "Patents") that may be granted for the Invention(s) in the United States or in any foreign countries.

For valuable consideration, the receipt and sufficiency of which we acknowledge, Assignors hereby sell, assign, and transfer to Assignee, its successors, legal representatives and assigns, the entire right, title, and interest in and to: the Invention(s), the Application, and any Patents; any divisions, continuations, and continuations-in-part of the Application; any reissues, reexaminations, or extensions of any and all Patents; the right to file foreign applications directly in the name of Assignee; and the right to claim priority rights deriving from the Application (collectively, the "Rights"). Assignors warrant that they are the sole owner of the Rights, and that the Rights are unencumbered. Assignors also agree to not sign any writing or do any act conflicting with this assignment and to sign all documents and do such additional acts

as Assignee deems necessary or desirable to perfect Assignee's enjoyment of the Rights; prepare and prosecute the Application or any other applications for Patents; conduct proceedings regarding the Rights, including any litigation or interference proceedings; or perfect or defend title to the Rights.

Assignors request the Commissioner of Patents to issue any Patent of the United States that may be issued on the Invention(s) to Assignee.

This Assignment may be executed in counterparts.

Assignors:

Date: November 9 2012 _______________
Bruce SCHARSCHMIDT

Date: Nov, 9, 2012 _______________
Masoud MOKHTARANI

Assignee:

Date: 11/9/12 _______________
By:
for HYPERION THERAPEUTICS, INC.

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Patrick D. Morris/Colleen Kirchner
Attorney Docket Number:	79532.8004.US01

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Utility filing Fee (Electronic filing)	4011	1	98	98
Utility Search Fee	2111	1	310	310
Utility Examination Fee	2311	1	125	125
Pages:				
Claims:				
Claims in excess of 20	2202	3	31	93
Independent claims in excess of 3	2201	1	125	125
Multiple dependent claims	2203	1	230	230

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous-Filing:				
Late filing fee for oath or declaration	2051	1	65	65
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1046**

Electronic Acknowledgement Receipt

EFS ID:	14290171
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Patrick D. Morris/Colleen Kirchner
Filer Authorized By:	Patrick D. Morris
Attorney Docket Number:	79532.8004.US01
Receipt Date:	21-NOV-2012
Filing Date:	11-SEP-2012
Time Stamp:	14:10:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1046
RAM confirmation Number	2054
Deposit Account	502586
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	8004US01_MPResponse.pdf	90992 f527355321e3724b34f4d0ba2fd74f161481e201	no	2
Warnings:					
Information:					
2		8004US01_PrelimAmendment.pdf	67028 4d61744ed56df1d491f8540297ff0b6780e8c2ff	yes	5

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	5

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
Warnings:					
Information:					
3	Oath or Declaration filed	8004US01_Declaration.pdf	570067 ad6ef309467e197cbf47f29944cd95668da9c172	no	2
Warnings:					
Information:					
4	Power of Attorney	8004US01_POA_Assignment.pdf	811694 0acd9fe7d3e40968cde46ea1e46930ce58861beb	no	4
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	41654 cafd151607d34ff082ff677a39c4c4c15f4bddec	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			1581435		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Electronic Acknowledgement Receipt

EFS ID:	14290171
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
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Time Stamp:	14:10:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1046
RAM confirmation Number	2054
Deposit Account	502586
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	8004US01_MPResponse.pdf	90992 f527355321e3724b34f4d0ba2fd74f161481e201	no	2
Warnings:					
Information:					
2		8004US01_PrelimAmendment.pdf	67028 4d61744ed56df1d491f8540297ff0b6780e8c2ff	yes	5

Multipart Description/PDF files in .zip description

Document Description	Start	End
Preliminary Amendment	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	5

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
Warnings:					
Information:					
3	Oath or Declaration filed	8004US01_Declaration.pdf	570067 ad6ef309467e197cbf47f29944cd95668da9c172	no	2
Warnings:					
Information:					
4	Power of Attorney	8004US01_POA_Assignment.pdf	811694 0acd9fe7d3e40968cde46ea1e46930ce58861beb	no	4
Warnings:					
Information:					
5	Fee Worksheet (SB06)	fee-info.pdf	41654 cafd151607d34ff082ff677a39c4c4c15f4bddec	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	1581435		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

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New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PTO/SB/06 (07-06)
Approved for use through 1/31/2007. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/610,580	Filing Date 09/11/2012	☐ To be Mailed

APPLICATION AS FILED – PART I

	(Column 1)	(Column 2)	SMALL ENTITY ☒		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE ($)	FEE ($)		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X $ =		OR	X $ =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X $ =			X $ =	
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $250 ($125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							
			TOTAL			TOTAL	

* If the difference in column 1 is less than zero, enter "0" in column 2.

APPLICATION AS AMENDED – PART II

AMENDMENT		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
	11/21/2012	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	* 25	Minus	** 40	= 0	X $31 =	0	OR	X $ =	
	Independent (37 CFR 1.16(h))	* 3	Minus	***6	= 0	X $125 =	0	OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE	**0**	OR	TOTAL ADD'L FEE	

AMENDMENT		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE ($)	ADDITIONAL FEE ($)		RATE ($)	ADDITIONAL FEE ($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	X $ =		OR	X $ =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X $ =		OR	X $ =	
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/CRYSTAL QUEEN/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**
If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPL. OF:	BRUCE SCHARSCHMIDT ET AL.	ART UNIT:	1765
APPLICATION NO.:	13/610,580	CONF. NO:	1957
FILED:	SEPTEMBER 11, 2012		
FOR:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS		

RESPONSE TO NOTICE TO FILE MISSING PARTS OF APPLICATION

Mail Stop Missing Parts
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In response to the Notice to File Missing Parts of Nonprovisional Application mailed on September 26, 2012, applicants submit the following:

☒ an executed Declaration of Inventorship;

☒ an executed Power of Attorney by Assignee; and

☒ a Preliminary Amendment.

1. Authorization for Extensions of Time Under 37 C.F.R. § 1.136 (a)(3)

Applicants petition for an Extension of Time if necessary for timely filing of this Response. The Commissioner is authorized to treat this or any future reply requiring a Petition for Extension of Time under 37 C.F.R. § 1.136 (a)(3) for its timely submission as incorporating a petition herefore for the appropriate length of time. Please charge all required extension of time fees in this application to Deposit Account No. 50-2586.

11/29/2012 VVAN11 00000029 502586 13610580

01 FC:2202 93.00 DA

2. Fee Calculation and Payment

For:	(Col. 1) No. Filed	(Col. 2) No. Extra	Small Entity			Other Than a Small Entity	
			Rate	Fee		Rate	Fee
Filing Fee			$95	$95.00	or	$380	$
Search Fee			$310	$310.00	or	$620	$
Examination Fee			$125	$125.00	or	$250	$
Total Claims	23 – 20	3	X $31=	$93.00	or	X $60=	$
Independent Claims	4 – 3	1	X $125=	$125.00	or	X $250=	$
☒ Multiple Dependent Claim Presented			+ $230=	$230.00	or	+ $450=	$
Application Size Fee – for each additional 50 sheets that exceeds 100 sheets			X $160=	$	or	X $310=	$
Missing Parts Surcharge			$65.00	$65.00		$130	$
Extension of Time Fee				$			$
*If the difference in Col. 1 is less than zero, enter "0" in Col. 2.			TOTAL	$1043.00	or	TOTAL	$

☒ Please charge Deposit Account No. 50-2586 in the amount of $1,043.00 for the requisite fees.

☒ Please charge any deficiency or credit to Deposit Account No. 50-2586.

Dated: November 21, 2012

Respectfully submitted,

PERKINS COIE LLP

By: /Patrick D. Morris/
Patrick D. Morris, Ph.D.
Reg. No. 53,351

Correspondence Address:
Customer No. 34055
Perkins Coie LLP
Patent - LA
P.O. Box 1208
Seattle, WA 98111-1208
Phone: (310) 788-9900
Fax: (206) 332-7198

79532-8004.US01/LEGAL25154605.1

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/610,580	09/11/2012	Bruce Scharschmidt	79532.8004.US01

CONFIRMATION NO. 1957

POA ACCEPTANCE LETTER

OC000000057852953

34055
PERKINS COIE LLP
POST OFFICE BOX 1208
SEATTLE, WA 98111-1208

Date Mailed: 12/04/2012

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/21/2012.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/ltaba/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

PATENT APPLICATION FEE DETERMINATION RECORD
Substitute for Form PTO-875

Application or Docket Number
13/610,580

APPLICATION AS FILED - PART I

	(Column 1)	(Column 2)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE($)	FEE($)		RATE($)	FEE($)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A	98		N/A	
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A	310		N/A	
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A	125		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	26 minus 20=	* 6	x 31 =	186	OR		
INDEPENDENT CLAIMS (37 CFR 1.16(h))	4 minus 3 =	* 1	x 125 =	125			
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is $310 ($155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).			0.00			
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))				230			
* If the difference in column 1 is less than zero, enter "0" in column 2.			TOTAL	1074		TOTAL	

APPLICATION AS AMENDED - PART II

AMENDMENT A		(Column 1)		(Column 2)	(Column 3)	SMALL ENTITY		OR	OTHER THAN SMALL ENTITY	
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

AMENDMENT B		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE($)	ADDITIONAL FEE($)		RATE($)	ADDITIONAL FEE($)
	Total (37 CFR 1.16(i))	*	Minus	**	=	x =		OR	x =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x =		OR	x =	
	Application Size Fee (37 CFR 1.16(s))									
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))							OR		
						TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
13/610,580	09/11/2012	1765	1139	79532.8004.US01	10	4

CONFIRMATION NO. 1957

34055
PERKINS COIE LLP
POST OFFICE BOX 1208
SEATTLE, WA 98111-1208

UPDATED FILING RECEIPT

Date Mailed: 12/04/2012

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Inventor(s)

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

Applicant(s)

Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

Power of Attorney: The patent practitioners associated with Customer Number 34055

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/636,256 04/20/2012

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see http://www.uspto.gov for more information.) - None.
Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

If Required, Foreign Filing License Granted: 09/24/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 13/610,580**

Projected Publication Date: 10/24/2013

Non-Publication Request: No

Early Publication Request: No
**** SMALL ENTITY ****

Title

METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Preliminary Class

528

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at http://www.uspto.gov/web/offices/pac/doc/general/index.html.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign AssetsControl, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

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The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage, facilitate, and accelerate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/610,580	09/11/2012	Bruce Scharschmidt	079532-8004.US01

CONFIRMATION NO. 1957

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

PUBLICATION NOTICE

Title:METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Publication No.US-2013-0281530-A1
Publication Date:10/24/2013

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently http://www.uspto.gov/patft/.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently http://pair.uspto.gov/. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Managment, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	079532-8004.US01	1957

34055 7590 10/09/2014
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

EXAMINER
TOWNSLEY, SARA ELIZABETH

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
10/09/2014	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentprocurement@perkinscoie.com

PTOL-90A (Rev. 04/07)

Office Action Summary	**Application No.** 13/610,580	**Applicant(s)** SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	**Art Unit** 1629	**AIA (First Inventor to File) Status** No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on ______.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ______.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ______; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1,2,5-7 and 9-13* is/are pending in the application.
5a) Of the above claim(s) ______ is/are withdrawn from consideration.
6)☐ Claim(s) ______ is/are allowed.
7)☐ Claim(s) ______ is/are rejected.
8)☐ Claim(s) ______ is/are objected to.
9)☒ Claim(s) *1, 2, 5-7, and 9-13* are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.
11)☐ The drawing(s) filed on ______ is/are: a)☐ accepted or b)☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:
1.☐ Certified copies of the priority documents have been received.
2.☐ Certified copies of the priority documents have been received in Application No. ______.
3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date ______.
3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. ______ .
4) ☐ Other: ______.

DETAILED ACTION

Election of Species

1. This application contains claims directed to patentably distinct species. For initial search and examination purposes, Applicant is required to elect

- a single, distinct nitrogen retention disorder, e.g., UCD, as recited in claim 7; and
- a single, distinct PAA prodrug, e.g., HPN-100, as recited in claim 13.

Each of these species must be identified so as to yield one single, distinct method species (i.e., a single, distinct embodiment).

2. The species are independent or distinct because claims to the different species recite the mutually exclusive characteristics of such species. In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1, 2, 5-7, and 9-13 are generic.

There is an examination and search burden for these patentably distinct species due to their mutually exclusive characteristics. The species require a different field of search (e.g., searching different classes/subclasses or electronic resources, or employing different search queries); and/or the prior art applicable to one species would not likely be applicable to another species; and/or the species are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement

may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election of the species may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species.

Should applicant traverse on the ground that the species are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the species to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the species unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103(a) of the other species.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SARA E. TOWNSLEY whose telephone number is 571-270-7672. The examiner can normally be reached on Mon-Fri from 9:00 am to 5:00 pm (EST). If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeff S. Lundgren, can be reached at 571-272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://portal.uspto.gov/external/portal. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SARA E. TOWNSLEY/
Examiner, Art Unit 1629

Index of Claims	Application/Control No. 13610580	Applicant(s)/Patent Under Reexamination SCHARSCHMIDT ET AL.
	Examiner SARA E TOWNSLEY	Art Unit 1629

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	10/05/2014								
	1	÷								
	2	÷								
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	12	÷								
	13	÷								

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of: **SCHARSCHMIDT, Bruce, et al.** **Serial No.:** 13/610,580 **Filed:** September 11, 2012 **For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC PRODRUGS**		**Examiner:** TOWNSLEY, Sara Elizabeth **Group Art Unit:** 1629 **Docket No.:** 079532.8004.US01 I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being deposited with the U.S. Patent and Trademark Office this 4th day of November 2014 via EFS-Web Electronic Filing. /Colleen Kirchner/ Colleen Kirchner

RESPONSE TO RESTRICTION REQUIREMENT

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

The following is in response to the Restriction Requirement mailed October 9, 2014 for the above-identified application.

The Restriction Requirement requests that Applicants elect a single, distinct nitrogen retention disorder as recited in claim 7. Applicants elect urea cycle disorder (UCD) without traverse. The Restriction Requirement also requests that Applicants elect a single distinct PAA prodrug as recited in claim 13. Applicants elect glyceryl tri-[4-phenylbutyrate] (HPN-100) without traverse. HPN-100 has the following structure:

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
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HPN-100 is a prodrug of phenylbutyrate (PBA) and a pre-prodrug of phenylacetic acid (PAA). As such, HPN-100 has the same active moiety as PBA and sodium PBA (i.e., PAA).

Pending claims 1, 2, 5-7, and 9-13 encompass the elected species.

If Applicants can do anything more to expedite this application, Applicants request that the Examiner contact the undersigned at (415) 344-7105.

Respectfully submitted,

Perkins Coie LLP

Date: November 4, 2014

/Patrick D. Morris/
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Registration No. 53,351

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Patent - LA
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Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 102 of 1354

Electronic Acknowledgement Receipt

EFS ID:	20606855
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Lara J. Dueppen/Colleen Kirchner
Filer Authorized By:	Lara J. Dueppen
Attorney Docket Number:	079532-8004.US01
Receipt Date:	04-NOV-2014
Filing Date:	11-SEP-2012
Time Stamp:	18:33:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	8004US01_Response.pdf	88104 c1354e2a46aab48111b6acef1ad71575a1071c8f	no	2

Warnings:

Information:

Total Files Size (in bytes):	88104

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

INFORMATION DISCLOSURE STATEMENT BY APPLICANT Form PTO-1449 (Modified) (Use several sheets if necessary)				*COMPLETE IF KNOWN*	
				Application Number	13/610,580
				Confirmation Number	1957
				Filing Date	September 11, 2012
				First Named Inventor	SCHARSCHMIDT, Bruce
				Group Art Unit	1765
				Examiner Name	
Sheet	1	of	11	Attorney Docket No.	79532.8004.US01

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.	U.S. Patent or Application NUMBER	Kind Code (if known)	Name of Patentee or Inventor of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	A1	4,284,647 A		BRUSILOW	8/1981	
	A2	5,968,979		BRUSILOW	10/19/1999	
	A3	6,060,510		BRUSILOW	5/2000	
	A4	6,083,984		BRUSILOW	7/2000	
	A5	6,219,567		EGGERS	4/17/2001	
	A6	2004/0229948		SUMMAR	11/2004	
	A7	2006/0135612		FERRANTE	6/2006	
	A8	2008/0119554		JALAN	5/2008	
	A9	2010/0008859		SCHARSCHMIDT	1/14/2010	
	A10	2012/0022157		SCHARSCHMIDT		
	A11	2012/0220661		LEE	08/30/2012	
	A12	2013/0210914		SCHARSCHMIDT	08/15/2013	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.	Office	NUMBER	Kind Code (if known)	Name of Patentee or Applicant of Cited Document	Date of Publication or Filing Date of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T
	B1	WO	2005/053607		Medicis Pharmaceuticla Corp.	6/16/2005		
	B2	WO	2006/056794		UCL Business PCL	6/01/2006		
	B3	WO	2007/005633		Navinta LLC	01/11/2007		
	B4	WO	2009/087474		Akthelia Pharmaceuticals	7/16/2009		
	B5	WO	2009/134460		Hyperion Therapeutics	11/05/2009		
	B6	WO	2010/025303		Hyperion Therapeutics	03/04/2010		
	B7	WO	2012/028620		INSERM	03/08/2012		

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume issue number(s), publisher, city and/or country where published.	T

EXAMINER	DATE CONSIDERED

*EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to application(s).

79532-8004.US01/LEGAL124080222.1

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				Group Art Unit	1765
				Examiner Name	
Sheet	2	of	11	Attorney Docket No.	79532.8004.US01

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	C1	AMBROSE, A.M., (1933) "Further Studies on the Detoxification of Phyenylacetic Acid." *J Biol Chem* 101:669-675.	
	C2	BATSHAW M.L. et al. (1980, December) "Treatment of Hyperammonemic Coma Caused by Inborn Errors of Urea Synthesis," *J Pediatr* 97(6):893-900.	
	C3	BATSHAW M.L. et al. (1982, June 10) "Treatment of Inborn Errors of Urea Synthesis: Activation of Alternative Pathways of Waste Nitrogen Synthesis and Excretion," *N Engl J Med* 306(23):1387-1392.	
	C4	BATSHAW, M.L. (1984) "Hyperammonemia," in Current Problems in Pediatrics, Lockhart, J.D. ed.: Year Book Medical Publishers, pp. 2-69.	
	C5	BATSHAW, M.L. et al. (1981, August) "New Approaches to the Diagnosis and Treatment of Inborn Errors of Urea Synthesis," *Pediatrics* 68(2):290-297.	
	C6	BERRY, G.T. et al., (2001) "Long-term Management of Patients with Urea Cycle Disorders." *J Pediatrics* 138:S56-S61.	
	C7	BRAHE, C., et al., (2005) "Phenylbutyrate Increases SMN Gene Expression in Spinal Muscular Atrophy Patients," *Eur J Hum Genet* 13:256-259.	
	C8	BRUNETTI-PIERRI, N., et al., (2011) "Phenylbutyrate Therapy for Maple Syrup Urine Disease," *Hum Mol Genet* 20(4):631-640.	
	C9	BRUSILOW, S.W., et al. (1979, September 1) "New Pathways of Nitrogen Excretion in Inborn Errors of Urea Synthesis," *Lancet* 2(8140):452- 454.	
	C10	BRUSILOW, S.W., et al. (1980, February 8) "Amino Acid Acylation: A Mechanism of Nitrogen Excretion in Inborn Errors of Urea Synthesis," *Science* 207:659-661.	
	C11	BRUSILOW, S.W., et al. (1984, June 21) "Treatment of Episodic Hyperammonemia in Children With Inborn Errors of Urea Synthesis," *N Engl J Med* 310(25):1630-1634.	
	C12	BRUSILOW, S.W., et al. (1991) "Phenylacetylglutamine May Replace Urea as a Vehicle for Waste Nitrogen Excretion. *Pediatric Res* 29(2):147-150.	
	C13	BRUSILOW, S.W., et al. (1991) "Treatment of Urea Cycle Disorders," Chapter 5 in Treatment of Genetic Diseases, Desnik, R.J. et al. eds, Churchill Livingstone, New York, New York, pp. 79-94.	

EXAMINER	DATE CONSIDERED

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Sheet	3	of	11	Attorney Docket No.	79532.8004.US01

OTHER PRIOR ART-NON PATENT LITERATURE DOCUMENTS

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	C14	BRUSILOW, S.W., et al. (1993) "Restoration of Nitrogen Homeostasis in a Man with Ornithine Transcarbamylase Deficiency." *J Metabolism* 42:1336-1339.	
	C15	BRUSILOW, S.W., et al. (1994, July 25 - Amendment Dated) "Protocols for Management of Intercurrent Hyperammonemia in Patients with Urea Cycle Disorders," FDA Application to Market a New Drug for Human Use or an Antibiotic Drug for Human Use, 14 pages.	
	C16	BRUSILOW, S.W., et al. (1995) "Urea Cycle Disorders: Clinical Paradigm of Hyperammonemic Encephalopathy." *Prog Liver Diseases* 12:293-309.	
	C17	BRUSILOW, S.W., et al. (1995) "Urea Cycle Enzymes," Chapter 32 in The Metabolic and Molecular bases of Inherited Diseases, Scriver, C.R. et al. eds., McGraw-Hill, Inc. New York, New York, pp.1187-1232.	
	C18	BRUSILOW, S.W., et al. (1996) "Urea Cycle Disorders: Diagnosis, Pathophysiology, and Therapy," *Adv Pediatr* 43:127-170.	
	C19	CALLOWAY, D.H. et al. (1971) "Sweat and Miscellaneous Nitrogen Losses in Human Balance Studies," *J Nutrition* 101:775-786.	
	C20	CALLOWAY, D.H. et al. (1971) "Variation in Endogenous Nitrogen Excretion and Dietary Nitrogen Utilization as Determinants of Human Protein Requirements," *J Nutrition* 101:205-216.	
	C21	CAMACHO, L.H. et al. "Phase I Dose Escalation Clinical Trial of Phenyl butyrate Sodium Administered Twice Daily to Patients With Advanced Solid Tumors," *Invest. New Drugs* 25:131-138 (2007, e-pub. October 20, 2006).	
	C22	CHANG, J. et al., (2001) "Treatment of Spinal Muscular Atrophy by Sodium Butyrate," *PNAS* 98(17):9808-9813.	
	C23	CHUNG, Y.L., et al., (2000) "A Novel Approach for Nasopharyngeal Carcinoma Treatment Uese Phenylbutyrate as a Protein Kinase C Modulator: Implications for Radiosensitization and EBV-Targeted Therapy," *Clin Cancer Res* 6:1452-1458.	
	C24	ClinicalTrials.Gov/Archive View of NCT00551200 on 2007_12_11 "Dose-Escalation Safety Study of Glyceryl Tri (4-Phenylbutyrate)(GT4P) to Treat Urea Cycle Disorders" [accessed 5 October 2009], 4 pages.	

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Sheet	4	of	11	Attorney Docket No.	79532.8004.US01

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	C25	COMTE, B. et al., (2002) "Identification of Phenylbutyrylglutamine, A new Metabolite of Phenylbutyrate Metabolism in Humans," *J Mass Spectrometry*, 37(6):581-590.	
	C26	CUDKOWICZ, ALS (2009) "Phase 2 Study of Sodium Phenylbutyrate in ALS," *Amyotrophic Lateral Sclerosis* 10:99-106.	
	C27	DEFERRARI, G. et al. (1981) "Brain Metabolism of Amino Acids and Ammonia in Patients with Chronic Renal Insufficiency," *Kidney International* 20:505-510.	
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(54) **Title:** METHOD FOR PREVENTING HEPATIC ENCEPHALOPATHIC EPISODES

(57) **Abstract:** A method for preventing an initial hepatic encephalopathic episode in persons at risk for hepatic encephalopathic episodes by administering to the person a therapeutically effective amount of at least one phenyl butyrate compounds or a salt, derivative or metabolite of phenyl butyrate in a pharmaceutically acceptable vehicle.

METHOD FOR PREVENTING HEPATIC ENCEPHALOPATHIC EPISODES

Cross References to Related Applications

[1] This application is a continuation-in-part of application Serial No. 10/122,445, filed April 12, 2002, which is incorporated herein by reference.

Background of the Invention

[2] This invention relates to the treatment or prevention of a class of brain disorders known as chronic hepatic encephalopathy. Hepatic encephalopathy is characterized by a progressive loss of brain and mental function, and is associated with disorders of liver function.

[3] Liver disorders that can be associated with hepatic encephalopathy vary widely in their causation and clinical presentation. Hepatitis, cirrhosis, drug or alcohol abuse, and a variety of other disorders can be associated with hepatic encephalopathy. Hepatic encephalopathies can also result from physical disruption of metabolite delivery to the liver.

[4] The loss of mental function associated with hepatic encephalopathies can be severe. Eventually, patients can lose their ability to carry out ordinary life functions, or even to recognize close relatives. The emotional toll taken by this disorder is heavy, as is the financial burden that it imposes on families and the community.

[5] Phenyl butyrate and its metabolite phenyl acetate are known chemical entities. Sodium phenyl butyrate has been approved for use in the United States to treat disorders of urea cycle metabolism, and is sold under the trademark Buphenyl® for that purpose. It has also been reported that certain of this class of components is effective as an anticancer agent (See, U.S. Patent No. 6,037,376), and as an anti-viral (See, U.S. Patent Nos. 5,877,213 and 5,710,178).

[6] There is also a patient population known to be at risk for hepatic encephalopathic episodes, including, without limitation, patients who are awaiting liver transplants, surgical and/or portal hypertension patients. These patients may suffer from the following, including but not limited to, congenital atresia or stenosis, thrombosis of portal vein, thrombosis of splenic vein, cirrhosis (including, but not limited to portal, postnecrotic, biliary, Wilson's disease, and hemochromatosis), acute alcoholic liver disease, congenital hepatic fibrosis, idiopathic portal hypertension (hepatoportal sclerosis), schistosomiasis, Budd-

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Chiari syndrome, constrictive pericarditis, arterial-portal venous fistula, Banti's syndrome and splenomegaly. Patients may also have surgical radiological shunts ("TIPS" or transjugular intrahepatic portosystemic shunt). TIPS patients also include, without limitation, Ascites patients. *See* Way, Current Surgical Diagnosis & Treatment (1994), 521.

[7] The following factors may also contribute, without limitation, to encephalopathic episodes for at risk patients: the extent of portal-systemic shunt, depressed liver function, intestinal protein load, intestinal flora, azotemia, constipation, the age of the patient, hypokalemia, alkalosis, diuretics, sedatives, narcotics, tranquilizers, infection, hypoxia, hypoglycemia and myxedema. *See* Current Surgical Diagnosis & Treatment, 535.

[8] Hepatic encephalopathy has the following proposed nomenclature in the art. Type A is encephalopathy associated with acute liver failure, Type B is encephalopathy associated with portal-systemic bypass and no intrinsic hepatocellular disease, and Type C is encephalopathy associated with cirrhosis and portal hypertension or portal systemic shunts. Type C has three subcategories: Episodic hepatic encephalopathy which may be precipitated, spontaneous or recurrent, Persistent hepatic encephalopathy which may be mild, severe or treatment dependent and Minimal hepatic encephalopathy. See Ferenci et al., Hepatic Encephalopathy- Definition, Nomenclature, Diagnosis, and Quantification: Final Report of the Working Party at the 11th World Congress of Gastroenterology, Vienna, 1998, Hepatology, vol. 35, Nov. 3, 2002.

[9] A person at risk for hepatic encephalopathic episodes is a person who has not suffered any hepatic encephalopathic episodes or has not suffered any hepatic encephalopathic episode for an extended period of time (about 12 weeks or longer), but has a disorder or medical condition which creates a risk of hepatic encephalopathic episodes. A hepatic encephalopathic episode is a clinical condition characterized by the presence of cerebral dysfunction in patients with liver disease or dysfunction with a West Haven Criteria grading of mental status of a Grade I or II.

[10] Hepatic encephalopathy has been divided into separate grades depending on the severity and symptoms in the West Haven Criteria. All grading in this specification refers to the West Haven Criteria. Grade I patients exhibit trivial lack of awareness, euphoria or anxiety, shortened attention span and impaired

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performance of addition. Grade II patients exhibit lethargy or apathy, minimal disorientation for time or place, subtle personality change, inappropriate behavior and impaired performance of subtraction. Grade III patients exhibit somnolence to semistupor (but responsive to verbal stimuli), confusion and gross disorientation. Grade IV patients are in a coma (unresponsive to verbal or noxious stimuli).

Summary of the Invention

[11] According to the present invention, phenyl butyrate compounds, their salts, derivatives and metabolites are used to treat chronic hepatic encephalopathy. Treatment according to this invention can arrest and even reverse the loss of mental function associated with chronic hepatic encephalopathies.

[12] In the practice of this invention, phenyl butyrate compounds, their salts, derivatives and metabolites are administered in an amount effective to achieve an optimum clinical result.

[13] In another embodiment of the invention, phenyl butyrate compounds, their salts, derivatives and/or metabolites are administered to a person at risk of hepatic encephalopathic episodes in amount effective to prevent, minimize (or lessen the severity of), or delay an initial hepatic encephalopathic episode. An initial hepatic encephalopathy episode is the first episode of the patient.

[14] In another embodiment of the invention, phenyl butyrate compounds, their salts, derivatives and/or metabolites are administered to a person at risk of hepatic encephalopathic episodes in amount effective to prevent, minimize (or lessen the severity of), or delay a hepatic encephalopathic episode, after the patient has not had an episode for at least 12 weeks.

[15] Patients with hepatic encephalopathy type A, B or C may have no recognizable clinical symptoms of brain dysfunction. Sometimes patients with grade I hepatic encephalopathy are described as having subclinical hepatic encephalopathy. However, administering phenyl butyrate compounds, their salts, derivatives and/or metabolites to one at risk of an episode before the clinical symptoms appear prevents the episodes or at least lessen the number and/or severity of episodes.

[16] In a prevention embodiment of the invention, the patient has never had an encephalopathic episode.

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[17] In another prevention embodiment of the invention, the patient has not had an encephalopathic episode in at least about 12 weeks.

[18] The risk of hepatic encephalopathic episodes for TIPS patients were noted in the following studies. In one study (Sanyal AJ, Freedman AM, Shiffman ML, et al., Portosystemic encephalopathy after transjugular intrahepatic portosystemic shunt: results of a prospective controlled study. Hepatology 1994; 20: 46-55, herein incorporated by reference), thirty TIPS patients were followed for 180 days and 9 of these patients experienced 24 episodes of hepatic encephalopathy; 6 of the 9 had a history of hepatic encephalopathy before TIPS and were receiving lactulose after the TIPS procedure. Fourteen of these 24 episodes occurred in the first 30 days after the TIPS procedure.

[19] In another study (Riggio O, Merli M, Pedretti G, et al., Hepatic encephalopathy after transjugular intrahepatic portosystemic shunt. Dig. Dis. Sci. 1996; 41: 578-84, herein incorporated by reference), 15 out of 47 TIPS patients experienced 20 hepatic encephalopathic episodes over a mean 17 month follow-up. Fourteen of the 20 episodes of hepatic encephalopathy occurred during the first 3 months of follow-up.

[20] In a more recent study (Thuluvath PJ, Bal JS, Mitchell S, et al. TIPS for management of refractory ascites: response and survival are both unpredictable. Dig. Dis. Sci. 2003; 48: 542-50, herein incorporated by reference), evaluated the use of TIPS in treatment of refractory ascites (effusion and accumulation of serous fluid in the abdominal cavity) in advanced cirrhosis. Mild hepatic encephalopathy was seen in 12% of patients and severe hepatic encephalopathy was seen in 25% immediately after TIPS.

Detailed Description of the Invention

[21] Sodium phenyl butyrate is conveniently available in a commercial preparation known as Buphenyl®, sold by Ucyclid Pharma, of Scottsdale, Arizona. Buphenyl® is prepared for oral delivery in tablet or powder form.

[22] Other related compounds which are useful in the current invention are the salts, derivatives and metabolites of phenyl butyrate. These are well known in the art. For example, phenyl butyrate compounds are defined to include but are not limited to phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and

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their acids, alcohols, salts, amines, esters, ethers and glycerides, salts, derivatives and metabolites.

[23] U.S. Pat. No. 4,456,942 discloses a group of phenyl acetate derivatives useful in the present invention. These compounds may be described by the following formula:

$$C_6H_5-CH_2-(CH_2)_n-C(=O)-OH$$

where n is 2, 4, 6 or 8.

[24] Another group of compounds useful in the present invention is disclosed in U.S. Pat. No. 5,968,979, which describes phenylalkanoic esters of glycerol according to the following formula:

$$\begin{array}{l} H_2C-O-R_1 \\ \;\;| \\ HC-O-R_2 \\ \;\;| \\ H_2C-O-R_3 \end{array}$$

where R_1, R_2 and R_3 are independently, H

$$HC(=O)-(CH_2)_n-CH_2-C_6H_5$$

or

$$HC(=O)-(C_mH_{2m-2})_n-CH_2-C_6H_5$$

where n is 0 or an even number from 2-24 and m is an even number from 2-24, provided that at least one of R_1, R_2 and R_3 is not H. Glyceryl-tri (4 phenyl butyrate) is an example of such a compound.

[25] Other compounds useful in the method of this invention include phenylacetic acid, its salts (especially sodium salts), halogenated analogs, and alkyl substituted analogs. Specific examples include sodium phenyl acetate and napthyl acetate.

[26] The use of sodium phenyl butyrate to treat chronic hepatic encephalopathy was demonstrated with a group of six patients. Each of these patients suffered from

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moderate to severe chronic hepatic encephalopathy, and had lost significant mental function as a consequence of the disorder.

[27] The patients in this group suffered from a variety of liver diseases, including Hepatitis C, cirrhosis, and damage caused by drug abuse. At least one patient suffered from a combination of these disorders.

[28] Each patient was given 6 gm/m^2/day of sodium phenyl butyrate, divided into three doses. This was done for seven days, during which time the patient's blood chemistry and overall health was monitored and evaluated.

[29] At the end of the seven day regimen, the patients' mental state was reported.

[30] One patient who had suffered significant impairment regained the ability to balance her checkbook, and her family reported a significant improvement in her ability to communicate with others. Another seriously impaired patient regained the ability to drive his car. All patients reported a recovery of mental function, although this benefit was reported to decrease after the use of the drug was terminated.

[31] The improvement in mental function achieved by the method of the present invention has been apparent, as is reported above. Other techniques for measuring improved mental function, such as the PHES score, and auditory nerve conduction studies can be used to demonstrate the effectiveness of this invention.

[32] The dose used in this study proved to be efficacious. However, the dose used in clinical practice will necessarily be adjusted in accordance with the good clinical judgment of the physician. Factors that will be ordinarily considered in this regard include the patient's tolerance for the drug (some of which are known to be difficult to take orally), the severity of the patient's hepatic encephalopathy, the patient's ability to absorb the drug, the patient's total sodium intake, and other factors. Occasionally, it may be necessary to measure the patient's blood levels of sodium phenyl butyrate and/or its metabolites or secondary markers (including but not limited to ammonia) which are known to one of ordinary skill in the art. Such ongoing clinical observation and dosage adjustment are commonplace in good medical practice.

[33] In the above described experiment, the method of this invention was carried out by administering the drug orally. It may be desirable in some circumstances to administer the drug parentally. Some compounds useful in the practice of this invention may be more effective when administered parentally, and others suffer from unpleasant side effects when admitted orally. Intravenous administration is particularly suitable for comatose patients who can be awakened from the comatose state by this method. Sodium phenyl acetate is well suited to parental administration, especially in combination with sodium benzoate. A suitable regimen consists of an initial loading dose and regular additional doses. For example, in infants, a loading dose of about 200-300 mg/kg (preferably about 250 mg/kg) given over 1-2 hours, followed by daily administration of about 200-300 mg/kg (preferably about 250 mg/kg), divided in three, is effective. In adults, a loading and daily dose of about 3.0 to about 8.0 g/m^2 (preferably about 5 to about 6 g/m^2) is effective.

[34] Generally, the orally administered daily dose of sodium phenyl butyrate used in this invention for treatment is between about 3 and about 12 g/m^2. More commonly, the daily dose will be between about 6 and about 9 g/m^2.

[35] In a separate embodiment, patients with advanced liver disease who have recently undergone the TIPS procedure and who may or may not be receiving non-absorbable antibiotics and/or lactulose on a chronic basis are given an oral daily dose of Buphenyl® (sodium phenylbutyrate) tablets 500 mg. The patients are equal to or over 18 years of age, have adequate liver function (ALT (alanine aminotransferase) and/or AST (aspartate aminotransferase) not more than 3 times ULN (upper limit of normal), creatinine clearance > 50 ml/min, and are not Grade II, III or IV hepatic encephalopathic. Patients are excluded due to the inability to obtain informed consent, pregnancy, a history of congestive heart failure requiring current therapy, any hospitalization in the previous 14 days, enrollment in another experimental protocol in the last 30 days, concomitant gastrointestinal disease, active gastrointestinal bleeding, clinical states manifest by sodium retention and edema, known hypersensitivity to sodium phenylbutyrate, use of probenecid, haloperidol, valproate and (non-topical) corticosteroids and if they are nursing mothers or women of childbearing age without adequate contraception. The Buphenyl® is administered over 12 weeks. Before receiving the Buphenyl®, patients in this target population are believed to have a risk of hepatic encephalopathic episode equal to or exceeding 30% (+/-10%) over a 12-week period. It is believed that this preventative treatment may

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reduce the risk by 50%, to a risk of about 15%. The clinical outcome is determined by prevention of a hepatic encephalopathic episode. Biochemical amounts are measured in the blood and/or urine by changes of phenyl butyrate and known metabolites, reduction in ammonia concentration, changes in liver enzymes and changes in branched amino acids concentrations. Neurological status and improvement in the quality of life are also be assessed.

[36] Doses for prevention of hepatic encephalopathic episodes may be dependent on the patient's liver function, and may be titrated as is known in the art, like other drugs products are titrated (e.g. human growth hormone). The dose used in clinical practice will necessarily be adjusted in accordance with the good clinical judgment of the physician. Factors that will be ordinarily considered in this regard include the patient's tolerance for the drug (some of which are known to be difficult to take orally), the patient's ability to absorb the drug, the patient's total sodium intake, and other factors. Occasionally, it may be necessary to measure the patient's blood levels of sodium phenyl butyrate. Such ongoing clinical observation and dosage adjustment are commonplace in good medical practice. These doses may range from about 0.1 g/m^2/day to about 15 g/m^2/day, preferably about 1 g/m^2/day to about 8 g/m^2/day, more preferably about 3 g/m^2/day to about 8 g/m^2/day. It may be beneficial to divide these doses into two or three smaller doses daily (totaling to the daily ranges specified). In several embodiments, these doses may be provided parentally, orally and/or intravenously.

[37] It is understood that while the invention has been described in conjunction with the detailed description thereof, that the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are evident from a review of the following claims.

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I claim:

1. A method of preventing an initial hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites.
2. The method of claim 1, wherein the compound is administered orally.
3. The method of claim 2, wherein the compound is administered in an amount from about 0.1 to about 15 g/m^2/day.
4. The method of claim 2, wherein the compound is administered in an amount from about 1 to about 8 g/m^2/ day.
5. The method of claim 2 wherein the compound is administered in an amount from about 3 to about 8 g/m^2/ day.
6. The method of claims 2, 3, 4 or 5 wherein the compound comprises sodium phenyl butyrate.
7. The method of claims 2, 3, 4 or 5 wherein the compound comprises glyceryl-tri (4 phenyl butyrate).
8. The method of claims 2, 3, 4 or 5 wherein the compound comprises sodium benzoate.
9. The method of claims 2, 3, 4 or 5 wherein the compound comprises sodium phenyl acetate.
10. The method of claims 2, 3, 4 or 5 wherein the compound comprises sodium phenyl acetate and sodium benzoate.
11. The method of claim 1, wherein the compound is delivered parentally.
12. The method of claim 11, wherein the compound is administered in an amount of about 0.1 to about 15 g/m^2/day.
13. The method of claim 11, when an initial loading dose of the compound of about 2 to about 13 g/m^2 is additionally administered to the person.

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14. The method of claim 11, wherein the compound is administered in an amount of about 1 to about 8 g/m^2/day.

15. The method of claim 11, wherein the compound is administer in an amount of about 3 to about 8 g/m^2/day.

16. The method of claims 11, 12, 13, 14 or 15 wherein the compound comprises sodium phenyl butyrate.

17. The method of claims 11, 12, 13, 14 or 15 wherein the compound comprises glyceryl-tri (4 phenyl butyrate).

18. The method of claims 11, 12, 13, 14 or 15 wherein the compound comprises sodium benzoate.

19. The method of claims 11, 12, 13, 14 or 15 wherein the compound comprises sodium phenyl acetate.

20. The method of claim 11, 12, 13, 14 or 15 wherein the compound comprises sodium phenyl acetate and sodium benzoate.

21. A method of lessening severity of an initial hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites and wherein the administering occurs before the hepatic encephalopathic episode.

22. A method of delaying an initial hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites and wherein the administering occurs before the hepatic encephalopathic episode.

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23. A method of lessening severity of a hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes, wherein at least 12 weeks has passed since the person had a prior episode, comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites and wherein the administering occurs before the hepatic encephalopathic episode.

24. A method of delaying a hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes, wherein at least 12 weeks has passed since the person had a prior episode, comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites and wherein the administering occurs before the hepatic encephalopathic episode.

25. A method of preventing a hepatic encephalopathic episode in a person at risk for hepatic encephalopathic episodes, wherein at least 12 weeks has passed since the person had a prior episode, comprising administering to the person a therapeutically effective amount of at least one phenyl butyrate compound in a pharmaceutically acceptable vehicle, wherein the compound comprises one or more of the group consisting of phenyl butyrate, phenyl acetate, sodium benzoate, glyceryl-tri (4 phenyl butyrate), phenylbutyrylglutamines, phenylalkanes, phenylalkenes, and their acids, alcohols, salts, amines, esters, ethers, glycerides, salts, derivatives, and metabolites and wherein the administering occurs before the hepatic encephalopathic episode.

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(54) **Title:** COMPOSITIONS COMPRISING ORNITHINE AND PHENYLACETATE OR PHENYLBUTYRATE FOR TREATING HEPATIC ENCEPHALOPATHY

(57) **Abstract:** The present invention relates to use of ornithine in the manufacture of a medicament for use in combination with at least one of phenylacetate and phenylbutyrate for preventing or treating liver decompensation or hepatic encephalopathy. The invention also relates to use of at least one of phenylacetate and phenylbutyrate in the manufacture of a medicament for use in combination with ornithine for preventing or treating liver decompensation or hepatic encephalopathy.

COMPOSITIONS COMPRISING ORNITHINE AND PHENYLACETATE OR PHENYLBUTYRATE FOR TREATING HEPATIC ENCEPHALOPATHY

Field of the invention

The present invention relates to the prevention or treatment of liver
5 decompensation or hepatic encephalopathy.

Background of the invention

Chronic liver disease is characterised by the gradual destruction of liver tissue over time, whereby healthy and regenerating liver tissue is slowly replaced with scar
10 and necrotic tissue. This is known as liver cirrhosis. Normal liver function is impaired and the scar tissue progressively diminishes blood flow through the liver. As normal regenerating liver tissue is lost, nutrients, hormones, drugs and toxins are no longer effectively processed.

This can result in symptoms including abnormal clearance of proteins absorbed
15 through the intestinal tract, leading to accumulation of ammonia; abnormal excretion, leading to an accumulation of bilirubin in the blood, producing jaundice; increased sinusoidal pressure, leading to fluid accumulation in the abdomen (ascites); and portal hypertension (and portosystemic shunting) wherein scarred liver tissue acts as a barrier to blood flow, leading to increased portal blood pressure and oesophageal varices.

20 Patients with chronic liver disease can be in a fairly stable clinical state and exhibit few or no symptoms. However, such patients are at risk of an abrupt deterioration in their condition which can lead to acute-on-chronic liver failure. This transition from a "compensated" state, where the liver is able to function, albeit at a reduced level, to a "decompensated" state, where liver function fails, involves the effect
25 of precipitating events. Precipitating events associated with chronic liver disease include gastrointestinal bleeding, infection (sepsis), portal vein thrombosis and dehydration.

For example, 50% of patients with cirrhosis of the liver have oesophageal varices and in a third of these patients, the oesophageal varices will burst and cause
30 gastrointestinal bleeding within two years of diagnosis (Grace ND (1992) Gastroenterol Clin North Am 21: 149-161). An upper gastrointestinal bleed is known to increase the susceptibility to life-threatening complications such as bacterial peritonitis, sepsis, renal failure and hepatic encephalopathy (Teran *et al.* (1997) Gastroenterology 112: 473-482;

Garden *et al.* (1985) Br J Surg 72: 91-95; Pauwels *et al.* (1996) Hepatology 24: 802-806; Bleichner *et al.* (1986) Br J Surg 73: 724-726) resulting in the death of about 30% of patients despite adequate control of bleeding (Grace 1992 *supra*).

Hepatic encephalopathy (HE) is a complex neuropsychiatric disorder that occurs in diverse clinical situations such as acute or chronic liver disease and spontaneous portosystemic venous shunting. In the early stages of hepatic encephalopathy subtle mental changes occur such as poor concentration, confusion and disorientation. In severe cases, hepatic encephalopathy can lead to stupor, coma, brain swelling (cerebral edema) and death. In the case of patients who develop HE as a result of chronic liver disease, the onset of HE is often the result of a clinically precipitating event such as gastrointestinal bleeding, sepsis (infection), portal vein thrombosis or dehydration.

Gastrointestinal bleeding and portosystemic shunting allows toxic substances, which are usually metabolised by the liver, to bypass the liver, enter the systemic circulation and cross the blood-brain barrier to exert direct or indirect neurotoxic effects on the central nervous system. Ammonia accumulation is thought to play an important role in the progression of hepatic encephalopathy and multiorgan failure (respiratory failure, cardiovascular failure, kidney failure). In addition to ammonia, septicaemia (or bacterial peritonitis) which develops soon after a gastrointestinal bleed is also likely to be a contributing factor to hepatic encephalopathy.

Liver decompensation can then lead to multiorgan failure and hepatic encephalopathy. In the early stages of hepatic encephalopathy subtle mental changes such as poor concentration or the inability to construct simple objects occurs. In severe cases, hepatic encephalopathy can lead to stupor, coma, brain swelling and death.

The prognosis for patients with chronic liver disease is difficult to estimate because the condition has many causes. Preventative measures to minimise progression from the compensated state to the decompensated state include avoidance of further causative agents which will worsen the condition, such as complete abstinence from alcohol and vaccination against hepatitis A and B.

However, once liver decompensation occurs, the chances of survival are reduced and liver transplantation is the only treatment that can extend life. Since it is liver decompensation that leads to a reduced life expectancy, it is highly desirable to prevent liver decompensation from occurring.

A common therapy for patients with hepatic encephalopathy involves strategies to reduce the concentration of ammonia. These include restriction of dietary protein intake; administration of lactulose, neomycin, L-ornithine L-aspartate (LOLA), or sodium benzoate; and cleansing enemas.

5

Summary of the invention

The present invention concerns the use of ornithine and at least one of phenylacetate and phenylbutyrate to prevent or treat liver decompensation or hepatic encephalopathy (HE) in patients. Isoleucine may also be administered to those patients further having an isoleucine deficiency attributable, for example to gastrointestinal bleeding. Accordingly, the invention provides:

10

- use of ornithine in the manufacture of a medicament for use in combination with at least one of phenylacetate and phenylbutyrate for preventing or treating liver decompensation or hepatic encephalopathy;

15

- use of at least one of phenylacetate and phenylbutyrate in the manufacture of a medicament for use in combination with ornithine for preventing or treating liver decompensation or hepatic encephalopathy;
- use of ornithine and at least one of phenylacetate and phenylbutyrate in the manufacture of a medicament for preventing or treating liver decompensation or hepatic encephalopathy;

20

- products containing ornithine and at least one of phenylacetate and phenylbutyrate for simultaneous, separate or sequential use for preventing or treating liver decompensation or hepatic encephalopathy;
- a pharmaceutical composition comprising ornithine and at least one of phenylacetate and phenylbutyrate;

25

- an agent for preventing or treating liver decompensation or hepatic encephalopathy, comprising ornithine and at least one of phenylacetate and phenylbutyrate; and
- a method of treating a patient having or at risk of having liver decompensation or hepatic encephalopathy, which method comprises administering an effective amount of ornithine and at least one of phenylacetate and phenylbutyrate to said patient.

30

Brief description of the Figures

Figure 1 shows that neutrophil function is altered in patients with cirrhosis and worsens with increasing severity of liver disease.

Figure 2 shows that ammonia reduces neutrophil phagocytosis.

5 Figure 3 shows that ammonia reduces neutrophil chemotaxis.

Figure 4 shows that the effect of ammonia on neutrophil phagocytosis can be reversed by interventions.

Figure 5 shows that a simulated gastrointestinal bleed reduces neutrophil chemotaxis which can be partially reversed by administration of isoleucine.

10 Figure 6 shows that a simulated bleed reduces protein synthesis and stimulates isoleucine oxidation inappropriately.

Figure 7 shows that administration of isoleucine during a simulated bleed enhances protein synthesis but does not reduce ammonia concentration.

Figure 8 shows that administration with LOLA reduces ammonia concentration

15 but allows ammonia to regenerate.

Figure 9 shows that active removal of glutamine prevents the secondary rise in ammonia concentration.

Figure 10 shows that phenylacetate binds glutamine to make an excretable compound and prevents the secondary rise in ammonia.

20 Figure 11 shows the effect of ornithine and phenylbutyrate on ammonia levels in patients with advanced cirrhosis.

Figure 12 shows the effect of ornithine and phenylbutyrate on glutamine levels in patients with advanced cirrhosis.

Figure 13 shows the changes in mental state of patients treated with placebo, O,

25 P or O+P.

Figure 14 shows the effect of ornithine, phenylbutyrate and isoleucine on ammonia levels in patients with advanced cirrhosis.

Figure 15 shows the effect of ornithine, phenylbutyrate and isoleucine on glutamine levels in patients with advanced cirrhosis.

30 Figure 16 shows the effect of ornithine, phenylbutyrate and isoleucine on glycine levels in patients with advanced cirrhosis.

Figure 17 shows the effect of ornithine, phenylbutyrate and isoleucine on isoleucine levels in patients with advanced cirrhosis.

Figure 18 shows the effect of ornithine, phenylbutyrate and isoleucine on ornithine levels in patients with advanced cirrhosis.

Figure 19 shows the effect of ornithine and phenylbutyrate on arterial ammonia in the bile duct ligated rat model.

5 Figure 20 shows the effect of ornithine and phenylbutyrate on plasma ornithine in the bile duct ligated rat model.

Figure 21 shows the effect of ornithine, phenylbutyrate and isoleucine on arterial plasma ammonia levels in a hyperammonaemic acute liver failure rat model.

Figure 22 shows muted arterial ammonia increase in the devascularized pig
10 model of acute liver failure with OP treatment.

Figure 23 shows that ammonia is being taken from the blood by the muscle in the O and the OP treated animals (samples were taken from the femoral vein –artery). In contrast, the placebo and the P alone animals shows an increase in ammonia production by the muscle.

15 Figure 24 shows that ammonia is produced by the gut in all animals except the OP treated animal (samples were taken from the portal drained viscera –artery).

Figure 25 shows that muscle glutamine release is increased by O but not P used in isolation. OP caused a markedly greater release of muscle glutamine (thereby trapping ammonia as glutamine in the muscle).

20 Figure 26 shows that gut glutamine uptake is enhanced by O, but reduced by OP (thereby reduced generation of ammonia in the gut).

Figure 27 shows that arterial ornithine levels increase in the two animals (O alone and OP groups) to which it is administered.

Figure 28 shows that arterial glutamine levels rise with O, but less so with OP.

25 Figure 29 shows that the combination of OP prevents the increase in the ammoniagenic amino acid glycine.

Figure 30 shows that ornithine alone caused an increase in brain water, phenyl acetate induced a small reduction in brain water, while in combination these agents bring about a substantial reduction in brain water (% control).

30

Detailed description of the invention

Throughout the present specification and the accompanying claims the words "comprise" and "include" and variations such as "comprises", "comprising", "includes"

and "including" are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The present invention is concerned with the early treatment of patients with liver
5 disease, before development of liver decompensation and thus before hepatic encephalopathy has occurred, to prevent or delay the onset of liver decompensation. Alternatively, the present invention is concerned with treatment of hepatic encephalopathy by effectively reducing ammonia concentration and maintaining neutrophil function.

10

Subjects to be treated

The present invention is concerned with the prevention or treatment of liver decompensation or hepatic encephalopathy. The subject's liver may therefore be in the compensated state. The subject may have chronic liver disease. The subject may have
15 liver cirrhosis. The subject may have acute liver failure. The subject to be treated may have hepatic encephalopathy.

The onset of both acute and chronic liver disease may be due to a xenobiotic cause. For example, the subject may have been exposed to a chemical, drug or some other agent which causes liver damage. The subject may have a reaction to an over-the-
20 counter, prescriptive or “recreational” drug which causes liver damage. The subject may have been taking Rezulin™ (troglitazone; Parke-Davis), Serzone™ (nefazodone; Bristol-Myers Squibb) or other drugs thought to cause liver damage. The subject may be one who has had an overdose of a particular drug or exceeded the recommended dosage of a drug capable of causing liver damage. For example, the subject may have taken an
25 overdose of paracetamol. The subject may have been exposed to chemicals which can cause liver damage such as, for example, at their place of work. For example, the subject may have been exposed to such chemicals in an industrial or agricultural context. The subject may have consumed plants which contain compounds which can cause liver damage, in particular this may be the case where the subject is an animal,
30 such as a herbivore. For example, the subject may have consumed a plant containing pyrrolizidine alkaloid such as ragwort. The subject may have been exposed to environmental toxins thought to cause liver disease.

Drug-related liver toxicity comprises more than 50% of all cases with acute liver disease (acute liver failure). Acetaminophen-(also known as paracetamol and *N*-acetyl-p-aminophenol) toxicity is the most common cause of acute liver failure in the United States and Great Britain. Long-term moderate to heavy alcohol users who take
5 acetaminophen in therapeutic or modestly excessive doses are at risk of severe hepatic injury and possibly acute liver failure. Alcohol use potentiates the toxic effects of acetaminophen. Idiosyncratic drug toxicity also contributes to acute liver failure. Idiosyncratic drug toxicity is thought to be a hypersensitivity response wherein the subject responds to a drug in a pharmacologically abnormal way. This abnormal
10 response can lead to acute liver failure.

The acute liver failure or chronic liver disease may be caused by infection with a pathogenic organism. For example, the liver disease may be due to viral infection. In particular, the subject may be infected, or have been infected, with a virus which causes hepatitis. The subject may have chronic viral hepatitis. The virus may, for example, be
15 hepatitis B, C or D virus. In some cases, and in particular where the subject has viral hepatitis, the subject may also be infected with HIV-I or II. The subject may have AIDS. It is possible that the subject may have been, or be, infected with other organisms which cause liver disease and in particular those which are present in the liver during some stage of their life cycle. For example, the subject may have, or have
20 had, liver fluke.

The subject may have an inherited disease which causes, or increases the risk of, chronic liver disease. For example, the subject may have one or more of hepatic hemochromatosis, Wilson's disease or α-1-antitrypsin deficiency. The subject may have an inherited disorder which causes some kind of structural or functional abnormality in
25 the liver which increases the likelihood of liver fibrosis. The subject may be genetically predisposed to develop an autoimmune disorder which damages the liver and hence which can contribute to liver fibrosis.

The chronic liver disease may be alcohol-induced. A man or woman to be treated may be, or have been, an alcoholic. He or she may be, or have been, consuming
30 on average 50 or more units of alcohol per week, 60 or more units of alcohol per week, 75 or more units of alcohol per week and even 100 or more units of alcohol per week. The man or woman may be, or have been, consuming on average up to 100 units of alcohol per week, up to 150 units of alcohol per week and even up to 200 units of

alcohol per week. The measurement of one unit of alcohol differs from country to country. Here, one unit equals 8 grams of ethanol in accordance with the United Kingdom standard.

The man or woman may have been consuming such levels of alcohol for 5 or more years, 10 or more years, 15 or more years or 20 or more years. The subject may have been consuming such levels of alcohol for up to 10 years, up to 20 years, up to 30 years and even up to 40 years. In cases of alcohol-induced liver cirrhosis the subject may be aged, for example, 25 years or over, 35 years or over, 45 years or over and even over 60 years.

The subject may be male or female. Women may be more susceptible to the adverse effects of alcohol than men. Women can develop alcoholic chronic liver disease in a shorter time frame and from smaller amounts of alcohol than men. There seems to be no single factor to account for increased susceptibility to alcoholic liver damage in females, but the effect of hormones on the metabolism of alcohol may play an important role.

In other embodiments of the invention, the subject may have one or more of a number of other conditions known to result in liver damage such as, for example, primary biliary cirrhosis, autoimmune chronic active hepatitis, and/or schistosomiasis (parasitic infection). The subject may have or have had a bile duct blockage. In some cases, the underlying cause of chronic liver disease may not be known. For example the subject may have been diagnosed as having cryptogenic cirrhosis. In one embodiment, the subject may be suspected of having any of the conditions listed herein.

Methods for diagnosing chronic liver disease, acute liver failure and hepatic encephalopathy are well known in the art and in particular to clinicians and veterinarians in the field. Preferably, the subject will have been diagnosed as having a liver disease and hepatic encephalopathy, for example by a medical or veterinarian professional. The subject may display one or more symptoms associated with liver disease such as one or more of jaundice, ascites, skin changes, fluid retention, nail changes, easy bruising, nose bleeds, oesophageal varices, and in male subjects may have enlargement of breasts. The subject may display exhaustion, fatigue, loss of appetite, nausea, weakness and/or weight loss. The subject may also display one or more symptoms associated with hepatic encephalopathy such as one or more of confusion, disorientation, dementia, stupor, coma, cerebral edema, multiorgan failure (respiratory

failure, cardiovascular failure or kidney failure), muscle stiffness/rigidity, seizures or speech impairment. The subject to be treated may or may not be taking other drugs to treat liver disease. The subject to be treated may be at risk of developing hepatic encephalopathy.

5 The liver disease may have been, or be, confirmed by physical examination including techniques such as ultrasound. Liver biopsies may have been taken to look for build up of fibrosis, necrotic cells, cellular degeneration and/or inflammation and other characteristic features of liver disease. Liver function may have been assessed in the subject to determine whether this is compromised in the subject. The nature and 10 underlying cause of the liver disease may be characterized. Any history of exposure to causative agents of liver disease may be determined.

The subject to be treated may be at risk for hepatic encephalopathic episodes, for example patients who are awaiting liver transplants, surgical and/or portal hypertension patients. A person at risk for hepatic encephalopathic episodes is a person who has not 15 suffered any hepatic encephalopathic episodes or has not suffered any hepatic encephalopathic episode for an extended period of time (about 12 weeks or longer), but has a disorder or medical condition which creates a risk of hepatic encephalopathic episodes. A hepatic encephalopathic episode is a clinical condition characterised by the presence of cerebral dysfunction in patients with liver disease or dysfunction. There is a 20 wide spectrum of mental disturbances in hepatic encephalopathy which range from minimal where the main effects are a reduction in the quality of life, to overt which leads to coma and ultimately death.

Scoring systems may be used to assess the severity of liver disease and hepatic encephalopathy and also the prognosis of subjects. The Child-Pugh, West Haven 25 Criteria, Glasgow Coma Scale or modified Child-Pugh scoring system may be used. Alternatively, the (APACHE) II scoring system may be used. Points are assigned to parameters including serum bilirubin levels, serum albumin levels and to signs including presence of ascites or encephalopathy. Subjects to be treated may be classified in Child-Pugh class A , B or C. Generally subjects to be treated are classified in Child- 30 Pugh class C.

A man or woman to be treated may be aged, for example from 25 to 80 years. In one embodiment, the man or woman is aged from 45 to 70 years. In another

embodiment, the man or woman is aged from 25 to 44 years. In a further embodiment, the man or woman is aged over 65 years.

The invention does have veterinary use, however. The subject to be treated may be a farm animal for example, a cow or bull, sheep, pig, ox, goat or horse or may be a 5 domestic animal such as a dog or cat. The subject may or may not be an animal model for liver disease. The animal may be any age, but will often be a mature adult subject.

Formulation

The amino acids used in the present invention may be pure crystalline amino 10 acids. In general, the amino acids are in the L-form, rather than the D-form, or a mixture of D and L. Isolated forms of the amino acids are typically used. Any active form of the amino acid may be used to prevent or treat the liver decompensation or hepatic encephalopathy. A pharmaceutically acceptable form of the amino acid may be used. The amino acids may be employed as free amino acids or amino acid salts or 15 derivatives.

Ornithine may be in pure crystalline amino acid form. In general, ornithine is in the L-form, rather than the D-form, or a mixture of D and L. Isolated forms of ornithine are typically used. Any active form of ornithine may be used or a pharmaceutically acceptable form of ornithine may be used. Ornithine may be employed as a free amino 20 acid or an amino acid salt or derivative.

Typically, ornithine is used as a single, monomeric amino acid. Ornithine may be used in salt form, for example ornithine hydrochloride may be used. Ornithine may be in the form of a physiologically acceptable salt in free form. Therefore, the ornithine or the ornithine salt are typically not chemically bound, or covalently linked to any 25 other agent.

Derivatives of ornithine may be used. For example, keto or hydroxy analogs of ornithine may be administered as sodium or calcium salts. Keto acids of ornithine include ornithine ketoglutarate, ornithine ketoleucine and ornithine ketovaline. Salts or derivatives of ornithine may be used in place of or in addition to free ornithine.

30 At least one of phenylacetate and phenylbutyrate may be used. Phenylacetate and/or phenylbutyrate may be in physiologically acceptable salt form, such as an alkali metal or alkaline earth metal salt. The salt may be sodium phenylacetate or sodium phenylbutyrate. The salt form of phenylacetate and phenylbutyrate may be in free form.

Therefore the phenylacetate and phenylbutyrate or phenylacetate salt and phenylbutyrate salt are typically not chemically bound, or covalently linked to any other agent.

Optionally isoleucine is used. Isoleucine may be in pure crystalline amino acid form. In general, isoleucine is in the L-form, rather than the D-form, or a mixture of D and L. Isolated forms of isoleucine are typically used. Any active form of isoleucine may be used or a pharmaceutically acceptable form of isoleucine may be used. Isoleucine may be employed as a free amino acid or an amino acid salt or derivative.

Typically, isoleucine is used as a single, monomeric amino acid. Isoleucine may be used in salt form, for example isoleucine hydrochloride may be used. Isoleucine may be in the form of a physiologically acceptable salt in free form. Therefore, the isoleucine or the isoleucine salt are typically not chemically bound, or covalently linked to any other agent.

Pharmaceutical compositions

The ornithine and the phenylacetate and/or phenylbutyrate are typically formulated for administration with a pharmaceutically acceptable carrier or diluent. The ornithine and the phenylacetate and/or phenylbutyrate may thus be formulated as a medicament with a standard pharmaceutically acceptable carrier(s) and/or excipient(s) as is routine in the pharmaceutical art. The exact nature of the formulation will depend upon several factors including the desired route of administration. Typically, ornithine and the phenylacetate and/or phenylbutyrate are formulated for oral, intravenous, intragastric, intravascular or intraperitoneal administration.

The pharmaceutical carrier or diluent may be, for example, an isotonic solution such as physiological saline. Solid oral forms may contain, together with the active compound, diluents, e.g. lactose, dextrose, saccharose, cellulose, corn starch or potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents; e.g. starches, gum arabic, gelatin, methylcellulose, carboxymethylcellulose or polyvinyl pyrrolidone; disaggregating agents, e.g. starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, such as lecithin, polysorbates, laurylsulphates; and, in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulations. Such pharmaceutical preparations may be manufactured in known

manner, for example, by means of mixing, granulating, tabletting, sugar-coating, or film-coating processes.

Liquid dispersions for oral administration may be syrups, emulsions or suspensions. The syrups may contain as carriers, for example, saccharose or saccharose with glycerine and/or mannitol and/or sorbitol.

Suspensions and emulsions may contain as carrier, for example a natural gum, agar, sodium alginate, pectin, methylcellulose, carboxymethylcellulose, or polyvinyl alcohol. The suspensions or solutions for intramuscular injections may contain, together with ornithine and at least one of phenylacetate and phenylbutyrate, a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

Medicaments of the invention can comprise ornithine as the only amino acid component. Medicaments of the invention can comprise ornithine and isoleucine as the only amino acid components. The medicament may consist essentially of ornithine and at least one of phenylacetate and phenylbutyrate. The medicament may consist essentially of ornithine, isoleucine and at least one of phenylacetate and phenylbutyrate.

The medicament may consist essentially of ornithine, phenylacetate and/or phenylbutyrate and a pharmaceutically acceptable carrier. Such a medicament therefore contains substantially no other amino acid in addition to ornithine. The medicament may consist essentially of ornithine, isoleucine, phenylacetate and/or phenylbutyrate and a pharmaceutically acceptable carrier. Such a medicament therefore contains substantially no other amino acid in addition to ornithine and isoleucine.

The phenylacetate may be present in an amount from 5 to 100%, for example from 10 to 50%, or 20 to 40%, by weight of the weight of ornithine. The phenylbutyrate may be present in an amount from 5 to 100%, for example from 10 to 50%, or 20 to 40%, by weight of the weight of ornithine.

However, the medicament may comprise free aspartate, glutamate or arginine in non-peptide form, typically in an insubstantial amount. Generally, the amount by weight of aspartate, glutamate or arginine does not exceed the amount by weight of ornithine. By an insubstantial amount, it is meant that the amount by weight of aspartate, glutamate or arginine, or a combination of these amino acids, does not exceed 20% by weight of ornithine. Therefore, the medicament may comprise substantially no aspartate. In one embodiment, the composition does not comprise aspartate, glutamate

or arginine. Trace amounts of aspartate, glutamate or arginine may be present in the composition. By trace amount, it is meant that the amount by weight of aspartate, glutamate or arginine, or a combination of these amino acids, does not exceed 1% by weight of ornithine. Preferably, the amount by weight of aspartate, glutamate or
5 arginine does not exceed 0.5% by weight of ornithine.

In another embodiment, the composition may comprise yet other amino acids in non-peptide form, typically as the free amino acid or a physiologically acceptable salt thereof in free form. The amount of these other amino acids generally does not exceed the amount by weight of ornithine. For example, the other amino acids may be present
10 in an amount by weight up to 20%, for example from 5 to 20%, of the weight of ornithine. Such other amino acids that may be present in the composition include essential and non-essential amino acids. The composition may comprise other branched chain amino acids (BCAAs). BCAAs include isoleucine, valine and leucine. Thus, a composition of the invention may further comprise isoleucine and/or valine and/or
15 leucine.

Treatment

Ornithine and at least one of phenylacetate and phenylbutyrate are administered in combination to a subject for preventing or delaying the onset of liver decompensation
20 or hepatic encephalopathy. Ornithine and at least one of phenylacetate and phenylbutyrate can thus be administered in combination to improve the condition of a subject, for example a subject suffering from chronic liver disease following a precipitating event. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination to alleviate the symptoms of a subject, for example the
25 symptoms associated with chronic liver disease in a subject following a precipitating event. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination to combat or delay the onset of liver decompensation or hepatic encephalopathy.

Ornithine and at least one of phenylacetate and phenylbutyrate may be
30 administered in combination to a subject for treatment of hepatic encephalopathy. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination to improve the condition of a patient suffering from hepatic encephalopathy. Ornithine and at least one of phenylacetate and phenylbutyrate may be

administered in combination to alleviate the symptoms associated with hepatic encephalopathy. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination to combat hepatic encephalopathy. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination to prevent
5 an initial hepatic encephalopathic episode in a person at risk of for hepatic encephalopathic episodes. Ornithine and at least one of phenylacetate and phenylbutyrate may be administered in combination lessen the severity of an initial hepatic encephalopathic episode in a person at risk of for hepatic encephalopathic episodes. Ornithine and at least one of phenylacetate and phenylbutyrate may be
10 administered in combination to delay an initial hepatic encephalopathic episode in a person at risk of for hepatic encephalopathic episodes.

Development of liver decompensation and hepatic encephalopathy involves "precipitating events" (or "acute attacks"). Such precipitating events include gastrointestinal bleeding, infection (sepsis), portal vein thrombosis and dehydration. The
15 onset of such an acute attack is likely to lead to hospitalisation. The patient may suffer one of these acute attacks or a combination of these acute attacks.

A subject who has had or is suspected of having had an acute attack is treated according to the invention with ornithine and phenylacetate and/or phenylbutyrate in combination to prevent progression of the liver to the decompensated state. The
20 invention can therefore prevent the medical consequences of liver decompensation such as hepatic encephalopathy. The ornithine and phenylacetate and/or phenylbutyrate may be used to preserve liver function. Use of ornithine and phenylacetate and/or phenylbutyrate may thus extend the life of a patient with liver disease. In one embodiment, the metabolic consequences of a gastrointestinal bleed such as
25 hyperammonemia, hypoisoleucemia and reduced protein synthesis in the post-bleeding period are prevented.

Typically, treatment of subjects may begin as soon as possible after the onset or the suspected onset of a precipitating event (acute attack). Preferably, treatment of the subject begins prior to repeated acute attacks. More preferably, treatment of the subject
30 begins following the first acute attack.

Treatment is typically given promptly after the start of an acute attack. Treatment may begin after the symptom(s) of an acute attack or suspected acute attack have been detected e.g. by a medic such as a physician, a paramedic or a nurse.

Treatment may begin upon hospitalisation of the subject. Treatment may thus begin within 6 hours, within 3 hours, within 2 hours or within 1 hour after the symptom(s) of an acute attack or suspected acute attack have been detected. Treatment of the subject may therefore begin from 1 to 48 hours, for example from 1 to 36 hours or from 1 to 24 5 hours after the symptom(s) of an acute attack or suspected acute attack have been detected.

Treatment may occur for up to 8 weeks, for example up to 6 weeks, up to 4 weeks or up to 2 weeks after the symptom(s) of an acute attack or suspected acute attack have been detected. Treatment may therefore occur for up to 48 hours, for example for 10 up to 36 hours or for up to 24 hours after the symptom(s) of an acute attack or suspected acute attack have been detected. Typically, treatment occurs to the time when recovery from the acute precipitating event is evident.

The subject is treated with the ornithine and the phenylacetate and/or phenylbutyrate. Ornithine and at least one of phenylacetate and phenylbutyrate may be 15 administered in combination in a single medicament, or separately in two or three different medicaments. Where ornithine and at least one of phenylacetate and phenylbutyrate are to be administered in a combined medicament, the combination may be prepared immediately before administration, or may be stored as a combined medicament.

20 Where the ornithine and the phenylacetate and/or phenylbutyrate are to be administered separately, the medicaments may be administered simultaneously or sequentially over a period of time. Two or three separate medicaments may be administered over a period of time.

Where two medicaments are administered, ornithine may be administered first, 25 followed by administration of the phenylacetate and phenylbutyrate, the phenylacetate or the phenylbutyrate. Alternatively, the phenylacetate and phenylbutyrate, the phenylacetate or the phenylbutyrate may be administered first, followed by ornithine. In another embodiment, a combination of ornithine and phenylacetate may be administered first, followed by administration of phenylbutyrate. Alternatively, a combination of 30 ornithine and phenylbutyrate may be administered first, followed by administration of phenylacetate. In another embodiment, phenylacetate may be administered first, followed by administration of a combination of ornithine and phenylbutyrate.

Alternatively, phenylbutyrate may be administered first, followed by administration of a combination of ornithine and phenylacetate.

Where three medicaments are administered, ornithine, phenylacetate and phenylbutyrate are administered at separate times. Ornithine may be administered first, second or third. Where ornithine is administered first, phenylacetate or phenylbutyrate may be administered second, followed by administration of phenylbutyrate or phenylacetate. Where ornithine is administered second, phenylacetate or phenylbutyrate are administered first, and phenylbutyrate or phenylacetate are administered third. Where ornithine is administered third, phenylacetate or phenylbutyrate are administered first, and phenylbutyrate or phenylacetate are administered second.

The second medicament may be administered up to 5 hours, such as up to 2 hours or up to 1 hour, following administration of the first medicament. The second medicament can thus be administered from 15 minutes to 5 hours, for example from 30 minutes to 4 hours or from 1 hour to 3 hours, following administration of the first medicament.

The third medicament may be administered up to 5 hours, such as up to 2 hours or up to 1 hour, following administration of the second medicament. The third medicament can thus be administered from 15 minutes to 5 hours, for example from 30 minutes to 4 hours or from 1 hour to 3 hours, following administration of the second medicament.

The medicaments of the invention may be administered at the same site or at different sites. The medicaments of the invention may be administered via the same route or by different routes. A medicament of the invention may be administered by any suitable route. Preferably it is administered by oral, intravenous, intragastric, intraperitoneal or intravasular routes. For example, when ornithine and at least one of phenylacetate and phenylbutyrate are administered separately, they may all be administered orally or they may all be administered intravenously or ornithine may be administered orally and the phenylacetate and/or phenylbutyrate may be administered intravenously, or the phenylacetate and/or phenylbutyrate may be administered orally and ornithine may be administered intravenously.

Therapeutically effective amounts of ornithine, the phenylacetate and/or phenylbutyrate and the optional isoleucine are administered to the subject. The doses of the ornithine, the phenylacetate and/or phenylbutyrate and the isoleucine can be

determined according to various parameters such as the age, weight and condition of the subject to be treated; the type and severity of the liver disease; the route of administration; and the required regimen.

A typical dose of ornithine, of phenylacetate or phenylbutyrate, or of isoleucine is from 0.02 to 1.25, for example from 0.1 to 0.5, g per kg of body weight, depending on such parameters. Consequently, a dosage of ornithine, of phenylacetate or phenylbutyrate, or of isoleucine may be from 1 g to 50 g such as from 5 g to 30 g. The dosage of ornithine may be 10 to 30 g. The dose of isoleucine may be 5 to 15 g. The ornithine and phenylacetate / phenylbutyrate may be administered in a weight ratio from 10:1 to 1:10 such as from 5:1 to 1:5 or from 2:1 to 1:2 or about 1:1. A physician will be able to determine the required dosage of ornithine and of phenylacetate or phenylbutyrate and of the optional isoleucine for any particular subject.

A single dose of ornithine and a single dose of phenylacetate and/or phenylbutyrate may be administered. Optionally, a single dose of isoleucine may also be administered. Alternatively multiple doses, for example two, three, four or five doses, of ornithine and/or of the phenylacetate and/or phenylbutyrate and/or of the optional isoleucine may be administered. Such multiple doses may be administered over a period of one month or two weeks or one week. In another embodiment, a single dose or multiple doses such as two, three, four or five doses of ornithine and/or of phenylacetate and/or phenylbutyrate may be administered daily.

Other amino acids may be administered to a subject as noted above. The or each such other amino acid may be administered in the same medicament as the ornithine and/or the phenylacetate and/or phenylbutyrate, or may be administered separately. When administered separately, the or each other amino acid may be given simultaneously with, or at a different time such as up to 5 hours, up to 2 hours or up to 1 hour before or after, the administration of ornithine and/or phenylacetate and/or phenylbutyrate. The or each other amino acid is typically administered orally or intravenously.

A therapeutically effective amount of the or each other amino acid is administered to the subject. The dose will be dependent upon various parameters such as those noted above for ornithine, phenylacetate and phenylbutyrate. A typical dose of the or each other amino acid is from 0.02 to 1.25, for example from 0.1 to 0.5, g per kg

of bodyweight. A dosage of the or each other amino acid may therefore be from 1 g to 50 g such as 5 g to 30 g.

A single dose of the or each other amino acid may be administered. Alternatively, multiple doses, for example two, three, four or five doses may be administered. Such multiple doses may be administered over a period of one month or two weeks or one week. In another embodiment, a single dose or multiple doses such as two, three, four or five doses may be administered daily.

The following Examples illustrate the invention.

Example 1: Neutrophil function is altered in patients with cirrhosis and worsens with increasing severity of liver disease

Methods for Measurement of Neutrophil Phagocytosis and oxidative burst

Phagotest: Heparinised whole blood was incubated with opsonised FITC-labelled *E coli* and CD16. The cells were then analysed by flow cytometry (FACScan Becton Dickinson), gated through forward and side scatter and subsequently assessed on the basis of R-phycoerythrin (PE) [Immunotech, Marseille, France] flurochrome expression to identify CD16 positive cells. The gated population was then assessed for the presence of FITC-labelled bacteria.

Phagoburst: Heparinised whole blood was incubated with opsonised *E coli* suspension to stimulate oxidative burst. A substrate solution was added to determine the conversion of dihydrorhodamine (DHR) 123 to the flurogenic compound Rhodamine (R) 123. The reaction was stopped and fixed before incubation with CD16 antibody for positive neutrophil identification. Analysis was then undertaken by flow cytometry.

Neutrophil Chemotaxis: Neutrophil chemotaxis was measured using a modified Boyden chamber method using interleukin-8 as chemo-attractant to stimulate chemokinesis.

Patients and Methods

We studied 30 patients with cirrhosis (Alcoholic cirrhosis; mean age 53.2 (SEM 4.6) and 20 healthy volunteers. Patients with cirrhosis were classified as those with superimposed alcoholic hepatitis (AH+) and those with decompensated or compensated

livers. Phagotest was used to determine the phagocytic capacity and Phagoburst was used to determine whether the cells were able to generate oxidative burst when exposed to *E coli*.

5 **Results**

We observed that neutrophils from cirrhotic patients had a significantly reduced ability to phagocytose bacteria. We also found that patients with cirrhosis had a reduced capacity to respond to stimulation of the neutrophils by *E coli* in terms of increasing the rate of generation of oxidative burst (Figure 1). This reduction in capacity correlated 10 with the severity of liver disease indicating that the more advanced the stage of liver disease, the less the ability to respond to and cope with infection.

Example 2: Ammonia reduces phagocytic capacity in neutrophils

15 **Methods for Measurement of Neutrophil Phagocytosis and oxidative burst**

As in Example 1.

Patients and methods

Blood was collected from healthy volunteers (n=15) and incubated for 1 hour 20 with increasing concentrations of ammonia. The ability of the neutrophils to phagocytose bacteria was measured using the Phagotest and Neutrophil chemotaxis assays. 10ng/ml IL-8 was used in the Neutrophil chemotaxis assay.

Results

25 With incubation of increasing concentrations of ammonia, there was a significant reduction in neutrophil phagocytosis (Figure 2) and also in neutrophil chemotaxis (Figure 3).

Example 3: The effect of ammonia on neutrophil phagocytosis can be reversed
30 **by interventions**

Methods for Measurement of Neutrophil Phagocytosis and oxidative burst

As in Example 1.

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Patients and methods

Blood was collected from healthy volunteers (n=15) and incubated for 1 hour with ammonia and selected amino acids. The ability of the neutrophils to phagocytose
5 bacteria was measured using the Phagotest assay.

Results

We observed that the ammonia-induced reduction in neutrophil phagocytosis could be partially reversed by ornithine and glutamine (Figure 4). However, neutrophil
10 phagocytosis was made worse by co-incubation of ammonia with aspartate, but remained unchanged with L-ornithine L-aspartate.

Example 4: A simulated gastrointestinal bleed reduces neutrophil chemotaxis which can be partially reversed by administration of isoleucine

15

Methods

Ten overnight fasted, metabolically stable patients with biopsy proven cirrhosis of the liver [9 males and 1 female; mean 49.6 years (SEM 9.1); mean Child-Pugh score of 7.8 (SEM 1.2)] were studied prior to and two hours after an oral administration of 75
20 grams of an amino acid mixture that mimics the hemoglobin molecule (Nutricia, Cuijk, Netherlands). In seven other patients [4 male and 3 female; mean 51.4 years (SEM 6.7); mean Child-Pugh score of 8.1 (SEM 1.4)], following administration of the amino acid mixture, isoleucine was administered intravenously over a 2 hour period (iso-osmotic solution containing 40mg/l of isoleucine at a rate of 100 ml/hr). Neutrophil chemotaxis
25 (see Example 1 for method) and plasma ammonia were measured in peripheral venous blood samples.

Results

Neutrophil chemotaxis was significantly lower in these cirrhotic patients
30 compared with age-matched controls (53.3 SEM 4.6) and was significantly reduced after simulated bleeding from 31 (±4.2) to 8 (±5.4) cells/high power field ($p<0.0001$) (Figure 5). Plasma concentration of ammonia increased significantly from 75.1 (±4.2) to 124 (±8.5) ($p<0.001$). The change in the concentration of ammonia correlated with the

change in neutrophil chemotaxis (r=0.65 and p <0.05). The reduction in neutrophil chemotaxis observed with the simulated bleed was abrogated in the group of patients treated with isoleucine 25.4 (±6.0) cells/high power field.

5 **Example 5: A simulated bleed reduces protein synthesis and stimulates isoleucine oxidation inappropriately**

Methods

Five overnight fasted patients with cirrhosis of the liver were recruited. A blood
10 sample was collected and expired air was sampled before the start of the infusion of the stable isotopes for the measurement of background isotope enrichment. Then the patients received a primed continuous intravenous infusion of [1-^{13}C]-isoleucine (1 mg/kg bw/h) until the end of the experiment (t=480 min).

15 **Results**

Figure 6 shows average whole body rate of appearance of isoleucine (Wb Ra) and isoleucine oxidation during the last hour of saline (black bars) and amino acid (grey bars) infusion (values in mean $\pm$ SEM; # represents p <0.05). An upper GI bleed in patients with cirrhosis resulted in a reduction in isoleucine and markedly decreased
20 whole body protein synthesis. The fraction of isoleucine flux used for oxidation did not change after the simulated bleed despite the marked reduction in isoleucine concentration, pointing to occurrence of BCAA antagonism.

Example 6: Administration of isoleucine during a simulated bleed enhances
25 **protein synthesis but does not reduce ammonia concentration**

Methods

Sixteen metabolically stable patients with biopsy-proven cirrhosis of the liver were studied. Patients were randomized either to supplementation with isoleucine
30 (40mg/L solution; 50 ml/hr) or placebo during a simulated bleed over a 4-hour period. Protein synthesis (measured using primed continuous infusion of L-[ring-$^2H_5$]phenylalanine), L-[ring-$^2H_4$]tyrosine and L-[ring-$^2H_2$]tyrosine) and ammonia.

Results

The results showed that infusion of isoleucine during a simulated bleed in patients with cirrhosis of the liver restores impaired protein synthesis of liver and muscle leading to a net anabolic state in these organs (Table 1). Ammonia concentration increased significantly in both groups but was not significantly different between those administered with isoleucine or placebo (Figure 7).

Example 7: Aspartate accumulation following infusion of L-ornithine L-aspartate in patients with advanced cirrhosis

Methods

5 patients with advanced cirrhosis who were awaiting liver transplantation (age: 59; 3 male, Child Class C disease, severe ascites, creatinine 102 umol/L) were undergoing treatment with 40 g/day of L-ornithine L-aspartate.

Results

Over a 3 day period there was a significant and progressive increase in the aspartate concentration increasing to 5 times the basal value (Table 2).

Table 2

	PRE	Day 1	Day 2	Day 3
ASPARTATE (μmol/L))	72 (11.8)	178 (23.2)	289 (27.1)	354 (31.1)

Table 1

Protein kinetics determined using the Phe model at t= 0 hours and at study end

		Time	Protein synthesis	P	Protein breakdown	P	Net Balance	P
Liver	SB-saline	0	415 ± 120		263 ± 50		152 ± 76	
		End	274 ± 250	0.445	108 ± 162	0.366	166 ± 231	0.836
	SB-isoleucine	0	218 ± 37		109 ± 25		98 ± 33	
		End	839 ± 221	0.038	157 ± 204	0.412	682 ± 165	0.010
Leg	SB-saline	0	117 ± 52		137 ± 51		-20 ± 19	
		End	372 ± 211	0.189	288 ± 175	0.232	87 ± 140	0.694
	SB-isoleucine	0	-31 ± 201		196 ± 61		-185 ± 152	
		End	377 ± 135	0.209	159 ± 100	0.535	261 ± 102	0.005

Data are mean ± SEM in nmol/kg body cell mass/min. End values represent the mean values of the final hour of the amino acid infusion. Protein synthesis data of liver and kidney are corrected for hydroxylation (see methods). Statistics: p values for Mann-Whitney U test for differences within groups; no significant differences were found between groups

Example 8: Administration with LOLA reduces ammonia concentration but allows ammonia to regenerate

Patients and Methods

5 Eight patients with cirrhosis (age 56 (5.6), 5M, ALD-6; Grade 2 HE: 4; Grade 3-4 HE: 4) were treated with an infusion of LOLA (40 g over 8 hours). Blood was sampled for the measurement of ammonia and glutamine.

Results

10 The results showed that administration of LOLA resulted in a significant reduction in ammonia concentration with a concomitant rise in glutamine concentration (Figure 8). This reduction in ammonia had beneficial effects upon the severity of HE. However, when LOLA was stopped, there was a rebound increase in the circulating ammonia levels, resulting in recurrence of HE in 3 of the 6 patients that had improved.

15

Example 9: Active removal of glutamine prevents the secondary rise in ammonia concentration

Patients and Methods

20 3 patients (age 45 (4.1) 2M, ALD, all HE grade 3, HRS all 3) that were undergoing heamofiltration (CVVH) were treated with an infusion of LOLA (40 g over 8 hours). Blood was sampled for the measurement of ammonia and glutamine.

Results

25 The results showed that LOLA resulted in a reduction in ammonia concentration but the addition of dialysis prevented the concomitant increase in glutamine concentration (Figure 9). Therefore, we believe there was a sustained reduction in ammonia concentration.

30 **Example 10: Phenylacetate binds glutamine to make an excretable compound and prevents the secondary rise in ammonia**

Patients and Methods

6 patients with acute liver failure (5 non-A non-B Hepatitis) and severe encephalopathy (Grade 3-4) were treated with LOLA and phenylacetate (40g/day over 8 hours).

5

Results

There was no significant increase in glutamine concentration and ammonia levels were reduced with the combined treatment (Figure 10). No rebound increase in ammonia was observed.

10

Example 11: The effect of ornithine and phenylbutyrate in human patients with hepatic encephalopathy

Patients

15 1. Groups-3 patients per group. Total 12.

2. Inclusion criteria

- adult patients aged 18-80 years, - liver cirrhosis documented by histology or clinical criteria

20 - HE type C, - ammonia concentration of > 80 umol/L, informed consent/assent

3. Exclusion criteria

- other concomitant neurological disorder, - use of another specific ammonia lowering drug, - respiratory failure requiring mechanical ventilation and sedation, - uncontrolled

25 gastrointestinal bleeding, - hypotension requiring inotropes, overt renal failure (creatinine >2 mg/dl), hemodialysis, - extracorporeal liver support, known hypersensitivity to any of the study drugs, - pregnancy.

Assessment of Mental State

30 Grading of hepatic encephalopathy (West Haven Criteria)

Grade 0 (minimal HE)	normal mental state (one or more quantifiable abnormalities on psychometric testing)
Grade 1	trivial lack of awareness euphoria or anxiety

	shortened attention span impaired performance of addition
Grade 2	lethargy or apathy minimal disorientation for time or place subtle personality change inappropriate behaviour impaired performance of subtraction
Grade 3	somnolence to semi-stupor, but responsive to verbal stimuli confusion gross disorientation
Grade 4	coma (unresponsive to verbal or noxious stimuli)

Methods

In an open labelled study, we included 8 patients with cirrhosis and hyperammonemia. They were matched for the severity of liver disease (see Table 3).
5 They were treated with one of the following regimes for a 3 day period and observations were made for 5 days. The study groups were:

(i) Placebo: 5% Dextrose over 4 hours;

(ii) Ornithine alone: 20g in 500 ml, 5% dextrose between 0800 and 1200;

(iii) Phenylbutyrate: 10g twice daily, orally (0800 and 1600); and

10 (iv) Ornithine + Phenylbutyrate: 20g in 500 ml, 5% dextrose between 0800 and 1200 + 10g twice daily, orally (0800 and 1600).

Patients were fasted overnight between 0000 midnight and 0800 am. They were fed intragastrically with a diet of 25KCal/Kg that included 1g/Kg protein diet starting at 0800 and finishing at midnight. Blood was sampled at 0730 am and then at 1800 hr for
15 the measurement of ammonia and glutamine. Patients were monitored closely for side effects. The drug was tolerated well in each of the groups and no adverse events were observed.

Table 3. Patient Demographics

20

	Placebo	Ornithine alone	Phenylbutyrate alone	OP
Age	P1: 47 P2: 57	P3: 46 P4: 40	P5: 56 P6: 48	P7: 52 P8: 52
Sex	P1: M P2: M	P3:F P4: F	P5:F P6: M	P7: M P8: F
Aetiology of Liver Disease	P1: HCV P2: HBV	P3: HBV P4: NASH	P5: NASH P6: HBV	P7: HBV P8: HBV
Severity of	P1: 9	P3: 13	P5: 14	P7: 14

Liver Disease (Pugh Score)	P2: 12	P4: 13	P6: 13	P8: 12
Precipitating Factor	P1: Infection P2: Infection	P3: SBP P4: Infection	P5: SBP P6: ?infection	P7: SBP P8: Infection
Severity of HE (West-Haven criteria)	P1: 2 P2: 3	P3: 3 P4: 3	P5: 3 P6: 3	P7: 3 P8: 3
Severity of HE (Glasgow coma score)	P1: 9 P2: 8	P3: 8 P4: 8	P5: 9 P6: 10	P7: 9 P8: 9
Other organ failure	P1: none P2: hypotension	P3: pre-renal, hypotension P4: hypotension	P5: none P6: pre-renal	P7: none P8: none
Dead/Alive	P1: A P2: A	P3: D P4: A	P5: A P6: A	P7: A P8: A
Complications	P1: infection, SBP P2: infection, variceal bleed	P3: HRS P4: rec. infection	P5: sepsis, ICU P6: recurrent SBP	P7: none P8: bleed, day 14

SBP: spontaneous bacterial peritonitis, Non alcoholic steatohepatitis, ICU: Intensive care support needed, HRS: hepatorenal syndrome

Results

5 Figure 11 shows that the mean ammonia levels remained largely unchanged over the period of treatment in the placebo group. In the L-Ornithine and the Phenylbutyrate group, the ammonia concentration increased from baseline values. In the group treated with both L-ornithine and Phenylbutyrate, there was a substantial reduction of ammonia. The postprandial increase in ammonia was reduced in the OP treated animals 10 in addition to the reduction in ammonia concentrations. Both patients in the OP group had improved their encephalopathy score by 2 grades by day 3, which was not observed in any of the other 6 patients.

Figure 12 shows that the mean glutamine levels remained largely unchanged over the period of treatment in the OP group despite a reduction in ammonia. There was 15 a reduction in glutamine in the Phenylbutyrate group, which may well be deleterious. In the L-Ornithine and placebo groups there was an increase in Glutamine concentrations which was markedly accentuated in the postprandial state.

Figure 13 shows the changes in mental state in the groups treated with Placebo, O, P and OP.

Example 12: The effect of ornithine, phenylbutyrate and isoleucine in human patients with hepatic encephalopathy

5 **Patients**

1. Groups- 2 patients per group. Total 6

2. Inclusion criteria

- Adult patients aged 18-80 years, liver cirrhosis documented by histology or clinical
10 criteria, Child B or C, recent Gastrointestinal bleed from varices (<6 hours after presentation), informed consent/assent.

3. Exclusion criteria

- other concomitant neurological disorder, use of another specific ammonia lowering
15 drug, respiratory failure requiring mechanical ventilation and sedation, uncontrolled gastrointestinal bleeding, hypotension requiring inotropes, overt renal failure (creatinine >2 mg/dl), hemodialysis, extracorporeal liver support, known hypersensitivity to any of the study drugs, pregnancy/lactation.

20 **Methods**

In an open labelled study, we included 6 patients with cirrhosis and who were admitted for management of variceal bleeding. They were matched for the severity of liver disease (see Table 4). They were treated with one of the following regimes for a 3 day period and observations were made for 5 days. The study groups were:

25 i. Placebo: 5% Dextrose over 4 hours (250 ml)

ii. Isoleucine alone: 10 gm IV in 250 ml 5% Dextrose over 2 hours in two divided doses.

iii. Isoleucine + Ornithine + Phenylbutyrate: Isoleucine:10 gm IV in 250 ml 5% Dextrose over 2 hours in two divided doses; Ornithine: 20g in 250 ml, 5% Dextrose
30 (t=0; 24, 48hr); Phenylbutyrate:10g twice daily, orally (t=0, 12, 24, 36, 48 hr).

Patients were fasted overnight between 0000 midnight and 0800 am. They were fed intragastrically with a diet of 25KCal/Kg that included 1g/Kg protein diet starting at 0800 and finishing at midnight. Blood was sampled at 0730 am and then at 1800 hr for

the measurement of ammonia and glutamine. Patients were monitored closely for side effects. The drug was tolerated well in each of the groups and no adverse events were observed. Because the patients received sedation for their initial endoscopy, the mental state assessment was impossible to interpret. One patient each in the Placebo and the
5 Isoelucine groups died from multiorgan failure in the hospital. The rest of the patients survived.

Table 4

	Placebo	Isoleucine alone	OIP
Age	P1: 43 P2: 62	P3: 57 P4: 42	P5: 43 P6: 45
Sex	P1: M P2: M	P3: F P4: M	P5: M P6: M
Aetiology of Liver Disease	P1: ALD P2: HCV	P3: HBV P4: ALD	P5: HBV P6: NASH
Severity of Liver Disease (Pugh Score)	P1: 13 P2: 14	P3: 13 P4: 11	P5: 14 P6: 10
Severity of HE (West-Haven criteria)	P1: 2 P2: 3	P3: 2 P4: 1	P5: 2 P6: 2
Estimated Blood Loss (u)	P1: 9 P2: 10	P3: 7 P4: 8	P5: 7 P6: 10
Dead/Alive	P1: D P2: A	P3: A P4: D	P5: A P6: A
Complications	P1: infection, rebleed P2: severe encephalopathy	P3: HRS P4: rec. infection	P5: chest infection P6: none

SBP: spontaneous bacterial peritonitis, Non alcoholic steatohepatitis, ICU: Intensive
10 care support needed, HRS: hepatorenal syndrome

Results

Figure 14 shows that no significant changes in ammonia concentrations in the placebo and the Isoleucine groups. In the group treated with OIP, there was a substantial
15 reduction in ammonia concentration.

Figure 15 shows that the glutamine levels are not significantly altered by administration of either Isoleucine, Placebo or OIP. Only in the OIP group the ammonia was reduced substantially.

Figure 16 shows an alternative by which OIP may act is through a reduction in the ammoniagenic amino acid, Glycine. Substantial reduction in Glycine is observed only in the OIP group.

Figure 17 shows the isoleucine levels are very low to start with in each of the
5 groups but increases to twice normal values in the Isoleucine treated groups. The concentration in the Placebo group remains low and unchanged.

Figure 18 shows the changes in the Ornithine levels in the patients over the course of treatment showing marked sustained increase in the concentrations of Ornithine which are significantly reduced to basal values on stopping the drug
10 indicating uptake in the different tissues.

Example 13: The effect of ornithine and phenylbutyrate in the bile duct ligated rat

15 **Methods**

Induction of cirrhosis by bile duct ligation (BDL)

Male Sprague-Dawley rats (200-250g) were used for this procedure. Following anaesthetisation, a mid-line laparotamy was performed, the bile duct was exposed, triply ligated with 4.0 silk suture, and severed between the second and third ligature. The
20 wound was closed in layers with absorbable suture, and the animal allowed to recover in a quiet room before being returned to the animal storage facility. Animals were kept at a constant temperature (20°C) in a 12 hour light/dark cycle with access to water and standard rodent chow *ad libitum.*

After five weeks post BDL (or sham procedure) the animals were switched from
25 rodent chow to a complete liquid diet (Liquidiet, Bio-Serv, Frenchtown NJ, USA) to which was added an amino acid mixture mimicking the composition of haemoglobin (2.8g/Kg/day, Nutricia Cuijk, The Netherlands,Product No. 24143). At six weeks, under anaesthesia a right carotid arterial catheter was inserted and used to collect repeated blood samples. Following this procedure a baseline sample was collected prior to
30 administration of the study formulations by IP injection. The study groups were: BDL control + Saline (n=5), BDL + ornithine (0.22g/Kg, n=6) in saline IP, BDL + phenylbutyrate (0.3g/Kg, n=7) in saline IP, BDL + OP (0.22g/Kg / 0.3g/Kg, n=7) in saline IP.

Blood samples were collected into pre-cooled heparinsed tubes and stored on ice prior to processing. Plasma was collected following centrifugation (3,000rpm, 10 mins) and stored at -80°C prior to analyses.

Ammonia, glucose, lactate and urea were measured using a COBAS Mira S
5 according to manufacturers instructions. Amino acids were quantified by HPLC with fluorescence detection.

Results

In the cirrhotic bile duct ligated rat model there is a substantial increase in the
10 arterial plasma ammonia level (205 ± 11 μmoles/L, mean ± SEM) compared with healthy controls (25.6 ± 2 μmoles/L, $p<0.001$ data, not shown). In this model we found that there was no change in the arterial ammonia levels over three hours in the saline treated placebo group.

Figure 19 shows the change in arterial plasma ammonia levels in BDL cirrhotic
15 rats following IP injections of saline (BDL control, n=5), ornithine (Orn, 0.22g/Kg, n=6), phenylbutyrate (PB, 0.3g/Kg, n=7) and ornithine phenylbutyrate (OP, 0.22g/Kg + 0.3g/Kg, n=7). * signifies $p<0.05$ for OP vs Orn at 3 hours (2 way ANOVA).

This figure shows that in the ornithine treated animals a slight decrease in ammonia concentration was detected, though this was not found to be different from
20 placebo. In the phenylbutyrate treated group a significant increase in plasma ammonia was found after 1 hour ($p<0.01$ vs all other groups), though this difference was found to be smaller at the three hour time point. This finding fits with the hypothesis that phenylbutyrate (phenylacetate) is only effective in subjects with raised glutamine concentrations. In the animals without ornithine supplementation which can be
25 metabolised to form glutamine the effects of P alone are undesirable and are potentially harmful. A significantly lower ammonia level was observed in the ornithine plus phenylbutyrate (OP) treated group. In these animals a sustained lowering of ammonia was measured over the three hour duration of the study the levels of which were found to be significantly less than those in the ornithine only group at the end of the study
30 ($p<0.05$).

This clearly demonstrates that the combination of OP has greater efficacy in reducing plasma ammonia than either O or P alone. Furthermore, the increased plasma levels of ammonia may be detrimental in the P alone treated animals.

In a subset of samples we examined the uptake of ornithine into the blood stream following IP injection of O or OP. Figure 20 shows the arterial ornithine concentration in the supplemented groups. It can be clearly seen that in both groups the plasma ornithine concentration is markedly increased at 1 hour following the IP injection,
5 which is subsequently reduced at 3 hours as this ornithine is metabolised in the body. No significant difference was found in plasma ornithine concentration between these groups at any time point.

This finding is important as it demonstrates that the chosen method of administration is effective in delivering ornithine in these animals. Furthermore, the
10 rapid uptake and observed decrease in plasma levels indicate that active metabolism of this amino acid is occurring.

Example 14: The effect of ornithine, phenylbutyrate and isoleucine in the bile duct ligated rat

15

Methods

Male Sprague-Dawley rats (200-250g) were used for this procedure. For the 48 hrs prior to sacrifice the animals were switched from standard rodent chow to a complete liquid diet (Liquidiet, Bio-Serv, Frenchtown NJ, USA) to which was added an
20 amino acid mixture mimicking the composition of haemoglobin (2.8g/Kg, Nutricia Cuijk, The Netherlands,Product No. 24143). Acute liver failure (ALF) was induced 24 hours prior to sacrifice by IP injection of galactosamine (1g/Kg, Sigma, Poole UK) in saline (n=5 in each group). Three hours prior to sacrifice animals were treated with either a formulation of OIP (ornithine 0.22g/Kg, isoleucine 0.25g/Kg, phenylbutyrate
25 0.3g/Kg, in saline IP) or saline control. At the termination of the experiment arterial blood was collected into pre-cooled heparinised tubes and stored on ice until processing. Plasma was collected and stored as above. Ammonia was determined as above.

Results

30 Arterial ammonia levels were found to be significantly reduced in acute liver failure rats treated with OIP compared with placebo controls (Fig. 21). This study was designed to test whether isoleucine in combination with ornithine and phenylbutyrate (phenylacetate) would be able to effectively lower plasma ammonia. It has been

previously demonstrated that isoleucine alone does not effect ammonia levels in human studies, though its efficacy in combination with O and P has not been previously tested.

Figure 21 shows arterial plasma ammonia levels in a hyperammonaemic acute liver failure model for saline placebo (ALF) and OIP treated (ALF + OIP). A significance level of $p<0.01$ was found between these two groups (T-Test).

This finding supports the hypothesis that isoleucine in combination with ornithine and phenylbutyrate is effective in reducing ammonia levels. These are in addition to the beneficial effects of isoleucine previously described for protein synthesis.

Example 15: The effect of ornithine and phenylbutyrate in the devascularized pig model

Methods

Five pigs were randomised into four groups: acute liver failure (ALF)+placebo+placebo (n=2); ALF+Ornithine+placebo; ALF+ Phenylbutyrate +placebo; ALF+Ornithine and Phenylbutyrate. Pigs had catheters inserted into the femoral artery and vein, portal vein, renal vein and pulmonary artery. The experiment started at time= -1hr, when placebo or treatment infusions were started.

1. Placebo: 5% Dextrose over 3 hours, oral water placebo
2. Ornithine alone: 0.3g/Kg, 5% dextrose over 3hours intravascular drip
3. Phenylbutyrate: 0.3g/Kg, 5% dextrose over 3hours intragastric feed

Ornithine + Phenylbutyrate: 0.3g/Kg, 5% dextrose over 3hours intravascular drip, 0.3g/Kg, 5% dextrose over 3hours intragastric feed.

ALF was induced by portal vein anastamosis to the inferior vena cava and subsequent hepatic artery ligation (devascularisation) at time= 0hr; infusions were stopped at t= +2hr and the experiment was terminated at time=8hr. Blood and urine samples were collected at time= 0, 1, 3, 5, 7 and 9hr for the measurement of regional ammonia and amino acid changes. At the end of the experiment a section of frontal cortex was removed for brain water measurements.

Results

Following ornithine infusion generating intracellular glutamate and the intragastric supply of conjugating phenylacetate results suggest profound alteration in overall ammonia levels and glutamine utilization in this catastrophic model of liver 5 failure.

There is a consistent rise in the arterial ammonia concentration with time from devascularisation in the placebo treated animal (Figure 22), with some muscle production (Figure 23) and a large amount of ammonia coming from the gut (Figure 24). This animal shows a modest muscle glutamine release (Figure 25) and appreciable 10 gut glutamine uptake (Figure 26).

In the case of the ornithine alone treated animal, the early ammonia rise is initially blunted, but rises thereafter to be the highest at termination of the experiment (Figure 22). There is a net uptake of ammonia by the muscle in this animal (Figure 24), with a comparable amount of glutamine being released from muscle – compared to the 15 placebo treated animal (Figure 25) with an increased gut uptake of glutamine (Figure 26).

Phenylbutyrate alone also shows an initial blunting of arterial ammonia levels, which quickly rises to levels comparable with ornithine alone at experiment termination (Figure 22) with little change in muscle ammonia uptake (Figure 23), but appreciable 20 gut production of ammonia (Figure 24). Interestingly, there is a net removal of glutamine by muscle with Phenylbutyrate alone treatment (Figure 25) with little overt effect on gut glutamine uptake, compared to placebo treated animal (Figure 26).

The combination of ornithine and Phenylbutyrate has the greatest impact on arterial ammonia levels with an impressive reduction in circulating levels at the end of 25 the experiment compared to all the other animals (Figure 22). Ammonia is actively removed from the blood by muscle in this animal (Figure 23) with a greatly reduced gut ammonia production (Figure 24). It is interesting to note that the muscle glutamine release is increased compared to both the placebo and ornithine alone treated animals (Figure 25). Despite this increased glutamine production in the muscle the gut 30 glutamine uptake is substantially reduced (Figure 26).

A demonstration of increased circulating levels of ornithine in the ornithine treated animals is shown in Figure 27.

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The impact of the devascularisation and treatment interventions on arterial glutamine are shown in Figure 28. There is an increase in the circulating level of glutamine in the ornithine treated animal, which is ameliorated by the co-administration of phenylacetate. An interesting finding was the substantial amelioration of the arterial
5 glycine levels that was found in the animal treated with both ornithine and phenylbutyrate (Figure 29).

At the end of the experiment the frontal cortex of the brain was removed and brain water content measured (Figure 30).

An independent pathologist reported on the cellular anatomy of the brain in
10 these experimental animals. His report is summarized below.

ALF: Microvessels with perivascular oedema with surrounding vesicles. Neuron with necrotic changes surrounded by vesicles.

ALF + O+P: Microvessels with perivascular oedema with surrounding vesicles (less than from ALF without any treatment). Intracellular edema.

15 Sham: Brain tissue with minimal ultrastructural changes=normal brain tissue.

Conclusions

The inventors have found that simulation of some of the symptoms of an acute attack associated with chronic liver disease, such as increasing the concentration of
20 ammonia or simulating a gastrointestinal bleed, results in reduction of neutrophil function and this reduction can be partially reversed by ornithine or isoleucine. Rescue of neutrophil function by both ornithine and isoleucine plays an important role in the prevention of sepsis which is a common precipitating factor in the progression of liver decompensation.

25 Furthermore, the inventors have found that isoleucine does not affect the rise in concentration of ammonia following a simulated gastrointestinal bleed. Therefore, contrary to the hypothesis that ammonia levels will decrease upon administration of isoleucine because of stimulation of protein synthesis, ammonia levels are unaffected. Thus, use of isoleucine in combination with ornithine, which is known to lower
30 ammonia levels, is particularly advantageous.

Therefore, administration of ornithine and isoleucine prevent the metabolic consequences of a gastrointestinal bleed. Rising ammonia levels are blunted, the deficiency in isoleucine is corrected and neutrophil function is rescued. The combined

use of ornithine and isoleucine therefore provides a new treatment for patients following a precipitating event to prevent liver decompensation from occurring.

The inventors have also found that L-ornithine L-aspartate (LOLA), which is used to reduce ammonia in patients with hepatic encephalopathy, does not reverse the
5 effect of ammonia on neutrophil function. Thus, use of ornithine alone is more advantageous than use of LOLA, since ornithine can both reduce ammonia and rescue neutrophil function. Also, the aspartate component of LOLA accumulates in the body. This accumulation of aspartate may actually by harmful to patients since aspartate worsens the effect of ammonia on neutrophil function, further reducing neutrophil
10 function. Accordingly, preventing or delaying the onset of liver decompensation can be achieved using ornithine in combination with isoleucine, preferably in the absence of aspartate.

Furthermore, the inventors have found that treatment of patients with hepatic encephalopathy (HE) with L-ornithine L-aspartate (LOLA) reduces ammonia levels and
15 as a consequence, increases glutamine levels. However, glutamine is only a temporary ammonia buffer as it can recycle and regenerate ammonia in the kidney and the small intestine. Therefore, treatment with LOLA alone can lead to a secondary rise in ammonia levels, further contributing to the pathology of hepatic encephalopathy.

Use of phenylacetate or phenylbutyrate in children with urea cycle disorders
20 reduces the abnormally high levels of glutamine. In contrast, patients suffering from HE have normal levels of glutamine unless, as shown in Example 1, they are being treated with LOLA which reduces levels of ammonia but increases levels of glutamine. Therefore, use of phenylacetate and/or phenylbutyrate allows for the removal of glutamine to prevent the secondary rise in ammonia levels in patients with HE.
25 Accordingly, an improved treatment for hepatic encephalopathy can be achieved by administration of ornithine in combination with at least one of phenylacetate and phenylbutyrate, preferably in the absence of aspartate.

Our extensive investigations in animal models and also in humans with cirrhosis support the view that the major organ removing ammonia in patients with cirrhosis is
30 the muscle, converting ammonia to glutamine, a reaction in which glutamate is utilised. In liver failure, the enzyme responsible for this reaction, glutamine synthetase is induced and the provision of glutamate would increase ammonia detoxification.

Ornithine, a precursor of glutamate, detoxifies ammonia by transformation to glutamine. However, our preliminary studies have shown that this glutamine, recirculates and regenerates ammonia. Our invention provides a novel method of not only detoxifying ammonia into glutamine but also eliminating the excess glutamine that is generated. Thus, OP reduces ammonia concentration in patients with cirrhosis and hyperammonemia significantly more markedly than either alone. The effect is clearly synergistic rather than additive. In addition, postprandial increase in ammonia is abolished by administration of OP. This may allow for feeding of patients with decompensated cirrhosis with protein-rich diets without the risk of hyperammonemia. The reduction in ammonia was associated with improvement in the mental state. It achieves reduction in ammonia concentration by preventing an increase in glutamine. This is consistent with the hypothesis that Ornithine is driving glutamine production in the muscle (thereby trapping 1 molecule of ammonia) but this glutamine is excreted (possibly as an adduct of phenylacetate) preventing a rise in systemic glutamine, thereby preventing rebound hyperammonemia.

The established wisdom that phenylacetate reduces ammonia in the hyperammonaemic infant presenting with urea cycle disorders is that the ammonia is trapped into glutamine and that the glutamine is shuttled to the kidneys for excretion as the phenylacetateglutamine adduct. These infants present with high ammonia and, importantly, high glutamine. Conversely the cirrhotic patient presents with high ammonia and normal to low glutamine. The pig model described above does not have a raised glutamine and the ammonia levels increase dramatically after the liver is isolated.

Treatment with ornithine alone increases blood glutamine whereas ammonia levels are unaffected. Phenylbutyrate alone marginally increases glutamine and again has insignificant effects on ammonia levels. In dramatic contrast, in this catastrophic model of escalating hyperammonaemia the combination of both ornithine and phenylbutyrate (OP) brings about an appreciable reduction in the circulating ammonia and ameliorates the increase in glutamine seen with ornithine alone. Glycine, an ammonia generating amino acid increased in all the animals, however, the rise in this amino acid was substantially blunted only in the OP treated animal, suggesting additional benefit for this form of intervention. An established consequence of elevated ammonia is brain swelling as water content of the brain increases. The brain from ornithine alone treated pig shows considerable increase in water content while the

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
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ornithine and phenylbutyrate combined reduces brain water content. Histologically, there is less apparent injury in the microstructure of the brain of the ornithine and phenylbutyrate combined treatment animal compared to the placebo treated animal.

CLAIMS

1. Use of ornithine in the manufacture of a medicament for use in combination with at least one of phenylacetate and phenylbutyrate for preventing or treating liver decompensation or hepatic encephalopathy.

2. Use of at least one of phenylacetate and phenylbutyrate in the manufacture of a medicament for use in combination with ornithine for preventing or treating liver decompensation or hepatic encephalopathy.

3. Use of ornithine and at least one of phenylacetate and phenylbutyrate in the manufacture of a medicament for preventing or treating liver decompensation or hepatic encephalopathy.

4. Use according to any one of the preceding claims wherein said liver decompensation is in a patient with chronic liver disease.

5. Use according to any one of the preceding claims wherein said prevention or treatment involves delaying the onset of liver decompensation.

6. Use according to any one of the preceding claims wherein the patient has had or is suspected of having had a precipitating event.

7. Use according to claim 6, wherein said precipitating event is gastrointestinal bleeding, infection, portal vein thrombosis or dehydration.

8. Use according to claim 6 or 7, wherein the medicament is administered within 6 hours of the symptom(s) of a said precipitating event or suspected precipitating event having been detected.

9. Use according to any one of the preceding claims wherein hepatic encephalopathy is treated in a patient with chronic liver disease or acute liver failure.

10. Use according to any one of the preceding claims wherein said ornithine is present as a free monomeric amino acid or physiologically acceptable salt.

11. Use according to any one of the preceding claims wherein the at least one of
5 phenylacetate and phenylbutyrate is present as sodium phenylacetate or sodium phenylbutyrate.

12. Use according to any one of the preceding claims wherein said medicament further comprises isoleucine.
10

13. Use according to claim 12 wherein said isoleucine is present as a free monomeric amino acid or physiologically acceptable salt.

14. Use according to any one of the preceding claims wherein said medicament
15 contains substantially no other amino acid.

15. Use according to any one of the preceding claims wherein the medicament is formulated for intravenous, intraperitoneal, intragastric, intravascular or oral administration.
20

16. Products containing ornithine and at least one of phenylacetate and phenylbutyrate as a combined preparation for simultaneous, separate or sequential use for preventing or treating liver decompensation or hepatic encephalopathy.

25 17. Products according to claim 16 which further comprise isoleucine.

18. Products according to claim 16 or 17 which comprises substantially no other amino acid.

30 19. A pharmaceutical composition comprising ornithine and at least one of phenylacetate and phenylbutyrate.

20. A pharmaceutical composition according to claim 19 which further comprises isoleucine.

21. A pharmaceutical composition according to claim 19 or 20 which comprises
5 substantially no other amino acid.

22. A pharmaceutical composition as defined in any one of claims 19 to 21 for use in a method of preventing or treating liver decompensation or hepatic encephalopathy.

10 23. An agent for preventing or treating liver decompensation or hepatic encephalopathy, comprising ornithine and at least one of phenylacetate and phenylbutyrate.

24. An agent according to claim 23 which further comprises isoleucine.

15

25. A method of treating a patient having or at risk of having liver decompensation or hepatic encephalopathy, which method comprises administering an effective amount of ornithine and at least one of phenylacetate and phenylbutyrate to said patient.

20 26. A method according to claim 25 which further comprises administering an effective amount of isoleucine to said patient.

27. A method according to claim 26 wherein said patient has an isoleucine deficiency attributable to gastrointestinal bleeding.

Figure 1

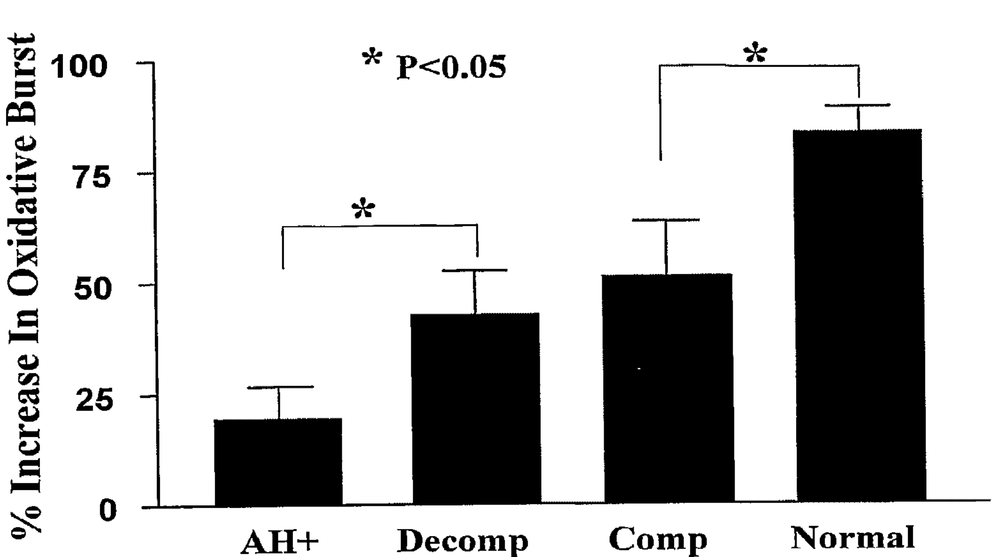
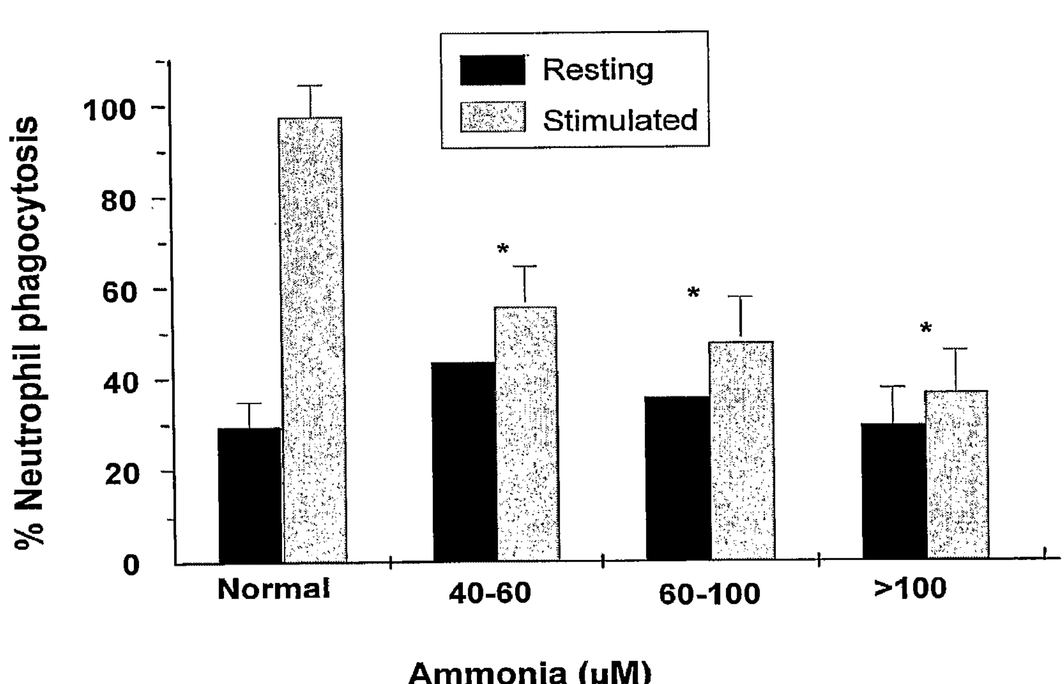


Figure 2



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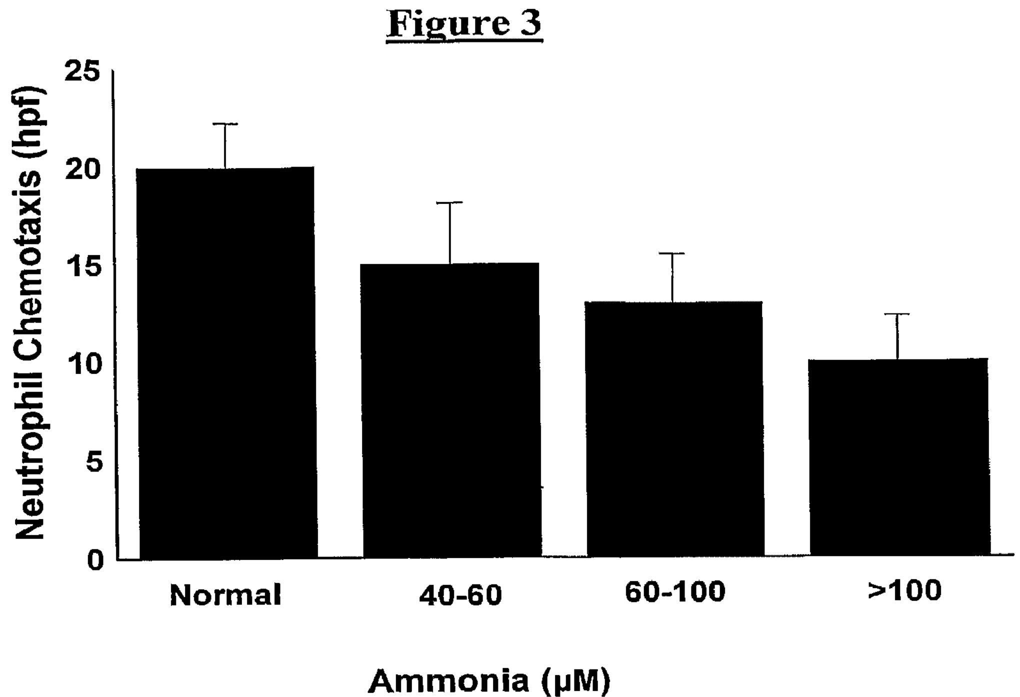

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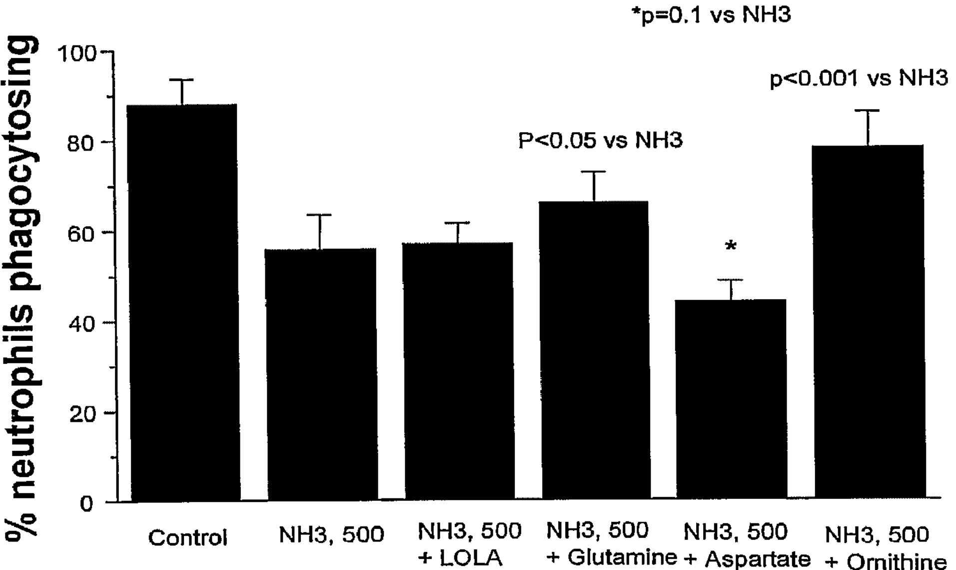

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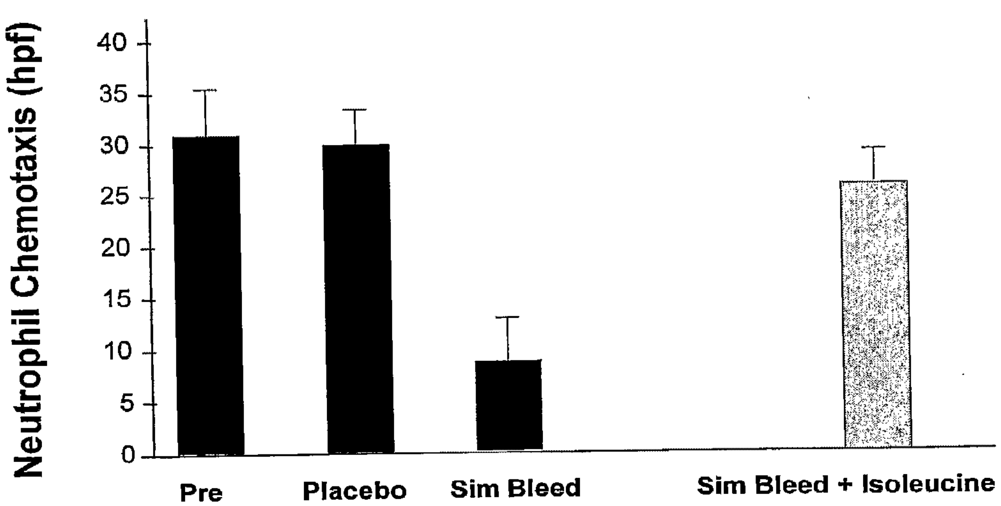

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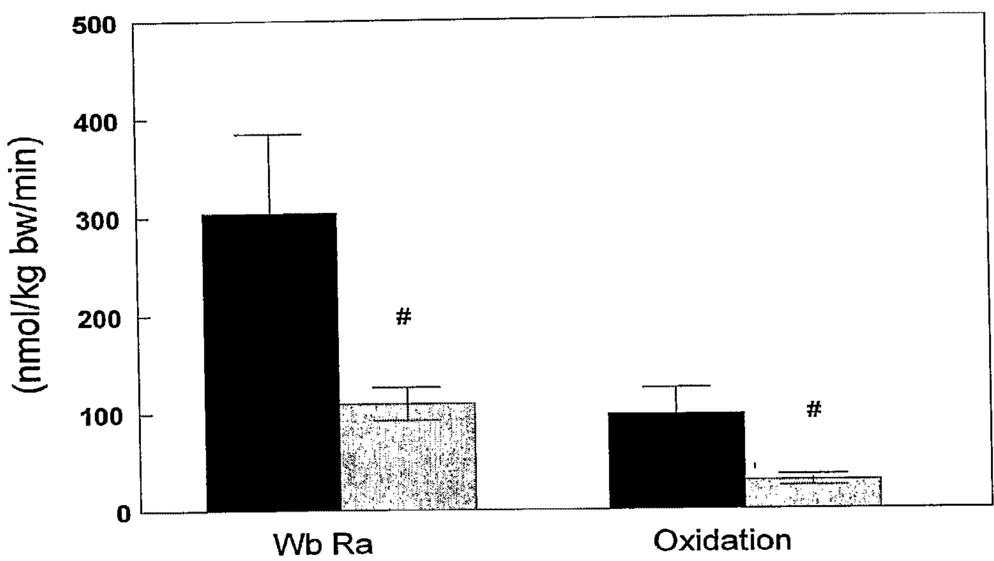

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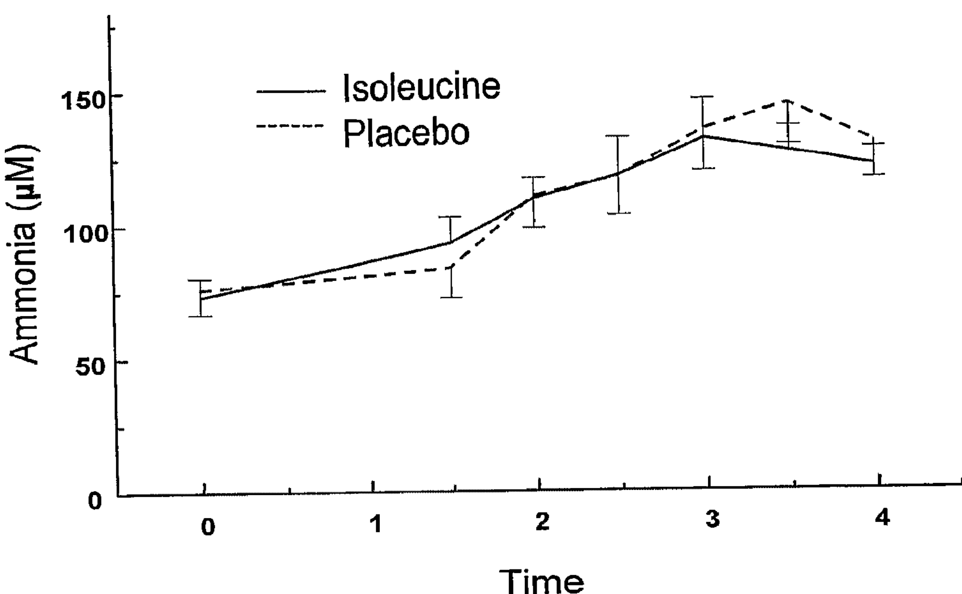

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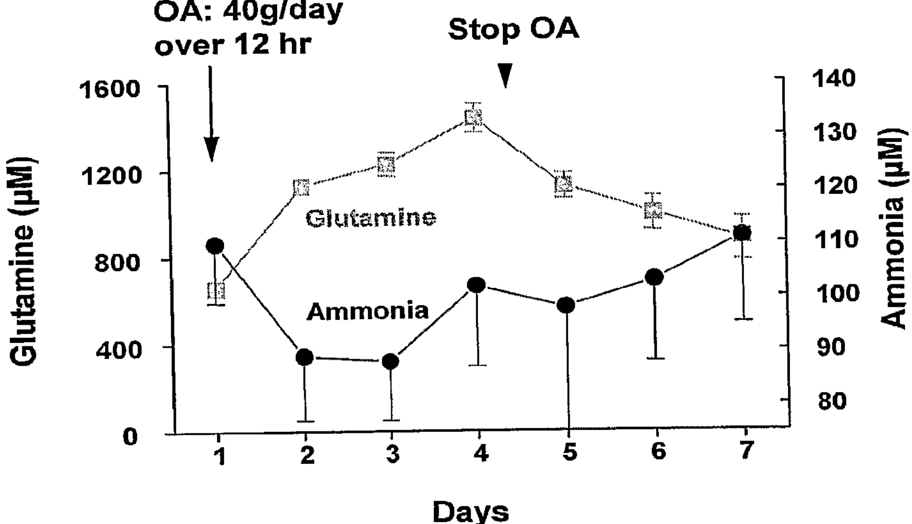

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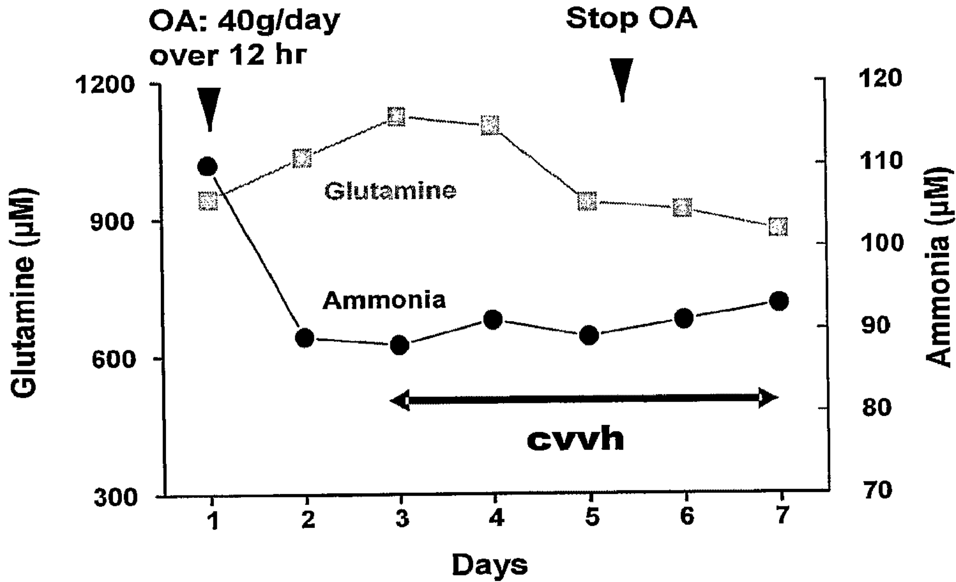

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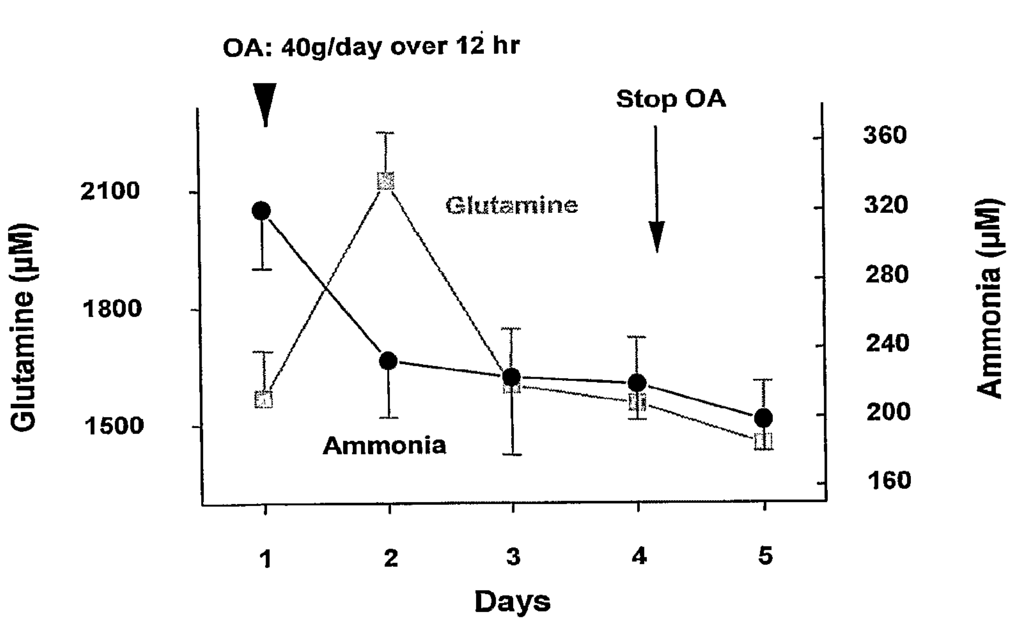

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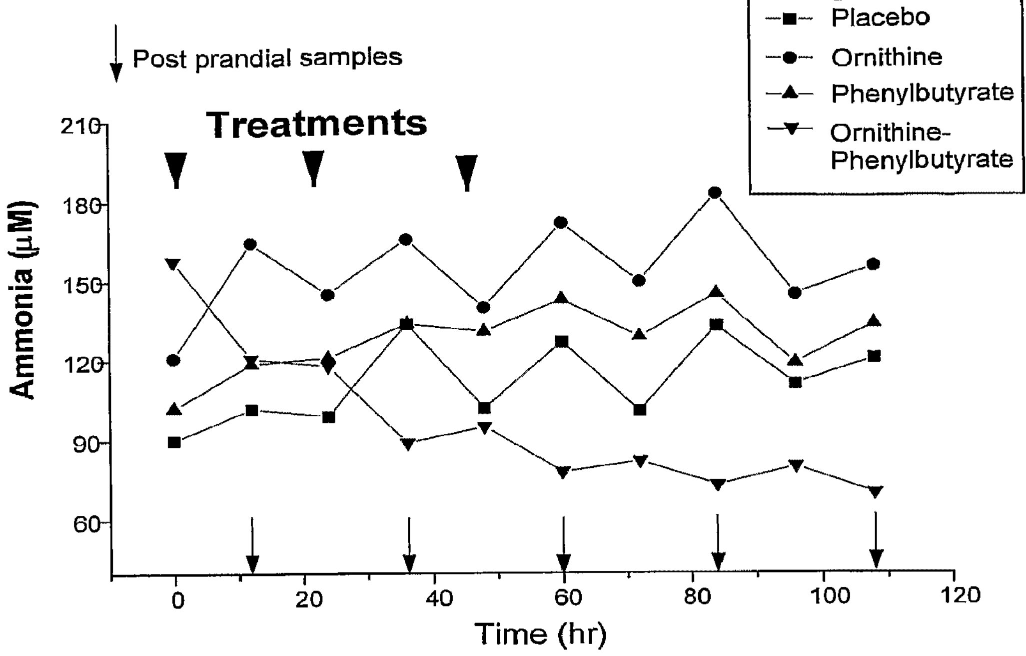

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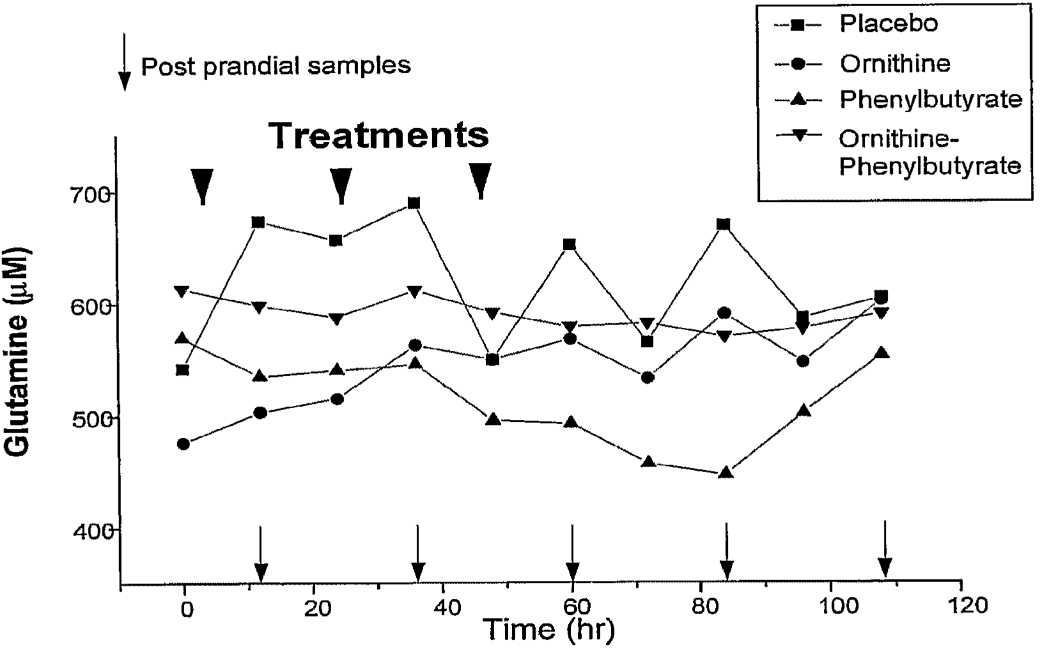

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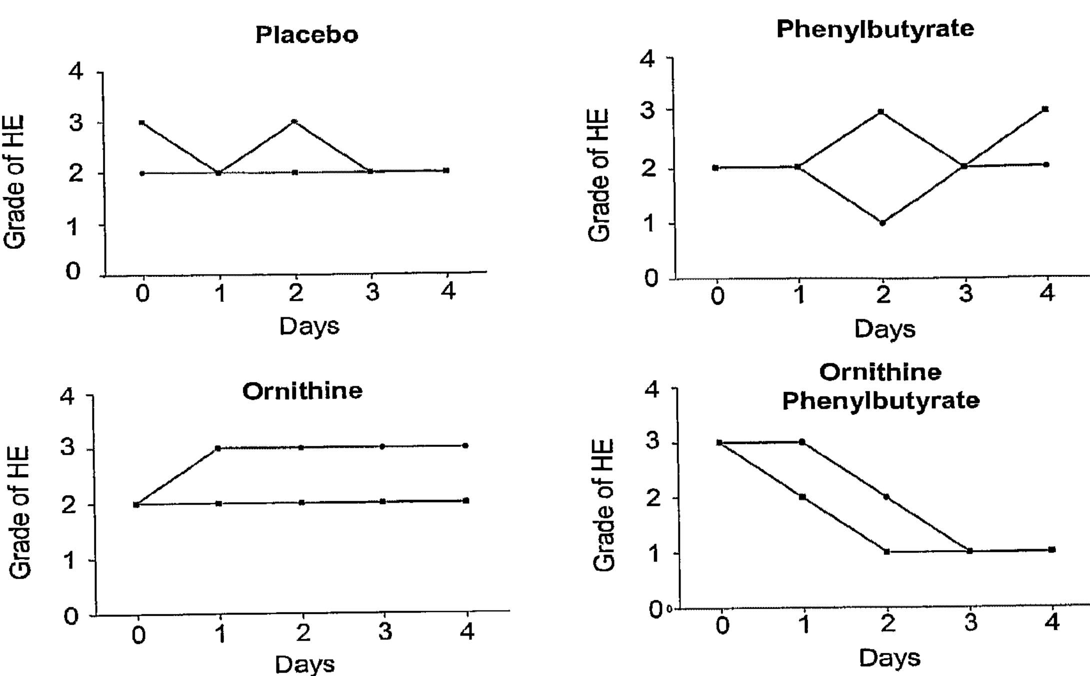

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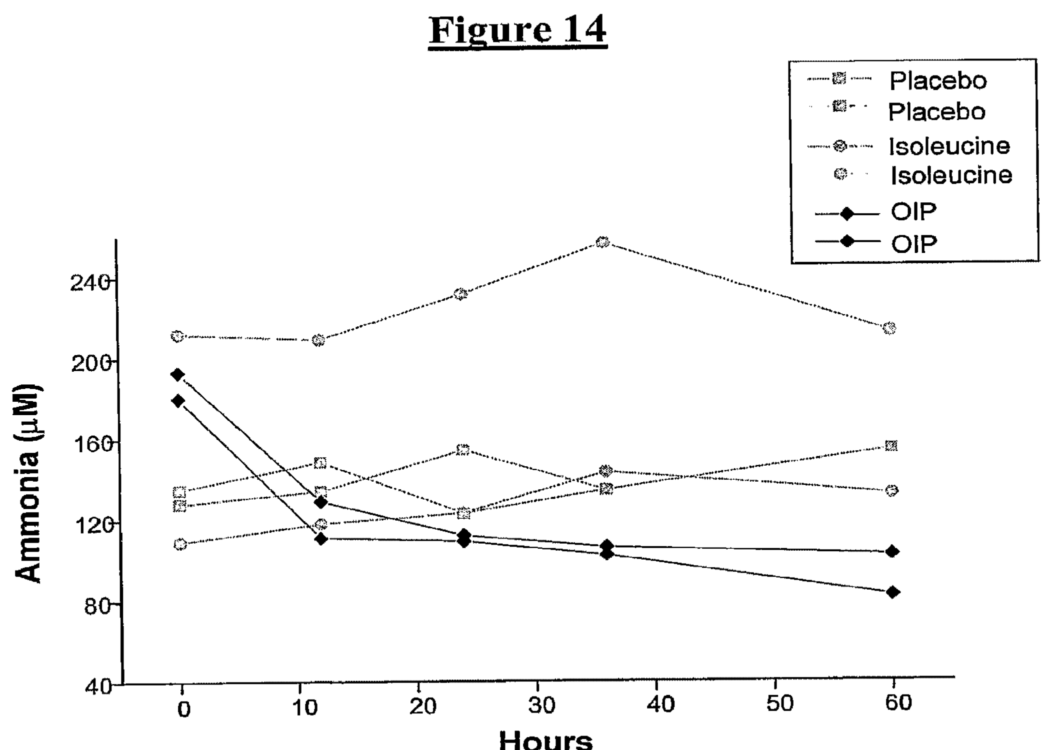

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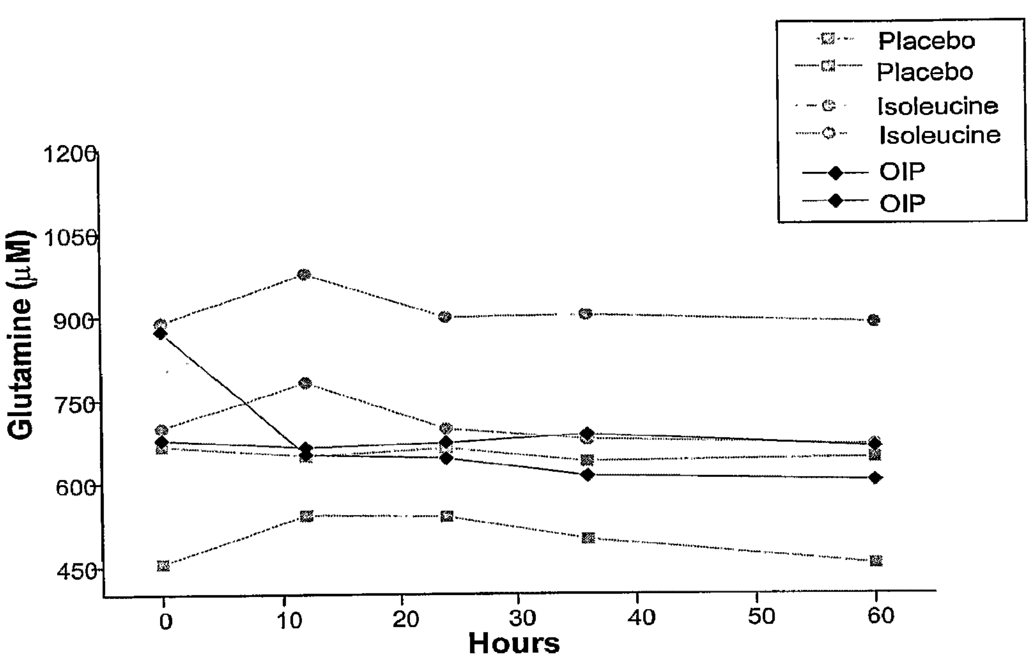

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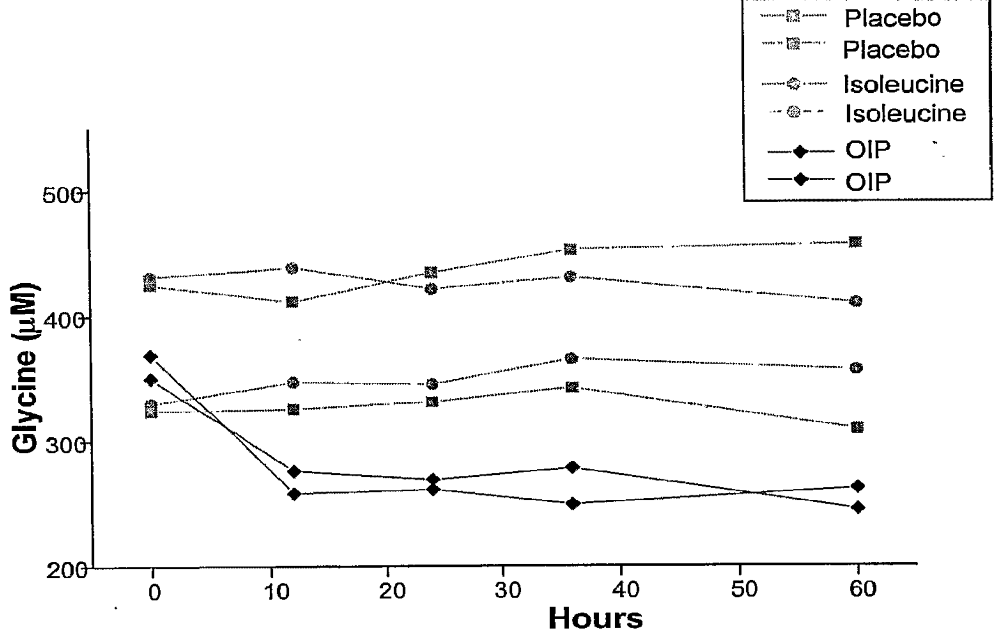

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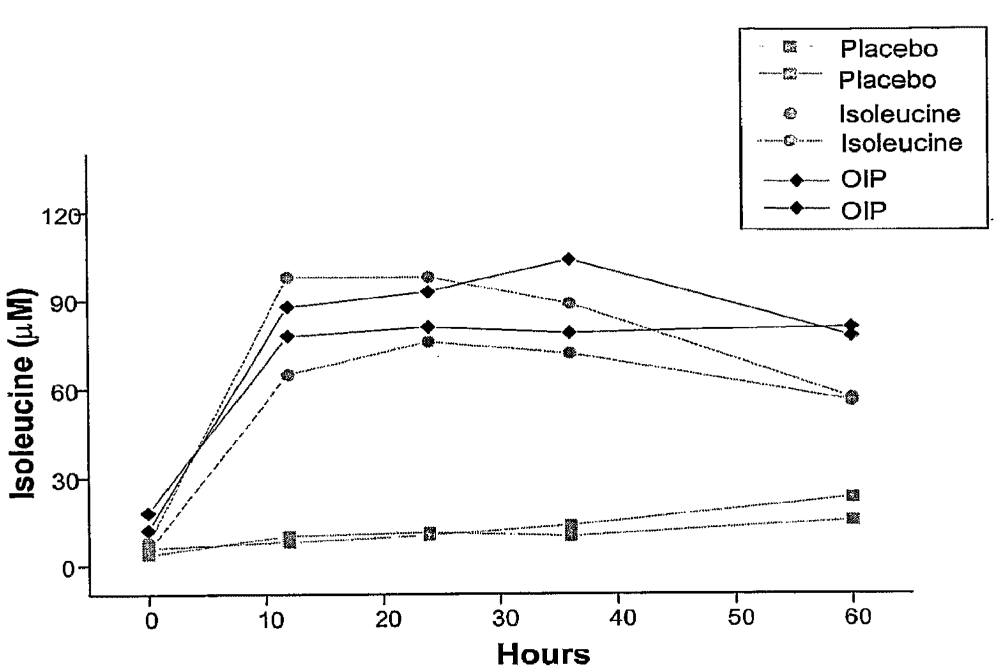

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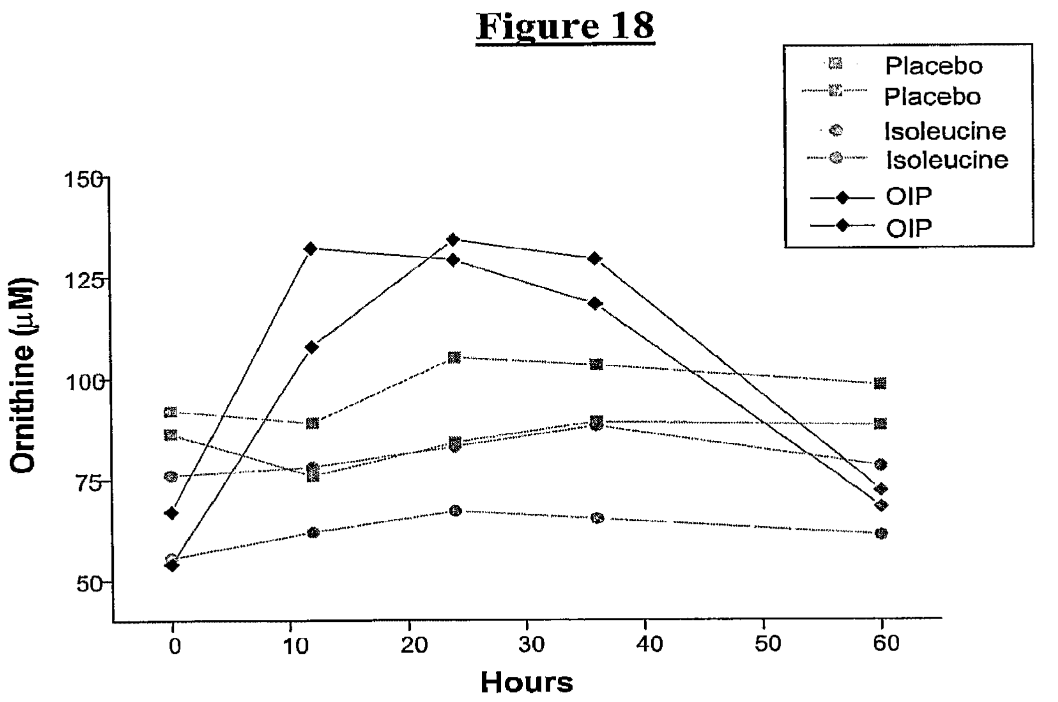

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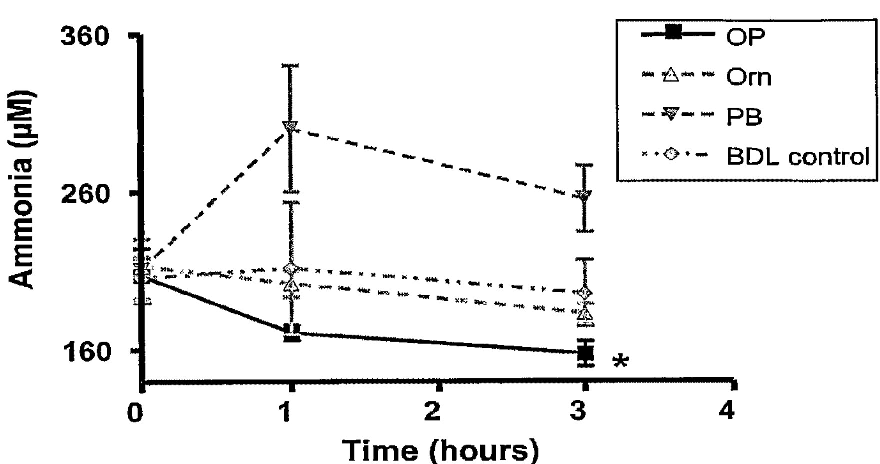

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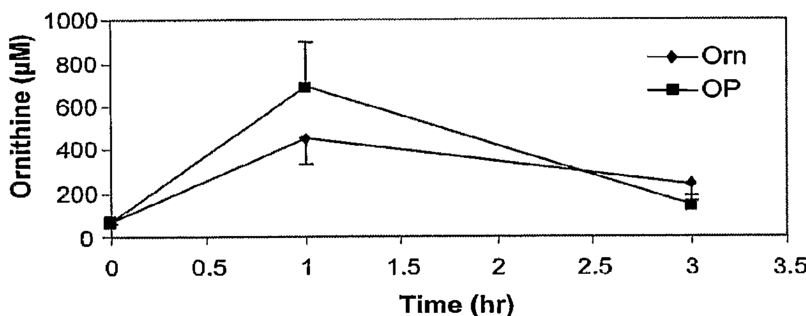

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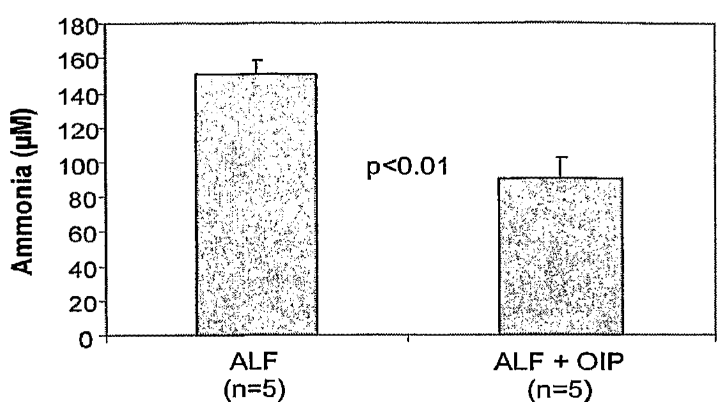

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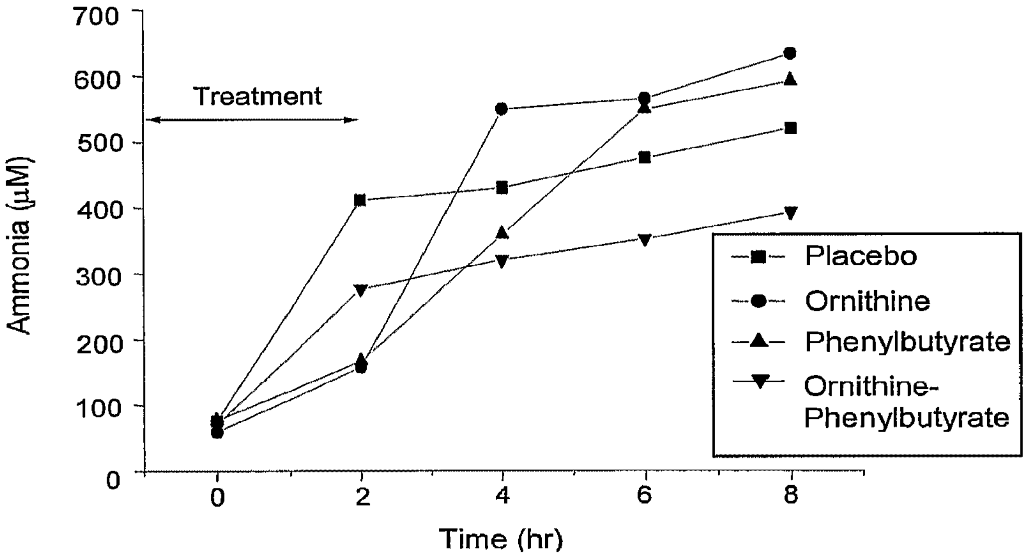

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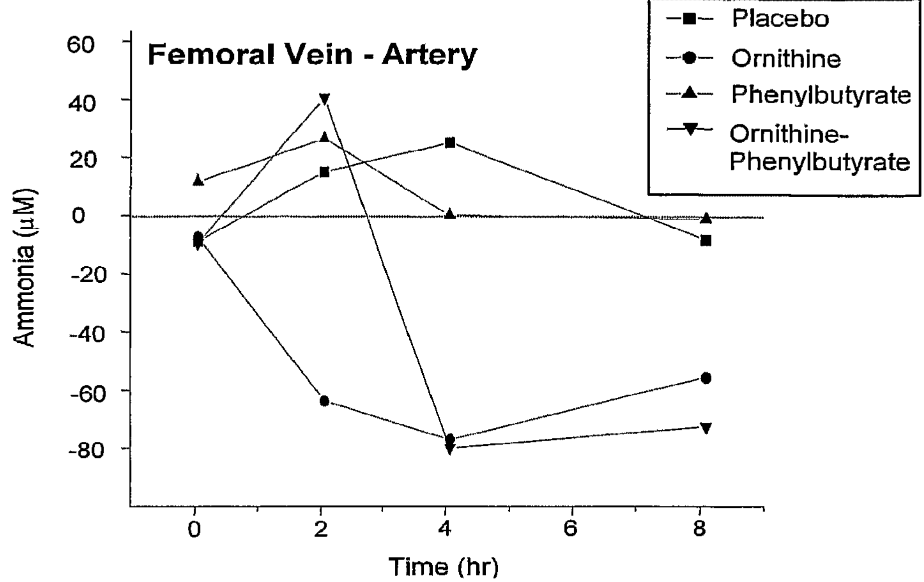

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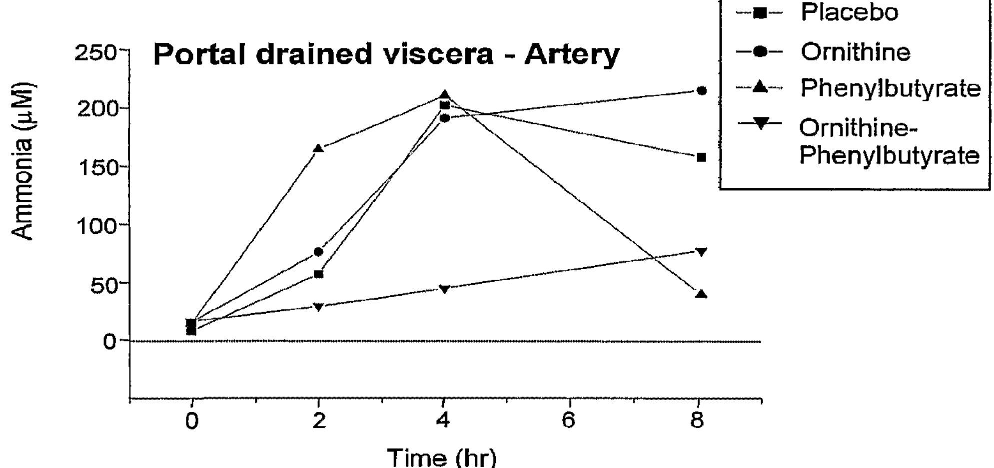

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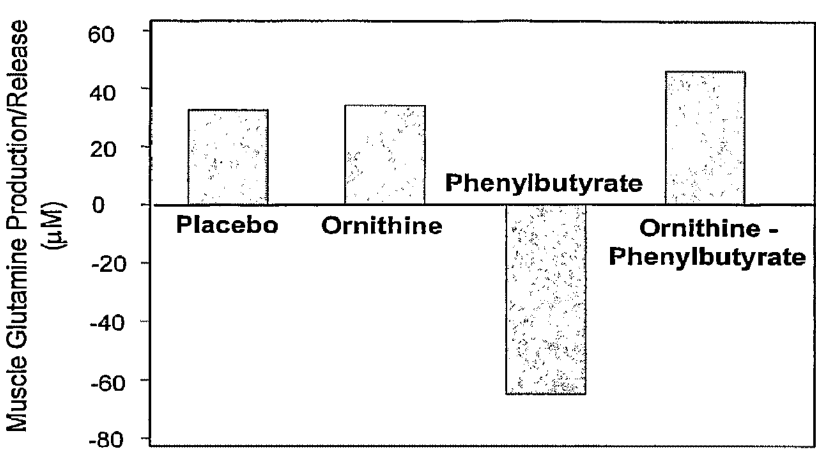

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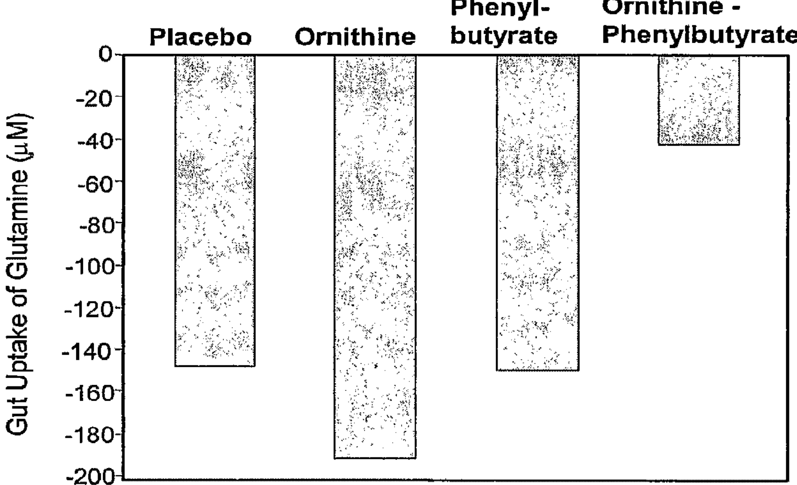

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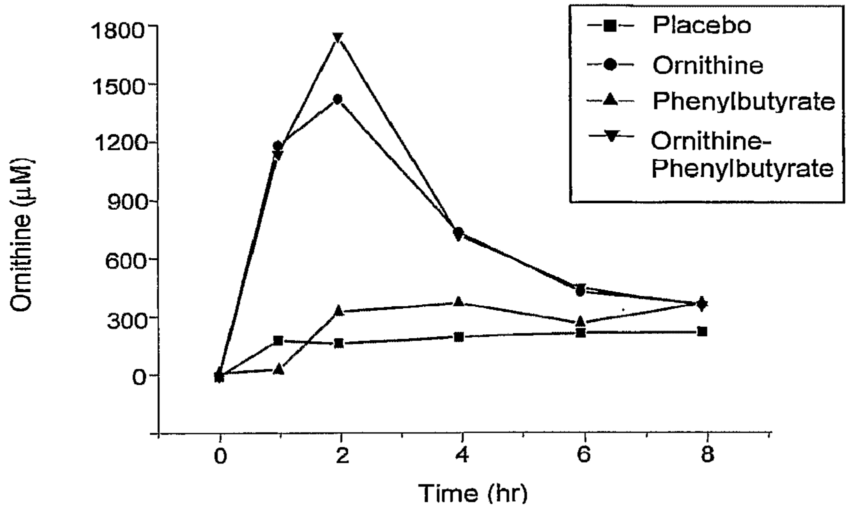

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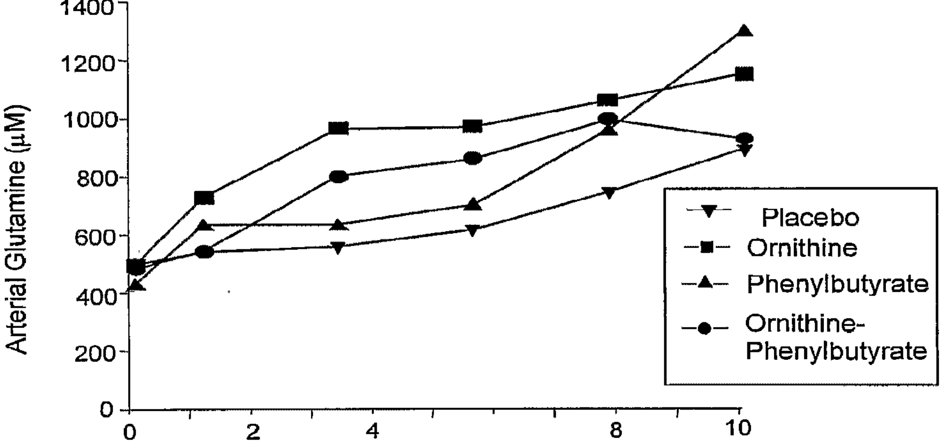

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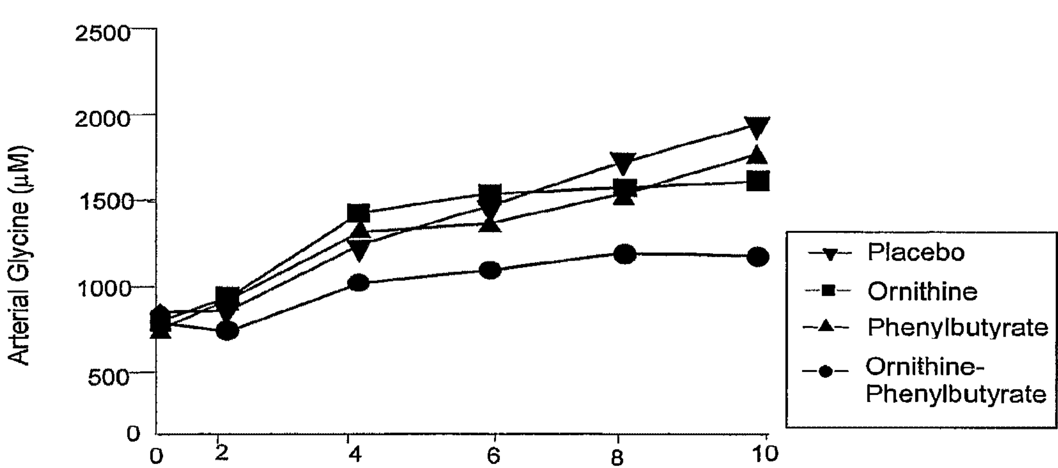

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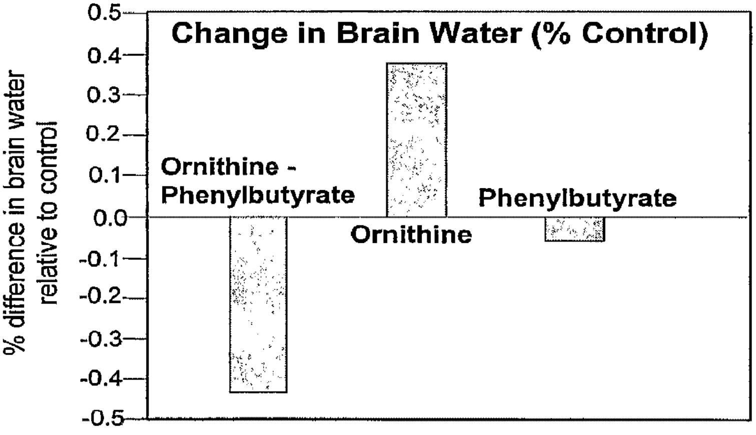

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2005/004539

A. CLASSIFICATION OF SUBJECT MATTER
A61P1/16 A61K31/192 A61K31/198

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 2005/182064 A1 (BURZYNSKI STANISLAW R) 18 August 2005 (2005-08-18) paragraph '0056! claims	16-24
A	US 4 228 099 A (WALSER ET AL) 14 October 1980 (1980-10-14) abstract	1-27
A	DATABASE WPI Section Ch, Week 200331 Derwent Publications Ltd., London, GB; Class B05, AN 2003-314385 XP002364873 & CN 1 383 815 A (LIU W) 11 December 2002 (2002-12-11) abstract	1-27

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 January 2006	08/02/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Skjöldebrand, C

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2005/004539

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2004/229948 A1 (SUMMAR MARSHALL L ET AL) 18 November 2004 (2004-11-18) the whole document -----	1-27

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/GB2005/004539

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 25-27 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Inte onal application No
PC1/GB2005/004539

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 2005182064	A1	18-08-2005	AU	2002352843	A1	10-06-2003
			BR	0214430	A	03-11-2004
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			WO	03086074	A1	23-10-2003
			US	2003195255	A1	16-10-2003

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(25) Filing Language: English

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(30) Priority Data:
11/174,026 1 July 2005 (01.07.2005) US

(71) Applicant *(for all designated States except US)*: **NAVINTA LLC** [US/US]; 1499 Lower Ferry Road, Ewing, NJ 08618-1414 (US).

(72) Inventor: **JOBDEVAIRAKKAM, Christopher, N.**; C/ONAVINTA LLC, 1499 Lower Ferry Rd., Ewing, NJ 08618 (US).

(74) Agent: **RUBEN, Bradley, Noell**; 463 1st Street, Suite 5a, Hoboken, NJ 07030-1859 (US).

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Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2007/005633 A2

(54) **Title:** PROCESS FOR PREPARATION OF LIQUID DOSAGE FORM CONTAINING SODIUM 4-PHENYLBUTYRATE

(57) **Abstract:** A process for preparing a stable aqueous dosage form of sodium 4-phenylbutyrate, including such dosage forms in a highly concentrated solution, as well as methods for making 4-phenylbutyrate and 4-phenylbutyric acid, and for using 4-phenylbutyrate. The stable aqueous dosage forms do not freeze at 0° C.

Process for preparation of liquid dosage form containing sodium 4-phenylbutyrate

Field of the Invention

This invention relates to a process of preparing a highly concentrated solution of sodium 4-phenylbutyrate in an aqueous medium useful as an alternative for present high dosage therapeutic treatments of urea cycle deficiencies, sickle-cell anemia, and cancer.

Back ground of the Invention

Sodium 4-phenylbutyrate is currently being prescribed to treat urea cycle deficiency in children; it is sold in the USA under the trademark BUPHENYL (Ucyclyd Pharma, Inc., Glen Burnie, MD), and in Europe under the trademark AMMONAPS (Orphan Europe). The urea cycle is the metabolic process by which the human body gets rid of nitrogen. There are six enzymes that take part in this process. A deficiency of any one of them upsets the process and causes excess nitrogen, in the form of ammonia, to accumulate in the body. The six urea cycle disorders are: carbamyl phosphate synthetase deficiency; n-acetylglutamate synthetase deficiency; ornithine transcarbamylase deficiency (the most common type); argininosuccinic acid synthetase deficiency (also called citrullinemia); argininosuccinase acid lyase deficiency; and arginase deficiency. Nitrogen accumulation is also present in patients with kidney or liver failure.

In children born with any of these rare enzyme deficiencies in the urea cycle, if the enzyme deficiency is severe, the condition leads to coma and death within a few days of birth. Such children are unable to excrete waste nitrogen as urea. Accordingly, the waste nitrogen accumulates as ammonium ions in the plasma leading to a condition known as hyperammonemia. Such genetic defects cannot be cured, but the condition can be treated by adherence to a life-long combination of a low protein diet and the administration of suitable medication. Presently, a combination of sodium phenylacetate and sodium benzoate is administered to children who have an N-acetylglutamine synthetase-1 deficiency, whereas sodium 4-phenylbutyrate (typically in a dosage of 450-600 mg/kg/day in three or more divided doses) is administered to children having an ornithine transcarbamoylase deficiency. In the

latter treatment, the sodium 4-phenylbutyrate is converted to 2-phenylacetate, which combines with the amino acid glutamine present in the plasma and the resulting combination (or conjugate) is excreted as phenylacetylglutamine in the urine. Thus, administration of sodium 4-phenylbutyrate provides an alternative to the urea pathway as a means of excreting waste nitrogen from the body.

The above-mentioned commercially available forms of 4-phenylbutyrate, BUPHENYL in the US and AMMONAPS in Europe, are marketed as a granular powder for making a solution for oral administration to infants and young children, and as 500 mg tablets for adults and children weighing over 20 kg. The powder dosage is measured in one of three differently sized measuring spoons, which always leads to an imprecise dosage level. For example, a six year old child suffering from ornithine transcarbamoylase deficiency and weighing19 kg has to take 3.8 g of powdered sodium 4-phenylbutyrate three times daily. The imprecise dosing measurement, and the need to mix the powder with a fluid for administration, leads to a lack of compliance in taking the prescribed dose at the required intervals. Consequently, it is invariably the case that children have to be admitted to hospital, sometime two or three times a year, because they feel nauseous, this being a first sign of hyperammonaemia caused by failure to maintain the dosing regimen. The symptom of nausea means the child patient cannot take the powder orally. Accordingly, in hospital the patient is treated with an intravenous infusion of sodium 4-phenylbutyrate (or sodium phenylacetate and sodium benzoate) to reduce the ammonium ion level to normal. When the nausea subsides, normal oral therapy is then resumed. Unfortunately, sometimes the delay in reaching a hospital leads to the patient being admitted in a hyperammonaemic coma; death may result or, on recovery, the child may be permanently brain-damaged.

Another important requirement for high dosage medications such as sodium 4-phenylbutyrate is the purity. High dosages such as 4 g per day or more require the purest of starting materials and good process control to bring all the impurities to less than 0.05% w/w.

WO 85/04805 discloses a process for waste nitrogen removal in human beings, wherein a compound having the formula $Ph\text{-}CH_2\text{-}(CH_2)_n\text{-}COOH$, wherein n is 2, such as 4-phenylbutyrate, is administered.

US Pat. App. 2004/0180962 discloses a delayed release methodology for using a low dosage of sodium 4-phenylbutyrate to treat urea cycle deficiency by

compounding in a tablet form with hydroxypropylmethylcellulose and a release-controlling excipient (a release retarder or a liberation controller). However, such delayed release methodologies are not the best approach for treating this particular disease because a sufficient amount of the metabolite (phenylacetate) must be present in the plasma to react with glutamine and then be excreted as phenylacetylglutamine.

US Pat. App. 2004/0152784 describes a pharmaceutical composition of sodium 4-phenylbutyrate with effective aromatic flavoring agent and at least one synthetic sweetening agent. This disclosure provides a dry granulated pharmaceutical composition that can be dissolved in water before administration. One of the examples provides a maximum concentration of sodium 4-phenylbutyrate in the reconstituted solution of 250 mg/mL at 10 °C. This reconstituted solution would require a relatively a large volume of solution for a suitable dosage, making it difficult to administer the drug to infants because of the large liquid volumes necessary upon dissolving the granules in water. Also, this particular pharmaceutical preparation is not stable biologically as it does not contain any preservative.

The '784 application also demonstrates that the sweetening agent (potassium aspartame) is not stable in the aqueous reconstituted solution of the dry powder containing sodium 4-phenylbutyrate because it loses its sweetness when stored for more than a few weeks. The drug 4-phenylbutyrate is a very bitter-tasting compound, so loss of sweetness leads to a lack of compliance with the dosing regimen. Accordingly, additional precautions are needed when using the formulation is the '784 application.

Sodium 4-phenylbutyrate is also useful for treating a variety of other medical indications, such as benign prostate hyperplasy, certain cancers, cystic fibrosis; HIV, spinocerebellar ataxia, kidney and liver failures, and thalasemia.

Another use for sodium phenylbutyrate is to induce fetal hemoglobin production in patients with sickle cell anemia; this has been described by George J. Dover (*Blood,* vol. 84, No. 1, Jul. 1, 1994: pp 339-343). This paper states that sodium phenylbutyrate in powdered form has a bitter taste that, despite many attempts, cannot be disguised. Two of the four subjects treated as outpatients reported an inability to maintain compliance with their dosing regimen because of the high dosage requirements (30 to 40 tablets per day).

DE 19,810,383 describes 4-phenylbutyrate as an apoptosis-inducing agent for neoplastic therapy.

WO 9937150 describes a transcription therapy for cancer using a retinoic acid and/or an inhibitor of histone deacetylase. For this treatment, 4-phenylbutyrate is classified as a histone deacetylase inhibitor.

WO 93/07866, WO 9510271, and EP 725635 all disclose compositions and methods using phenylacetic acid (a metabolite of 4-phenylbutyrate) and its derivatives for therapy and prevention of a number of pathologies, Including cancer, AIDS, anemia, and severe beta-chain hemoglobinopathies. A number of U.S. patents describe the use of phenylacetic acid as an anticancer agent (*e.g.*, 6,037,376) and as an anti-viral agent (*e.g.*, 5,877,213 and 5,710,178).

WO 9856370 and US 6,207,195 describe therapeutic sodium 4-phenylbutyrate containing nanospheres for the treatment of cystic fibrosis by CFTR gene therapy.

US Pat. App. 2003/0195255 describes a method of administering sodium 4-phenylbutyrate orally to treat loss of mental function associated with chronic hepatic encephalopathies, recommending a high dosage of about 200-300 mg//kg initially over one to two hours, and then divided into three equal dosages daily; for adults the dose is described as 3 to 12 g/m^2. With regard to the synthetic of sodium 4-phenylbutyrate and related compounds, some of the methods involve using substituted malonic esters.

WO 9901420 and WO 9503271 each describes a process of preparing substituted amino malonic acid and α-amino substituted propanoic acid from its ethyl ester. Preparation of substituted butyric acid from substituted malonic esters using various reagents is reported in several published research papers. *J. Med. Chem.*, 47 (12), 3282-3294, 2004; *Bioorg & Med Chem.*, 11(1), 113 -121, 2003; *J. Med. Chem.*, 46 (10), 2008-2016, 2003; *Enantiomer*, 7(1), 1-3, 2002; *J. Med. Chem.*, 45 (2), 263-274, 2002; *J. Het. Chem.*, 25(6), 1689–1695, 1988.

In addition, 4-phenylbutyrate has been shown as useful for protecting againt cerebral ischemic injury. (X. Qi, *et al.*, *Mol. Pharmacol.*, 66(4), 899-908 (2004).)

Commercial manufacturing of 4-phenylbutyric acid involves the potential carcinogen benzene as one of the raw materials. US Pat. No. 6,372,938 Burzynski *et al.; J. Am. Chem. Soc.*, 74, 1591 (1952); *J. Am. Chem. Soc.* 74; 4721 (1952); *Bull.Acad.Sci.USSR Div.Chem.Sci.*(Engl.Transl.), EN, 36, 2, 327-330 (1987); *Akad. Nauk SSSR Ser. Khim.*; RU, 2; 367-371 (1987).

Summary and Objects of the Invention

Sodium 4-phenylbutyrate is a very bitter-tasting compound and so it is very difficult for patients to comply with their dosing regimen, especially children who have to take large amounts of the medicine every day. It would be of immense benefit to the children and their parents if the oral dosage were more palatable, easier to administer, and/or have a lower volume liquid dosage form, and preferably a combination of all three. The treatment works, but non-compliance with the present dosing regimen causes incomplete treatment leading to occasional hospitalization.

Accordingly, one object of this invention is to provide an improved pharmaceutical composition containing sodium 4-phenylbutyrate for the use by patients presently administered with a high dosage and high volume dose of this drug. To accomplish this, one embodiment of this invention provides a process for preparing a liquid dosage of sodium 4-phenylbutyrate in a more concentrated aqueous solution than provided by the present art, preferably containing at least one of a preservative and a sweetening agent, and preferably both, in addition to a flavoring agent; a fragrance can also be added. The supersaturated solution can have a concentration up to 500 mg/mL of sodium 4-phenylbutyrate or more; preferably the concentration ranges from about 300 mg/mL to about 700 mg/mL. A preservative such as sodium benzoate can be present, preferably at about 2.5 mg/mL. In other embodiments, the dosage can include a sweetening and/or other flavoring agent, such as about 2 mg/mL of sodium saccharine, 0.01 mg/mL of sucralose, and/or about 2 mg/mL of raspberry flavoring. This highly concentrated liquid dosage is more concentrated and more palatable, leading to easier administration to young patients and facilitating improved compliance to the dosing regimen. This concentrated solution is effective and very easy to administer to babies because it requires only a few milliliters at any one dosing time; and it is easy to administer to children because each dosage is only a few milliliters of solution at any one time.

In another embodiment, this invention provides a process of preparing a supersaturated solution of sodium 4-phenylbutyrate in water by adding sufficient water to a known quantity of sodium 4-phenylbutyrate at an elevated temperature of about 30° to about 80° C to produce a concentration of about 600 mg/mL.

Yet another object of this invention is to provide a process for manufacturing sodium 4-phenylbutyrate with impurities at a level less than 0.05% (weight/weight

basis). The general process provided by this invention is to treat $Ph\text{-}(CH_2)_2\text{-}CH(COOEt)_2$ (*i.e.,* diethyl 2-phenylethylmalonate) with acetic acid and aqueous hydrochloric acid to produce 4-phenylbutyric (or 4-phenylbutanoic) acid. In another and continuing embodiment, conversion of 4-phenylbutyric acid to its sodium salt is accomplished in an organic solvent medium with an inorganic base.

The present invention is a novel method of synthesis of 4-phenyl butyrate without benzene.

In summary this invention provides a pharmaceutical liquid composition, comprising a solution of sodium 4-phenylbutyrate in an aqueous medium at a concentration of at least about 300 mg//mL, including generally at a concentration of 300 mg/mL to about 700 mg/mL, and more preferably at a concentration of 400 mg/mL to about 600 mg/mL. As a dosage the composition preferably further comprises at least one or more of a flavoring agent, including sweeteners, a preservative, and compatible mixtures thereof. The composition may also include an inorganic base.

This invention also provides a process for making a highly concentration solution of 4-phenylbutyrate by dissolving the same in water, preferably at an elevated temperature.

This invention also provides a process for making 4-phenylbutyrate from 4-phenylbutyric acid by dissolving the same in an organic medium, treating with an inorganic alkali, heating, adding a second solvent to precipitate the product, and isolating/purifying the product.

This invention also provides a process for making 4-phenylbutyric acid from a diester of the formula $Ph\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}(COOR)_2$ wherein R is an alkyl of not more than four carbons, aryl, or aralkyl wherein the alkyl portion has not more than four carbons, treating the same with a mineral acid, precipitating the product, and thereafter isolating and/or purifying the same.

This invention also provides a method of treating a patient suffering from a urea cycle deficiencies, sickle-cell anemia, cancer, or potential cerebral ischemic injury, comprising providing an oral aqueous solution of 4-phenylbutyrate having a concentration of at least about 300 mg/mL and orally administering said solution to a patient in need thereof.

Detailed description of the specific embodiments

This invention relates to an oral liquid pharmaceutical multiple dosage form of sodium 4-phenylbutyrate in a supersaturated solution in an aqueous medium, preferably containing at least one preservative. The drug concentration in the formulation is achieved to a maximum of about 700 mg/mL, and at 600 mg/mL the solution does not freeze at 0° C.

Thermodynamically, the solubility of a species is dependent upon temperature and the interaction between the species and the solvent through various types of intermolecular and intramolecular interactions. The solute--solvent intermolecular interactions are the prime reason for the change in solubility at different temperatures. For a true solution, at a relatively higher temperature the solute–solvent intermolecular interaction is more pronounced than at a relatively lower temperature, and thus it is typically observed the solubility of a compound soluble in a given solvent increases as the temperate increases.

In this invention it has been found that the solubility of sodium 4-phenylbutyrate has been found to be exceptionally higher than that reported in the prior art (for example, the above-mentioned US application 2004/0152784 reports a maximum solubility of sodium 4-phenylbutyrate of 250 mg/mL at 10° C.). This art-reported solubility is believed to pertain to the maximum solubility of the monomeric form of sodium 4-phenylbutyrate in water.

As described in more detail below, this invention describes a process of preparing a highly concentrated solution of sodium 4-phenylbutyrate, having a concentration 500 mg /mL in water by dissolving 5 g of sodium 4-phenylbutyrate in about 3.5 mL water to yield a solution volume of about 10 mL. The temperature can be room temperature (25° C) or an elevated temperature, preferably in the range of up to about 80° C. We found it is more difficult to make this solution at room temperature, but the solution can be made at a higher temperature and then cooled to room temperature without precipitating resulting. The solution thus made was believed to be a supersaturated, non-ideal solution that does not obey the van't Hoff equation (a plot of $-\ln K$ versus $1/T$ giving a straight line, where K is the solubility constant and T is the absolute temperature). While not desirous of being constrained to a particular theory, these results suggest to us that that the solution so formed is a micellar kinetic phase where sodium 4-phenyl butyrate is the micelle in an aqueous bulk phase. Therefore, due to likely micelle formation of sodium 4-phenylbutyrate (which we term the self-associated polymeric form), the high concentration of about 500 mg/mL can

be achieved in solution. Even further, this high concentration solution did not freeze or precipitate out upon storage, even at 0 °C for two days, and only on further cooling to -4° C is precipitation observed. This novel invention thus provides a dosage form better able to help the patients presently administered with a high volume dosage of sodium 4-phenylbutyrate. This invention is not intended to be limited by this discussion of micellar phases, or the presence or absence of other high concentration phases (such as sponge or L3, worm-like micelles, sheets and other laminar phases) that may be formed depending on the particular processing conditions and/or materials used. In the follow description the term “solution” is used without regard to whether a micellar phase is present.

In another embodiment this invention provides a process for preparing 4-phenylbutyric acid by the scheme shown below, where an organic ester is treated with an acid in a solvent, optionally concentrating the product, purifying the product, and optionally further purifying the product.

R_1COOH, H^+ / Solvent

R_1 = Methyl, Ethyl, Propyl, Chloromethyl, Bromomethyl,
R = Methyl, Ethyl

In this process, an organic ester of the formula $Ph\text{-}CH_2\text{-}CH_2\text{-}CH\text{-}(COOR)_2$ is treated with a mineral acid in a water miscible organic solvent medium at a desired temperature. Each R is independently an alkyl group containing up to four carbon atoms, or an aryl or aralkyl group wherein the alkyl portion has up to four carbon atoms. The resulting product may be concentrated, such as by evaporation (vacuum and/or temperature induced). Thereafter, the product 4-phenylbutyric acid is precipitated from solution with the aid of a non-polar solvent. This crude 4-phenylbutyric acid product may also be is purified by vacuum distillation. Finally, if desired, the crude 4-phenylbutyric acid is purified by recrystallization using a combination of non-polar solvents. In this process, the mineral acid is preferably hydrochloric acid or sulfuric acid, and the solvent contains a carboxylic acid of less than four carbon atoms in the main chain.

In another embodiment we provide a process of preparing sodium 4-phenylbutyrate including the steps of dissolving 4-phenylbutyric acid in an organic medium, treating the solution with in inorganic alkali such as sodium hydroxide or sodium carbonate, heating the resulting mixture, optionally concentrating the heated mixture by distilling out the solvent, adding a suitable solvent to the mixture to precipitate sodium 4-phenylbutyrate from the mixture, and isolating the product by filtration and drying under vacuum at a selected temperature. The organic medium is selected from one or more organic solvents preferably chosen from the group consisting of alkyl alcohols (such methanol, ethanol, and isopropanol), alkyl esters (such ethyl acetate), and tetrahydrofuran, and compatible mixtures thereof. The preferred temperature at which the solution is first heated is in the range of about 30° C to about 95° C. In the precipitation step, the organic solvent is preferably chosen from the group consisting of dialkyl ethers (such as isopropyl ether and diethyl ether), dialkyl acetates (such as ethyl acetate), dialkyl ketones (such as acetone or ethyl methyl ketone), and other solvents, such as 1,4-dioxan, and compatible mixtures thereof.

Practice of this invention is illustrated by the non-limiting examples provided herein.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 500 mg/mL

Example 1

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was added about 10 mL of water, and the mixture was agitated to dissolve the butyrate and form a solution. To the solution was added about 0.05 g of sodium saccharin, 0.05 g of sodium benzoate, and the solution was mixed well. This solution was compounded with water to yield 25 mL of a liquid oral dosage form.

Example 2

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. About 10 mL of water was added to the flask and the mixture was agitated to dissolve the butyrate. To the solution was added about 0.05 g of raspberry flavor (*e.g.*, raspberry XBF-700194, available from IFF International Flavors & Fragrances, New York, NY), 0.05 g of sodium benzoate, and then mixed well. This mixture was

compounded to 25 mL with water. Any flavoring that is dispersible in water is generally suitable for this invention.

Example 3

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was added about 10 mL of water and agitated to dissolve. To the mixture was added about 0.05 g of sodium benzoate and mixed well. This mixture was compounded to 25 mL with water.

Example 4

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. Added about 10 mL of water and agitated to dissolve. To the mixture added about 0.05 g of raspberry flavoring, 0.05 g of sodium benzoate, 0.05 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 5

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask, to which was added about 10 mL of water and then agitated to dissolve. To the mixture was added about 0.15 g of raspberry flavor, 0.05 g of sodium benzoate, 0.25 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 6

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. To that was added about 10 mL of water and the mixture agitated to dissolve. To the solution was then added about 100 mg of sodium carbonate, 0.15 g of raspberry flavor, 0.05 g of sodium benzoate, 0.25 g of sodium saccharin and mixed well. This mixture was compounded to 25 mL with water.

Example 7

About 12.5 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask, about 10 mL of water was added, and the mixture agitated to dissolve. Then were added about: 100 mg of sodium carbonate, 0.15 g of raspberry flavor, 0.05 g of

sodium benzoate, and 0.25 g of sucralose; and the combination mixed well. This mixture was compounded to 25 mL with water.

Example 8

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask. About 9 mL of water was added and the mixture agitated with heating to a temperature of about 70° C to dissolve. The solution was then left to cool to room temperature and about 0.05 g of raspberry flavor, 0.05 g of sodium benzoate, and 0.05 g of sodium saccharin were added with good mixing. This mixture was compounded to 25 mL with water.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 640 mg/mL

Example 9

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask and about 9 mL of water was added and the mixture, which was then agitated with heating at a temperature of about 70° C to dissolve the butyrate. The solution was then cooled to 25° C and 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added with good mixing. This solution was compounded to 25 mL with water.

Example 10

About 16 g of sodium 4-phenylbutyrate was transferred to a 25 mL volumetric flask to which was then added about 9 mL of water. The mixture was agitated to dissolve the butyrate at an elevated temperature of about 70° C . The solution was cooled to 25° C and 0.05 g of sodium benzoate was added and the solution mixed well. This solution was compounded to 25 mL with water.

Example 11

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask. About 90 mL of water was added and the mixture agitated with heating at a temperature 70° C to dissolve. The solution was then cooled to 25° C and 0.5 g of sodium benzoate and 0.5 g of sodium saccharin were added and mixed well. This solution was compounded to 250 mL with water.

Example 12

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask. To the flask was added about 90 mL of water and the mixture agitated with heating at a temperature 70° C to dissolve. The mixture was cooled to 25° C and 0.5 g of sodium benzoate was added and mixed well. This mixture was compounded to 250 mL with water.

Example 13

About 160 g of sodium 4-phenylbutyrate was transferred to a 250 mL volumetric flask to which was then added about 90 mL of water and agitated with heating at a temperature 70° C to dissolve. The mixture was cooled to 25° C and 0.5 g of sodium benzoate was added and mixed well. This solution was compounded to 250 mL with water. This solution was then kept at 0°C for about 48 hours and was no precipitation or freezing of the solution was found to have occurred. Further cooling of this solution to about –4° C caused precipitation.

Preparation of a liquid oral pharmaceutical composition of sodium 4-phenylbutyrate with a strength of 500 mg/mL starting with 4-phenylbutyric acid.

Example 14

About 10.9 g of 4-phenylbutyric acid was transferred to a 25 mL volumetric flask. About 10 mL of water was added and then about 2.9 g of sodium hydroxide was added. This mixture was agitated with heating at a temperature 70° C for about 20 min. until a clear solution resulted. The solution was cooled to 25° C and 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added and mixed well. This solution was compounded to 25 mL with water.

Example 15

About 10.9 g of 4-phenylbutyric acid was transferred to a 25 mL volumetric flask to which was added about 10 mL of water, and about 3.9 g of sodium carbonate was added. This mixture was agitated with heating at a temperature of about 90° C for about 30 min. until a clear solution was obtained. The solution was cooled to 25 ° C and then 0.05 g of sodium benzoate and 0.05 g of sodium saccharin were added and

mixed well. This mixture solution compounded to 25 mL with water to provide the liquid oral composition.

Preparation of 4-phenylbutyric acid

Example 16

To a mixture of 2000 mL of acetic acid and 1500 mL of 6N hydrochloric acid was added 500 g of Diester {$PhCH_2CH_2CH(COOEt)_2$). The temperature of the mixture was raised to the range of about 95° to 110° C and refluxed for about 20 hrs. The progress of the reaction was monitored by chromatography, and at completion the acetic acid and water were removed by distillation at atmospheric pressure. The residue was dissolved in water using 10% sodium hydroxide. The aqueous solution was then washed with methylene chloride and the pH was adjusted with concentrated hydrochloric acid to a pH of about 1. The product was extracted with 1700 ml of hexane and the eluate was cooled to -10° C. The resulting precipitated crude 4-phenylbutyric acid was isolated by filtration and dried under vacuum at about 30 °C. Yield 280 g (90%). The crude 4-phenyl butyric acid so isolated was dissolved in 1500mL hexane at a temperature of about 30° to 50° C and then cooled to about –10° C and then stirred for about one hour to precipitate. The pure 4-phenyl butyric acid was then isolated by filtration and dried under vacuum without heating. (Purity >99%.)

Example 17

To a mixture of 2000 mL of acetic acid and 1500 mL of 6N hydrochloric acid added 500 g of Diester {$PhCH_2CH_2CH(COOEt)_2$}. The temperature of the mixture was raised to between about 95° to about 110° C and refluxed for about 20 hrs. The progress of the reaction mixture was monitored by chromatography and at completion the acetic acid and water were removed by distillation at atmospheric pressure. The residue was dissolved in water using 10 % sodium hydroxide. The aqueous solution was washed with methylene chloride and the pH was adjusted with concentrated hydrochloric acid to about one. The product was extracted with 1700 ml of hexane and the solution was cooled to -10° C. The precipitated crude 4-phenylbutyric acid was isolated by filtration and dried under vacuum at about 30 °C . Yield 280 g (90%). The crude 4-phenyl butyric acid was then fractionally distilled under vacuum at about 170 °C. (Purity > 99 %.)

Preparation of Sodium 4-phenylbutyrate

Example 18

About 200 g of 4-phenylbutyric acid was dissolved in 1200 mL of methanol, then 65 g sodium carbonate was added and the mixture heated to about 60 °C for about 45 min. The solution is concentrated to about 1/10th of its original volume and 7000 mL of acetone was added with stirring for about 40min at about 0° C. The precipitated sodium4-phenylbutyrate was filtered and washed with acetone, and dried under vacuum at 30 ° C.

The foregoing description is meant to be illustrative and not limiting. Various changes, modifications, and additions may become apparent to the skilled artisan upon a perusal of this specification, and such are meant to be within the scope and spirit of the invention as defined by the claims.

What is claimed is:

1. A pharmaceutical liquid composition, comprising: a solution of sodium 4-phenylbutyrate in an aqueous medium at a concentration of at least about 300 mg//mL.
2. The composition of claim 1, further comprising a preservative.
3. The composition of claim 1, further comprising a flavoring agent.
4. The composition of claim 1, further comprising a preservative and a flavor.
5. The composition of claim 3, wherein the flavoring agent is a sweetening agent.
6. The composition of claim 4, wherein the flavoring agent is a sweetening agent.
7. The composition of claim 1, further comprising at least two flavoring agents, at least one of said flavoring agents being a sweetening agent, and a preservative.
8. The composition of claim 1, wherein the concentration of sodium 4-phenylbutyrate ranges from about 300 mg/mL to about 700 mg/mL.
9. The composition of claim 8, wherein the concentration of sodium 4-phenylbutyrate is in the range from about 400 mg / mL to about 600 mg/mL.
10. The composition of claim 9, wherein the concentration is about 500 mg/mL.
11. The composition of claim 2, 4, 6, or 7, wherein the preservative is sodium benzoate.
12. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is sodium saccharine.
13. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is sucralose.
14. The composition of claim 3, 4, 5, or 7, wherein the sweetening agent is a mixture of sodium saccharine and sucralose
15. The composition of claim 3, 4, 5, or 7, wherein the flavoring agent is a raspberry flavor.
16. The composition of claim 1, further comprising a base.
17. The composition of claim 14, wherein the base is sodium carbonate.
18. The composition of claim 14, wherein the base is sodium hydroxide.
19. The composition of claim 1, further comprising 4-phenylbutyric acid
20. The composition of claim 17, further comprising sodium carbonate.

21. The composition of claim 1, wherein the weight fraction of water is less than the weight fraction of sodium 4-phenylbutyrate.
22. A process for preparing an aqueous solution of 4-phenylbutyrate, comprising the steps of: adding water to sodium 4-phenylbutyrate powder; and dissolving the powder in the water by agitation at temperature ranging from about 25° C to about 80° C to obtain a solution having a concentration of at least about 300 g/mL of 4-phenylbutyrate.
23. The process of claim 20, wherein weight fraction of water in the solution is less than the weight fraction of 4-phenylbutyrate.
24. A process for making of sodium 4-phenylbutyrate, comprising the steps of:
    (A) dissolving 4-phenylbutyric acid in a first organic solvent medium;
    (B) treating the solution of step (A) with a inorganic alkali;
    (C) heating the treated solution of step (B) to a predetermined temperature;
    (D) adding a second solvent to the heated mixture effective to precipitate sodium 4-phenylbutyrate therefrom; and
    (E) isolating the precipitate product by filtration and drying under vacuum at a predetermined temperature.
25. The process of claim 22, further comprising concentrating the solution obtained after step (C) by distilling out the organic solvent medium.
26. The process of claim 22, wherein the inorganic alkali is sodium carbonate.
27. The process of claim 22, wherein the inorganic alkali is sodium hydroxide.
28. The process of claim 22, wherein the first organic solvent comprises two or more organic solvents.
29. The process of claim 22 or 26, wherein the first organic solvent is selected from the group consisting of methanol, ethanol, isopropanol, ethyl acetate, tetrahydrofuran, and compatible mixtures thereof.
30. The process of claim 22, the second solvent is two or more organic solvents.
31. The process of claim 22 or 28, wherein the second solvent is selected from the group consisting of Isopropyl ether, diethylether, ethyl acetate, ethyl methyl ketone, 1,4-dioxan, acetone, and compatible mixtures thereof.
32. The process of claim 22, wherein the predetermined temperature in each of step (C) and (E) is independently selected to be in the range of about 30°C to about 95° C.

33. A process for making 4-phenylbutyric acid, comprising:
    (i) treating an organic ester of the formula Ph-CH_2-CH_2-CH-$(COOR)_2$, wherein each R is independently an alkyl containing up to four carbon atoms, an aryl group, or an aralkyl group wherein the alkyl portion has up to four carbon atoms, with a mineral acid in a water miscible organic solvent at a predetermined temperature; and
    (ii) precipitating 4-phenylbutyric acid using a non-polar solvent..
34. The process of claim 31, further comprising concentrating the solution by evaporation between steps (i) and (ii).
35. The process of claim 31, further comprising step of purifying the crude 4-phenylbutyric acid obtained in step (ii) by vacuum distillation.
36. The process of claim 31, further comprising purifying the crude 4-phenylbutyric acid obtained in step (ii) by recrystallization using one or more solvents.
37. The process of claim 31, wherein each R is independently methyl, ethyl or propyl.
38. The process of claim 34, wherein the water miscible organic solvent contains one or more carboxylic acids having less than 4 carbons in the main chain.
39. The process of claim 36, wherein the carboxylic acids selected from propanoic acid, substituted propanoic acid, acetic acid, substituted acetic acid, and formic acid.
40. The process of claim 36 or 37, wherein the mineral acid is hydrochloric acid.
41. The process of claim 36 or 37, wherein the mineral acid is sulfuric acid.
42. A method of treating a patient suffering from a urea cycle deficiencies, sickle-cell anemia, cancer, or potential cerebral ischemic injury, comprising providing an oral aqueous solution of 4-phenylbutyrate having a concentration of at least about 300 mg/mL and orally administering said solution to a patient in need thereof.
43. The method of claim 40, wherein the solution further comprises a preservative, a flavoring agent, a fragrance, or a mixture thereof.
44. The method of claim 41, wherein the solution further comprises a preservative and a flavoring agent.

45. The method of claim 42, wherein the solution further comprises a fragrance and a sweetener as the flavoring agent.

46. The composition of any of claims 1-10 and 14-19, wherein the solution does not freeze at 0° C.

47. The process of claim 20 or 21, wherein the solution does not freeze at 0° C.

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(54) Title: AGONISTS FOR ANTIMICROBIAL PEPTIDE SYSTEMS

(57) Abstract: Short chain fatty acids (SCFAs) and glycerol esters of SCFAs not previously used for that purpose are provided for use as a medicament for treating, preventing or counteracting microbial infections in animals, including humans, by stimulating the innate antimicrobial peptide defense system . Preferred compounds include phenyl substituted short chain fatty acids (SCFAs) derivatives and. Also provided are methods and compositions for treating, preventing or counteracting microbial infections, including bacterial, viral, fungal, and parasitic infections, by administration of medicaments comprising a secretagogue-effective amount of the compounds of the invention.

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Agonists for antimicrobial peptide systems

Technical field

5 The invention relates to compounds which are active as drugs for stimulating the innate antimicrobial peptide system and can be used as antimicrobial drugs.

Background art

10 Antimicrobial peptides and proteins play an important role in innate host defenses and are believed to be particularly important at mucosal surfaces that form the initial barrier between the host and the external environment. Such peptides are found in large quantities in the colonic epithelium. The peptides can be considered as endogenous antibiotics and are widespread in nature as immediate defense effectors. They are mainly 15 stored in vacuoles of granulocytes ready for activation upon stimulation or secreted directly onto mucosal and other surfaces by epithelial cells.

A human antimicrobial peptide has been identified and is referred to as LL-37, a 37-residue peptide present in neutrophils, epithelial cells and lymphocytes. Both isolated and 20 chemically synthesised LL-37 show antimicrobial activity *in vitro*.

Certain bacteria have evolved mechanisms to overcome the antimicrobial peptide barrier, such as *Shigella* bacteria which down-regulate LL-37 expression in the colon epithelium.

25 Rabbani *et al. (Short-Chain Fatty Acids Improve Clinical, Pathologic, and Microbiologic Features of Experimental Shigellosis. The Journal of Infectious Diseases 1999;179:390–7)* investigated that naturally occurring short chain fatty acids (SCFAs; acetate, propionate, and butyrate in 60:30:40 ratio) which occur as fermentation products in the gut. The authors used a rabbit model of shigellosis. They reported that the mixture, given 30 by colonic infusion into the rabbits with acute shigellosis, improved clinical, pathologic, and bacteriologic characteristics.

Hase et al. (*Cell Differentiation Is a Key Determinant of Cathelicidin LL-37/Human Cationic Antimicrobial Protein 18 Expression by Human Colon Epithelium. INFECTION 35 AND IMMUNITY, Feb. 2002, vol 70, No 2 p. 953–963*) reported that infection *in vitro* of HCA-7 cells with *Salmonella enterica* serovar Dublin or enteroinvasive *Escherichia coli*

modestly upregulated LL-37/hCAP18 mRNA expression. The authors concluded that differentiated human colon epithelium expresses LL-37/hCAP18 as part of its repertoire of innate defense molecules.

5 Schauber et al. (*Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways.* Gut 2003; 52:735-741.) investigated the effect of naturally occuring SCFAs on LL-37 expression in vivo and in vitro. These authors report that following exposure to butyrate, isobutyrate and propionate, expression of the LL-37 mRNA increases *in vitro* in colonocytes. The 10 authors are cautious about the possible consequences of increased antimicrobial peptide expression on the commensal intestinal flora, which is critical for protection of the mucosa against enteropathogenic microbes. They note a pathological increase in the activity of endogenous antibiotics would not then be beneficial to the host but might have deleterious consequences.

15

Raqib *et al.* (*Improved outcome in shigellosis associated with butyrate induction of an endogenous peptide antibiotic.* Proc. Natl. Acad. Sci. 2006; 103: 9178-9183.) reported that butyrate treatment of rabbits resulted in reduced clinical illness and bacterial load in the stool and significant upregulation of CAP-18 (the rabbit homologue of LL-37) in the 20 surface epithelium.

Other molecules have also been investigated for their possible utility in stimulating natural defensins.

25 WO2000-09137 (Magainin Pharmaceuticals) describes newly isolated aminosterol compounds and pharmaceutical compositions based on the aminosterol compounds are described. Methods for the treatment of various disorders, for example, a microbial infection, are also described

30 US2002-0076393 (Fehlbaum *et al.*) describe the use of isoleucine or active isomers or analogs thereof for stimulating production of defensin. It should be noted that the claims refer, *inter alia*, to one such analog being butyrate or an active derivative thereof. However where butyrate was tested and it appeared to be less active than isoleucine at similar concentrations (see Figure 7 therein).

35

US2003-0109582 (Zasloff) describe the use of isoleucine compounds for stimulating Paneth cells to release natural antimicrobial agents including peptides, to reduce or eliminate pathogenic organisms in the GI tract of mammalian bodies, including humans, utilizing an active isoleucine compound as a secretagogue. "Isoleucine compounds" are 5 defined as including 'isoleucine butyrate' though this compound is not described or tested.

US7311925 (Zasloff) describes methods of blocking microbial adherence to a eukaryotic cell surface in a mammal by applying a pharmacologically acceptable composition 10 containing at least one compound selected from the group consisting of isoleucine, an active isomer thereof, and an active analog thereof, to said surface in a microbial blocking quantity. Active analogs of isoleucine are defined as including 'isoleucine butyrate' though this compound is not described or tested.

15 US20080038374 (Stahle) describes use of a vitamin D compound, which is able to specifically and directly up-regulate hCAP18, for the manufacturing of a medicament with antimicrobial effect for treatment of conditions deficient in LL-37, such as chronical ulcers, and atopic dermatitis.

20 WO/2008/073174 (GALLO) describes methods and compositions for modulating gene expression and cathelicidin the innate immune response by 1,25$(OH)_2$ vitamin D3 (1,25D3). That compound is tested alongside non-specific histone deacetylase inhibitors (HDACi) including butyrate or trichostatin A.

25 Hata *et al.* (2008) "*Administration of oral vitamin D induces cathelicidin production in atopic individuals*" J ALLERGY CLIN IMMUNOL, VOLUME 122, NUMBER 4, described a study in which 14 normal controls and 14 atopic subjects with moderate to severe atopic dermatitis were treated with oral vitamin D3 to see if this could overcome the relative deficiency in induction of cathelicidin in the atopic patients. After supplementation with 30 4000 IU/d oral vitamin D for 21 days, AD lesional skin showed a statistically significant increase in cathelicidin expression.

Despite the above disclosures, it will be appreciated that the provision of compounds or combinations of compounds for use in enhancing the innate immune response, for 35 example in the gut, would provide a contribution to the art.

Summary of the invention

As can be seen from the discussion above, the publications in the art had been cautious about the possible deleterious consequences of SCFA compounds which stimulate the effect of endogenous antibiotics in the human gut, because of their potential effect on commensal intestinal flora. Additionally, it was known that butyrate, for example, had practical drawbacks, in particular the unpleasant odour and taste, that made it unsuitable for pharmaceutical use. These reasons may account for the fact that the effect of SCFAs had not been investigated in the art in humans but greater interest has apparently been given to the use of vitamin D in the skin.

The present inventors have found that a number of pharmaceutically acceptable SCFA-derivatives and prodrugs are active as drugs to stimulate the innate antimicrobial peptide system in human cell lines and can be used as preventive and curative antimicrobial drugs in animal models of disease. These pharmaceutically acceptable SCFA-derivatives may be more acceptable (in terms of odour and\or taste) than butyrate. These findings have profound implications for the use of these compounds on replacing or supplementing existing antibiotics or other antimicrobial strategies in treating human disease.

An abstract has previously been made available stating that an unidentified drug stimulated cathelcidin antimicrobial peptide (CAMP) and human beta-defensin 1 (hBD-1) gene expression in the bronchial epithelial cell line VA10 ("Induction of Antimicrobial Peptide Gene Expression by a approved drug in a Bronchial Epithelial Cell Line"; Jónas Steinmann and Guðmundur Hrafn Guðmundsson, Institute of Biology, University of Iceland, Sturlugata 7, 101 Reykjavik, Iceland).

After the presently claimed priority date, a poster was presented showing for the first time that 4-phenylbutyrate (PBA) stimulates cathelicidin antimicrobial peptide gene expression in a bronchial epithelial cell line ("Induction of Antimicrobial Peptide Gene Expression in a Bronchial Epithelial Cell Line"; Jonas Steinmann and Guðmundur Hrafn Guðmundsson Institute of Biology, University of Iceland, 101 Reykjavik, Iceland; 15th March 2008).

Sodium phenylbutyrate is a known medicament. For example it has been marketed by Ucyclyd Pharma (Hunt Valley, USA) under the trade name Buphenyl and by Swedish Orphan International (Sweden) as Ammonaps. It has been used to treat urea cycle

disorders (Batshaw *et al.* (2001) *J. Pediatr.* 138 (1 Suppl): S46–54; discussion S54–5). Scandinavian Formulas, Inc. Sellersville, PA supplies sodium phenylbutyrate worldwide for clinical trials. Sodium phenylbutyrate is also under investigation for the treatment of some sickle-cell disorders (Blood Products Plasma Expanders and Haemostatics) and for use as a potential differentiation-inducing agent in malignant glioma and acute myeloid leukaemia. It has also been investigated in respect of cystic fibrosis pathology due to its capacity to traffic DeltaF508-cystic fibrosis transmembrane conductance regulator (CFTR) to the cell membrane and restore CFTR chloride function at the plasma membrane of CF lung cells in vitro and in vivo (Roque *et al.* J Pharmacol Exp Ther. 2008 Sep;326(3):949-56. Epub 2008 Jun 23). It is believed in the literature that phenylbutyrate is a prodrug which is metabolized in the body by beta-oxidation to phenylacetate.

Notwithstanding the above, prior to the present invention, PBA was not known or suggested for the uses claimed herein.

Detailed disclosure of the invention

Thus in a first aspect, the present invention provides compounds as defined by formula I for use as a medicament for treating, preventing or counteracting microbial infections in humans and animals by stimulating the innate antimicrobial peptide defense system,

Compounds of the invention

In a first aspect, the present invention provides compounds as defined by formula Ia for use as a medicament for treating, preventing or counteracting microbial infections in humans and animals by stimulating the innate antimicrobial peptide defense system,

(Ia)

wherein

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R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or
5 an amino acid group $CONHCR^7COOH$ or a salt thereof;

m and *n* are each independently 0 or 1;

R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a} and R^{3b} each independently represent hydrogen, halide, amino,
10 hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group; and/or
R^{2a}, together with an adjacent R^{3a} or R^{1a}, may represent a carbon-carbon π bond; and/or
15 R^{2b}, together with an adjacent R^{3b} or R^{1b}, may represent a carbon-carbon π bond;

R^4 may be hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group;

20

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group;

25 R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group; and

R^7 is a side chain of a naturally occurring amino acid or is selected from
30 $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, or $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms;

and wherein, if R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is selected from halide, amino, hydroxyl, carbonyl, a linear or branched substituted
35 or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group.

In some embodiments the compound may be a compound of formula I:

(I)

5 wherein, preferably, R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

10 R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or

15 substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

20 x represents a single, double or triple bond,

or x-$R^{3a}R^4$ together represent hydrogen in which case R^1 is preferably $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or

25 nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

30 R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms.

Compounds of formula I are compounds of formula Ia in which R^{1a} and R^{1b} are both hydrogen, *m* and *n* are both 1, and R^{2b} and R^{3b} are *either* both hydrogen *or* together form a π bond in position 'x'. If R^{2a} and R^{3a} also together form a π bond, then position 'x' represents a double bond.

Compounds of formula Ia in which R^{1a}, R^{1b} and R^{2b} are all hydrogen, *m* is 0, *n* is 1, and R^4 is hydrogen can also be represented as compounds of formula I where x-$R^{3a}R^4$ together represent hydrogen.

In compounds of formula I, 'x' is preferably a single bond.

Preferences for R^1

In certain preferred embodiments, the compound of the invention is a carboxylic acid, in these cases R^1 represents a carboxyl group, or a pharmaceutically acceptable salt thereof. If R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen. In other preferred embodiments, R^1 is a carboxylic acid derivative, such as an ester or an amide.

In some such embodiments, as represented by formula IIa, R^1 is an ester group of formula $COOR^5$ where R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, and preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl. Particularly preferred R^5 groups are methyl and ethyl.

R^{3a} R^{3b} R^{1a} R^{1b} OR^5 R^4 m n R^{2a} R^{2b} O

(IIa)

In some preferred embodiments R^1 is an ester selected from a triglyceride ester moiety or diglyceride ester moiety.

If R^1 is a triglyceride moiety the compounds of the invention are of the following general formula (IIb):

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(IIb)

If R^1 is a diglyceride moiety, the compounds of the invention are of the following general formula (IIc):

5

(IIc)

Embodiments of particular interest include glyceryl tributyrate or glyceryl tripropionate. Other preferred embodiments make use of corresponding glycerol esters of one or more
10 phenyl substituted fatty acids or other short chain fatty acids such as the above mentioned. Such glyceryl triesters include for example but not limited to glyceryl tributyrate wherein one or more of the butyrate acyl chains are substituted with phenyl, e.g. 1-butanoyloxy-3-(4'-phenylbutanoyloxy)propan-2-yl butanoate, 1,3-(4',4"-diphenyl)-di(butanoyloxy)propan-2-yl butanoate, and 1,3-di(butanoyloxy)propan-2-yl-4-
15 phenylbutanoate.

Further embodiments which are carboxylic derivatives embodiments include amides of formula (IId), wherein R^1 is a group of formula $CONR^5R^6$, wherein R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to
20 10 carbon atoms, preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl, and R^6 is selected from hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, preferably 3 to 5 carbon atoms, or a substituted or nonsubstituted aryl group such as for example phenyl, or benzyl.

25

(IId)

In certain embodiments R^1 is an amino acid group, in which case the compounds of the invention may be represented as compounds of the following general formula (IIe):

(IIe)

or a salt thereof, in which R^7 is an amino acid side chain. In some embodiments R^7 is the side chain of a naturally occurring amino acid.

For example, R^7 may be a side chain of leucine ($CH_2CH_2CH_2CH_3$), isoleucine ($CH(CH_3)CH_2CH_3$), methionine ($-CH_2CH_2SCH_3$), lysine ($-CH_2CH_2CH_2CH_2NH_2$), or arginine ($-CH_2CH_2CH_2NHC(=NH)NH_2$). In some embodiments, particularly if R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are all hydrogen and *m* and *n* are 1, R^7 is preferably not an isoleucine side chain ($CH(CH_3)CH_2CH_3$).

Alternatively, R^7 may be a derivative or analogue of a naturally occurring amino acid side chain, such as a lysine side chain derivative ($-CH_2CH_2CH_2CH_2NHR^8$), an arginine side chain derivative ($-CH_2CH_2CH_2NHC(=NH)NHR^8$), or a group such as $-CH_2CH_2CH_2NHR^8$, wherein R^8 represents hydrogen, a linear or branched substituted or unsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group.

In certain embodiments found to be useful, the compounds of the invention are relatively small SCFA derivatives. For example, compounds of formula I wherein R^{2a} and R^4 represent hydrogen. In these embodiments R^{3a} is preferably hydrogen, hydroxyl, or a substituted or nonsubstituted aryl group including phenyl, or benzyl, with the above

limitation applying to R^{3a} in the case where R^1 is carboxyl or a salt thereof. Substituted aryl can be hydroxyl or amino-substituted phenyl, or benzyl.

Preferred chain lengths

In some preferred compounds of the invention, *m* and *n* are each 1. These compounds may be described as butyric acid/butyrate derivatives and are of general formula (IIIa):

(IIIa)

where R^1, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are as previously defined.

In other preferred compounds, *m* is 1 and *n* is 0. These compounds may be described as propionic acid/propionate derivatives and are of general formula (IIIb):

(IIIb)

where R^1, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 are as previously defined. It can be seen that if *m* were 0 and *n* were 1, this would also result in propionic acid derivatives.

In some embodiments, both *m* and *n* may be 0. This results in compounds which may be described as acetic acid/acetate derivatives, of general formula (IIIc):

(IIIc)

Preferred substituents

Preferred embodiments of the invention include compounds which are substituted butyric, propionic or acetic acid derivatives of general formulae (IIIa) to (IIIc), wherein R^1 is carboxylate or a derivative thereof as defined above and wherein one or more of R^{1a}, R^{1b},
5 R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen, preferably selected from an alkyl group or an aryl group. It is preferred that one or more, preferably one, of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group, most preferably a phenyl or substituted phenyl group. When one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group, it is preferred that the others are selected from hydrogen or an alkyl group, the alkyl group being preferably
10 methyl.

Most preferably, R^4 is an aryl group, preferably phenyl or substituted phenyl. Certain preferred compounds according to these embodiments are of general formula (IVa):

R^{3a} R^{3b} R^{1a} R^{1b} m n R^{2a} R^{2b} O O^-

15

(IVa)

Preferred butyric acid derivatives are therefore of general formula (IVb):

R^{3a} R^{3b} R^{1a} R^{1b} R^{2a} R^{2b} O O^-

(IVb)

20 preferred propionic acid derivatives are of general formula (IVc):

R^{1a} R^{1b} R^{3a} R^{3b} O O^-

(IVc)

and preferred acetate derivatives are of general formula (IVd):

25

(IVd)

In formulae (IVa) to (IVd), the phenyl ring may optionally be substituted with one or more substituents, as further defined below. Preferred substituents are alkyl, halide, hydroxyl and amino.

The carboxylate group may optionally be derivatised as an ester or amide, as set out above. In these embodiments, R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} are preferably hydrogen or an alkyl group with 1 to 10 carbon atoms, the alkyl group being preferably methyl or ethyl.

In alternative embodiments, R^4 may be hydrogen, and one or more, preferably one, of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} may be an aryl group such as phenyl or substituted phenyl.

Substituents α to the carboxylate

R^{1a} and R^{1b} are preferably selected from hydrogen and an alkyl group having from 1 to 10 carbon atoms, the alkyl group being preferably methyl or ethyl. In some embodiments, R^{1a} and R^{1b} may both be alkyl, but it is preferred that at least one of R^{1a} and R^{1b} is hydrogen.

In particular, the following compounds are useful in accordance with the invention: 4-phenylbutyric acid, 3-phenylbutyric acid, 2-phenylbutyric acid, 3-phenylpropionic acid, 2-phenylpropionic acid, 2-methyl-3-phenylpropionic acid *[ST7]*, 2-methyl-4-phenylbutyric acid, or a pharmaceutically acceptable salt of any of said compounds, methyl 4-phenylbutyrate, ethyl 4-phenylbutyrate, methyl 3-phenylbutyrate, ethyl 3-phenylbutyrate, methyl 2-phenylbutyrate, ethyl 2-phenylbutyrate, methyl 3-phenylpropionate, ethyl 3-phenylpropionate, methyl 2-phenylpropionate, ethyl 2-phenylpropionate, methyl 2-methyl-3-phenylpropionate, ethyl 2-methyl-3-phenylpropionate, methyl 2-methyl-4-phenylbutyrate, and ethyl 2-methyl-4-phenylbutyrate.

Metabolites of these compounds may also be useful in the invention, in particular phenyl acetate.

Substituents β to the carboxylate (where present)

In embodiments, one or both of R^{2a} and R^{2b} may optionally be hydroxyl. This may be
5 preferred where it is desired that the compound of the invention have increased resistance to metabolism such as beta oxidation, and hence in principle a longer half-life.

Definitions and further preferences

10 Alkyl:

As used herein the term "alkyl", unless otherwise specified, refers to a C_{1-10} alkyl group, that is to say a monovalent moiety obtained by removing a hydrogen atom from a hydrocarbon compound having from 1 to 10 carbon atoms, which may be aliphatic or alicyclic, or a combination thereof, which may be linear or branched, and which may be
15 saturated, partially unsaturated, or fully unsaturated. In certain instances C_{1-4}, C_{1-5}, C_{1-6} or C_{1-7} alkyl groups may be preferred.

Examples of saturated linear C_{1-10} alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, n-pentyl (amyl) and n-hexyl.

20

Examples of saturated branched C_{1-10} alkyl groups include, but are not limited to, iso-propyl, iso-butyl, sec-butyl, tert-butyl, and neo-pentyl.

Examples of saturated alicyclic C_{1-10} alkyl groups (which may also be referred to as "C_{3-10}
25 cycloalkyl" groups) include, but are not limited to, groups such as cyclopropyl, cyclobutyl, cyclopentyl, and cyclohexyl, as well as substituted groups (e.g., groups which comprise such groups), such as methylcyclopropyl, dimethylcyclopropyl, methylcyclobutyl, dimethylcyclobutyl, methylcyclopentyl, dimethylcyclopentyl, methylcyclohexyl, dimethylcyclohexyl, cyclopropylmethyl and cyclohexylmethyl.

30

Unsaturated alkyl groups contain one or more double or triple bonds i.e. one or more carbon-carbon π bonds. Examples of unsaturated C_{1-10} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{2-10}alkenyl" groups) include, but are not limited to, ethenyl (vinyl, $-CH=CH_2$), 2-propenyl (allyl, $-CH-CH=CH_2$), isopropenyl
35 ($-C(CH_3)=CH_2$), butenyl, pentenyl, and hexenyl.

Examples of unsaturated C_{1-10} alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C_{2-10} alkynyl" groups) include, but are not limited to, ethynyl (ethinyl) and 2-propynyl (propargyl).

5 Examples of unsaturated alicyclic (carbocyclic) C_{1-10} alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C_{3-10}cycloalkenyl" groups) include, but are not limited to, unsubstituted groups such as cyclopropenyl, cyclobutenyl, cyclopentenyl, and cyclohexenyl, as well as substituted groups (e.g., groups which comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

10

Aryl:

As used herein the term "aryl", unless otherwise specified, refers to a C_{5-20} aryl group, that is to say a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C_{5-20} aromatic compound, said compound having one ring, or two or more

15 rings (e.g., fused), and having from 5 to 20 ring atoms, and wherein at least one of said ring(s) is an aromatic ring. Preferably, each ring has from 5 to 7 ring atoms.

The ring atoms may be all carbon atoms, as in "carboaryl groups", in which case the group may conveniently be referred to as a "C_{5-20} carboaryl" group.

20

Examples of C_{5-20} aryl groups which do not have ring heteroatoms (i.e. C_{5-20} carboaryl groups) include, but are not limited to, those derived from benzene (i.e. phenyl) (C_6), naphthalene (C_{10}), anthracene (C_{14}), phenanthrene (C_{14}), naphthacene (C_{18}), and pyrene (C_{16}).

25

Examples of aryl groups which comprise fused rings, one of which is not an aromatic ring, include, but are not limited to, groups derived from indene and fluorene.

Alternatively, the ring atoms may include one or more heteroatoms, including but not

30 limited to oxygen, nitrogen, and sulphur, as in "heteroaryl groups". In this case, the group may conveniently be referred to as a "C_{5-20} heteroaryl" group, wherein "C_{5-20}" denotes ring atoms, whether carbon atoms or heteroatoms. Preferably, each ring has from 5 to 7 ring atoms, of which from 0 to 4 are ring heteroatoms.

35 Examples of C_{5-20} heteroaryl groups include, but are not limited to, C_5 heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole),

pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, and oxatriazole; and C_6 heteroaryl groups derived from isoxazine, pyridine (azine), pyridazine (1,2-diazine), pyrimidine (1,3-diazine; e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine), triazine, tetrazole, and oxadiazole (furazan).

5

Examples of C_{5-20} heteroaryl groups which comprise fused rings, include, but are not limited to, C_9 heterocyclic groups derived from benzofuran, isobenzofuran, indole, isoindole, purine (e.g., adenine, guanine), benzothiophene, benzimidazole; C_{10} heterocyclic groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine,
10 quinoxaline; C_{13} heterocyclic groups derived from carbazole, dibenzothiophene, dibenzofuran; C_{14} heterocyclic groups derived from acridine, xanthene, phenoxathiin, phenazine, phenoxazine, phenothiazine.

Optional Substitution:
15 The above alkyl and aryl groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

20

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkoxy group, discussed below), a C_{3-20} heterocyclyl group (also
25 referred to as a C_{3-20} heterocyclyloxy group), or a C_{5-20} aryl group (also referred to as a C_{5-20} aryloxy group), preferably a C_{1-7} alkyl group.

C_{1-7} alkoxy: -OR, wherein R is a C_{1-7} alkyl group. Examples of C_{1-7} alkoxy groups include, but are not limited to, $-OCH_3$ (methoxy), $-OCH_2CH_3$ (ethoxy) and $-OC(CH_3)_3$ (tert-butoxy).

30

Oxo (keto, -one): =O; carbonyl (>C=O). Examples of cyclic compounds and/or groups having, as a substituent, an oxo group (=O) include, but are not limited to, carbocyclics such as cyclopentanone and cyclohexanone; heterocyclics, such as pyrone, pyrrolidone, pyrazolone, pyrazolinone, piperidone, piperidinedione, piperazinedione, and
35 imidazolidone; cyclic anhydrides, including but not limited to maleic anhydride and succinic anhydride; cyclic carbonates, such as propylene carbonate; imides, including but

not limited to, succinimide and maleimide; lactones (cyclic esters, -O-C(=O)- in a ring), including, but not limited to, β-propiolactone, γ-butyrolactone, δ-valerolactone, and ε-caprolactone; and lactams (cyclic amides, -NH-C(=O)- in a ring), including, but not limited to, β-propiolactam, γ-butyrolactam (2-pyrrolidone), δ-valerolactam, and
5 ε-caprolactam.

Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, C_{1-7} alkyl group, a C_{3-20}heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, =NH, =NMe, =NEt, and
10 =NPh.

Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C_{1-7}alkyl group
15 (also referred to as C_{1-7} alkylacyl or C_{1-7} alkanoyl), a C_{3-20} heterocyclyl group (also referred to as C_{3-20} heterocyclylacyl), or a C_{5-20} aryl group (also referred to as C_{5-20} arylacyl), preferably a C_{1-7} alkyl group. Examples of acyl groups include, but are not limited to, $-C(=O)CH_3$ (acetyl), $-C(=O)CH_2CH_3$ (propionyl), $-C(=O)C(CH_3)_3$ (butyryl), and -C(=O)Ph (benzoyl, phenone).
20

Carboxy (carboxylic acid): -COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl
25 group, preferably a C_{1-7}alkyl group. Examples of ester groups include, but are not limited to, $-C(=O)OCH_3$, $-C(=O)OCH_2CH_3$, $-C(=O)OC(CH_3)_3$, and -C(=O)OPh.

Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7}alkyl
30 group. Examples of acyloxy groups include, but are not limited to, $-OC(=O)CH_3$ (acetoxy), $-OC(=O)CH_2CH_3$, $-OC(=O)C(CH_3)_3$, -OC(=O)Ph, and $-OC(=O)CH_2Ph$.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): $-C(=O)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of
35 amido groups include, but are not limited to, $-C(=O)NH_2$, $-C(=O)NHCH_3$, $-C(=O)N(CH_3)_2$, $-C(=O)NHCH_2CH_3$, and $-C(=O)N(CH_2CH_3)_2$, as well as amido groups in which R^{N1} and

R^{N2}, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

5 Acylamido (acylamino): $-NR^{A1}C(=O)R^{A2}$, wherein R^{A1} is an amide substituent, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group, and R^{A2} is an acyl substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. Examples of acylamide groups include, but are not limited to,
10 $-NHC(=O)CH_3$, $-NHC(=O)CH_2CH_3$, and -NHC(=O)Ph. R^{A1} and R^{A2} may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl and phthalimidyl:

succinimidyl maleimidyl phthalimidyl

Acylureido: $-N(R^{U1})C(O)NR^{U2}C(O)R^{A3}$ wherein R^{U1} and R^{U2} are independently ureido
15 substituents, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably hydrogen or a C_{1-7} alkyl group. R^{A3} is an acyl group as defined for acyl groups. Examples of acylureido groups include, but are not limited to, -NHCONHC(O)H, -NHCONMeC(O)H, -NHCONEtC(O)H, -NHCONMeC(O)Me, -NHCONEtC(O)Et, -NMeCONHC(O)Et, -NMeCONHC(O)Me, -NMeCONHC(O)Et, -
20 NMeCONMeC(O)Me, –NMeCONEtC(O)Et, and -NMeCONHC(O)Ph.

Carbamate: $-NR^{N1}-C(O)-OR^{O2}$ wherein R^{N1} is an amino substituent as defined for amino groups and R^{O2} is an ester group as defined for ester groups. Examples of carbamate groups include, but are not limited to, -NH-C(O)-O-Me, -NMe-C(O)-O-Me, -NH-C(O)-O-Et,
25 -NMe-C(O)-O-t-butyl, and -NH-C(O)-O-Ph.

Thioamido (thiocarbamyl): $-C(=S)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, $-C(=S)NH_2$, $-C(=S)NHCH_3$, $-C(=S)N(CH_3)_2$, and $-C(=S)NHCH_2CH_3$.

30

Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom,

5 Amino: $-NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7}alkyl group, or, in the case of a "cyclic" amino group, R^{N1} and R^{N2}, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring
10 atoms. Examples of amino groups include, but are not limited to, $-NH_2$, $-NHCH_3$, $-NHC(CH_3)_2$, $-N(CH_3)_2$, $-N(CH_2CH_3)_2$, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.

15 Imino: =NR, wherein R is an imino substituent, for example, for example, hydrogen, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group.

Amidine: $-C(=NR)NR_2$, wherein each R is an amidine substituent, for example, hydrogen,
20 a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably H or a C_{1-7} alkyl group. An example of an amidine group is $-C(=NH)NH_2$.

Carbazoyl (hydrazinocarbonyl): $-C(O)-NN-R^{N1}$ wherein R^{N1} is an amino substituent as defined for amino groups. Examples of azino groups include, but are not limited to, -
25 C(O)-NN-H, -C(O)-NN-Me, -C(O)-NN-Et, -C(O)-NN-Ph, and $-C(O)-NN-CH_2-Ph$.

Nitro: $-NO_2$.

Nitroso: -NO.

30

Azido: $-N_3$.

Cyano (nitrile, carbonitrile): -CN.

Isocyano: -NC.

Cyanato: -OCN.

5 Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

Isothiocyano (isothiocyanato): -NCS.

10

Sulfhydryl (thiol, mercapto): -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C_{1-7} alkyl group (also referred to as a C_{1-7} alkylthio group), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl
15 group, preferably a C_{1-7} alkyl group. Examples of C_{1-7} alkylthio groups include, but are not limited to, $-SCH_3$ and $-SCH_2CH_3$.

Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group (also referred to
20 herein as C_{1-7} alkyl disulfide). Examples of C_{1-7} alkyl disulfide groups include, but are not limited to, $-SSCH_3$ and $-SSCH_2CH_3$.

Sulfone (sulfonyl): $-S(=O)_2R$, wherein R is a sulfone substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.
25 Examples of sulfone groups include, but are not limited to, $-S(=O)_2CH_3$ (methanesulfonyl, mesyl), $-S(=O)_2CF_3$ (triflyl), $-S(=O)_2CH_2CH_3$, $-S(=O)_2C_4F_9$ (nonaflyl), $-S(=O)_2CH_2CF_3$ (tresyl), $-S(=O)_2Ph$ (phenylsulfonyl), 4-methylphenylsulfonyl (tosyl), 4-bromophenylsulfonyl (brosyl), and 4-nitrophenyl (nosyl).

30 Sulfine (sulfinyl, sulfoxide): -S(=O)R, wherein R is a sulfine substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfine groups include, but are not limited to, $-S(=O)CH_3$ and $-S(=O)CH_2CH_3$.

35 Sulfonyloxy: $-OS(=O)_2R$, wherein R is a sulfonyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group.

Examples of sulfonyloxy groups include, but are not limited to, $-OS(=O)_2CH_3$ and $-OS(=O)_2CH_2CH_3$.

Sulfinyloxy: $-OS(=O)R$, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, $-OS(=O)CH_3$ and $-OS(=O)CH_2CH_3$.

Sulfamino: $-NR^{N1}S(=O)_2OH$, wherein R^1 is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to, $-NHS(=O)_2OH$ and $-N(CH_3)S(=O)_2OH$.

Sulfinamino: $-NR^{N1}S(=O)R$, wherein R^{N1} is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfinamino groups include, but are not limited to, $-NHS(=O)CH_3$ and $-N(CH_3)S(=O)C_6H_5$.

Sulfamyl: $-S(=O)NR^{N1}R^{N2}$, wherein R^{N1} and R^{N2} are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, $-S(=O)NH_2$, $-S(=O)NH(CH_3)$, $-S(=O)N(CH_3)_2$, $-S(=O)NH(CH_2CH_3)$, $-S(=O)N(CH_2CH_3)_2$, and $-S(=O)NHPh$.

Sulfonamino: $-NR^{N1}S(=O)_2R$, wherein R^{N1} is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably a C_{1-7} alkyl group. Examples of sulfonamino groups include, but are not limited to, $-NHS(=O)_2CH_3$ and $-N(CH_3)S(=O)_2C_6H_5$. A special class of sulfonamino groups are those derived from sultams – in these groups one of R^1 and R is a C_{5-20} aryl group, preferably phenyl, whilst the other of R^1 and R is a bidentate group which links to the C_{5-20} aryl group, such as a bidentate group derived from a C_{1-7} alkyl group. Examples of such groups include, but are not limited to:

2,3-dihydro-tenzo[d]isothiazole-1,1-dioxide-2-yl 1,3-dihydro-benzo[c]isothiazole-2,2-dioxide-1-yl

3,4-dihydro-2H-benzo[e][1,2]thiazine-1,1-dioxide-2-yl

Phosphoramidite: -OP(OR^{P1})-NR$^{P2}{}_2$, where R^{P1} and R^{P2} are phosphoramidite substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidite groups include, but are not limited to, -OP(OCH_2CH_3)-N(CH_3)$_2$, -OP(OCH_2CH_3)-N(i-Pr)$_2$, and -OP(OCH_2CH_2CN)-N(i-Pr)$_2$.

Phosphoramidate: -OP(=O)(OR^{P1})-NR$^{P2}{}_2$, where R^{P1} and R^{P2} are phosphoramidate substituents, for example, -H, a (optionally substituted) C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group, preferably -H, a C_{1-7} alkyl group, or a C_{5-20} aryl group. Examples of phosphoramidate groups include, but are not limited to, -OP(=O)(OCH_2CH_3)-N(CH_3)$_2$, -OP(=O)(OCH_2CH_3)-N(i-Pr)$_2$, and -OP(=O)(OCH_2CH_2CN)-N(i-Pr)$_2$.

In many cases, substituents may themselves be substituted. For example, a C_{1-7} alkoxy group may be substituted with, for example, a C_{1-7} alkyl (also referred to as a C_{1-7} alkyl-C_{1-7}alkoxy group), for example, cyclohexylmethoxy, a C_{3-20} heterocyclyl group (also referred to as a C_{5-20} aryl-C_{1-7} alkoxy group), for example phthalimidoethoxy, or a C_{5-20} aryl group (also referred to as a C_{5-20}aryl-C_{1-7}alkoxy group), for example, benzyloxy.

Preferred substituents for an aryl or alkyl group may include C_{1-10} alkyl groups, C_{5-20} aryl groups, hydroxyl, C_{1-7}alkoxy groups, nitro, amino, substituted amino (-NR^{N1}R^{N2} as defined above) and halides.

Isomers, Salts, Solvates, and Protected Forms

Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diasteriomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r- forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms; α- and β-forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and

halfchair-forms; and combinations thereof, hereinafter collectively referred to as "isomers" (or "isomeric forms").

Note that, except as discussed below for tautomeric forms, specifically excluded from the
5 term "isomers", as used herein, are structural (or constitutional) isomers (i.e. isomers which differ in the connections between atoms rather than merely by the position of atoms in space). For example, a reference to a methoxy group, $-OCH_3$, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, $-CH_2OH$. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural
10 isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C_{1-7} alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

15 The above exclusion does not pertain to tautomeric forms, for example, keto-, enol-, and enolate-forms, as in, for example, the following tautomeric pairs: keto/enol (illustrated below), imine/enamine, amide/imino alcohol, amidine/amidine, nitroso/oxime, thioketone/enethiol, N-nitroso/hyroxyazo, and nitro/aci-nitro.

H O OH H^+ O^-
—C—C ⇌ C=C ⇌ C=C
H^+

keto enol enolate

20 Note that specifically included in the term "isomer" are compounds with one or more isotopic substitutions. For example, H may be in any isotopic form, including ^{1}H, ^{2}H (D), and ^{3}H (T); C may be in any isotopic form, including ^{12}C, ^{13}C, and ^{14}C; O may be in any isotopic form, including ^{16}O and ^{18}O; and the like.

25 Unless otherwise specified, a reference to a particular compound includes all such isomeric forms, including (wholly or partially) racemic and other mixtures thereof. Methods for the preparation (e.g. asymmetric synthesis) and separation (e.g., fractional crystallisation and chromatographic means) of such isomeric forms are either known in the art or are readily obtained by adapting the methods taught herein, or known methods,
30 in a known manner.

Unless otherwise specified, a reference to a particular compound also includes ionic, salt, solvate, and protected forms of thereof, for example, as discussed below.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding salt of the active compound, for example, a pharmaceutically-acceptable salt. Examples of pharmaceutically acceptable salts are discussed in Berge, *et al., J. Pharm. Sci.*, 66, 1-19 (1977).

For example, if the compound is anionic, or has a functional group which may be anionic (e.g., -COOH may be $-COO^-$), then a salt may be formed with a suitable cation. Examples of suitable inorganic cations include, but are not limited to, alkali metal ions such as Na^+ and K^+, alkaline earth cations such as Ca^{2+} and Mg^{2+}, and other cations such as Al^{3+}. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH_4^+) and substituted ammonium ions (e.g., NH_3R^+, $NH_2R_2^+$, NHR_3^+, NR_4^+). Examples of some suitable substituted ammonium ions are those derived from: ethylamine, diethylamine, dicyclohexylamine, triethylamine, butylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine, benzylamine, phenylbenzylamine, choline, meglumine, and tromethamine, as well as amino acids, such as lysine and arginine. An example of a common quaternary ammonium ion is $N(CH_3)_4^+$.

If the compound is cationic, or has a functional group which may be cationic (e.g., $-NH_2$ may be $-NH_3^+$), then a salt may be formed with a suitable anion. Examples of suitable inorganic anions include, but are not limited to, those derived from the following inorganic acids: hydrochloric, hydrobromic, hydroiodic, sulphuric, sulphurous, nitric, nitrous, phosphoric, and phosphorous. Examples of suitable organic anions include, but are not limited to, those derived from the following organic acids: acetic, propionic, succinic, glycolic, stearic, palmitic, lactic, malic, pamoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicyclic, sulfanilic, 2-acetyoxybenzoic, fumaric, phenylsulfonic, toluenesulfonic, methanesulfonic, ethanesulfonic, ethane disulfonic, oxalic, pantothenic, isethionic, valeric, lactobionic, and gluconic. Examples of suitable polymeric anions include, but are not limited to, those derived from the following polymeric acids: tannic acid, carboxymethyl cellulose.

It may be convenient or desirable to prepare, purify, and/or handle a corresponding solvate of the active compound. The term "solvate" is used herein in the conventional sense to refer to a complex of solute (e.g. active compound, salt of active compound) and solvent. If the solvent is water, the solvate may be conveniently referred to as a hydrate, for example, a mono-hydrate, a di-hydrate, a tri-hydrate, etc.

It may be convenient or desirable to prepare, purify, and/or handle the active compound in a chemically protected form. The term "chemically protected form", as used herein, pertains to a compound in which one or more reactive functional groups are protected from undesirable chemical reactions, that is, are in the form of a protected or protecting group (also known as a masked or masking group or a blocked or blocking group). By protecting a reactive functional group, reactions involving other unprotected reactive functional groups can be performed, without affecting the protected group; the protecting group may be removed, usually in a subsequent step, without substantially affecting the remainder of the molecule. See, for example, Protective Groups in Organic Synthesis (T. Green and P. Wuts, Wiley, 1999).

For example, a hydroxy group may be protected as an ether (-OR) or an ester (-OC(=O)R), for example, as: a t-butyl ether; a benzyl, benzhydryl (diphenylmethyl), or trityl (triphenylmethyl) ether; a trimethylsilyl or t-butyldimethylsilyl ether; or an acetyl ester (-OC(=O)CH_3, -OAc).

For example, an aldehyde or ketone group may be protected as an acetal or ketal, respectively, in which the carbonyl group (>C=O) is converted to a diether (>C$(OR)_2$), by reaction with, for example, a primary alcohol. The aldehyde or ketone group is readily regenerated by hydrolysis using a large excess of water in the presence of acid.

For example, an amine group may be protected, for example, as an amide or a urethane, for example, as: a methyl amide (-NHCO-CH_3); a benzyloxy amide (-NHCO-$OCH_2C_6H_5$, -NH-Cbz); as a t-butoxy amide (-NHCO-$OC(CH_3)_3$, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-$OC(CH_3)_2C_6H_4C_6H_5$, -NH-Bpoc), as a 9-fluorenylmethoxy amide (-NH-Fmoc), as a 6-nitroveratryloxy amide (-NH-Nvoc), as a 2-trimethylsilylethyloxy amide (-NH-Teoc), as a 2,2,2-trichloroethyloxy amide (-NH-Troc), as an allyloxy amide (-NH-Alloc), as a 2(-phenylsulphonyl)ethyloxy amide (-NH-Psec); or, in suitable cases, as an N-oxide (>NO•).

For example, a carboxylic acid group may be protected as an ester for example, as: an C_{1-7} alkyl ester (e.g. a methyl ester; a t-butyl ester); a C_{1-7} haloalkyl ester (e.g., a C_{1-7} trihaloalkyl ester); a triC_{1-7} alkylsilyl-C_{1-7} alkyl ester; or a C_{5-20} aryl-C_{1-7} alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether ($-S-CH_2NHC(=O)CH_3$).

Prodrugs

5

It is contemplated that some of the active compounds of the invention act in the form of prodrugs, that means that they are metabolised in the body to the active form. Among these compounds are esters such as glyceryl tributyrate, glyceryl tripropionate, glyceryl tri(4-phenylbutyrate) and methyl 4-phenylbutyrate.

10

Further aspects and embodiments

In the following aspects or embodiments of the invention the compound of the invention is any as defined above e.g. as in formula Ia or formula I, or IIIa.

15

Preferably the compound is a butyric acid/butyrate derivative such as an acid salt, ester or amide such as is defined by any of formula IIa, IIb, IIc, IId, IIe.

Preferably it comprises at least one aryl substituent, which is preferably at R^4, such as is

20 defined by any of formula IVb.

In particular aspects of the invention there are provided methods for treating, preventing or counteracting a microbial infection in a patient in need of the same, by administering to the patient an effective amount of a compound of the invention as described herein.

25

The effective amount is sufficient to demonstrate antimicrobial activity *in vivo* e.g. by stimulating (e.g. derepressing or inhibiting down-regulation of) synthesis of the cathelicidin LL-37. Stimulation may be towards, equal to, or above basal levels (i.e. normal levels in the absence of the infection).

30

By the term "antimicrobial activity" as used herein, is meant the ability to inhibit the growth of or actually kill a population of microbes which can be bacteria, viruses, protozoa or fungal microbes. Thus "antimicrobial activity" should be construed to mean both microbistatic as well as microbicidal activities. Antimicrobial activity should also be

35 construed to include a compound which is capable of inhibiting infections, i.e. disease-causing capacity of microbes.

The compounds of the present invention exhibit an antimicrobial effect by stimulating the innate antimicrobial peptide defense system.

5 Generally the use of the present invention will be such as to lead to secretion of the relevant peptide same onto an epithelial surface (e.g. in the gastrointestinal tract). This in turn will lead to increased antimicrobial activity at the surface (and hence improvement of its barrier function) and treatment of the microbial infection and disease caused by it.

10 The microbial targets and diseases targeted by the present invention may be any believed to benefit therefrom, but a preferred target is infectious colitis e.g. as caused by Clostridium difficile colitis.

The compounds of the invention are particularly useful against infections of bacterial 15 strains that are tolerant against conventional antibiotics. Nevertheless use of the compounds described herein in conjunction with conventional antibiotics may be preferred and forms one part of the present invention.

Other combination treatments of the present invention include the use of compounds 20 described herein with other other compounds believed to have antimicrobial effect. These include: aminosterol type compounds, for example which include spermidine, spermine or other polyamines (see WO2000-09137); isoleucine or active isomers or analogs thereof (see US2002-0076393 or US2003-0109582 or US7311925); and vitamin D type compounds (see US20080038374 or WO/2008/073174). The disclosure of all 25 these references, in respect of these compounds, their definition, and their provision, is hereby specifically incorporated herein by cross-reference.

Preferred dosages and dosage forms are described in more detail below. A preferred daily dosage may be between 250 µg to about 25 g, preferably up to around 5g, more 30 preferably less than 3 g per day, which may be split into doses given e.g. 1, 2 or 3 times daily.

Said compound is preferably administered in an oral dosage form such as but not limited to a tablet, a capsule, a solution, a suspension, a powder, a paste, an elixir, and a syrup. 35 Other administration forms are also useful, these include but not are limited to topical administration forms, which are in particular useful against infections of the skin, these

include for example creams, oils, lotions, and ointments. Yet further dosage forms include dosage forms for delivery to the respiratory system including the lungs, such as aerosols and nasal spray devices.

5 Aspects of the invention include a method for treating, preventing or counteracting microbial infections, including bacterial, viral, fungal and parasitic infections (also including infections by bacterial strains resistant to currently used antibiotics), by administering a medicament comprising a secretagogue-effective amount of at least one compound of the invention as defined above.

10

In yet a further aspect, the invention provides a pharmaceutical composition for use in the methods described herein e.g. for treating, preventing or counteracting a microbial infection, including the above mentioned types, comprising an active ingredient being at least one compound of the invention, and typically at least one pharmaceutically
15 acceptable excipient.

In yet a further aspect, the invention provides use of compounds of the invention in the preparation of a medicament for use in the methods described herein.

20 Some of these aspects and embodiments will now be discussed in more detail:

Secretion of host defense peptides

The gastrointestinal tract (GI tract) of mammals is covered by a continuous sheet of
25 epithelial cells that is folded into villus projections and crypts. Within the base of the crypts, where the stem cells of the GI tract can be found, there are specialized, granular cells called Paneth cells. Both enterocytes and Paneth cells produce antimicrobial peptides. The enterocytes synthesize and secrete antimicrobial peptides into the gut lumen both constitutively and upon induction. The Paneth cells at the base of the
30 intestinal crypts, secrete alpha-defensins into the cryptal well, resulting in concentrations estimated at mg/mL levels, which eventually flush into the gut lumen.

Both systems contribute to bowel health. In children and adults suffering from diarrhea caused by Shigella, synthesis of the cathelicidin LL-37 and the colonic enterocyte beta-
35 defensin HBD-1 is markedly depressed; expression recovers in time during resolution of the illness. Similarly, mice which lack the proteolytic enzyme required for processing

cryptdins (the murine Paneth cell alpha-defensins) lack functional cryptdins and exhibit increased susceptibility to orally administered Salmonella.

Other epithelial surfaces of the mammalian body also have such host defense secretion systems, including but not limited to the cornea, the lung, the kidney and the skin.

The use of the compositions and methods of the present invention result in the stimulation of epithelial cells and Paneth cells of the gastrointestinal tract and other epithelial surfaces of man and in other animals to secrete large quantities of naturally occurring broad-spectrum antimicrobial agents, including antimicrobial peptides such as defensins, cryptdins, LL-37, HBD1, and HBD2, and antimicrobial proteins such as lysozyme, transferrin, lactoferrin, phospholipases, and SLPI (secretory leukocyte protease inhibitor). The substances stored by the Paneth cells exhibit activity against a wide range of infectious agents including bacteria, protozoa, viruses, and fungi.

The epithelial cells targeted by the present invention may be any of these. Preferably however the invention is utilise for the treatment of microbial infections of the GI tract.

Microbial infections and diseases

As mentioned, an important aspect of the invention provides methods for treating, preventing or counteracting microbial infections by administering a medicament comprising a secretagogue-effective amount of at least one compound of the invention.

In useful embodiments, infections and other conditions that benefit from treatment according to the invention are in particular those relating to organs having epithelial surfaces with host defense peptide secretion systems such as the above mentioned.

Such infections, conditions and diseases include but are not limited to traveller's diarrhoea, endemic diarrhoea, dysentery, viral gastroenteritis, parasitic enteritis, Crohn's disease, ulcerative colitis, irritable bowel syndrome, precancerous states of the gastrointestinal tract, cancer of the gastrointestinal tract, diverticulitis, post-antibiotic diarrhoea, Clostridium difficile colitis, lactose intolerance, flatulence, gastritis, esophagitis, heartburn, gastric ulcer, ulcers associated with Helicobacter pylori, duodenal ulcer, short bowel syndrome, dumping syndrome, gluten enteropathy, or food intolerance.

Also included in the methods of the inventions are infections of the skin, including but not limited to boils, carbuncles, furuncles, cellulitis, abscesses, impetigo, and erysipelas; infections of the eye including but not limited to conjunctivitis, stye, blepharitis, cellulitis, keratitis, corneal ulcer, trachoma, uveitis, canaliculitis and dacryocystitis, infections to the respiratory system and infections in the kidneys. Also included are infections caused by bacterial strains resistant to classical antibiotic treatment, including infections by multidrug resistant strains.

A preferred target for the present invention is infectious colitis. As is well known in the art, microbial species causing this include Yersenia enterocolitica, Salmonella, Shigella, Campylobacter, Clostridium and E. Coli. Some bacteria, such as Clostridium difficile, may elaborate a toxic substance that leads to the development of pseudomembranous colitis.

The compounds of the invention are particularly useful against infections of bacterial strains that are tolerant against conventional antibiotics, and it follows from the secretagogue action of the compounds in the context herein, that it is not foreseen that bacterial strains can develop resistance against treatment in accordance with the invention.

As illustrated in the accompanying Examples, selected representative compounds have been tested and found to exhibit the desired activity.

Combination treatments

As noted above, use of the use of the compounds described herein in conjunction with conventional antibiotics may be preferred and forms one part of the present invention. Example antibiotics include Penicillins, Penicillin G, Phenoxymethyl– penicillin, Flucloxacillin, Amoxycillin, Metronidazole, Cefuroxime, Augmentin, Pivmecillinam, Acetomycin, Ciprofloxacin and Erythromycin. Where these specific antibiotics are named, it will be appreciated that commonly available analogs may be used.

As demonstrated in the accompanying Examples (see Examples 4-6) it has been found that a combinatorial effect is achieved when compounds of the invention are administered together with vitamin D. Accordingly, the invention also encompasses the above methods, further comprising the co-administration of vitamin D, with one or more

compounds of the invention. Other compounds which may be co-administered include aminosterol type compounds; isoleucine or active isomers or analogs thereof; vitamin D type compounds.

5 Also provided are pharmaceutical compositions comprising, in addition to one or more of the compounds of the invention, vitamin D or one of the other aforementioned compounds as a further ingredient. Such compositions can be formulated in any of the above mentioned formulations and dosage forms.

10 Oral dosage forms are preferred, as described below.

Preferred dosages

In the methods and compositions of the present invention, the active compound is
15 administered/present in an amount which is effective to stimulate and/or activate this system. Such amount is also referred to herein as a "secretagogue-effective" amount, where the term secretagogue refers to a substance which increases the levels of active antimicrobial peptides in epithelial surfaces.

20 As noted hereinbefore, PBA has previously been marketed for treatment of hyperammonaemia related to hereditary urea cycle disorders. According to the SPC of Buphenyl (tablet or powder) the drug is dosed at 9.9 to 13.0 g/m^2/day divided into three portions. This amounts to 16 – 23 g daily, or ca. 5.5 to 8.0 g three times daily.

25 In different studies, topical dosages for PBA used in various studies ranged from 528 mg/day to 1.12 g/day, which corresponds to 35-60% of the normal daily intracolonic production of butyrate. None of these studies reported any adverse effect or reactions. According to one study, daily oral dose of 4g of sodium butyrate given as colonic-targeted tablets for 6-weeks in IBD patients and was also found safe and well tolerated without any
30 adverse effects.

Rabbit studies performed at ICDDRB in Dhaka (see below) showed that dosing about 7.5-22.5 mg/kg was sufficient for therapeutic effect in shigellosis. Scaling this dose to a 70 kg human suggests that a maximally 720 mg daily dose would be effective for the
35 treatment of, for example, shigellosis.

Based on these examples it will be appreciated that a practical upper limit for treatment would be of the order of 20 g/daily (based on urea cycle treatment) and the lower limit may be expected to be lower than 700 mg, e.g. equal to or around 600, 500, 400, 300, 200, 100 mg daily. Potentially even lower amounts may be utilised e.g. 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mg.

It will nevertheless be understood that the suitable amount of the compound to be administered can vary depending on the selected specific compound(s), the specific location of the infection and condition(s) to be treated and/or prevented. In some embodiments, the amount to be administered can be in the range of about 10 μg to about 25 g. A suitable dosage form can be selected and formulated accordingly. For example, for treatment of diseases and conditions in the gastro-intestinal system a dose in the range of 250 μg to about 25 g may be suitable, including the range of about 1 g to about 25 g, e.g. in the range of about 1 g to 10 g, such as about 1 g, 2 g, 5 g or 10 g.

All dosages may be split or given e.g. 1, 2 or 3 times daily.

Administration and formulation

Preferably, the medicament is administered orally but other administration routes are within the scope of the invention and may be more suitable for certain conditions. Such other administration routes include topical, buccal nasal, parenteral, including rectal and vaginal administration.

Inhaled dosage forms include aerosol, inhaler & metered dose inhaler. Ophthalmic dosage forms include eye drops (solution or suspension), ophthalmic gels, and ophthalmic ointments. Otic dosage forms include ear drops (solution or suspension). Rectal dosage forms include enema and suppository. Vaginal dosage forms include douches and pessaries (vaginal suppositories) and vaginal tablets.

Examples of suitable formulations for topical use include creams, ointments, gels, or aqueous or oily solutions or suspensions. Parenteral administration can be accomplished for example by formulating the compound as a sterile aqueous or oily solution for intravenous, subcutaneous, or intramuscular dosing or as a suppository for rectal dosing.

Compositions for oral use may be in the form of hard gelatin capsules in which the active

ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

5 The compositions can be formulated in various suitable forms, depending on which conditions they are primarily aimed at. In certain embodiments, the compositions are for oral administration. Such compositions include but are not limited to tablets, capsules, a solution, a suspension, a powder, a paste, an elixir, or a syrup.

10 Compositions may be delayed-release or colonic-targeted compositions such as are well known in the art.

Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium
15 carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within
20 the gastrointestinal tract, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions

25 Another aspect of the invention relates to a pharmaceutical composition for treating, preventing or counteracting any of the above mentioned conditions or diseases. The compositions comprise at least one of the compounds described herein together with at least one pharmaceutically acceptable excipient.

30 The oral composition of the invention may be formulated for delayed and/or extended release and may be enteric coated by means well known to the skilled person, to be released in the lower intestinal tracts.

Functional foods

35

It will also be appreciated, in particular when it is desired to administer a large amount of active compound, such as, in the range of 1-25 g that the compounds of the invention can be (isolated and then) formulated and comprised in functional food or feed products. Such functional food products include but are not limited to fermented food products including fermented bean products, e.g. soy bean products such as tempeh, products from fermented oat, germinated barley, and similar products. Such products, generally produced by microbial fermentation which breaks down betaglucans, will have a natural content of short chain fatty acids that can boost the effect of the compounds of the present invention. The form of functional food product in accordance with the invention can be any form suitable for the chosen food type, including crackers, pastry, spread or paste, a purée, a jelly, a yoghurt, a drink concentrate, or any other suitable food product in which the selected active compound(s) can be readily formulated in.

Other species

The methods and compositions of the present invention have application in the treatment of both humans as well as other animals, including veterinary and animal husbandry applications for companion animals, farm animals, and ranch animals. These applications include but are not limited to treating, preventing or counteracting diseases and conditions in dogs, cats, cows, horses, deer and poultry including hen, turkey ducks, geese; as well as in household pets such as birds and rodents. For large animals, a suitable dose can be larger than the above mentioned amounts.

Any sub-titles herein are included for convenience only, and are not to be construed as limiting the disclosure in any way.

The invention will now be further described with reference to the following non-limiting Figures and Examples. Other embodiments of the invention will occur to those skilled in the art in the light of these.

The disclosure of all references cited herein, inasmuch as it may be used by those skilled in the art to carry out the invention, is hereby specifically incorporated herein by cross-reference.

Figures

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
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Figure 1: Fold-induction of *CAMP* mRNA (encoding LL-37) levels in lung epithelial cells (VA10), upon treatment with different agents of the invention. Column c represents a control (untreated cells), Column 3 represents a positive control of vitamin D3 (1,25-dihydroxyvitamin D_3 or 1,25(OH)2D_3) treated cells, column 1 is sodium butyrate and column 2 is sodium 4-phenylbutyrate treated cells. Cells were harvested 24 hours after sodium 4-phenylbutyrate and vitamin D stimulation, and mRNA was isolated. Real time reverse transcription PCR results show how expression of the human cathelicidin gene is affected by sodium 4-phenylbutyrate and vitamin D treatment.

Figure 2: Induction of *CAMP* mRNA expression by butyrate (BA) and PBA derivates. *A)* Structures of utilized chemicals butyrate (BA) 4 mM, 4-phenyl butyrate (PBA) 4 mM, α-methyl hydrocinnamate (ST7) 4 mM, and 2,2-dimethyl-butyrate (ST20) 4 mM. *B)* Induction of *CAMP* mRNA expression by indicated chemicals for 24 hours.

Figure 3: Induction of *CAMP* gene mRNA expression by PBA. *A)* VA10 cells were stimulated with the indicated concentrations of PBA or solvent (Control) for 24 hours. *B)* VA10 cells were stimulated with 4 mM PBA or treated with solvent alone and harvested after the indicated period of time. C) A498, HT-29 and U937 cells were stimulated with 4 mM PBA or solvent only and harvested after the indicated period of time. *CAMP* mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. The normalized data is plotted as mean + SE from at least three independent experiments.

Figure 4: Combinatorial effects of vitamin D and sodium 4-phenylbutyrate stimulation on *CAMP* mRNA expression in lung epithelial VA10 cells, determined as described above for Figure 1. The columns are as follows: C = control; 1 = sodium 4-phenylbutyrate alone; 2 = vitamin D alone; 3 = treatment of sodium 4-phenylbutyrate together with vitamin D.

Figure 5: Further demonstrations of synergetic induction of *CAMP* mRNA and pro-LL-37 expression by PBA (4 mM) and 1,25$(OH)_2D_3$. (20 nM) *A)* VA10 cells were stimulated with PBA (4 mM), 1,25$(OH)_2D_3$ (20 nM) or solvent (Control) for 24 hours. *CAMP* mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Normalized data is plotted as mean + SE from three independent experiments. The differences observed are significant ($P < 0.05$). *B)* VA10

cells were stimulated with PBA (4 mM), $1,25(OH)_2D_3$ (20 nM) or solvent (Control) for 24 hours. Total cell lysates and supernatants analyzed by Western blot for LL-37. One representative blot out of three is shown.

5 Figure 6A: Induction of the gene encoding LL-37 with sodium 4-phenylbutyrate and vitamin D is affected by the inhibitor U0126 which inhibits the MEK/ERK kinase pathway. C = control; 1 = sodium 4-phenylbutyrate alone; 2 = vitamin D alone. The open columns represent treatment with the inhibitor U0126. The black columns show treatment without the inhibitor. This indicates that the signaling pathways are affected differently by vitamin
10 D and phenylbutyrates.

Figure 6B. Further demonstration of inhibition of PBA induced CAMP gene expression by MAP kinase inhibitors as shown in the Figure, VA10 cells were treated with 4 mM PBA in the presence or absence of 20 µM of the indicated inhibitors. *CAMP* mRNA levels were
15 determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Normalized data is plotted as mean + SE from three independent experiments. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

20 Figure 7: Immunohistochemistry showing that CAP-18 (the rabbit homologue to LL-37) is expressed in surface epithelial cells of healthy rabbits, that Shigella infection results in downregulation of the peptide and that this downregulation can be counteracted by oral intake of tributyrylglycerol.

25 Figure 8: Inhibition of PBA induced CAMP gene expression by cycloheximide shows that translation is necessary. VA10 cells were treated with 4 mM PBA or butyrate (BA) in the presence or absence of 20 µg/ml cycloheximide. CAMP mRNA levels were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression incontrol samples (solvent) where controls were given the
30 arbitrary value of one. Normalized data is plotted as mean plus standard error of the mean from at least three independent experiments. *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

Figure 9: VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24
35 hours. Acetylation of histone H3 and H4 was analyzed by quantitative ChIP using antibodies against the respective acetylated histones. Results were normalized to normal

rabbit IgG and total input and plotted as fold precipitation over IgG. Normalized data is plotted as mean + SE from independent experiments (n=3). No significant differences were observed in acetylation of histones.

5 Figure 10: PBA induced expression does not involve the co-activators of VDR. VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24 hours. mRNA levels of the respective VDR co-activators were determined by real time RT-PCR. Individual samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Data is normalized 10 to control and plotted as mean + SE from three independent experiments.

Figure 11: Induction of hBD-1 mRNA expression by PBA. VA10 cells were stimulated with 4 mM of PBA or solvent alone (Control) for 24 hours. hBD-1 mRNA levels were determined by real time RT-PCR, *CAMP* induction shown for comparison. Individual 15 samples were normalized to total RNA input. Results were normalized to expression in control samples where controls were given the arbitrary value of one. Data is normalized to control and plotted as mean + SE from at least three independent experiments.

Figure 12: Schematic illustration of proposed mechanism for action of PBA treatment in 20 Shigella infected epithelia.

Examples

Example 1

25 LL-37 expression in lung epithelial cells treated with different agents

Lung epithelial cells (VA 10) were grown to confluency under standard conditions and the agents to be tested added at the indicated concentrations (see below). mRNA was isolated 24 hours after treatment and measured by real time reverse transcription PCR.

30 Results are shown in Figure 1, where column C represents control (untreated cells), column 3 represents a positive control of vitamin D3 (1,25-dihydroxyvitamin D_3 or 1,25(OH)2D3) (100 nM) treated cells, column 1 is sodium butyrate (2 mM) and column 2 is sodium 4-phenylbutyrate (2 mM) treated cells.

35 The results show that sodium 4-phenylbutyrate is a more effective inducer of LL-37 mRNA expression than butyrate or vitamin D in VA10 cells, but does not have does not

have the foul smell associated with butyrate. Prior to our studies there were no compounds known to induce LL-37 to the same degree as butyrate let alone without the smell and taste problem. It is particularly surprising that the the deviation from the structure of butyrate can be as substantial as adding an aromatic ring (i.e. doubling the
5 molecular weight). In the light of the present disclosure it may therefore be concluded that that butyrate derivatives, such as aromatic derivatives, will also be active.

In a further experiment, the ability of two other PBA analogs to induce CAMP gene expression was tested (see Figure 2). VA10 cells were stimulated with 4 mM of α-
10 methylhydrocinnamate (ST7), a PBA analog or 2,2-dimethylbutyrate (ST20), a butyrate analog. After 24 hours of incubation, total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. ST7 significantly increased CAMP mRNA expression, while ST20 stimulation had no apparent effect on CAMP mRNA expression levels. Thus it can be seen that quaternary carbon atoms, at least
15 proximal to the carboxyl group, would appear to be undesirable. Conversely, in aryl-butyrate derivatives, it appears that analogs including different chain or branched chains, remain active.

Real time PCR

20

Six-well plates were seeded with 1.0 x 10^6 cells per well and grown for two days. Medium was then exchanged and different wells were left untreated, supplemented with 2 mM sodium butyrate or 2 mM sodium 4-phenylbutyrate. The cells were incubated for 48 h and total RNA was prepared using the RNEasy kit (Qiagen). Total RNA concentrations were
25 measured using the Quant-iT RiboGreen RNA assay kit (Invitrogen). Superscript III first-strand synthesis system (Invitrogen) was used to synthesize cDNA using random primers according to the protocol of the manufacturer. The expression of the *CAMP* gene, encoding LL-37 was analyzed on the 7500 Real Time PCR System (Applied Biosystems) using the fluorescent probe (5′-6-FAM -TGTTATCCTTATCACAACTGAT-3′ with MGB
30 quencher) and forward and reverse primers specific for the CAMP cDNA (5'-ACCCAGCAGGGCAAATCTC-3' and 5'-GAAGGACGGGCTGGTGAAG-3', respectively). Results were normalized to total RNA quantity, presented as relative fold induction of untreated control cells.

35 **Example 2**

LL-37 expression in lung epithelial cells treated with different dose of sodium 4-phenylbutyrate

Figure 3 shows the dose-response of *CAMP* mRNA expression in VA10 lung epithelial
5 cells upon treatment with increasing concentrations of sodium 4-phenylbutyrate. To determine time and dose dependence of PBA induced expression of CAMP mRNA, VA10 cells were stimulated with 4mM PBA over different time points and with different concentrations for 24 hours. Total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. Increase of CAMP mRNA expression
10 was dependent on PBA dose and increased over time.

In earlier experiments it appeard that at higher concentrations, which were non-physiologically relevant (8 mM) the response ceased to be dose-dependent (results not shown).

15

In earlier experiments in which controls were not measured at the relevant time points, artefacts were seen after long incubations (48 hours; results not shown). Therefore in the experiment shown, controls were measured at the relevant time point and normalised to 1.

20

The example indicates that successful treatment can be envisaged with a once-daily dosage regimen.

Example 3

25 Induction of CAMP gene expression by PBA in other cell lines

In order to investigate the effect of PBA on other cell lines, HT-29 (Human colonic adenocarcinoma cell line), A497 (Human renal carcinoma cell line) and U937 (Human leukemic monocyte lymphoma cell line) were stimulated with 4 mM PBA for 8, 24 and 48
30 hours. Total RNA was isolated from the cells and CAMP mRNA expression levels analyzed by real time RT-PCR. CAMP mRNA expression was signifcantly increased in all cell lines tested (Figure 3C).

Example 4

35 Synergistic effects of sodium 4-phenylbutyrate and vitamin D on LL-37 expression in lung epithelial cells

A further test shows that sodium 4-phenylbutyrate and vitamin D have combinatorial effects on *CAMP* mRNA expression. VA10 lung epithelial cells were grown as before and treated with sodium 4-phenylbutyrate alone at 2 mM vitamin D alone at 100 nM, and both together, at 2 mM and 100 nM respectively. Treatment with butyrate (at 2 mM) was included as control. Cells were harvested at different timepoints and mRNA was isolated and analysed with real-time reverse transcription PCR.Treatment with both sodium 4-phenylbutyrate and vitamin D clearly show combinatorial effects on mRNA expression level as the effects of the combination are 6-fold higher than of either chemical alone.

In Figure 4, column c shows *CAMP* mRNA levels in the control (untreated cells), column 1 represents treatment with sodium 4-phenylbutyrate alone, column 2 shows treatment with vitamin D alone, and column 4 shows the treatment of sodium 4-phenylbutyrate together with vitamin D.

This is further shown in Figure 6A and 6B. VA10 cells were incubated with a low dose of 20 nM of $1,25(OH)_2D_3$ and 4 mM PBA together and with the respective compounds alone. Expression of CAMP mRNA was found to be higher than the added fold induction of PBA and $1,25(OH)_2D_3$, indicating a synergistic effect (Figure 5).

Example 5

Stimulation by sodium 4-phenylbutyrate and vitamin D acts through different signaling pathways

Epithelial lung cells were treated with sodium 4-phenylbutyrate or vitamin D. For each agent two samples were treated, with and without MAP kinase inhibitor U0126 (concentration of 20 μM) which is specific for inhibiting MEK1 and MEK2 protein kinases.

Results are shown in Figure 6A, where column C represents control (untreated cells), column 1 shows treatment with sodium 4-phenylbutyrate at 2 mM, and column 2 shows treatment with vitamin D (100 nM) for 24 h. The open columns represent treatment with the MAP kinase inhibitor U0126, whereas the black columns show treatment without the inhibitor.

The results shown indicate that different signaling pathways are involved in the induction by sodium 4-phenylbutyrate and vitamin D; this may explain the combined effects of the chemicals on the induction of the *CAMP* gene.

5 The effect of inhibitors for c-Jun N-terminal kinase (JNK), p38 kinase and extracellular signal-regulated kinase 1/2 (ERK1/2) on PBA induced CAMP gene expression were also investigated as shown in Figure 6B. One hour prior to stimulation with 4 mM PBA, VA10 cells were pre incubated with 20 μM SP600125, SB203580 or U0126 to inhibit the respective kinases. After 24 hours of incubation, total RNA was isolated and analyzed by
10 real time RT-PCR for CAMP mRNA. Inhibitors for the ERK1/2 and JNK pathways significantly reduced PBA induced CAMP gene expression.

Example 6

Shigella infected rabbits treated with glyceryl tributyrate

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It has been confirmed by immunohistochemistry that CAP-18 (the rabbit homologue to LL-37) is expressed in surface epithelial cells of healthy rabbits (Figure 7A) and that Shigella infection results in downregulation of peptide production (Figure 7B). Furthermore, upon treatment with tributyrylglycerol, the downregulation of gene
20 expression by Shigella is reverted and/or prevented (Figure 7C).

Animal model: Inbred New Zealand White rabbits of either gender weighing 1.8 to 2 kg were used for the study. The animals were individually caged in a room maintained at 22-25°C. Before inclusion in the study, health status of the rabbits was determined by
25 physical examination, culture of stool and rectal swab specimens and fecal parasitic examination. Healthy coccidia-free rabbits that were also free of enteric pathogens (e.g. *Salmonella, Shigella, Vibrio cholera*) were studied. Rabbits were infected with *Shigella* and divided into two groups, one group was treated orally with glyceryl tributyrate and the other with saline. Expression of the CAP-18 peptide and its proform in colonic and rectal
30 tissue specimens were analyzed in healthy rabbits, in untreated infected rabbits, in infected and healthy rabbits treated with glycerol tributyrate. For analyses of toxicity effects of glycerol tributyrate healthy rabbits were also treated with this compound.

Bacterial strain and inoculum preparation: The *Shigella flexneri* 2a strain was isolated
35 from stool of a patient. The strain was positive for the Serény test and Congo red binding, reflecting invasive properties (Berkhoff, H.A. and Vinal, A.C., 1986, Avian Dis. 30, 117-

121)) From this stock, bacteria were subcultured on trypticase soya agar (TSA; Becton Dickinson, Sparks, MD) plates and cultured overnight at 37°C. Three to five smooth colonies were inoculated in trypticase soya broth and cultured for 4 h with shaking at 37°C. The broth was then washed in normal saline at 7000 rpm for 10 min and bacterial pellet was suspended in normal saline to a concentration of 1 x 10^9 cfu in 7 mL that were given to the rabbits.

A non-surgical rabbit model of shigellosis was used in this study as described previously with slight modifications (Etheridge, M.E. et al., 1996, Lab. Anim. Sci. 46, 61-66). Briefly, rabbits were fasted for 36 hours and given a single oral dose of a tetracyclin hydrochloride (250 mg/kg; Novartis, Dhaka, Bangladesh) suspension. After that, rabbits were anesthetized with sodium pentobarbitol (33 mg/kg; Sigma, Chemical Co, St Louis, MO) and given 37.5 mg/kg weight of G-cimetidine (Gonoshasthoya Pharmaceuticals, Dhaka, Bangladesh) intravenously via the marginal car vein to inhibit gastric secretion. Fifteen minutes later, 7 ml of 5% sodium bicarbonate solution was administered orally with a sterile plastic feeding tube (3.33 x 465 mm, Tycohealthcare Ireland Ltd., Tullamore, Ireland), which was followed 15 minutes later by a second 15-ml dose of 5% sodium bicarbonate solution and a 7-ml dose of the bacterial suspension (10^9 cfu in 7 ml normal saline (0.9% w/v, pH 7.2)) immediately thereafter. Twenty minutes after inoculation of the bacterial suspension, 7 ml of Loperamide HCl (0.02 mg/kg body weight) in normal saline was introduced orally to reduce intestinal motility. Thereafter, rabbits were allowed to eat and drink regular food. Usually rabbits developed dysentery within 24 hours of bacterial inoculation. Time of bacterial inoculums was considered as 0 hr. After development of dysenteric symptoms, rabbits were given glyceryl tributyrate (47 µmol/kg body weight, i.e., 140 µmol butyrate equiv./kg) by an orogastric feeding tube twice daily at twelve hours interval for 3 days. Four days after bacterial inoculation, rabbits were given an overdose of intravenous sodium pentobarbitol (66 mg/kg; Sigma) for euthanasia.

To evaluate the presence of the CAP-18 peptide immunohistochemical staining was performed by using the chicken polyclonal antibody specific to CAP-18 (Innovagen). Briefly, paraffin sections were deparaffinized, hydrated and given microwave treatment in retrieval buffer (Dako laboratories A/S, Glostrup, Denmark) for 12 minutes followed by washing in phosphate buffer (pH 7.2). After cooling, endogenous peroxidase activity was quenched and sections were incubated overnight with the CAP-18-specific antibody (2 µg/ml) at room tempture. After washing, sections were incubated with horse-radish-peroxidase conjugated donkey anti-chicken antibody (1:200; Jackson ImmunoResearch

Laboratories, Inc.) for 1 hr at room temperature. This was followed by washing and development of the color was with diaminobenzidine (DAB, brown). As a control, specific antibodies were replaced by irrelevant isotype-matched-antibodies. In addition, synthetic CAP-18 was incubated at 10-fold higher concentration with the CAP-18 antibody overnight at 4°C and the mixture was used as above for immunostaining. This served as control for the specific staining. After counter-staining in hematoxylin and eosin, slides were mounted in paramount (BDH Chemicals, Poole, England).

Clinical recovery of the rabbits from shigellosis was established by disappearance of blood from stool, reappearance of formed stool, normalization of weight, body temperature, return of normal appetite and playful activity.

Example 7

Inhibition of PBA induced CAMP gene expression by cycloheximide

In order to assess whether the PBA and butyrate induction pathways of CAMP gene expression are direct, VA10 cells were treated with PBA or butyrate in the presence and absence of cycloheximide (CHX). After 24 hours of incubation, total RNA was isolated and CAMP mRNA levels measured using real time RT-PCR. Pre-incubating the cells with 20 µg/ml of CHX for one hour prior stimulation effectively blocked both PBA and butyrate induced CAMP gene expression

This suggests that that PBA induced CAMP gene expression is induced through a secondary effect. This secondary induction pathway may depend on MAP kinase signaling through JNK and ERK1/2 as it was shown in VA10, a bronchial epithelial cell line (see Figures 6A and 6B).

Example 8

The effect of PBA on histone acetylation at the CAMP gene promoter

The effect of PBA on acetylation of histone H3 and H4 by quantitative chromatin immunoprecipitation was assessed. No significant change in histone acetylation could be observed at the CAMP gene proximal promoter (1000 bp upstream of transcription start site) after treatment with 4 mM PBA for 24 hours (Figure 9)

Earlier it has been assumed that induction of CAMP gene expression by histone deacetylase inhibitors occurs through an increase of histone acetylation and relaxation of chromatin structure, facilitating the binding of other transcription factors. The present data speaks against this hypothesis. Assessing acetylation of H3 and H4 at the CAMP
5 proximal promoter using quantitative chromatin immunoprecipitation, a significant change in acetylation was detectable after treatment with PBA. Furthermore, it was previously shown (see Example 7) that inhibiting protein synthesis using cycloheximide blocks both butyrate and PBA induced expression of CAMP gene expression. These results rule out that an increase of histone acetylation at the CAMP proximal promoter by these
10 compounds directly facilitates CAMP gene expression. Without wishing to be bound by theory, it is believed that an increase of histone acetylation facilitates the expression of other genes, which then increase CAMP gene expression as a secondary effect.

15 **Example 9**

The effect of PBA on vitamin D co-activator expression

Hypothesizing that the synergistic effect between PBA and $1,25(OH)_2D_3$ was due to an induction of VDR co-activator genes by PBA, we analyzed the effect of PBA
20 on mRNA levels of several known VDR co-activator genes in VA10. None of the genes were significantly upregulated after treatment with 4 mM PBA for 24 hours (see Figure 10). These co-activators are therefore not involved in the PBA-induced effects on gene expression.

25 **Example 10**

Induction of hBD-1 mRNA expression by PBA

CAMP is not the only antimicrobial defense gene that is induced by PBA. Another well-known peptide is also induced, although at lower level than CAMP (See Figure 11). This
30 suggests that PBA has a general effect on mucosal defenses.

Example 11

Synthesis of glyceryl tributyrate

35 Butanoic anhydride (164 ml, 1.0 mol) was added during 10 min to glycerol (7.34 ml, 100 mmol) in Pyridine (300 ml) at 0°C. The mixture was stirred at 0°C for 10 min and at room

temperature for 18 h. Water (200 ml) was added and the mixture was heated at 60°C for 15 min. Evaporation of solvent gave a residue that was partitioned between dichloromethane (DCM, 400 ml) and $NaHCO_3$ (20 % in water, 400 ml). The aqueous layer was further extracted with DCM (50 ml). The combined organic extracts were washed first with saturated aqueous $NaHCO_3$ (400 ml) and then with HCl (1M in water, 400 ml). The organic layer was collected and dried with Na_2SO_4 and then concentrated *in vacuo* to afford 29.6 g (98 %) of glyceryl tributyrate ^{1}H NMR ($CDCl_3$), 0.95 (t; *J*=7.4 Hz; 2 X CH_3), 0.96 (t; *J*=7.4 Hz; CH_3), 1.60-1.73 (m; 3 X CH_2), 2.31 (t; *J*=7.4 Hz; 2 X CH_2), 2.32 (t; *J*=7.35 Hz; CH_2), 4.16 (dd + AB; *J*=11.9, 6.0 Hz; 2 X CH_a), 4.31 (dd + AB; *J*=11.9, 4.3 Hz; 2 X CH_b), 5.29 (m; 5.26-5.31; *CH*).

Example 12

Synthesis of N-Butanoylglycine ethyl ester

Glycine ethyl ester hydrochloride (13.96 g, 100 mmol) and triethylamine (34.65 ml, 250 mmol) in dichloromethane (DCM, 500 ml) was stirred for 2 h at room temperature, which resulted in a fine white precipitate. Butanoic anhydride (19.63 ml, 120 mmol) in DCM (100 ml) was added over 5 min and the reaction mixture turned to a clear solution. After 30 min at room temperature, and subsequent removal of solvent (*in vacuo*), water was added (18 ml, 1 mol) followed by pyridine (23.73 g, 24.26 ml, 300 mmol). The solution was heated at 60°C for 30 min. The mixture was partitioned between DCM (200 ml) and aqueous HCl (2.4 M, 200 ml, saturated with NaCl). The aqueous layer was separated and extracted with DCM (50 ml). The combined organic extract was washed with HCl (*aq.*, 1 M, 250 ml) and the water layer was extracted with an additional portion of DCM (50 ml). The combined organic extracts was washed with $NaHCO_3$ (*aq.*, 4.2 %, 200 ml) and the water layer extracted once more with DCM (50 ml). The combined organic extracts was dried with Na_2SO_4 and concentrated *in vacuo* yielding 16.3 g (94 %) of N-butanoylglycine ethyl ester. ^{1}H NMR ($CDCl_3$), 0.97 (t; *J*=7.4 Hz; CH_3), 1.30 (t; *J*=7.1 Hz; CH_3), 1.65-1.74 (m; CH_2), 2.23 (t; *J*=7.5 Hz; CH_2), 4.05 (d; 4.9 Hz; CH_2), 4.23 (q; 7.2 Hz; CH_3), 5.9 (broad; *NH*).

Example 13

Synthesis of N-Butanoylglycine

N-Butanoylglycine ethyl ester (16.3 g, 94.16 mmol) was dissolved in aqueous NaOH (1 M, 282 ml, 282 mmol) and then stirred for 15 h at room temperature. Aqueous HCl (12 M,

15.7 ml, 188 mmol) was added to pH=5. The water was then evaporated (*in vacuo*) and the residue was dissolved in aqueous HCl (1 M, 175 ml) which gave a pH of 1. The solution was saturated with NaCl and extracted with tetrahydrofuran (3 X 100 ml). The combined organic extracts was dried with Na_2SO_4 and evaporated *in vacuo* yielding 13 g
5 (95%) of N-butanoylglycine. ^{1}H NMR ($CDCl_3$), 0.97 (t; *J*=7.4 Hz; *CH_3*), 1.64-1.74 (m; *CH_2*), 2.27 (t; *J*=7.5 Hz; *CH_2*), 4.09 (d; *J*=5.1 Hz; *CH_2*), 6.24 (broad; *NH*), 8.1 (broad; *COOH*).

Example 14

Synthesis of N^{α},N^{ε}-dibutanoyllysine

10 Lysine (1g, 6.1 mmol) was dissolved in 160 ml tetrahydrofuran(THF)-water (1:1), whereupon butanoic anhydride (2.89g 18.3 mmol) was added. The solution was kept stirring at room temperature and after 1h 80 ml of THF was added and after standing overnight sodium carbonate decahydrate was added (5.23 g, 18.3 mmol). After this mixture was stirred for ca 30 min another portion of butanoic anhydride (2.89g 18.3 mmol)
15 was added and the mixture was again kept stirring overnight. The mixture was saturated with sodium chloride and made acidic with concentrated HCl (to about pH 1). The top layer was separated and the solvent was evaporated. To the residue 400 ml 0.125 M NaOH (*aq*) and 100 ml THF was added. After ca 15 the THF was evaporated and the solution was washed with chloroform (2x200 ml). The aqueous phase was then acidified
20 with 7 ml conc. HCl (*aq*) and extracted with chloroform-methanol (4:1, 2X250 ml). The organic phase was dried with sodium sulfate, filtered and concentrated under reduced pressure. The remaining butanoic acid was removed by repeated evaporation of added formic acid-water (3:1) under reduced pressure to give 1.32 g (79%) of product. ^{1}H NMR ($CDCl_3$), 0.92-0.98 (m, 6H; 2x*CH_3*), 1.3-1.48 (m, 2H; *CH_2*), 1.54 (qv, 2H, *J*=6.8 Hz; *CH_2*),
25 1.62-1.70 (m, 4H; 2x*CH_2*), 1.75-1.83 (m, 2H; *CH_2*), 1.85-1.95 (m, 2H; *CH_2*), 2.18 (t, 2H, *J*=7.3 Hz; *CH_2*), 2.25 (t, 2H, *J*=6.2 Hz; *CH_2*), 3.17-3.22 (m, 1H; ε-*CH_{2a}*), 3.31-3.37 (m, 1H; ε-*CH_{2b}*), 4.52-4.58 (m, 1H; α*CH*), 6.08 (bs, 1 H; ε-NH), 6.86 (d, 1 H, *J*=7.3 Hz; α-NH).

Example 15

30 Demonstration of effectiveness of butyrate-class compounds in human infectious colitis (shigellosis)

The following trial is performed with sodium butyrate enema but may be performed correspondingly using PBA for oral administration.

35

Requirement of a population

Sodium butyrate enemas have been applied in inflammatory bowel diseases, including ulcerative colitis, diversion colitis, Crohn's Diseases but never in an infectious colitis.

5 Adult patients with shigellosis have been selected to assess the efficacy in infectious colitis which may be later conducted in children.

Selection of butyrate enema over oral tablets

10 A large body of evidence is available to show that sodium butyrate enema given over a range of 2 –6 weeks in adult patients with inflammatory bowel disease (IBD) is safe with no obvious side effects. The topical dosage used in various previous studies ranged from 528 mg/day to 1.12 g/day, which corresponds to 35-60% of the normal daily intracolonic production of butyrate. None of these studies reported any adverse effect or reactions.
15 According to one study, daily oral dose of 4g of sodium butyrate given as colonic-targeted tablets for 6-weeks in IBD patients and was also found safe and well tolerated without any adverse effects. The present study utilised enema.

Study design: A double blind randomized clinical trial with subsequent follow-up.

20

Study Subjects: Adult male and female patients attending the Dhaka Hospital and Matlab Hospital of ICDDR,B are screened for participation in the study.

Inclusion criteria:

25
- 18-45 years of age
- Males & females
- duration of diarrhoea 0-3 days
- culture-confirmed *Shigella spp* (all Shigella *spp*) in stool on enrolment

30 *Exclusion criteria:*

- who received antimicrobial treatment before attending the ICDDR,B hospital
- clinical symptoms of other concomitant infections (such as chronic respiratory infections, other concomitant gastrointestinal infections)

Randomization

According to a computer-generated randomization list, patients full filling the entry criteria is randomized to either intervention group (Pivmecillinam plus butyrate enema) or control/placebo group (Pivmecillinam plus normal saline enema).

5

Composition of enema and procedure for enema

Butyrate enema contains 80 mmol/L of butyrate in normal saline (pH 7.2).
Placebo enema contains 30 mmol/L NaCl (pH 7.2).

10 The patient is instructed to lie on a bed (cholera cot) in left lateral position. A soft rectal catheter is introduced by a nurse/physician, through which 80 ml of butyrate solution is instilled slowly with a 50 ml plastic syringe. The patient is asked to retain the enema for at least ½ hour by remaining supine for 30 minutes after the administration. However, if a patient cannot retain the enema for 30 minutes, he is given a second round of enema
15 immediately after defecation.

Case Management

After enrolment, the patients are admitted in the study ward of ICDDRB Dhaka and Matlab hospital. A standard clinical history and clinical examination is performed by the
20 study physician. All patients receive Pivmecillinam, 400 mg, 8 hourly for 5 days. The intervention group receives butyrate enema 80 ml of 80 mM sodium butyrate, 12 hourly for 72 hours while the placebo group gets 80 ml of normal saline 12 hourly for 72 hours. All patients receive the usual hospital food three times a day (breakfast, lunch and supper). The patients remain in the study ward for 5 days to enable identification of any
25 relapse cases.

Sample size

In a study by Kabir I et al (1984) (*Kabir I, Rahaman MM, Ahmed SM, Akhter SQ, Butler T. Comparative efficacies of pivmecillinam and ampicillin in acute shigellosis.*
30 *Antimicrob Agents Chemother. 1984 May;25(5):643-5.*), it has been shown with 3.2 ± 1.8 (mean ±SD) duration of diarrhoea of patients with shigellosis while treated with pivmecillinam. Expecting a 30% reduction in duration of diarrhoea when treated with butyrate enema along with pivmecillinam, considering 5% level of significance and 80% power the sample size will be 55 per group. Considering a dropout of 10%, the sample
35 size in each group will be 61.

Clinical Parameters measured / recorded

1. Appetite
2. Abdominal cramps
3. Rectal tenesmus
4. Body temperature, 8 hourly
5. Daily frequency of stool (No. of times of defecation)
6. Stool output (in grams)
7. Presence of RBC, pus cells and macrophages in stool by RME
8. Weight at admission, daily during hospitalization and after 14 days (at follow-up)
9. Sigmoidoscopic findings

Other analysis

1. Stool culture by serial dilution method for bacterial count (twice daily) for 4 days.
2. Stool for detection of LL-37 by Western blot
3. Stool for determination of LL-37 by ELISA
4. Rectal biopsy (from Dhaka patients only) for histologic grading of inflammation.
5. Rectal biopsy for immunohistochemical staining of LL-37 and image analysis.
6. Rectal biopsy for assessing transcripts of LL-37 in tissue by realtime PCR.
7. Serum for measuring butyrate

Data analysis

For normally distributed data, it is intended to use appropriate parametric tests (eg. t test) to compare the results between groups. In case the data is skewed, nonparametric tests will be used. Statistical analysis can then be done using two-factor ANOVA to determine significant interactions between time and treatment and in case of any significant interactions post hoc Tukey procedure will be performed. For data that are not normally distributed, ANOVA on ranks will be applied. For within group (between days) comparisons, one-way ANOVA will be done. Statistical calculations will be performed using the statistical software SigmaStat® 3.1 (Jandel Scientific, San Rafael, Calif.) and SPSS 13.

CLAIMS

1. A compound of formula Ia for use as a medicament for treating, counteracting or preventing microbial infection in an animal by stimulating the innate antimicrobial peptide defense system:

(Ia)

wherein

R^1 represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2CH(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2CH(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof;

m and *n* are each independently 0 or 1;

R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a} and R^{3b} each independently represent hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group; and/or

R^{2a}, together with an adjacent R^{3a} or R^{1a}, may represent a carbon-carbon π bond; and/or

R^{2b}, together with an adjacent R^{3b} or R^{1b}, may represent a carbon-carbon π bond;

R^4 may be hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group;

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group;

5 R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group; and

R^7 is a side chain of a naturally occurring amino acid or is selected from
10 $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, or $CH_2CH_2CH_2NHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms;

and wherein, if R^1 is carboxyl or a salt thereof, at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is selected from halide, amino, hydroxyl, carbonyl, a linear or branched substituted
15 or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group.

2. A compound as claimed in claim 1 wherein:

20 R^{1a} and R^{1b} are both hydrogen,
m and *n* are both 1, and R^{2b} and R^{3b} are *either* both hydrogen *or* together form a π bond in position 'x' whereby if R^{2a} and R^{3a} also together form a π bond, then position 'x' represents a double bond,

25 or wherein:

R^{1a}, R^{1b} and R^{2b} are all hydrogen,
m is 0, *n* is 1,
and R^4 is hydrogen,

30

such that the compound has formula I.

R^4 R^{3a} x R^1 R^{2a}

wherein

R^1 represents a carboxyl group, phospate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde,
5 imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or
10 substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

15 R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

x represents a single, double or triple bond,

or x-$R^{3a}R^4$ together represent hydrogen in which case R^1 is $COOR^5$, $CONH_2$,
20 $CONR^5R^6$, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

25 R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CNHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group
30 with three to five carbon atoms.

3. A compound of formula I for use as a medicament for treating, counteracting or preventing microbial infections in an animal, including humans, by stimulating the innate antimicrobial peptide defense system

$$R^{3a}-C(R^4)\overset{x}{-}CH(R^{2a})-CH_2-R^1$$

I

wherein

5 R^1 represents a carboxyl group, phospate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, $COOR^5$, $CONH_2$, $CONR^5R^6$, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$, or an amino acid group $CONHCR^7COOH$ or a salt thereof,

10 R^{2a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^{3a} represents hydrogen, hydroxyl, carbonyl, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or 15 substituted or nonsubstituted aryl group, except when R^1 is carboxyl or a salt thereof R^{3a} is not hydrogen,

R^4 represents hydrogen, or a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

20 x represents a single, double or triple bond,

or $x\text{-}R^{3a}R^4$ together represent hydrogen in which case R^1 is $COOR^5$, $CONH_2$, $CONR^5R^6$, or a triglyceride moiety $COOCH_2(OOCR^5)CH_2(OOCR^6)$ or diglyceride moiety $COOCH_2(OOCR^5)CH_2OH$,

R^5 represents a linear or branched substituted or nonsubstituted saturated or 25 nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group,

R^6 represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or substituted or nonsubstituted aryl group, and

30 R^7 represents $CH_2CH_2SCH_3$, $CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CH_2NHR^8$, $CH_2CH_2CH_2CNHC(=NH)NHR^8$, where R^8 is hydrogen or a linear or branched acyl group with three to five carbon atoms.

4. The compound of any one of claims 2 to 3 wherein R^1 represents a carboxyl group or a pharmaceutically acceptable salt thereof.

5. The compound of any one of claims 2 to 4 wherein R^1 represents an ester group of formula $COOR^5$.

6. The compound of any one of claims 2 to 5 wherein R^{2a} and R^4 represent hydrogen.

7. The compound of claim 6, wherein R^{3a} represents a substituted or nonsubstituted aryl group.

8. The compound of any of the aforementioned claims wherein R^5 and R^6 independently represent a linear or branched acyl chain with three to five carbon atoms.

9. The compound of claim 1 wherein at least one of *m* and *n* is 1, R^1 represents a carboxyl group or a pharmaceutically acceptable salt thereof and at least one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is a substituent other than hydrogen, or R^1 is a carboxylic acid derivative selected from: ester, amide.

10. The compound of claim 9 wherein R^1 is an ester selected from a triglyceride ester moiety or diglyceride ester moiety.

11. The compound of claim 9 wherein R^1 is an amide of an amino acid group such that the compound has the general formula (IIe):

R^{3a} R^{3b} R^{1a} R^{1b} R^4 m n H N CO_2H R^{2a} R^{2b} O R^7

(IIe)

or a salt thereof, in which R^7 is a naturally occuring amino acid side chain.

12. The compound of any one of claims 9 to 11 wherein one of R^{1a}, R^{1b}, R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl group and the others are selected from hydrogen or an alkyl group.

13. The compound of claim 12 wherein one of R^{2a}, R^{2b}, R^{3a}, R^{3b} and R^4 is an aryl
5 group and the others are selected from hydrogen or an alkyl group.

14. The compound of any one of claims 9 to 13 wherein at least one of R^{1a} and R^{1b} is hydrogen.

10 15 The compound of any of claims 1 to 14 wherein R^5 and R^6, if present, are independently represent propanoyl, *n*-butanoyl, or *iso*-butanoyl.

16 The compound of any of claims 1 to 15 wherein R^8, if present, represents propanoyl, *n*-butanoyl, or *iso*-butanoyl.

15

17 The compound of any of claims 1 to 16 whereinselected from the group consisting of: 4-phenylbutyric acid, 3-phenylbutyric acid, 2-phenylbutyric acid, 3-phenylpropionic acid, 2-phenylpropionic acid, 2-methyl-3-phenylpropionic acid *[ST7]*, 2-methyl-4-phenylbutyric acid, or a pharmaceutically acceptable salt of any of said compounds,
20 methyl 4-phenylbutyrate, ethyl 4-phenylbutyrate, methyl 3-phenylbutyrate, ethyl 3-phenylbutyrate, methyl 2-phenylbutyrate, ethyl 2-phenylbutyrate, methyl 3-phenylpropionate, ethyl 3-phenylpropionate, methyl 2-phenylpropionate, ethyl 2-phenylpropionate, methyl 2-methyl-3-phenylpropionate, ethyl 2-methyl-3-phenylpropionate, methyl 2-methyl-4-phenylbutyrate, and ethyl 2-methyl-4-
25 phenylbutyrate.

18 The compound of any of claims 1 to 17, wherein said microbial infection is selected from the group consisting of bacterial, viral, protozoal and fungal infections.

19 The compound of claim 18, wherein said microbial infection is caused by a
30 microbial species selected from: Yersenia enterocolitica, Salmonella, Shigella, Campylobacter, Clostridium and E. Coli.

20 The compound of any one of claims 1 to 19, wherein said microbial infections results in gastrointestinal disorders selected from the list consisting of: traveller's diarrhoea, endemic diarrhoea, dysentery, viral gastroenteritis, parasitic enteritis, Crohn's
35 disease, ulcerative colitis, irritable bowel syndrome, precancerous states of the

gastrointestinal tract, cancer of the gastrointestinal tract, diverticulitis, post-antibiotic diarrhoea, Clostridium difficile colitis, lactose intolerance, flatulence, gastritis, esophagitis, heartburn, gastric ulcer, ulcers associated with Helicobacter pylori, duodenal ulcer, short bowel syndrome, dumping syndrome, gluten enteropathy and food intolerance; eye
5 infections optionally selected fromconjunctivitis, stye, blepharitis, cellulitis, keratitis, corneal ulcer, trachoma, uveitis, canaliculitis and dacryocystitis; urinary tract and genital infections optionally selected from pyelonephritis, cystitis, gonorrhoea and urethritis; infections of the respiratory system optionally selected from bronchitis, pneumonia, rhinosinusitis, sinusitis, pharyngitis/tonsillitis, laryngitis and influenza; skin infections
10 optionally selected from boils, carbuncles, furuncles, cellulitis, abscesses, impetigo, and erysipelas; infections caused by bacterial strains resistant to classical antibiotic treatment.

21. The compound of any of claims 1 to 20 wherein the microbial infection in the animal has lead to down-regulation of the innate antimicrobial peptide defense system,
15 and whereby stimulation of the innate antimicrobial peptide defense system upto or above basal levels leads to secretion of the relevant peptide onto an epithelial surface which is optionally in the gastrointestinal tract such as to enhance the antimicrobial activity thereof.

22. The compound of any of claims 1 to 21 for use in a combination treatment for
20 treating, counteracting or preventing microbial infection in an animal, wherein the compound is used in combination with any one or more of: an antibiotic; an aminosterol-type compound; isoleucine or active isomers or analogs thereof; a vitamin D type compound.

25 23. A pharmaceutical composition for treating, preventing or counteracting a microbial infection comprising as an active ingredient at least one compound of any one of claims 1 to 21 and at least one pharmaceutically acceptable excipient.

24 The pharmaceutical composition of claim 23, formulated as an oral dosage form.

30

25 The pharmaceutical composition of claim 24, wherein said oral dosage form is selected from a tablet, a capsule, a solution, a suspension, a powder, a paste, an elixir, a syrup.

26 The pharmaceutical composition of any one of claims 23 to 25, wherein a unit dose of said composition comprises in the range of about 10-1000 mg of said active ingredient.

5 27 The pharmaceutical composition of any one of claims 23 to claim 26 further comprising any one or more of: an antibiotic; an aminosterol-type compound; isoleucine or active isomers or analogs thereof; a vitamin D type compound.

28 A functional food or feed product comprising an amount of at least one compound
10 of any one of claims 1 to 21, which amount is effective for treating, counteracting or preventing bacterial infections in an animal being fed with said food or feed.

29 The functional food or feed product of claim 28, comprising in the range of about 0.1 to 20 mg of the active ingredient per g of food product.

15

30 A method for treating, preventing or counteracting microbial infection in an animal, wherein the effects of the microbial infection are diminished or reduced by upregulation of the innate antimicrobial peptide system, said method comprising administration of a medicament comprising a secretagogue-effective amount of at least one compound of
20 formula I as defined in any one of claims 1 to 22.

31 The method of claim 30, comprising administration of said medicament in an oral dosage form.

25 32 The method of claim 31, wherein the daily dosage is between 250 μg to about 25 g which is optionally split into doses given 1, 2 or 3 times daily.

33 A compound, composition, food, or method as claimed in any one of the preceding claims wherein the animal is a human.

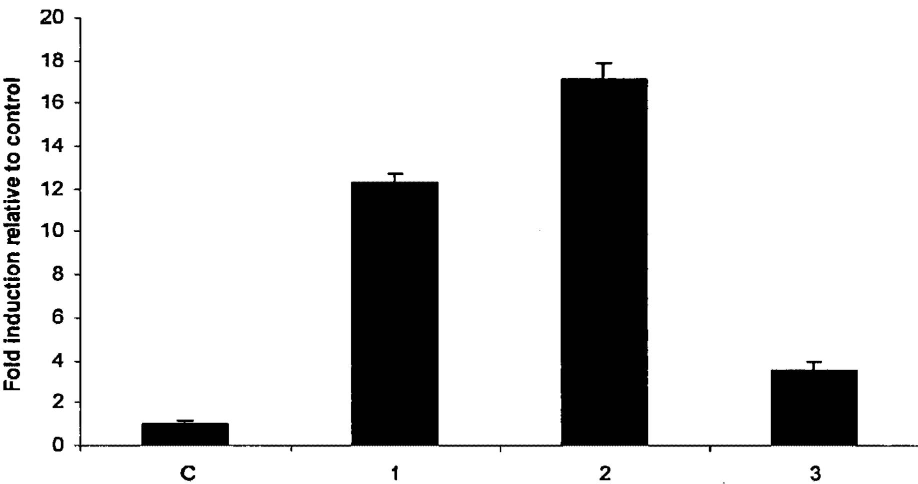

Figure 1.

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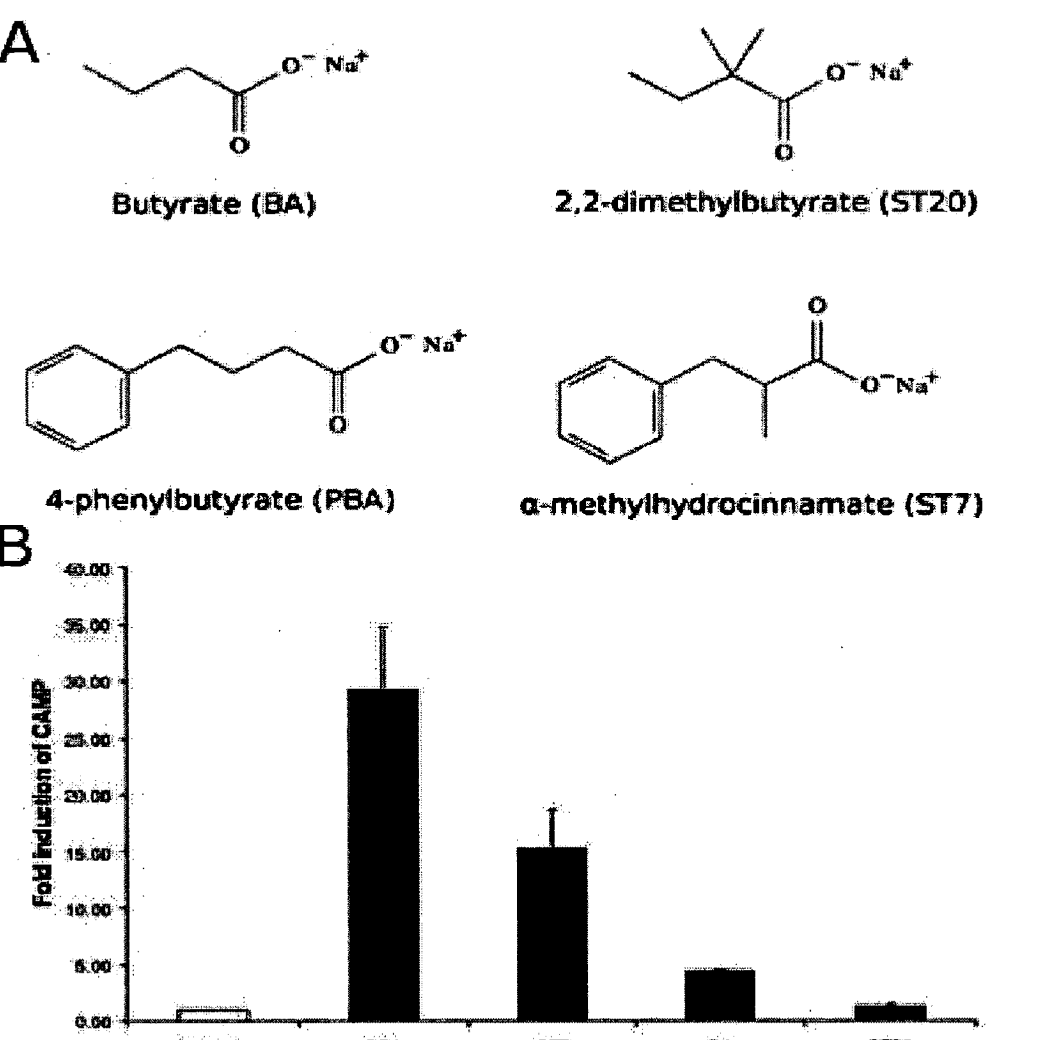

Figure 2

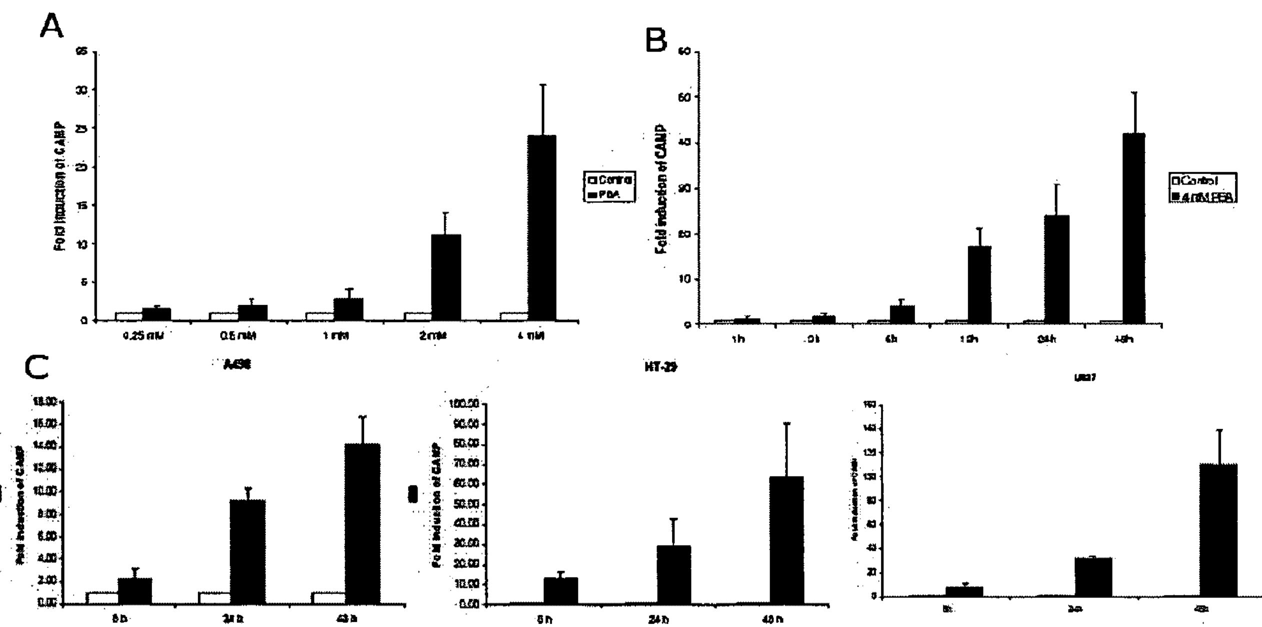

Figure 3

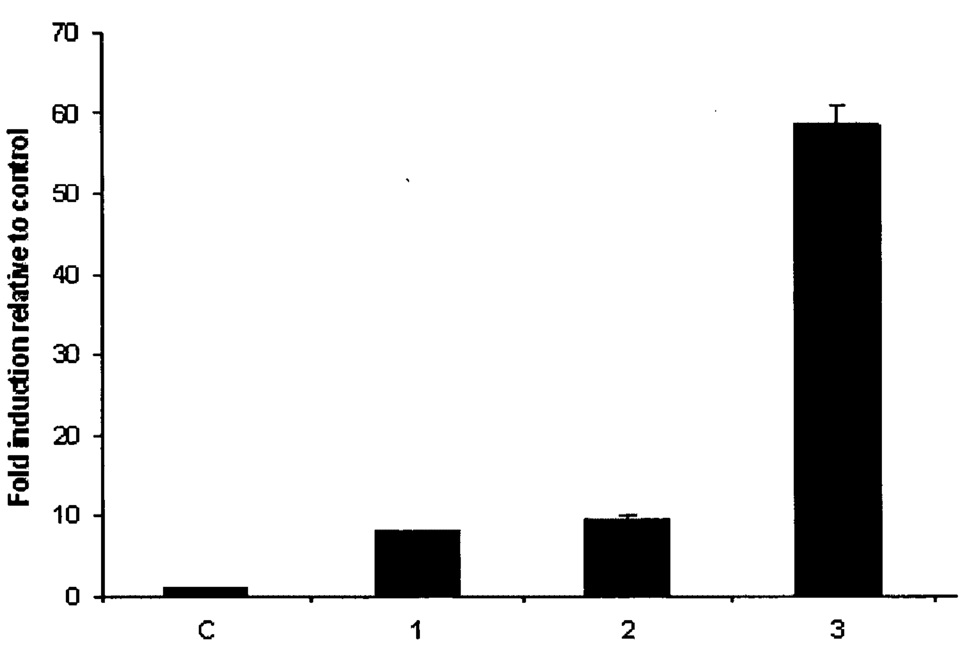

Figure 4

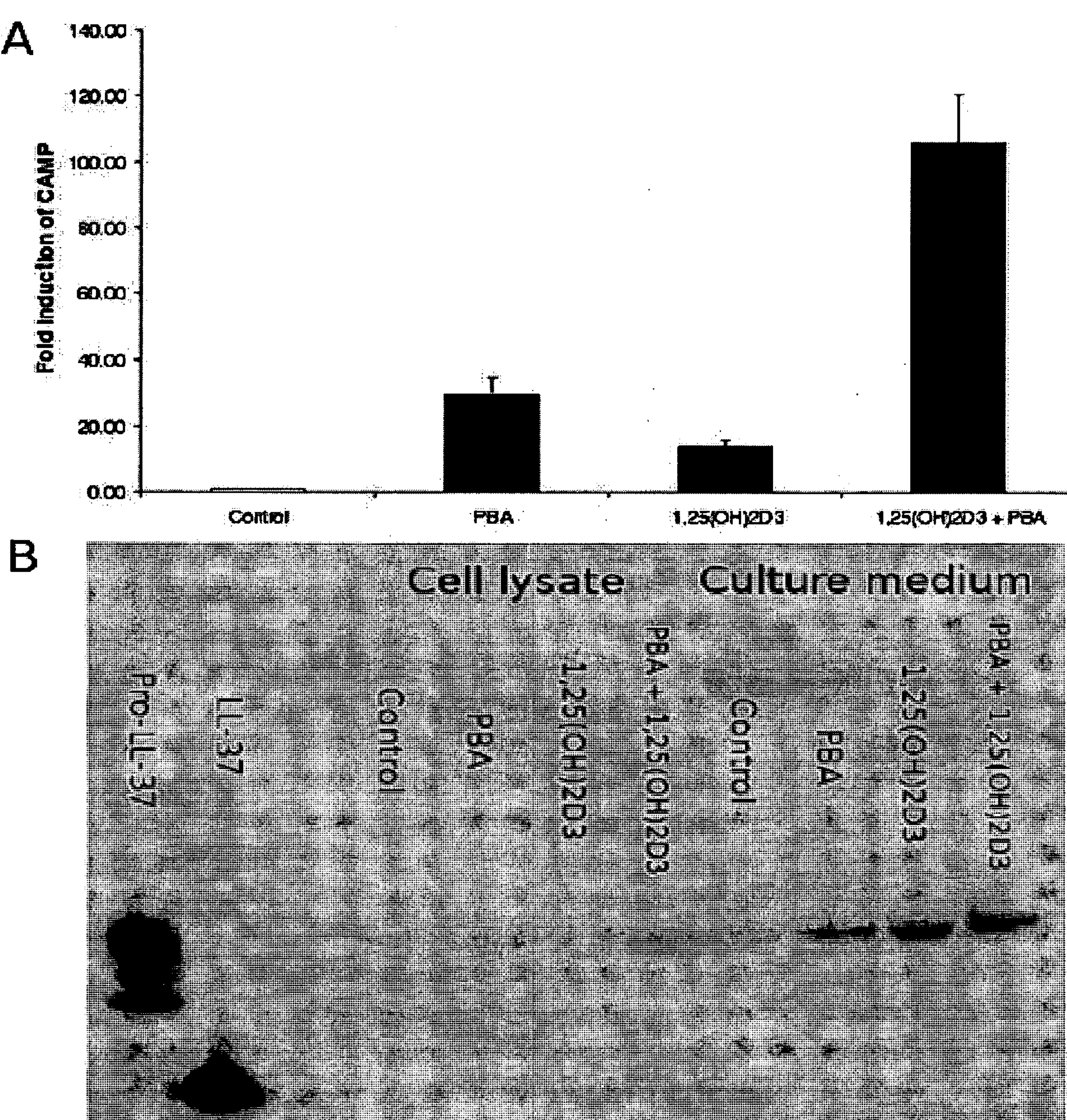

Figure 5

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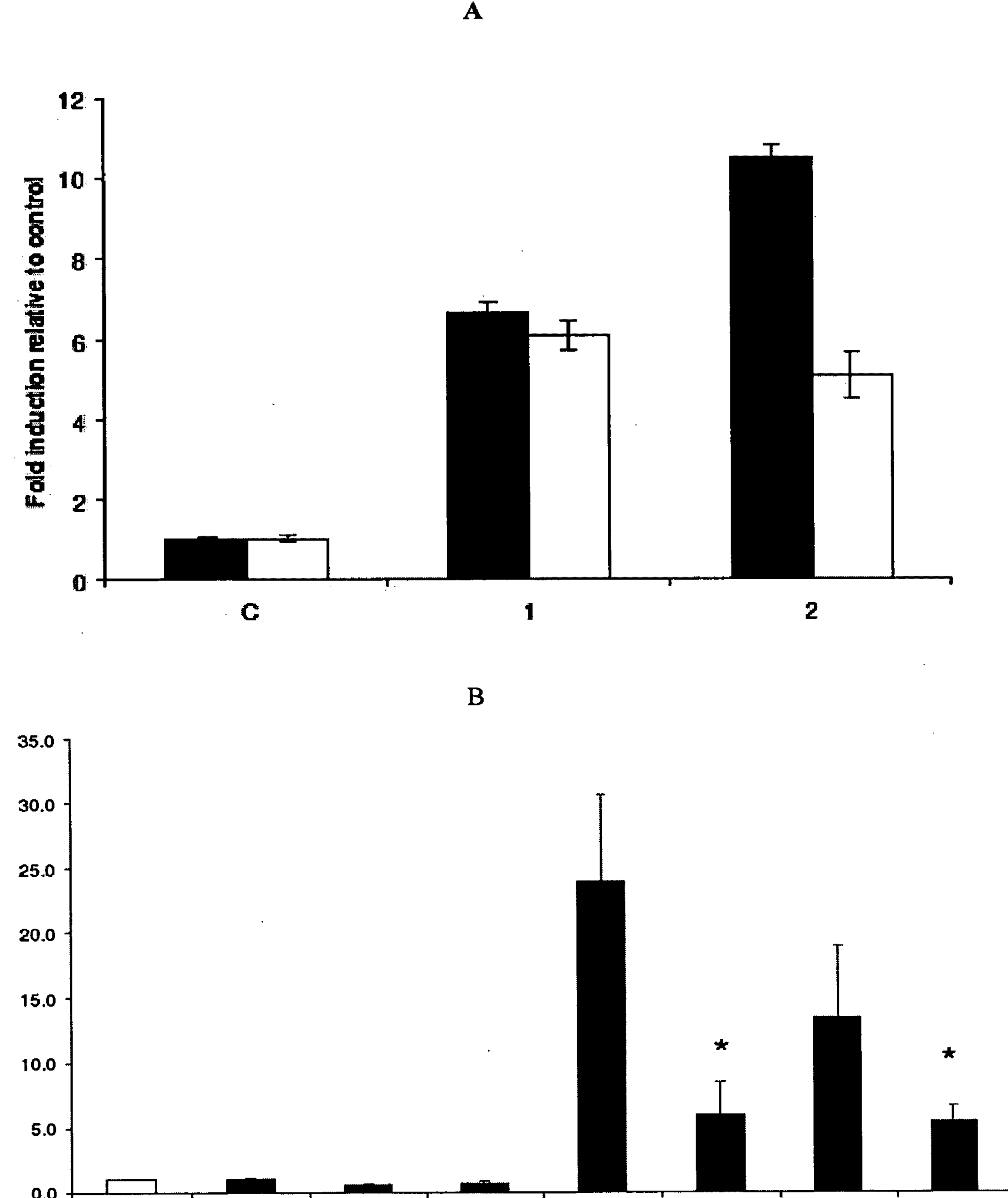

Figure 6

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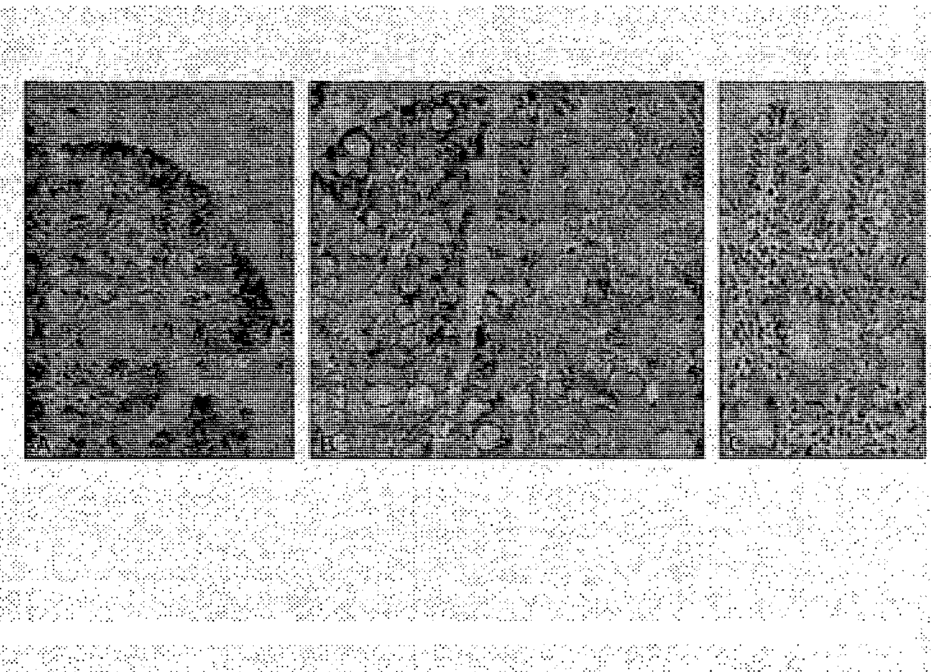

Figure 7

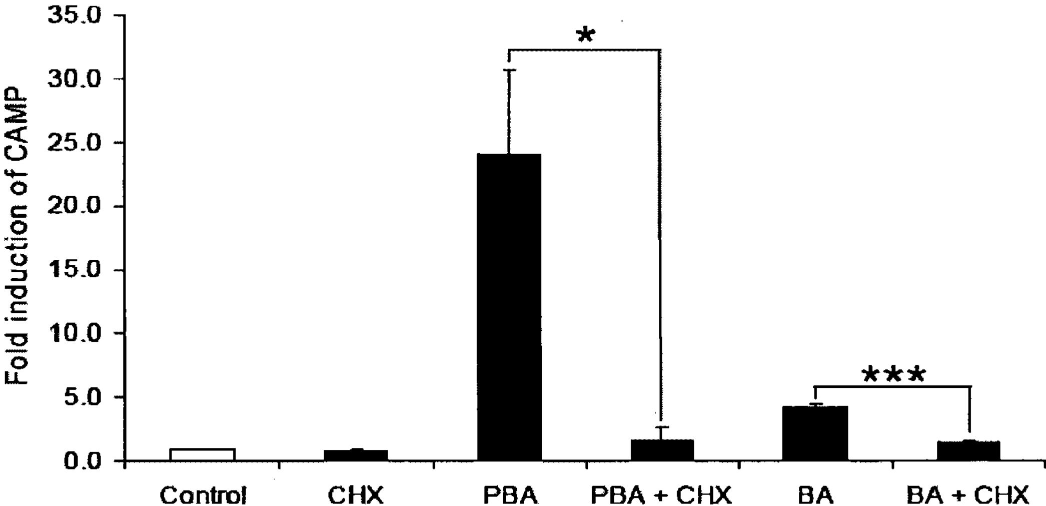

Figure 8

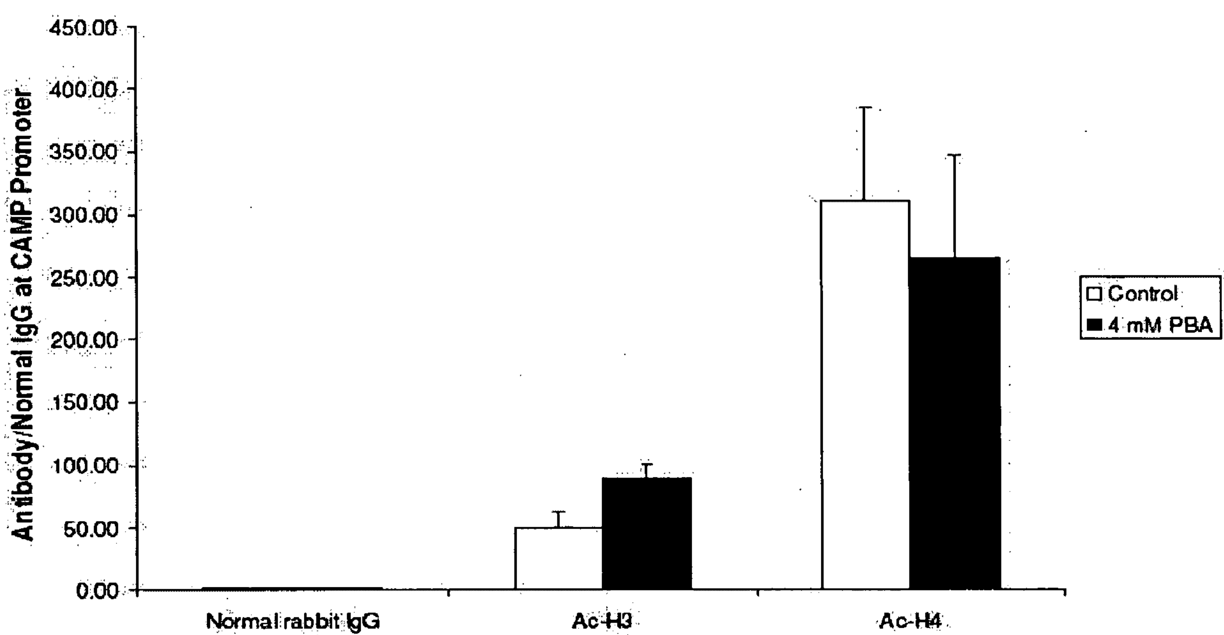

Figure 9

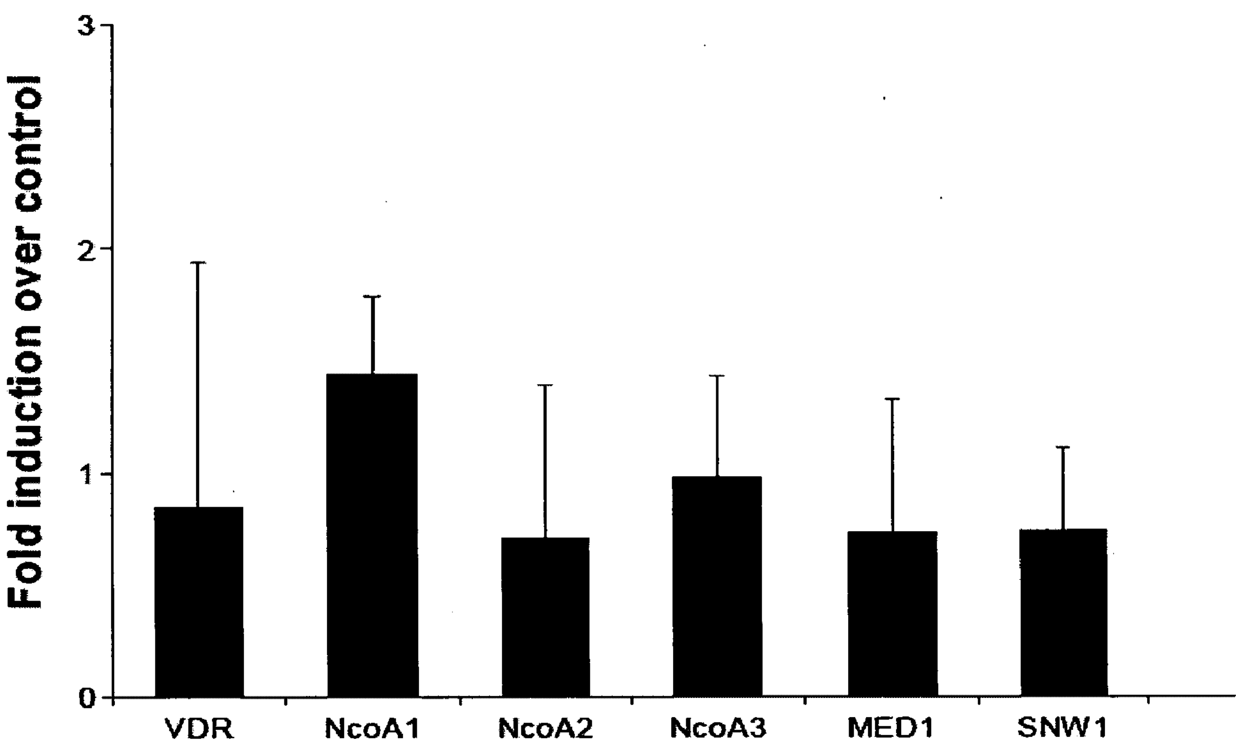

Figure 10

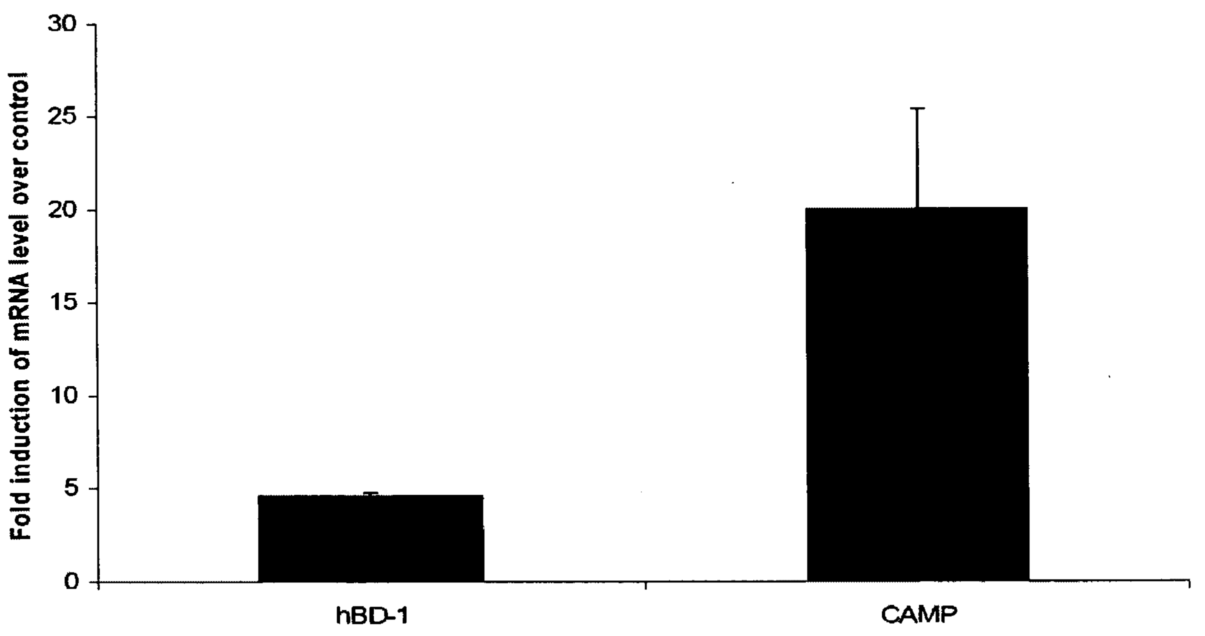

Figure 11

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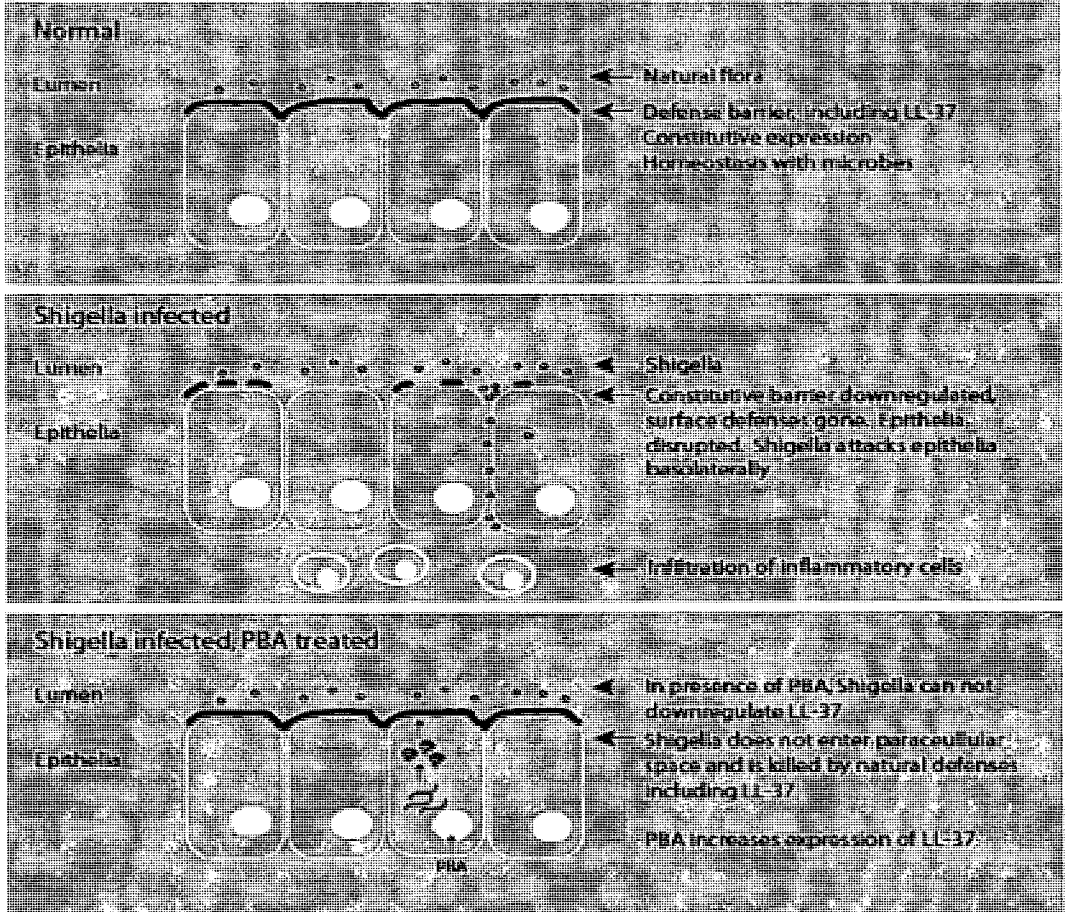
Figure 12



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(54) Title: METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

FIGURE 4

BUPHENYL® (3g/m²) HPN-100 (3g/m²)

Mean Plasma Concentration (uM)

PBA
PAA
PAGN

Time (hours) Time (hours)

In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving BUPHENYL® (sodium phenylbutyrate) (sodium PBA) at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100. In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately12 hours, and fall generally close to the PAGN curves up to that time.

(57) Abstract: The invention provides a method for determining a dose and schedule and making dose adjustments of PBA prodrugs used to treat nitrogen retention states, or ammonia accumulation disorders, by measuring urinary excretion of phenylacetylglutamine and/or total urinary nitrogen. The invention provides methods to select an appropriate dosage of a PBA prodrug based on the patient's dietary protein intake, or based on previous treatments administered to the patient. The methods are applicable to selecting or modifying a dosing regimen for a subject receiving an orally administered ammonia scavenging drug.

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METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Cross-Reference to Related Applications

[0001] This application claims benefit of priority to U.S. Provisional application serial number 61/093,234, filed August 29, 2008, which is incorporated herein by reference in its entirety. This application is also related to the U.S. provisional patent application entitled "Treating special populations having liver disease with nitrogen-scavenging compounds," naming Sharron Gargosky as inventor, serial number 61/048,830, filed on April 29, 2008.

Technical Field

[0002] This invention relates to treatment of patients with nitrogen retention states, in particular urea cycle disorders (UCDs) and cirrhosis complicated by hepatic encephalopathy (HE), using administered compounds that assist in elimination of waste nitrogen from the body. The compounds can be orally administered small-molecule drugs, and the invention provides methods for delivering these compounds and selecting suitable dosages for a patient.

Background Art

[0003] Drug dosing is usually based upon measurement of blood levels of the active drug species in conjunction with clinical assessment of treatment response. However, the present invention is based on evidence that for certain prodrugs of phenylacetic acid (PAA), measuring the blood level of the prodrug (e.g. PBA) or of PAA formed from it is unreliable. In addition, assessment of treatment effect by measuring levels of ammonia in the blood is inconvenient, because it requires withdrawing multiple blood samples under carefully controlled conditions. Because blood ammonia levels are affected by various factors including dietary protein, they also fail to provide a direct measure of how much ammonia the drug is mobilizing for elimination. The invention demonstrates that prodrugs of phenylbutyric acid (PBA) behave similarly to sodium PBA, in that measuring PBA levels is unreliable for assessing their effectiveness. This invention provides a novel method for dosing in patients with nitrogen retention states, in particular patients with liver disease and clinical manifestations of hepatic encephalopathy and patients with UCDs. It is particularly applicable to prodrugs that liberate or are metabolized to form phenylacetic acid, i.e., prodrugs of PAA, and those prodrugs that are metabolized to form PBA.

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[0004] Hepatic encephalopathy refers to a spectrum of neurologic signs and symptoms which frequently occur in patients with cirrhosis or certain other types of liver disease.

[0005] Urea cycle disorders comprise several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia. The urea cycle is depicted in Figure 1, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. The enzymes including their Enzyme Commission (EC) numbers and modes of inheritance include the following:

- Carbamyl phosphate synthetase (CPS; EC Number 6.3.4.16; autosomal recessive),
- ornithine transcarbamylase (OTC; EC Number 2.1.3.3; X-linked),
- argininosuccinate synthetase (ASS; EC Number 6.3.4.5; autosomal recessive),
- argininosuccinate lyase (ASL; EC Number 4.3.2.1; autosomal recessive),
- arginase (ARG; EC Number 3.5.3.1; autosomal recessive), and
- N-acetyl glutamine synthetase (NAGS 1; EC Number 2.3.1.1; autosomal recessive)

[0006] Mitochondrial transporter deficiency states which mimic many features of urea cycle enzyme deficiencies include the following:

- Ornithine translocase deficiency (hyperornithinemia, hyperammonemia, homocitrullinuria or HHH Syndrome)
- Citrin (aspartate glutamate transporter) deficiency

[0007] The common feature of UCD and hepatic encephalopathy that render them treatable by methods of the invention is an accumulation of excess waste nitrogen in the body, and hyperammonemia. In normal individuals, the body's intrinsic capacity for waste nitrogen excretion is greater than the body's waste nitrogen production, so waste nitrogen does not accumulate and ammonia does not build up to harmful levels. For patients with nitrogen retention states such as UCD or HE, the body's intrinsic capacity for waste nitrogen excretion is less than the body's waste nitrogen production based on a normal diet that contains significant amounts of protein. As a result, nitrogen builds up in the body of a patient having a nitrogen retention disorder, and usually results in excess ammonia in the blood. This has various toxic effects; drugs that help eliminate the excess ammonia are an important part of an overall management strategy for such disorders.

[0008] To avoid build-up of ammonia to toxic levels in patients with nitrogen retention states, dietary intake of protein (a primary source of exogenous waste nitrogen) must be balanced by the patient's ability to eliminate excess ammonia. Dietary protein can be limited, but a healthy diet requires a significant amount of protein, particularly for growing

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children; thus in addition to controlling dietary protein intake, drugs that assist with elimination of nitrogen are used to reduce ammonia build-up (hyperammonemia). The capacity to eliminate excess ammonia in treated patients can be considered the sum of the patient's endogenous capacity for nitrogen elimination (if any) plus the amount of additional nitrogen-elimination capacity that is provided by a nitrogen scavenging drug. The methods of the invention use a variety of different drugs that reduce excess waste nitrogen and ammonia by converting it to readily-excreted forms, such as phenylacetyl glutamine (PAGN). In some embodiments, the invention relates to methods for determining or adjusting a dosage of an oral drug that forms PAA *in vivo*, which is converted into PAGN, which is then excreted in urine and thus helps eliminate excess nitrogen.

[0009] Based on prior studies in individual UCD patients (e.g. Brusilow, Pediatric Research, vol. 29, 147-50 (1991); Brusilow and Finkelstien, J. Metabolism, vol. 42, 1336-39 (1993)) in which 80-90% of the nitrogen scavenger sodium phenylbutyrate was reportedly excreted in the urine as PAGN, current treatment guidelines typically either assume complete conversion of sodium phenylbutyrate or other PAA prodrugs to PAGN (e.g. Berry et al., J. Pediatrics, vol. 138, S56-S61 (2001)) or do not comment on the implications of incomplete conversion for dosing (e.g. Singh, Urea Cycle Disorders Conference Group '*Consensus Statement from a Conference for the Management of Patients with Urea Cycle Disorders*', Suppl to J Pediatrics, vol. 138(1), S1-S5 (2001)).

[0010] Current treatment guidelines recommend 4 times per day dosing, based on the fact that PBA is absorbed rapidly from the intestine when administered in the form of sodium PBA and exhibits a short half life in the bloodstream (Urea Cycle Disorders Conference Group 'Consensus Statement' 2001)

[0011] Current recommendations for sodium phenylbutyrate dosing indicate that dosage should not exceed 600 mg/kg (for patients weighing up to 20 kg) or in any case 20 grams total.

Disclosure of Embodiments of the Invention

[0012] The invention provides a novel approach for determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs, including sodium phenylbutyrate and glyceryl tri-[4-phenylbutyrate] (HPN-100), based upon the urinary excretion of the drug metabolite phenylacetylglutamine (PAGN) and/or total urinary nitrogen. It is based in part on the discoveries that bioavailability of these drugs as conventionally assessed based on systemic blood levels of the drugs themselves or of the active species produced *in vivo* from these drugs does not accurately predict removal of

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waste nitrogen or reduction of plasma ammonia in healthy human volunteers, adults with liver disease, or patients with UCDs receiving ammonia scavenging drugs as defined below and that conversion of orally administered sodium phenylbutyrate (NaPBA, or sodium PBA) to PAGN to urinary PAGN is incomplete, typically about 60-75%. Prodrugs of phenylbutyrate (PBA, the active ingredient in BUPHENYL®(sodium phenylbutyrate), which is the sodium salt of PBA along with small amounts of inert ingredients), which is itself a prodrug of phenylacetic acid (PAA), are especially subject to the effects described herein.

phenylbutyrate

Phenylacetic acid

Phenylacetylglutamine

[0013] As used herein “ammonia scavenging drugs” is defined to include all orally administered drugs in the class which contain or are metabolized to phenylacetate. Thus, the term includes at least phenylbutyrate, BUPHENYL® (sodium phenylbutyrate), AMMONAPS®, butyroyloxymethyl-4-phenylbutyrate, glyceryl tri-[4-phenylbutyrate] (HPN-100), esters, ethers, and acceptable salts, acids and derivatives thereof. These drugs reduce high levels of endogenous ammonia by providing phenylacetic acid in vivo, which is metabolized efficiently to form phenylacetyl glutamine (PAGN). PAGN is efficiently excreted in urine, carrying away two equivalents of nitrogen per mole of PAA converted to PAGN. References herein to sodium phenylbutyrate are understood to include reference to the drug product BUPHENYL®, and BUPHENYL® was used for the Examples herein wherever test subjects were treated with sodium phenylbutyrate. Thus the sodium PBA dosages used in the Examples generally refer to a dosage of BUPHENYL®, and the amounts of sodium phenylbutyrate in those Examples should be interpreted accordingly. Note that the terms ‘ammonia scavenger’ and ‘nitrogen scavenger’ are used interchangeably in this

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invention, reflecting the fact that the drugs described herein lower blood ammonia through elimination of waste nitrogen in the form of PAGN.

[0014] In some embodiments, the invention uses prodrugs that can be converted into PAA within the body. Sodium phenylbutyrate (sodium PBA) is one such drug; it is converted by oxidative mechanisms into PAA in the body. HPN-100 is another such drug: it can be hydrolyzed to release PBA, which in turn can be oxidized to form PAA. Thus, HPN-100 is a prodrug of PBA, and also a prodrug of PAA. Clinical evidence demonstrates that HPN-100 is converted into PAA in the body as expected, and that PAA is then linked to a molecule of glutamine and converted into PAGN, which is eliminated in the urine as predicted. This process can be summarized as follows:

HPN-100 → 3 PBA → 3 PAA

PAA + glutamine → PAGN.

[0015] PAGN is mainly excreted in the subject's urine, and removes two molecules of ammonia per molecule of excreted PAGN. Each HPN-100 molecule forms three PAA molecules, so each molecule of HPN-100 can promote excretion of six molecules of ammonia. The clinical results suggest that conversion of HPN-100 into PBA and PAA is efficient and fairly rapid, but surprisingly suggest that some conversion of HPN to PAGN may occur before the HPN-100 (or PBA, or PAA derived from PBA) enters systemic circulation. As a result, systemic levels of PAA or PBA are not reliably correlated with the efficacy of HPN-100 as an ammonia scavenger.

[0016] In some embodiments, the invention uses a prodrug of PBA, including HPN-100 and other esters of phenylbutyrate. The PBA prodrug is thus a prodrug of a prodrug, since PBA acts to scavenge ammonia after it is converted to PAA and is thus considered a prodrug of PAA. In some embodiments, the PBA prodrug is an ester of phenylbutyrate, such as those described below; a preferred PBA prodrug for use in the invention is HPN-100. These compounds can be made and used by methods disclosed in U.S. Patent No. 5,968,979, which is incorporated herein by reference for its description of these compounds and methods for their administration.

[0017] Where an 'equal molar' or 'equimolar' amount of a second drug is to be used along with or instead of a certain amount of a first drug, the amount of each drug is calculated on a molar basis, and the equimolar amount of the second drug is the amount that produces an equal molar amount of active drug *in vivo*. Where one of the drugs is a prodrug, the amount of prodrug will typically refer to the molar amount of the active species

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formed from that prodrug. That active species is usually PAA for the prodrugs described herein, and the molar amount of a prodrug corresponds to the amount of PAA that would form in the body from that amount of the prodrug, assuming complete conversion into PAA occurs *in vivo*. Thus, for example, a molecule of HPN-100 can be metabolized by ester hydrolysis followed by oxidation to form three molecules of PAA, so a mole of HPN-100 would be considered equimolar to three moles of PAA. Similarly, since HPN-100 hydrolyzes to form three molecules of PBA (and one molecule of glycerin), an equimolar amount of HPN-100 would be one-third of the molar amount of PBA.

[0018] The following Table sets forth amounts of HPN-100 that correspond to equimolar amounts of certain relevant doses of BUPHENYL® (sodium phenylbutyrate). Note that the conversion of the dose of sodium PBA to the dose of HPN-100 involves correction for their different chemical forms [i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium; (sodium PBA [g] x 0.95 = HPN-100 [g])] as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

BUPHENYL® (sodium PBA)	**HPN-100 PBA Equivalent Dose (mg)**	**HPN-100 PBA Equivalent Dose (mL)**
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[0019] The present invention can use prodrugs of the formula (I):

$$\begin{array}{c} H \\ | \\ H-C-O-R_1 \\ | \\ H-C-O-R_2 \\ | \\ H-C-O-R_3 \\ | \\ H \end{array} \quad (I)$$

wherein R_1, R_2, and R_3 are independently, H,

$$-C(=O)-(CH_2)_n-CH_2-C_6H_5 \quad \text{or} \quad -C(=O)-(C_mH_{2m-2})-CH_2-C_6H_5$$

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and n is zero or an even number, m is an even number and at least one of R_1, R_2, and R_3 is not H. For each R_1, R_2, or R_3, n or m is independently selected, so the R_1, R_2, and R_3 groups in a compound of formula I do not have to be identical. The preferred compounds are those wherein none of R_1, R_2, and R_3 is H, and frequently each n or m for a particular embodiment is the same, i.e., R_1, R_2, and R_3 are all the same. The advantage over the prior art of decreased dosage is greater with such triesters, and having all three acyl groups the same reduces issues related to mixtures of isomers. Moreover, the triol backbone liberated by hydrolysis of the esters is glycerol, a normal constituent of dietary triglyceride which is non-toxic.

[0020] The present invention also utilizes phenylbutyrate and phenylacetate prodrugs of the formula II:

R—O—C(=O)—R_4 (II)

wherein R is a C_1-C_{10} alkyl group,

R_4 is

$(CH_2)_n$—Ph or (C_mH_{2m-2})—Ph

and n is zero or an even number, and m is an even number.

[0021] In Formula II, R can be, for example, ethyl, propyl, isopropyl, n-butyl, and the like.

[0022] The compounds of the invention are esters of the congeners of phenylalkanoic and phenylalkenoic acids having an even number of carbon atoms in the alkanoic acid portion, which include phenylacetic acid esters and those of phenylbutyric acid, etc., which can be converted by efficient beta-oxidation processes to phenylacetic acid in the body. They are thus prodrugs for phenylacetic acid. Where n is 2 or 4, the esters are also prodrugs for phenylbutyric acid. Preferably the alkylene or alkenylene carboxylate group contains 24 or fewer carbon atoms, so n or m is less than 24. In some embodiments, n and m are 0, 2, 4 or 6, and in some preferred embodiments n or m is 2.

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[0023] Certain preferred embodiments of the invention use HPN-100 (Formula III):

(III)

[0024] Total daily dosage of prodrugs like sodium PBA can often be selected according to the amount needed to provide an appropriate amount of the active species, if that amount is known or can be determined. PBA is a prodrug for PAA; therefore, an initial dose of PBA could be selected if an effective dosage of PAA were known, taking into account the fraction of PBA that is converted into PAA and ultimately into PAGN. If a subject has been treated with PAA or a prodrug that forms PAA in the body, the amount of the previously used drug that was effective provides a possible starting point for selecting a dosage of a new prodrug of PAA. In this same patient, after the new prodrug is administered at the expected PAA dose equivalence, the PAA levels in the subject could be monitored and the dose of the prodrug adjusted until the same plasma level of PAA that was effective with the previous treatment is achieved. However, the current invention is based in part on finding that plasma PAA and PBA levels are not well correlated with the dose of a PBA prodrug administered or with ammonia elimination; for monitoring a dosing level of a PBA prodrug, one should not rely upon these parameters to assess the effectiveness of the prodrug. While not bound by the underlying theory, explanations for this effect (i.e. the inconsistent relationship between ammonia scavenging and PBA and/or PAA blood levels) are provided herein.

[0025] The following Table provides data from three clinical test groups showing the inconsistent relationship between plasma PAA and PBA levels among healthy volunteers, patients with cirrhosis and UCD patients, despite that fact that, as described in detail below,

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all groups exhibited similar ammonia scavenging activity based on urinary excretion of PAGN. Overall, this shows that urinary PAGN provides a convenient method for monitoring ammonia elimination induced by the administered drug, which does not require drawing blood and directly relates to the actual nitrogen elimination provided by the administered nitrogen scavenging drug without being influenced by the many other factors that can affect plasma ammonia levels.

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Plasma Pharmacokinetics of PBA, PAA, and PAGN Comparison across Studies

Analyte	Treatment	C_{max} (μg/mL)	T_{max} (h)	T½ (h)	AUC_{24} (μg·h/mL)
Healthy Volunteers (Single Dose – 3 g/m²/day PBA Mole Equivalent)					
PBA	Sodium PBA	221.0	0.9	0.7	542.6
	HPN-100	37.0	2.4	1.9	137.2
PAA	Sodium PBA	58.8	3.9	1.2	279.8
	HPN-100	14.9	4.0	NC	70.9
PAGN	Sodium PBA	63.1	3.2	1.7	395.1
	HPN-100	30.2	4.0	NC	262.1
Healthy Volunteers and Cirrhotic Patients (100 mg/kg BID) [1]					
PBA	Child-Pugh A	42.8	2.3	1.2	131.7
	Child-Pugh B	41.8	2.9	3.4	189.5
	Child-Pugh C	44.3	3.1	1.9	192.1
	Volunteers	29.8	3.0	2.1	132.7
PAA	Child-Pugh A	33.2	3.8	1.8	168.8
	Child-Pugh B	30.8	4.5	2.8	252.4
	Child-Pugh C	53.1	4.8	7.7	579.9
	Volunteers	25.5	3.6	1.9	130.5
PAGN	Child-Pugh A	37.7	3.9	5.0	335.1
	Child-Pugh B	38.1	4.0	7.5	466.99
	Child-Pugh C	43.1	5.3	4.0	578.4
	Volunteers	46.3	4.3	7.2	550.9
UCD Subjects (Multiple Dose – PBA Mole Equivalent)					
PBA	Sodium PBA	141.0	2.1	NC	739.0
	HPN-100	70.1	6.1	NC	540.0
PAA	Sodium PBA	53.0	8.1	NC	595.6
	HPN-100	40.5	8.0	NC	574.6
PAGN	Sodium PBA	83.3	7.2	3.9	1133.0
	HPN-100	71.9	8.0	4.8	1098.0

C_{max}= maximum plasma concentration; T_{max} = time of maximum plasma concentration; AUC_{24} = AUC from time 0 to 24 hours; NC = not calculated

[1]Study did not include a sodium phenylbutyrate comparator arm, values represent HPN-100 dosing only. AUC values represent the AUC from time 0 to the last measurable plasma concentration.

[0026] One embodiment of the invention is a method for determining and/or adjusting the dose of ammonia scavenging drugs in patients with UCDs, whereby dose would be based on the amount of dietary protein the patient is consuming, the anticipated percentage conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. Dose adjustments, if necessary, would be based on the observed urinary excretion of PAGN and/or total urinary nitrogen (TUN), the difference between the two reflecting the patient's endogenous capacity for waste nitrogen excretion. This endogenous capacity may be absent in certain patients having innate urea cycle disorders due to inborn metabolic deficiencies,

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but patients with later-onset nitrogen accumulation disorders generally have some endogenous capacity, referred to sometimes as their residual urea synthesis capacity. See Brusilow, PROGRESS IN LIVER DISEASES, Ch. 12, pp. 293-309 (1995). The subject's plasma ammonia level may also be determined; this is a critical parameter for tracking effectiveness of an overall treatment program, but reflects a variety of factors such as dietary protein and physiological stress, as well as the effect of a drug used to promote nitrogen excretion.

[0027] Once the patient's residual endogenous capacity for waste nitrogen excretion has been determined, either as the difference between PAGN output and total nitrogen output or as total urinary nitrogen output in the absence of an ammonia scavenging drug, the tolerable amount of dietary protein can be calculated for that patient according to the dosage of the ammonia scavenging drug being administered, or the dosage of the ammonia scavenging drug can be adjusted or calculated to compensate for an estimated protein intake.

[0028] Another embodiment is a method for determining and adjusting the dose of an ammonia scavenging drug to be administered to a patient with liver disease, including hepatic encephalopathy, whereby the starting dose would be based on the amount of dietary protein the patient is consuming, the anticipated conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. While the urea synthetic capacity in patients with liver disease would generally be greater than for patients with UCDs, considerable patient to patient variability would be expected among both groups depending, respectively, on the severity of their liver disease and the severity of their inherited enzymatic defect. Dose adjustments based on the observed urinary excretion of PAGN and total waste nitrogen would adjust for these individual patient characteristics.

[0029] Another embodiment is a method for determining or adjusting allowable dietary protein in the diet of a patient with UCD or with hepatic encephalopathy, who is being treated with an oral PAA-forming ammonia scavenging drug, whereby the amount of allowable protein would be determined by the amount of PAGN and total nitrogen in the urine. The difference between total waste nitrogen in the urine and the amount of PAGN excreted is indicative of the patient's endogenous waste nitrogen processing capacity. Once the patient's endogenous nitrogen processing capacity is known, the patient's endogenous nitrogen processing capacity can be used to adjust dietary protein intake while administering a fixed dosage of an ammonia scavenging drug, or the dosage of the ammonia scavenging drug can be determined according to the amount needed to facilitate elimination of the waste nitrogen from the patient's dietary protein. Dietary protein intake should be determined or adjusted according to how much nitrogen the subject can eliminate above the amount that is

eliminated as PAGN, which results from the PAA-forming ammonia scavenging drug being administered. When making these calculations or adjustments, it is suitable to assume that about 47% of nitrogen in protein will become waste nitrogen that needs to be excreted in the urine (the amount may be less for growing patients, who retain a greater fraction of ingested nitrogen to support body growth), and that about 16% of protein, on average, is nitrogen (see Brusilow 1991).

[0030] It has generally been assumed for such determinations that a prodrug would be converted with 100% efficiency into PAGN for elimination [see, e.g., Berry et al., J. Pediatrics 138(1), S56-S61 (2001) where Figure 1 assumes 100% conversion]; and one report found that about 80-90% of PAA or PBA was excreted from a specific individual as PAGN. Brusilow, Pediatric Research 29(2), 147-150 (1991). It has now been found that HPN-100 and phenylbutyrate are both converted into urinary PAGN at an overall efficiency of about 60% to about 75% on average (about 60% conversion efficiency was seen in UCD patients and about 75% conversion was seen in cirrhotic patients, for example); consequently, this efficiency factor can be used to more accurately calculate or determine initial dosing levels for these drugs, or dietary protein levels acceptable for patients who use these drugs. Given this conversion rate, each gram of HPN-100 can facilitate elimination of waste nitrogen from about a gram (~1.3 grams) of dietary protein per day. Note that PAGN carries away two molecules of ammonia per molecule of PAGN. Examples of calculations based on these parameters are provided in Examples 9 and 10 herein.

[0031] In one aspect, the invention provides a method for transitioning a patient from phenylacetate or phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate, and is adjusted according to the levels of excreted PAGN that result when the prodrug is administered.

[0032] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of ammonia scavenging during the transition (e.g. for clinically 'fragile' patients with a propensity for frequent hyperammonemia). The methods can use two, three, four, five, or more than five steps as judged clinically prudent. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate, amount (i.e. the amount necessary to deliver an equimolar

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amount of PBA) of HPN-100 or other prodrug of phenylbutyrate, e.g., if the transition is to be done in three steps, about one-third of the phenylbutyrate would be replaced with a prodrug at each step.

[0033] Another embodiment of the invention is based on observations that delivery of PBA in the form of a glyceryl tri-ester or other prodrug imparts slow release characteristics that allow greater flexibility in dosing schedule. Sodium phenylbutyrate (sodium PBA), for example, is typically dosed every 4 to 8 hours, or even more frequently, in order to maintain a suitable plasma level of PAA. This regimen reflects the rapid absorption of phenylbutyrate from the gastrointestinal tract and quick metabolic conversion to PAA. HPN-100, by contrast, which is a glyceryl tri-ester of phenylbutyrate, has been found to be absorbed only 40% as rapidly as sodium PBA, enabling dosing three times daily, such as with meals, or even twice daily, such as morning and evening. This dosing flexibility is further enhanced by the fact that the pharmacokinetic (PK) and pharmacodynamic (PD) properties of HPN-100 are indistinguishable in the fed or fasted states. It is thus not critical for the frequency of administration to be rigidly maintained with the PBA prodrugs in the form of an ester; the number of doses per day can be reduced for greater convenience, and the dosages do not have to be linked to meal schedules as is recommended in the label for sodium PBA. Indeed, pharmacokinetics for utilization of HPN-100 were very similar when HPN-100 was taken with food or without food, after a day of fasting, so HPN-100 can be taken with food or without food. This translates into a more convenient treatment protocol and potentially higher patient compliance upon substituting HPN-100 for phenylbutyrate or phenylacetate. Surprisingly, even though HPN-100 and sodium PBA are both prodrugs of PAA, HPN-100 is effective when administered less frequently than sodium PBA. While it is typically necessary to administer smaller doses of sodium PBA 3-6 times per day to maintain a stable level of plasma ammonia, similar results can be achieved with only 2-3 doses of HPN-100 per day. In some embodiments discussed in greater detail below, HPN-100 is administered in two doses per day (BID), and in some embodiments it is administered in three doses per day (TID).

[0034] It has also been found that because of the slow-release characteristics of HPN-100, a patient taking HPN-100 has more sustained and often lower plasma levels of PBA and PAA than a patient taking sodium PBA itself. This is believed to be consistent with the greater flexibility in dosing that is discussed in more detail elsewhere in this application (plasma levels of PBA rise and fall more quickly after administration of sodium PBA than after administration of HPN-100).

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[0035] Other aspects of this invention relate to the observation that there is apparently no saturation in the ability of the body to convert sodium PBA or HPN-100 to urinary PAGN over a several-fold dose range up to and including, the maximum doses of sodium PBA recommended to date. This should enable a patient to take a higher dose of HPN-100 than an equimolar amount compared to the patient's dosage of PBA. It suggests a patient can receive a higher dosage of HPN-100 than those dosages of sodium PBA that have been recommended to date, which is especially useful for patients whose ammonia levels were not adequately controlled by the highest labeled dosages of sodium PBA. Such patients can receive doses of HPN-100 that are higher than previously recommended sodium PBA dosages.

[0036] Other aspects of the invention will be apparent from the following detailed description and the examples provided herein.

[0037] For convenience, the amounts of PAA (phenylacetic acid), PBA (phenyl butyric acid), or HPN-100 to be administered to a subject as discussed herein refer to a total daily dosage. Because these compounds are used in relatively large daily amounts, the total daily dosage may be taken in two, three, four, five, or six, or more than six daily doses, and different drugs may be administered on different schedules. Thus the total daily dosage better describes a treatment regimen with one drug for comparison to treatments with related drugs.

Brief Description of the Drawings

[0038] Figure 1 shows waste nitrogen disposal via the urea cycle and by the auxiliary pathway involving PAGN.

[0039] Figure 2 depicts a conventional model to describe pharmacokinetic (PK) behavior of a prodrug, which, in the case of phenylbutyrate, assumes that PBA and PAA must reach the systemic circulation in order to be active; i.e., in order to be converted to PAGN and effect ammonia scavenging.

[0040] Figure 3 depicts an adapted model to describe PK behavior of sodium PBA or other drugs such as HPN-100 that can be converted to PBA and PAA, informed by the observations described herein showing that metabolism of HPN-100 results in lower plasma levels of PAA and PBA while providing equivalent pharmacological effect. Unlike the conventional model, this model allows for 'pre-systemic' conversion of PBA/PAA to PAGN and explains inconsistent relationship between blood levels of these metabolites and PAGN-mediated excretion of waste nitrogen

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[0041] Figure 4 shows how plasma levels of PAA, PBA, and PAGN change over time following administration of a single dose of either PBA or HPN-100. It shows that the peak level of PAA is lower when the PBA prodrug, HPN-100, is used, and the PAA level at 24 hours post-administration is higher with the prodrug. Thus the prodrug provides a more sustained level of plasma PAA.

[0042] Figure 5 presents data on ammonia levels from the tests in Example 3.

[0043] Figure 6 presents an anatomic explanation for the observations that the prodrug (PBA) can be converted to PAGN prior to reaching the systemic circulation (corresponds to the model depicted in Figure 3).

[0044] Figure 7 shows that PBA levels fluctuate relatively rapidly after dosing in healthy adults, while PAA and PAGN levels reach a fairly stable state after a few days of treatment with sodium phenylbutyrate.

[0045] Figure 8 shows that PBA, PAA and PAGN levels reach steady states at different times in healthy adults and that PAA takes longer to reach a steady state level in cirrhotics

[0046] Figures 9a, 9b, and 9c show that in subjects treated with HPN-100, there is little or no correlation between the dose of HPN-100 and plasma levels of either PBA or PAA in the subject. However, it also shows that urinary excretion of PAGN correlates well with dosage of HPN-100.

[0047] Figure 10 shows plasma ammonia levels [time-normalized area under the curve, or TN-AUC or Area under the curve (AUC)] during the day and night for 10 UCD patients treated for seven days with either sodium PBA or an equimolar dosage of HPN-100, and illustrates that HPN-100 provided better control of ammonia levels than PBA: both the AUC (area under the curve), which is an index of total ammonia exposure, and Cmax, which measures the peak concentration of ammonia, were lower in subjects receiving HPN-100 than in subjects receiving an equimolar dosage of PBA.

[0048] Figure 11 shows that HPN-100 did a better job than PBA of managing plasma levels of nitrogen overnight.

[0049] Figure 12 demonstrates that in patients whose ammonia levels were well controlled on sodium PBA, HPN-100 maintained control. By contrast, patients whose ammonia levels were elevated despite treatment with sodium PBA exhibited the greatest benefit in terms of improved ammonia control from HPN-100.

[0050] Figure 13 summarizes the data from Figure 12 and provides a statistical comparison of ammonia levels for patients on sodium PBA and those on HPN-100. It also shows the normal range for each set of patients.

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Modes of Carrying Out the Invention

[0051] In one aspect, the invention is reduced to practice in determining the dose, dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy. The starting dose and schedule would be based upon the theoretical considerations including the estimated percentage conversion of the drug to PAGN, the waste nitrogen resulting from the patient's dietary protein and the percentage of drug converted to and excreted as PAGN. Following initiation of treatment, further dose adjustments would then be made if necessary, upon the actual measurement of urinary PAGN output, or a well-correlated parameter like total urinary ammonia or the ratio of PAGN to creatinine.

[0052] In another aspect, the invention provides a method to transition a patient from phenylbutyrate or phenylacetate to a prodrug of phenylbutyrate (which is a prodrug of PAA), such as HPN-100, or other ester or prodrugs such as compounds of Formula I and II as shown herein. For a number of reasons, HPN-100 is considered a more desirable drug than sodium PBA for many patients who have high ammonia levels and require treatment with an ammonia scavenging drug. In particular, it avoids the unpleasant taste associated with sodium PBA, and it reduces potentially harmful sodium intake, since phenylbutyrate is administered as a sodium salt. A large majority of patients (nine out of ten UCD patients who participated in the clinical study described in example 3) preferred HPN-100 over sodium PBA in clinical testing. Thus many patients who have been treated with phenylbutyrate as an ammonia scavenging drug may want to transition from it to HPN-100.

[0053] It would seem logical for a physician to transition a patient from phenylbutyrate to a prodrug of phenylbutyrate by calculating the amount of the prodrug that would produce an amount of PBA that corresponds to the dosage of phenylbutyrate previously administered to the patient. This would be expected to produce about the same blood plasma level of the active ingredient, PBA. Efficacy of the new treatment with the prodrug could then be assessed by monitoring levels of phenylbutyrate in the blood, to establish the same levels achieved when PBA was administered. As discussed below, however, that approach is not appropriate because, surprisingly, plasma levels of PBA do not correlate well with administered dosages of HPN-100 or with the effectiveness of a dose of HPN-100 or sodium PBA. (Note that sodium PBA is the acid form of phenylbutyrate, which is the common name for the drug BUPHENYL®, and is typically administered as BUPHENYL®, which is a

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sodium salt of PBA. References to treatment with PBA herein encompass administration of the phenylbutyrate neutral compound or a salt of phenylbutyrate. Typically, and in all of the working examples herein, PBA is administered as BUPHENYL®.)

[0054] Alternatively, since PBA is a prodrug for PAA, the dosage of a phenylbutyrate prodrug could be calculated according to the theoretically formed amount of PAA, which should be the same amount as what would be calculated from the PBA dosage, since one molecule of PBA is expected to produce one molecule of PAA. The molecular weight of sodium PBA, the registered drug form of PBA (the sodium salt of PBA), is 186; the molecular weight of HPN-100 is 530, and of course HPN-100 provides three equivalents of PBA per molecule, so only one-third as many moles of HPN-100 would be needed to replace a molar quantity of either PBA or PAA. Thus each gram of sodium PBA could be replaced by 0.95 grams of HPN-100; and since HPN-100 is a liquid having a density of 1.1 g/mL, each gram of sodium PBA would be replaced by 0.87 mL of HPN-100, assuming HPN-100 is used as an undiluted liquid. This can be used to select a starting dosage of HPN-100 for patients being transitioned from sodium PBA to HPN-100. Alternatively, a starting dose of HPN-100 in a patient not already taking BUPHENYL® (sodium phenylbutyrate) would need to take into account the surprising observation described in more detail below (see examples 2 and 3) that conversion of the PBA, when administered as HPN-100, into urinary PAGN is incomplete and averages about 60-75%.

[0055] Alternatively, the physician could measure plasma levels of either PBA or PAA in a subject receiving an effective amount of PBA, and determine a dosage of a PBA prodrug by administering enough of the prodrug to produce the same plasma levels of PBA or PAA. The physician could then monitor the amount of either PBA or PAA in the blood to ensure that the appropriate amount of active drug was being produced in the body. It might be expected that a prodrug of phenylbutyrate would provide a slightly lower blood plasma concentration of PAA or PBA than phenylbutyrate, and thus a lower nitrogen-scavenging effect, since conversion of the prodrug to the active drug might be less than 100% efficient. Thus monitoring PAA or PBA plasma levels and increasing the prodrug dosage to bring levels up to those obtained by administering phenylbutyrate might be expected to produce the same physiological effect as the phenylbutyrate dosage. However, it was found that it is not necessary for the plasma level of PAA or PBA observed upon administration of a prodrug of phenylbutyrate to match that produced by an effective

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amount of phenylbutyrate, in order to achieve the same ammonia-scavenging effect. Rather, efficacy of the prodrug HPN-100 correlates with urinary PAGN levels, not with plasma levels of PAA or PBA.

[0056] Models have been developed to describe how ammonia-scavenging drugs or prodrugs are expected to behave *in vivo*. One model, shown in Figure 2, reflects conventional approaches to assessing drug effectiveness as applied to HPN-100 based on blood levels of PAA or PBA. Clinical testing has shown that HPN-100 does not produce the plasma levels of PAA and PBA that might be expected from this model, though, even though it is at least as effective on an equimolar basis as PBA for controlling blood ammonia levels, and for eliminating ammonia as PAGN via the urine. Thus the conventional model fails to account for some important metabolic differences between PBA and HPN-100. It was hypothesized that, as compared with sodium PBA, a greater percentage of PBA derived from HPN-100 is converted into PAGN for elimination (or PAA or PBA derived from it) before entering the systemic circulation (the "central compartment" in Figure 2). Recognition of this important and unexpected difference underlies certain aspects of the present invention.

[0057] A refined working model based upon the observations described herein and as outlined in this disclosure is depicted in Figure 3. It supports the conclusion that PBA derived from HPN-100 as well as from sodium PBA can be converted into PAGN without entering into systemic circulation; presumably, HPN-100 or its initial metabolic products (*e.g.*, a compound of formula I wherein one or two of R_1-R_3 represent phenylbutyryl groups, and the remaining one or two of R_1-R_3 represent H—the expected products of partial hydrolysis of HPN-100) may reach the liver and be converted into PAGN there, prior to reaching the systemic circulation. Moreover, the fractional conversion of PBA derived from HPN-100 is greater than for PBA absorbed when PBA is administered as the salt, an observation which explains the lower blood levels of PBA following administration of HPN-100 as compared with sodium PBA despite equivalent or potentially superior ammonia scavenging activity. This observation led to the recognition that plasma levels of PAA or PBA are not reliable indicators of the effectiveness of a PBA prodrug like HPN-100, and should not be relied upon to set or adjust dosages of such PBA prodrug compounds. Data presented herein, e.g. as summarized in Figure 9, demonstrate this effect. Alternative methods for monitoring a subject treated with HPN-100 are needed, and are provided herein.

[0058] In addition, PK/PD modeling, as reflected by considerations and depicted in figures 3 and 6, demonstrate that HPN-100 is absorbed only about 40% as rapidly as PBA

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when dosed orally. As a result, HPN-100 provides a slow-release delivery effect, even though it appears to metabolize to PBA rapidly once absorbed. This provides greatly flexibility in dosing and explains why HPN-100 can be dosed, e.g., three times per day or even twice per day to provide similarly stable ammonia levels that require four or more doses of PBA to achieve.

[0059] In view of these observations of unexpected pharmacokinetic behavior, plasma PAA and PBA levels should not be used to evaluate or monitor treatment of a subject with HPN-100 or sodium PBA. Alternative methods are needed, and are provided herein, for monitoring a subject treated with HPN-100. For one, it has been found that between 50 and 85% of HPN-100 is converted into urinary PAGN, typically about 60% to about 75%. This conversion efficiency for HPN-100 and sodium PBA in UCD patients is surprising in light of previous references that have generally assumed the conversion efficiency of sodium PBA to be about 100%. Urinary PAGN has been shown to be inversely correlated with levels of waste nitrogen, e.g. ammonia, in the blood, thus efficacy of HPN-100 can be evaluated by measuring urinary PAGN. It has also been found that HPN-100 has little to no effect on creatinine levels. Moreover, because creatinine levels in healthy adults and patients with nitrogen retention states are typically rather stable, either measuring PAGN output in urine over time, or measuring the ratio of the concentrations of PAGN to creatinine, which can be conveniently done in spot testing, provides a way to monitor HPN-100's effectiveness. In one aspect, the invention thus provides a method to assess the effectiveness of a treatment with HPN-100, comprising determining the ratio of PAGN to creatinine in a 'spot urine' test. Clinical studies show that urinary excretion of PAGN, and the ratio of PAGN to creatinine in urine, correlate well with blood ammonia levels: an increase of PAGN or of the PAGN / creatinine ratio correlates with decreasing plasma ammonia levels. Accordingly, in one method, HPN-100 treated patients are monitored by measuring urinary PAGN output, or by measuring the ratio of PAGN to creatinine in spot urine testing. This method can be used to monitor treatment of a treatment-naïve patient, or of a patient being transitioned from PBA to HPN-100, or a patient being treated with HPN-100. Increasing levels of urinary PAGN output, or an increase in the ratio of PAGN to creatinine in spot testing provides a way to determine whether a dosing regimen that utilizes HPN-100 or another PBA prodrug is promoting elimination of excess ammonia, and to compare two treatment methods to determine which is more effective for the particular subject.

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[0060] While plasma ammonia levels are often used to assess disease control in UCD patients, it is often inconvenient to rely upon plasma ammonia levels for optimizing the dosing of HPN-100 outside of a clinical setting. Moreover, plasma ammonia levels are affected by many factors and might be elevated regardless of how well a drug treatment works; it reflects dietary and other factors as well as the adequacy of a drug dosage being used. Plasma ammonia varies a good deal even when relatively well-controlled, based on meal timing, drug timing, and various other factors. Thus to meaningfully reflect drug effect, the plasma ammonia levels need to be monitored over time by repeated blood samplings, which is not practical for routine monitoring of some patients and which does not provide direct information about whether an ammonia scavenging drug is working. Measurements of urinary PAGN, on the other hand, can be done more conveniently as a routine monitoring method because they do not require medical assistance to collect the samples for testing. Moreover, urinary PAGN specifically measures the waste nitrogen clearance provided by the scavenging agent, while many other factors affecting ammonia levels may cause ammonia control to be misleading with regard to the actual effect of the nitrogen scavenging drug. Thus, even though in theory a number of different parameters could be measured to assess effectiveness of a dosage of HPN-100, only measurements based on urinary PAGN are both convenient and reliable as a direct measurement of the nitrogen scavenging drug's effect.

[0061] Thus in one embodiment, the invention provides a method to monitor the effectiveness of treatment of a UCD patient with HPN-100, where monitoring consists essentially of monitoring the patient's urinary PAGN excretion, and optionally checking plasma ammonia levels. Urinary PAGN levels comparable to those achieved with a previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level of less than about 40 μmol/L, or of not greater than 35 μmol/L would indicate the treatment was effective. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test.

[0062] In another aspect, the invention provides a utilization efficiency factor for HPN-100 or for sodium PBA of about 60% to about 75%, which can be used to more accurately determine an initial starting dose of either drug and/or correlate dietary protein intake with projected urinary PAGN.

[0063] In one aspect, the invention provides a method for transitioning a patient from phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method

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involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylbutyrate. For example, the amount of HPN-100 needed to provide an equal molar amount of PBA would be calculated (an equimolar amount), and this equimolar amount would be administered to the patient. Urinary excretion of PAGN or plasma ammonia levels would be monitored, and the dosage of HPN would be increased or decreased as needed to establish a level of PAGN excretion that is about the same as that provided by a previously used effective amount of phenylbutyrate or another nitrogen scavenging drug. Typically, a subject being transitioned from PAA or another PAA prodrug onto HPN-100 using this method would be tested for urinary PAGN output prior to the transition and afterwards, and the dosage of HPN-100 would be adjusted as needed to match the urinary PAGN output from this patient when treated with the previous PAA drug or prodrug, assuming the previous PAA prodrug treatment was considered effective. This provides a safer and more effective transition to the new prodrug than methods that rely upon using an equimolar amount without monitoring the *in vivo* effects of that amount of the new drug. It also avoids the risk of inaccurate dosing and potential overtreatment that could result if one monitored PAA or PBA and tried to adjust the prodrug (i.e. HPN-100) dosage to match the PAA or PBA level to the corresponding level provided by administering sodium phenylbutyrate itself.

[0064] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of ammonia scavenging during the transition. In some embodiments, a patient taking an initial dosage of phenylbutyrate is transitioned from phenylbutyrate to a prodrug of phenylbutyrate in steps. The methods can use two, three, four, five, or more than five steps. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate amount of HPN-100 or other prodrug of phenylbutyrate. The appropriate amount for each step can be approximately an amount sufficient to provide an equal molar amount of PBA if it is assumed that the prodrug is quantitatively converted into PBA. Note, too, that BUPHENYL® (sodium phenylbutyrate) contains about 6% inactive ingredients, so it is appropriate to base calculations upon the PBA content of the drug rather than on the weight of the formulated drug. The patient is then monitored to determine how much ammonia scavenging effect has been provided. The amount of HPN-100 (or prodrug) can then be

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adjusted to produce about the same amount of ammonia excretion in the form of excreted PAGN that was achieved by the initial dosage of phenylbutyrate, if the patient was well controlled.

[0065] A physician who is switching a patient from PBA to HPN-100 or another ester of phenylbutyrate should be aware that an effective amount of HPN-100 does not necessarily produce a PAA or PBA level that is as high as those seen when sodium phenylbutyrate is administered. It is reported that PAA exhibits some toxicity at high plasma concentrations. Thibault, et al., *Cancer Research*, 54(7):1690-94 (1994) and *Cancer*, 75(12):2932-38 (1005). Given this, and given the unique properties of HPN-100 described above, it is particularly important that a physician not use plasma levels of PAA or PBA to measure the efficacy of HPN-100. If one administers HPN-100 in amounts sufficient to match the plasma PBA or PAA levels provided by administering phenylbutyrate, for example, the dose of HPN-100 may be unnecessarily high.

[0066] The treatment-naïve patient is one not presently receiving an ammonia-scavenging drug treatment to manage nitrogen levels. While there are recommended dosage levels for the nitrogen scavenging drugs in many cases, the right dosage for a naïve patient may be lower than those ranges, for example, and, less commonly, it may be above an equimolar amount when compared to the dosages recommended for sodium PBA. The initial dosage of PAA or a PAA prodrug can be calculated by methods known in the art once a patient's dietary intake of protein is known, and assuming the patient has a relatively normal liver function. Saul W Brusilow, "Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion," *Pediatric Research* 29:147-150, (1991). Methods are also know for measuring the total amount of nitrogen excreted in the urine; in the case of a subject taking a drug that acts by providing PAA, the total waste nitrogen will include PAGN excreted.

[0067] It is estimated that about 47% of nitrogen in proteins consumed will be converted into waste nitrogen, and that about 16% of protein on average is nitrogen. Using these figures, and assuming HPN-100 is efficiently converted to PAGN, a daily dosage of about 19 g of HPN-100 would provide a vehicle to excrete the waste nitrogen from about 43 g of dietary protein; each gram of HPN-100 would thus be able to carry away waste nitrogen from about 2 g of dietary protein. In addition, if it is estimated that HPN-100 utilization efficiency is between about 50% and 85% in various individual patients (as disclosed herein, it has been found that about 60-75% of HPN-100 is converted into urinary PAGN on average), which is consistent with clinical observations to date, and these factors can be

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used to further refine the relationship between dietary protein intake and HPN-100 dosing levels for a given subject. With this refinement, each gram of HPN-100 would assist with removal of waste nitrogen for about 1 gram (~ 1.3 grams) of dietary protein. This factor can be used to calculate a suitable dosage of HPN-100 if dietary protein intake is known or controlled, and it can be used to calculate a tolerable dietary protein intake for subject receiving HPN-100.

[0068] This method can also be used to establish a recommended daily dietary protein intake for a patient, by determining the patient's endogenous nitrogen elimination capacity, calculating an amount of dietary protein that this endogenous capacity permits the patient to process without assistance from a nitrogen scavenging drug, and adding to the amount of dietary protein the patient can process on his/her own an amount of protein that the patient would be able to process when using a particular dosage of PBA or a PBA prodrug like HPN-100. Using HPN-100 as an example, a maximum daily dosage of about 19 grams of HPN-100, utilized at an estimated efficiency of 60%, would enable the treated patient to eliminate waste nitrogen corresponding to about 40 g of dietary protein. Thus the invention provides a method to establish a suitable dietary protein level for a patient having a urea cycle disorder or HE, by adding this amount of protein to the amount the patient's endogenous nitrogen elimination capacity can handle.

[0069] In some embodiments, it is also useful to measure PAGN excretion, which accounts for some of the total waste nitrogen excreted when PAA or a PAA prodrug is working. The total waste nitrogen excreted minus the amount of PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via the urea cycle or other mechanisms, and is helpful in determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific. Dosage of HPN-100 can then be established by determining the subject's endogenous capacity to eliminate waste nitrogen; subtracting the amount of dietary protein corresponding to the subject's endogenous nitrogen elimination capacity; and providing a dosage of HPN-100 sufficient to permit the subject to handle the balance of waste nitrogen, based on the subject's dietary protein intake.

[0070] The plasma or blood level of ammonia is optionally also determined, in addition to measuring urinary PAGN, to assess the effectiveness of the overall drug and dietary regimen for a particular patient. If the ammonia control is inadequate, the dosage of the

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nitrogen scavenging drug may need to be increased if that can be done, or the patient's dietary protein intake can be decreased if that is feasible.

[0071] In some instances, the dosage of HPN-100 may be limited to dosages that do not exceed recommended dosing levels for phenylbutyrate, adjusting for the fact that each mole of HPN-100 can produces three moles of phenylbutyrate. The label for the use of sodium PBA for the chronic treatment of UCDs recommends a daily dosage not to exceed 20 g; a daily dosage in a range of 9.9-13.0 g/m^2 set according to the subject's size for subjects over 20 kg in weight; and a dosage within a range of 450-600 mg/kg for subjects weighing less than or equal to 20 kg is indicated. While lower doses of HPN-100 may provide comparable ammonia scavenging to PBA on a molar equivalent basis, it may be suitable to select a higher dosage of HPN-100 to achieve adequate ammonia control for certain subjects. Typically, that dose will not exceed the recommended ranges for dosages of phenylbutyrate for a given indication. Thus it may be appropriate to administer HPN-100 at a daily dosage not to exceed an amount of HPN-100 that corresponds to the molar amounts of phenylbutyrate described above (and correcting for the fact that HPN-100 can provide three molecules of PBA). For a subject weighing more than 20 kg, a dosage range for HPN-100 would be between 8.6 and 11.2 mL/m^2. For a subject weighing less than 20 kg, a dosage range of about 390 to 520 μL/kg per day of HPN-100 would be appropriate, based on the use of an equimolar amount compared to the recommended doses of HPN-100. There is no evidence to suggest that HPN-100 would produce adverse effects at a rate in excess of that from an equimolar amount of sodium PBA, so the daily recommended upper limit of 20 g per day of sodium PBA suggests that a daily dose limit of HPN-100 based on the recommendations for sodium PBA would correspond to an equimolar amount of HPN-100, or about 19 g or 17.4 mL.

[0072] Thus in one embodiment, the invention provides a method to monitor the effectiveness of a treatment of a UCD patient with HPN-100, where monitoring consists of, or consists essentially of, monitoring the patient's urinary PAGN excretion and/or plasma ammonia levels. Urinary PAGN levels comparable to those achieved with a previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level that was normal, e.g., a level of less than about 40 μmol/L, or of not greater than 35 μmol/L, would indicate the treatment was effective. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test.

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[0073] However, it has also been found that HPN-100 exhibits no indications of toxicity at equimolar doses when compared to the approved PBA dosage of 20 g / day and a dose 2-3 times the equivalent of 20 grams of PBA is unlikely to produce PAA blood levels leading to AEs. Moreover, tolerability of taking HPN-100 is much higher than for PBA and a linear relationship has been observed between HPN-100 dose and PAGN output up to doses of 17.4 mL. In some patients or clinical settings, HPN-100 doses well above the approved PBA dosage are expected to be beneficial; for example, in UCD patients who exhibit recurrent hyperammonemia even on maximal doses of sodium PBA, in UCD patients who need increased dietary protein to support body requirement, or in patients with other nitrogen retaining states.

[0074] Thus in another embodiment, the invention provides methods to treat a subject having HE or UCD, with a dosage of HPN-100 that corresponds to between 100 and 300% of the equimolar amount of the recommended highest dose of PBA. In some embodiments, the suitable dosage will be between about 120% and 180% of the highest recommended dose of PBA; in other embodiments it will be between 120-140% or from 140-160% or from 160-180% of the equimolar amount of the recommended highest dosage of PBA. In accordance with this aspect, the daily dosage of HPN-100 could be as much as 57 g, or up to about 38 g, or up to about 33 g, or up to about 30g, or up to about 25g.

[0075] In one aspect, the invention provides a method to identify the starting dose or dose range and to individually adjust the dose or dose range of a nitrogen scavenging drug comprising PAA or a PAA prodrug (including HPN-100) used for the management of a treatment-naïve patient, which method comprises the steps of:

a) administering an initial dosage of the drug estimated according to the patient's dietary protein load, taking into account the expected percentage conversion to PAGN

b) measuring the amount of total waste nitrogen excreted following administration of the nitrogen scavenging drug comprising PAA or a PAA prodrug;

c) measuring blood ammonia to determine if the increase in urinary excretion of total waste nitrogen is sufficient to control blood ammonia levels; and

d) adjusting the initial dosage to provide an adjusted dosage of the nitrogen scavenging drug comprising PAA or a PAA prodrug based upon ammonia control, dietary protein, and the amount of total waste nitrogen excreted by the patient, or the amount of waste PAGN excreted. Either or each of these parameters can be monitored to assess the dosage of HPN-100 or other nitrogen scavenging drug being administered. Optionally, the

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method also includes determining the subject's endogenous nitrogen eliminating capacity (residual urea synthesis capacity) to further help determine an initial dose of HPN-100.

[0076] The initial dosage of the HPN-100 for a treatment naïve patient can be calculated as the amount of waste nitrogen that needs to be eliminated based on the patient's dietary protein intake. This amount can be reduced by an amount equivalent to the waste nitrogen the patient can eliminate using the patient's endogenous waste nitrogen elimination capacity, which can be measured as described herein. The suitable starting dose of HPN-100 can be calculated by estimating dietary protein intake that needs to be managed via the nitrogen scavenging drug, and providing a dose of drug amounting to about 1 g of HPN-100 per 1-2 grams of dietary protein in excess of the amount the patient's endogenous nitrogen elimination capacity can handle, taking into account the expected percentage conversion of the administered PBA to urinary PAGN. The method optionally further includes assessing urinary PAGN output to see if it accounts for the expected amount of waste nitrogen, and optionally may include measuring plasma levels of ammonia in the subject to ensure that an acceptable level of ammonia has been achieved. Checking the patient's plasma ammonia levels provides a measure of the effectiveness of the overall treatment program, including diet and drug dosing.

[0077] The table below summarizes the amount of dietary protein that doses of HPN-100 below (dose 1), within (dose 2) and above (dose 3) those corresponding to the recommended dosages of sodium PBA would be expected to 'cover' (i.e. mediate resulting waste nitrogen excretion), given the following assumptions: 1 gram of PAA mediates the excretion of ~0.18 grams of waste nitrogen if completely converted to PAGN; 60% of the PAA delivered as the PBA prodrug released from HPN-100 is converted to PAGN; 47% of dietary protein is excreted as waste nitrogen, and 16% of dietary protein consists of nitrogen (Brusilow 1991; Calloway 1971). These factors can be used when relating dietary protein intake, drug dosing and waste nitrogen elimination for purposes of the present invention.

HPN-100 Doses and Expected Waste Nitrogen Excretion Based on Dietary Protein

Dose 1	3 mL BID	Corresponds to ~0.47x the dose administered in Example 2, for a 70 kg adult and ~0.35x the amount of PBA (~6.1 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~8.5 g of dietary protein
Dose 2	9 mL BID	Corresponds to ~1.42x the dose administered in Example 2, for a 70 kg adult and ~01.1x the amount of PBA (~18.2 g) delivered in the maximum

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		approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~26 g of dietary protein
Dose 3	15 mL BID	Corresponds to ~2.36x the dose administered in Example 2, for a 70 kg adult and ~1.73 x the amount of PBA (~30.3 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~43 g of dietary protein

[0078] As used herein, plasma levels of ammonia are acceptable when they are at or below a level considered normal for the subject, and commonly this would mean plasma ammonia level is below about 40 μmol/L. In certain clinical tests described herein the upper limit of normal for the subjects was between 26 and 35 μmol/L, and it is recognized in the art that a normal ammonia level will vary depending upon exactly how it is measured; thus as used to describe ammonia levels herein, 'about' means the value is approximate, and typically is within ± 10% of the stated numeric value.

[0079] In other aspects, the invention provides a method to identify a suitable starting dose or dose range for a UCD or HE patient and to individually adjust the dose or dose range of a new nitrogen scavenging drug used for the management of a patient already treated with a previous nitrogen scavenging drug, which method comprises the steps of:

a) administering an initial dosage of the new nitrogen scavenging drug (which can be estimated according to the patient's dietary protein load and/or the dose of the new drug expected to yield the same amount of urinary PAGN excretion as a previously used nitrogen scavenging drug);

b) measuring the amount of total waste nitrogen and/or of PAGN excreted following administration of the new drug;

c) optionally measuring blood ammonia to determine if the initial dosage is sufficient to control blood ammonia levels, or to establish a suitable average ammonia level; and

d) adjusting the initial dosage of the new drug as needed to provide an adjusted dosage based upon ammonia control, dietary protein, and the amount of total waste nitrogen excreted by the patient. The adjusting of the initial dosage is done based on the amount of urinary PAGN, without relying upon plasma levels of PAA, PBA, or PAGN, and preferably without relying upon plasma levels of ammonia.

[0080] Where the patient has previously been treated with PAA or a PAA prodrug, the treating physician may rely, wholly or in part, upon the previous treatment to set a dosage

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for a new PAA prodrug, or a PBA prodrug, to be administered to the same patient. If the previous drug was reasonably effective for managing the patient's condition, the physician may set the dosage for a new PAA or PBA prodrug by reference to the previous one, so that the new drug is administered at a dosage that provides the same dosage of PAA to the patient, assuming complete conversion of each prodrug into PAA.

[0081] Again, as discussed above, it is sometimes desirable to measure PAGN excreted in addition to total waste nitrogen excreted. The total waste nitrogen excreted minus the amount of PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms, and is helpful in determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific.

[0082] In another aspect, the invention provides a method to identify the amount of dietary protein that could be safely ingested by a subject with a nitrogen accumulation disorder, including hepatic encephalopathy and UCD, where the patient is taking an ammonia-scavenging drug that comprises PAA or a PAA prodrug, which method comprises the steps of:

a) measuring the amount of total waste nitrogen excreted following administration of the drug,

b) determining the amount of dietary protein calculated to yield an amount of waste nitrogen less than or equal to urinary waste nitrogen; and

c) adjusting dietary protein and/or drug dosage as appropriate based upon measurement of blood ammonia and total waste nitrogen excretion.

[0083] Where the subject is receiving treatment with a nitrogen-scavenging drug, it may be necessary to reassess the patient's dietary intake of protein periodically, since many factors will affect the balance between nitrogen intake, nitrogen excretion, and dosage of a nitrogen scavenging drug. The invention provides methods to determine how much dietary protein a patient can handle, based on measuring the patient's nitrogen excretion levels. It may further be useful to measure the patient's PAGN level as discussed above, to help determine the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms.

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[0084] In the above methods, the patient may be one having a urea cycle disorder, or other nitrogen accumulation disorders. In many embodiments, the methods are applicable to patient's having a urea cycle disorder, but relatively normal liver function.

[0085] The above methods can be practiced with a variety of prodrugs of PAA or PBA. In some embodiments, HPN-100 is the PBA prodrug of choice for these methods.

[0086] In another aspect, the invention provides a method to transition a patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the portion of phenylacetate or phenylbutyrate; and

c) monitoring the amount of PAGN excreted by the patient to assess the effectiveness of the replacement amount of the prodrug.

[0087] Optionally, this method comprises adjusting the amount of the prodrug and administering an adjusted amount of the prodrug, then further monitoring PAGN excretion to assess the effectiveness of the adjusted amount of the prodrug. The replacement amount of the PBA prodrug can be about an equimolar amount to the amount of PBA being replaced.

[0088] For reasons discussed extensively herein, it is misleading to rely upon PAA levels when moving a patient to a prodrug (or a new prodrug) of PAA or PBA. The availability of liver-based mechanisms for rapid conversion of a prodrug into PAGN without necessarily entering the systemic system renders plasma levels of PAA and PBA insufficient as predictors of efficacy, so the method relies upon the excreted PAGN for assessing and monitoring treatment with a PAA or PBA prodrug that is to be given to the patient.

[0089] In many cases, it will be possible to transition a patient directly from, e.g., phenylbutyrate to HPN-100 or another PBA prodrug in a single stage, rather than in incremental steps. Thus all of the previously used PAA or PAA prodrug may be replaced with a suitable substitution amount of the new drug (PBA prodrug). However, in some situations (e.g. 'fragile patients', patients taking dosages at or near the recommended limits of PAA or PAA prodrug, and for patients having very limited endogenous capacity for excreting nitrogen wastes, or in situations where the ability of the patient to metabolize or

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excrete the drug is uncertain), it may be preferable to transition from the initial drug to a new PBA prodrug like HPN-100 in two or more stages or steps. Thus the transition may be made in 2, 3, 4 or 5 steps, and at each step a fraction of the original drug (e.g, about half for a two-step transition, about a third for a three-step transition, etc.) is replaced by the new PBA prodrug to be administered. This approach might be appropriate for a 'fragile' UCD patient known to be susceptible to repeated episodes of hyperammonemia while receiving treatment or while taking a large amount of drug that promotes nitrogen elimination.

[0090] Thus in another aspect, the invention provides a method to transition a UCD patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the phenylacetate or phenylbutyrate; and

c) monitoring plasma level of ammonia in the patient to assess the effectiveness of the replacement amount of the prodrug.

[0091] In some embodiments, the replacement amount of the prodrug is an equimolar amount compared to the amount of PBA being replaced

[0092] During the monitoring step, the patient is being treated with a mixture of phenylacetate or phenylbutyrate plus the new prodrug. The proportion depends upon what step of the transition the patient is in. The physician can also use information about the effects of a first step in setting the replacement amount of the prodrug for use in subsequent steps; thus if the prodrug is significantly more effective than predicted when the estimated amount used as a replacement amount is administered in a first step, the replacement amount used in a subsequent step of the transition can be proportionally reduced.

[0093] In another aspect, the invention provides a method to initiate treatment with phenylacetate, phenylbutyrate or a PBA prodrug in a step-wise fashion, as might be appropriate for a 'fragile patient' (a UCD patient with a history of frequent symptomatic hyperammonemia and/or neonatal onset disease who presumably has no urea synthetic capacity, or a patient with severely compromised liver function whose ability to metabolize the drug may be uncertain). This process may be more complex, since the prodrug will rely

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upon liver function to be activated and to function; thus the method is preferably done in a stepwise fashion, exemplified by the following steps:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as excreted PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

[0094] The methods also include optionally measuring total urinary nitrogen and urinary PAGN after at least 3 days of drug administration, at which point a steady state has been achieved. It also can include calculating the amount of drug converted to PAGN, which would be expected to be at least 50%, to determine if the drug is having the desired effect. A suitable dosage of the drug would be identified as one where the amount of excreted PAGN is sufficient to clear the expected amount of waste nitrogen from the dietary intake of protein, which can be adjusted to account for the patient's endogenous nitrogen elimination capacity.

[0095] The fraction of nitrogen waste to be cleared in a single step can be selected with due regard to the severity of the patient's condition (nitrogen accumulation disorder). In some embodiments, it will be appropriate to target removal of about 50% of the waste nitrogen for which clearance assistance is needed. In some embodiments, the method will target removal of about 100% of the waste nitrogen.

[0096] In another aspect, the invention provides a method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

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c) determining the level of excreted PAGN for the subject to make sure it has not decreased; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

[0097] If it is found that the amount of excreted PAGN decreases, additional HPN-100 or additional PBA would be administered to reestablish a level of PAGN excretion that is suitable for the patient, and the replacement steps would then be continued until all of the PBA was replaced by HPN-100.

[0098] Here again, the portion of phenylbutyrate to be replaced in an initial step can be 100%, about 1/2, about 1/3, or about ¼, or some value between these. During a stepwise process, where less than all of the phenylbutyrate is replaced in a first step, the patient will receive both HPN-100 and phenylbutyrate. As demonstrated herein, the appropriate method for determining a suitable dose of HPN-100 will take account of the excreted PAGN, rather than being based only on less reliable criteria for evaluating the orally delivered PBA prodrug.

[0099] In another embodiment, the invention provides a method to administer a phenylbutyrate prodrug to a patient, comprising determining the rate of PAGN excretion for the subject following administration of at least one phenylbutyrate prodrug, and selecting or adjusting a dose administration schedule based on the PAGN excretion rate. The compound can be a compound of Formula I, Formula II or Formula III as described above. Advantageously, the compounds used herein as prodrugs of PBA achieve nitrogen scavenging comparable to that of PBA but exhibit a slow-release kinetic profile that produces a more stable ammonia level in the treated subject. In some embodiments, the methods of the invention include administering a prodrug as described herein to a subject at a dosage that provides comparable ammonia level control to that achieved by PBA, but with significantly lower exposure of the subject to systemic PBA. In some embodiments, the subject experiences pharmacokinetic parameters for PBA that demonstrate lower exposure to PBA, including a lower AUC and Cmax for PBA, while maintaining a plasma ammonia level comparable to or better than that provided by treatment with a dosage of PBA within the normal dosing range. When HPN-100 and PBA were administered to UCD patients at equimolar dosages, the patient receiving HPN-100 had overall lower plasma ammonia levels, and also lower PBA exposure:

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	AUC (NH_3) μg-hr/mL	C_{max} (NH_3) μg-hr/mL	AUC (PBA) μg-hr/mL	C_{max} (PBA) μg-hr/mL
PBA	38.4(20)	79.1(40)	739(49)	141(44)
HPN-100	26.1(10)	56.3(28)	540(60)	70(65)

[00100] While a larger data set is needed to demonstrate statistical significance, limited amounts of data are available in part due to the rarity of these conditions. Nevertheless, the data indicates that PBA treatment resulted in less effective ammonia level control and greater exposure to PBA, while the PBA prodrug HPN-100 at equimolar dosing provided better ammonia level control and lower PBA exposure levels. Accordingly, in one aspect the invention provides a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces better ammonia level control than PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA, when compared to treatment with an equimolar amount of PBA. In some embodiments, the treatment uses HPN-100 as the prodrug, and in some embodiments the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20%; or the exposure to PBA upon treatment with the prodrug is lower by at least about 30% compared to treatment with PBA; or both of these conditions are met to demonstrate reduced exposure to PBA. In some embodiments, the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the prodrug is administered. Preferably, the prodrug provides plasma ammonia levels that average less than about 40 μmol/L or not more than 35 μmol/L.

[00101] The advantageous slow-release kinetic profile of compounds used herein as prodrugs of PBA permits less frequent and more flexible dosing in selected patients as compared with sodium PBA. While all patients with UCDs and a propensity for elevated ammonia levels should in principle be able to benefit from the ammonia scavenging activity of HPN-100, UCD patients with substantial residual urea synthetic capacity (e.g. UCD whose first manifestations occur at several years of age or older; i.e. patients who do not exhibit neonatal onset) would be the best candidates for three times daily or even twice daily dosing with PBA prodrugs such as HPN-100. Patients with cirrhosis and HE would also be candidates for less frequent dosing, as even patients with severe liver disease have significant residual urea synthetic capacity (Rudman et al., J. Clin. Invest. 1973).

[00102] Specific embodiments of the invention include the following:

A. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an

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initial dosage of HPN-100, wherein monitoring the effect consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output.

In this method, the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and urinary PAGN output can be determined as a ratio of urinary PAGN to urinary creatinine, since it has been demonstrated by others that creatinine, the daily excretion of which tends to be constant for a given individual, can be used as a means to normalize measures of urinary parameters while correcting for variations in urinary volume. In these methods, the nitrogen retention disorder can be chronic hepatic encephalopathy or a urea cycle disorder. Plasma ammonia levels may also be monitored to adjust the overall treatment program and dietary protein intake, but as discussed above, urinary PAGN provides a preferred way to assess the drug's role in waste nitrogen elimination.

B. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100, wherein the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and wherein monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. In these methods, administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level in the patient. This can be a level of about 35 or about 40 μmol/L.

C. A method to determine a starting dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a utilization efficiency of about 60% to about 75%. In such methods, the dosage of HPN-100 can be calculated from the patient's dietary protein intake, or it can be estimated from the patient's body weight and approximate growth rate. In such methods, the dosage of HPN-100 is sometimes reduced to account for the patient's residual urea synthesis capacity, by adjusting the amount of HPN-100 to reflect the amount of ammonia scavenging needed in view of the patient's endogenous capacity for nitrogen elimination.

D. A method to determine a dosage of a PAA prodrug for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

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c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN.

In these methods, the PAA prodrug can be phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof, or it can be HPN-100.

E. A method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN; and

e) administering to the patient the suitable dosage of the PAA prodrug.

In these methods, the PAA prodrug is often phenylbutyrate or a pharmaceutically acceptable salt thereof, or HPN-100.

G. A method to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, comprising:

a) determining a replacement amount of HPN-100 to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the HPN-100 for the phenylacetate or phenylbutyrate; and

c) monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the HPN-100.

In these methods, an increase the amount of urinary PAGN may indicate that the amount of HPN-100 can be reduced, and a decrease in urinary PAGN may indicate the amount of HPN-100 needs to be increased.

H. A method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

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a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted urinary PAGN for the subject; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

I. A method to initiate treatment with phenylacetate, phenylbutyrate or a HPN-100 in a step-wise fashion, comprising:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion based upon diet and urea synthetic capacity; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as urinary PAGN taking into account the expected percentage conversion of the administered PBA to urinary PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

J. A method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA when the patient receives the PBA prodrug, when compared to the AUC and Cmax observed when the patient receives an equimolar amount of PBA.

In these methods, the PBA prodrug is often HPN-100.

The methods include a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA exposure can be lower with the prodrug than with PBA by at least about 20%, or by at least about 30% compared to treatment with PBA. This is believed to be related to the slow absorption or uptake

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characteristics of HPN-100, which provide a more stable level of PBA exposure and provide an unexpected advantage of HPN-100 to be effective with less frequent dosing when compared to sodium phenylbutyrate.

K. A method to determine a suitable dietary protein level for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's endogenous nitrogen elimination capacity;
b) calculating from the endogenous nitrogen elimination capacity an amount of dietary protein the patient can process without the aid of a nitrogen scavenging drug;
c) then adding an amount of protein that the patient should be able to process with the assistance of selected dosage of a nitrogen scavenging drug to arrive at an amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, taking into account the amount of protein required for health and body growth.

In this method, the nitrogen scavenging drug can be HPN-100. Commonly, the selected dosage of HPN-100 is not more than about 19 grams per day, and the amount of dietary protein the patient should be able to process with the assistance of this amount of HPN-100 is about 1 grams (~1.3 g) of protein per gram of HPN-100.

L. A method to treat a patient with a PBA prodrug, comprising administering HPN-100 at a daily dose in excess of 19 g per day to a subject having HE or UCD. Optionally, the daily dose of HPN-100 is between about 20 g and about 57 g.

M. A method for determining the dosing schedule of a PBA prodrug wherein the patient retains substantial residual urea synthetic capacity, as would be the case for most patients with cirrhosis and HE or most UCD patients who do not exhibit symptoms within the first two years of life.

[00103] In the foregoing methods that utilize HPN-100, the exposure to PBA upon treatment with the prodrug HPN-100 is lower by at least about 30% compared to treatment with PBA. Also, commonly the AUC for PBA is less than about 600 and the Cmax for PBA

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is less than about 100 when the prodrug is administered. Also, in the foregoing methods, when the subject is treated with the prodrug, which can be HPN-100, the subject will typically achieve and maintain normal plasma ammonia levels.

[00104] The following examples are offered to illustrate but not to limit the invention.

[00105] The data below from three human studies and one preclinical study illustrate that the conventional approach of assessing drug exposure and effect by measuring blood levels does not correlate with nitrogen scavenging as assessed by urinary excretion of PAGN or by reduction of plasma ammonia. These data demonstrate that, surprisingly, the plasma level of PBA or PAA seen with an effective amount of a prodrug can be far less the plasma level of PBA or PAA seen with a similarly effective amount of phenylbutyrate. Moreover, they demonstrate the need to allow for incomplete conversion of sodium PBA or HPN-100 into PAGN in selecting starting dosage, the delayed release behavior and implications for dosing schedule of delivering PBA as a triglyceride rather than as a salt, and the possibility of administering HPN-100 in doses greater than those currently recommended for sodium PBA. These are followed by a biological explanation for the findings.

Example 1

Single dose safety and PK in healthy adults

[00106] To assess its pharmacokinetic (PK) and pharmacodynamic (PD) profile, HPN-100 was administered as a single dose to 24 healthy adults. Pharmacokinetic samples were taken pre-dose and at 15 and 30 minutes post-dose and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours post-dose. As discussed below, plasma levels of the major HPN-100 metabolites PBA, PAA and PAGN were many fold lower after administration of HPN-100 than after sodium PBA. By contrast, urinary excretion of PAGN was similar between the two groups (4905 +/- 1414 mg following sodium PBA and 4130 +/- 925 mg following HPN-100) and the differences that were observed were determined to be largely an artifact of incomplete collection due to stopping urine collection at 24 hours (note that PAGN excretion following administration of sodium PBA was largely complete at 24 hours but continued beyond 24 hours following administration of HPN-100). Thus, the plasma metabolite concentrations did not accurately reflect the comparative ammonia scavenging activity of sodium PBA and HPN-100.

[00107] Three healthy adult volunteers were treated with a single dose of either sodium PBA or HPN-100 at a dosage of 3 g/m^2. Plasma levels of PAA, PBA, and PAGN were

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monitored periodically for 12-24 hours by known methods. Results of this are shown in Figure 4, which shows a curve for each subject (note the log scale).

[00108] In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving sodium PBA at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100.

[00109] In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately 12 hours, and were generally close to the PAGN curves up to that time.

Example 2

Administration of HPN-100 to patients with liver disease

[00110] To determine its pharmacokinetic (PK) and pharmacodynamic (PD) profile in patients with liver disease, clinical testing was conducted in which HPN-100 was administered orally as a single dose (100 mg/kg/day on day 1), and twice daily for 7 consecutive days (200 mg/kg/day on days 8 through 14, in two doses of 100 mg/kg per dose), to subjects with hepatic impairment with cirrhosis (Child-Pugh scores of A, B, or C) and to a gender and age-matched control group of healthy adults with normal hepatic function. On day 15, subjects received a single dose of HPN-100 (100 mg/kg). PK blood samples were taken pre-dose, at 15 and 30 minutes post-dose, and at 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose on days 1, 8, and 15, and at 48 hours after dosing on days 1 and 15. On days 9–14, blood samples were taken pre-morning dose and at 2 hours post-morning dose. Urine was collected 0–4, 4–8, 8–12, and 12–24 hours post-dose on days 1, 8, and 15, and at 24–48 hours post-dose on days 1 and 15.

[00111] HPN-100 was metabolized via the predominant pathway in all subject groups, and the alternative HPN-100 metabolites PAG (phenylacetyl glycine), PBG (phenylbutyryl glycine), and PBGN (phenylbutyryl glutamine) were below the limit of quantification in all plasma samples. Both the extent of systemic exposure (AUC_{0-t}) and C_{max} for PBA and PAA tended to be higher in Child-Pugh group B or C than in Child-Pugh group A or the healthy volunteer group, although there were no significant differences in these variables on day 15.

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As described below, plasma PAA levels did correlate with Childs-Pugh classification (i.e. were higher in patients with more severe liver disease). However, the average conversion of HPN-100 to PAGN was ~ 75%, and no difference were seen between patients with cirrhosis and normal healthy volunteers, demonstrating that hepatic impairment did not affect the subjects' ability to activate the PBA prodrug HPN-100 or to utilize it for elimination of excess ammonia. Thus, as summarized in more detail below, plasma metabolite levels did not correlate well with the HPN-100 dosage and, just as for healthy adults, plasma metabolite levels did not accurately reflect the nitrogen scavenging effect of HPN-100. Moreover, the mean conversion of administered PAA to PAGN averaged ~75% in this patient population.

Analyte	Subject group	Geometric mean ratio	90% CI	P value for group effect
PBA	**AUC_{0-t}**			0.40
	Child-Pugh A	0.92	0.58–1.43	
	Child-Pugh B	1.26	0.80–1.97	
	Child-Pugh C	1.37	0.87–2.14	
PBA	**C_{max}**			0.52
	Child-Pugh A	1.42	0.87–2.31	
	Child-Pugh B	1.35	0.83–2.21	
	Child-Pugh C	1.50	0.92–2.45	
PAA	**AUC_{0-t}**			0.64
	Child-Pugh A	1.22	0.48–3.06	
	Child-Pugh B	1.53	0.61–3.85	
	Child-Pugh C	1.94	0.77–4.88	
PAA	**C_{max}**			0.72
	Child-Pugh A	1.33	0.70–2.52	
	Child-Pugh B	1.16	0.61–2.20	
	Child-Pugh C	1.52	0.80–2.88	

AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum observed plasma concentration; PAA, phenylacetic acid; PBA, phenylbutyric acid.

[00112] During multiple dosing (days 8–15), there was a trend for higher systemic concentrations of PBA and PAA in subjects with greater hepatic impairment (Child-Pugh B

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or C) compared with Child-Pugh group A and the healthy volunteers. Unlike PBA, PAA did accumulate significantly in plasma during multiday dosing. Differences between single (day 8) and multiple dosing (day 15: steady state) were significant for AUC_{0-12} and C_{max} of PAA for all subjects combined ($p < 0.001$), but not for PBA. After dosing on day 15, extent of exposure to PAA, but not PBA, significantly correlated with hepatic impairment.

[00113] The clinical efficacy of HPN-100 is dependent on its ammonia scavenging capabilities, through conjugation of glutamine with PAA to form PAGN. After dosing on each day, PAGN was the major metabolite excreted: 42–49% of the HPN-100 dose administered was excreted as PAGN on day 1, 25–45% on day 8, and 58–85% on day 15. Very low amounts of PBA and PAA were excreted in the urine (≤ 0.05% of the total HPN-100 dose). There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. Urinary PAGN excretion is also an indication of the ammonia-scavenging capacity of HPN-100, as 2 moles of ammonia combine with 1 mole of PAA to produce PAGN. Hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study. There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. The observations that hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study but was associated with accumulation of PAA in plasma underscores the importance of utilizing urinary PAGN rather than metabolite blood levels to guide drug effect and, as a corollary, the importance of the invention, as does the fact that the mean percentage conversion of administered PAA into urinary PAGN among the 4 treatment groups was ~75%.

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Urinary PAGN Excretion After Dosing on Day 15 (0-48 Hours).

	Child-Pugh A (8)	Child-Pugh B (8)	Child-Pugh C (8)	Healthy Adults (8)
Amount excreted (μmol) Mean (SD) Range	31431 (15291) 16016–65229	25152 (11426) 13643–41635	30752 (20860) 6331–60139	28716 (8223) 17203–41092
Molar % of dose excreted Mean (SD) Range	79.6 (30.5) 48.9–138.2	58.2 (29.2) 26.5–99.6	85.0 (65.1) 23.1–221.1	68.6 (21.9) 30.6–96.
Molar % of dose ammonia scavenged Mean (SD) Range	159.2 (60.9) 97.9–276.4	116.3 (58.3) 53.0–199.2	169.9 (130.1) 46.3–442.3	137.2 (43.9) 61.3–193.4

[00114] Of particular note, there was no relationship between the plasma levels of PBA and PAA, which exhibited a non-statistically significant directional change toward higher plasma levels in patients with liver disease than healthy adults, and urinary excretion of PAGN.

EXAMPLE 3

Administration of HPN-100 To Adults With UCDs

[00115] To further explore its pharmacokinetic (PK) and pharmacodynamic (PD) profile in clinical states associated with nitrogen retention, 10 adult UCD patients were switched from sodium PBA to a PBA equimolar dose of HPN-100. Subjects were required to be on a stable dose of sodium PBA before enrolment. Upon enrolment, all subjects received sodium PBA for 7 days and were then admitted to a study unit (Visit 2-1) for overnight observation and 24-hour PK and ammonia measurements and urine collections. Subjects were then converted to the PBA equimolar dose of HPN-100, either in a single step or in multiple steps depending on the total dose of sodium PBA; 9 out of 10 patients converted in a single step. Subjects stayed on the 100% HPN-100 dose for one week and were then re-admitted to the study unit for repeated PK (Visit 11-1), ammonia and urine collections.

[00116] The findings from this study, summarized in detail below, demonstrate that, just as in healthy adults and patients with liver disease, plasma metabolite levels do not correlate well with ammonia scavenging activity as reflected by urinary PAGN excretion and

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corroborated by plasma ammonia results. Moreover, the findings demonstrate considerable inter-individual variability in the percentage of both sodium PBA and HPN-100 that is converted to urinary PAGN.

[00117] Pharmacokinetic, ammonia and safety analyses: As summarized in the table below, 7 days of HPN-100 administration resulted in comparable PAA and plasma PAGN levels but slightly lower PBA levels compared to the PBA molar equivalent dose of sodium PBA.

Comparison of Pharmacokinetic Parameters at Steady State – sodium PBA vs. HPN-100

PK Parameter	Arithmetic Mean (CV%)	
	Sodium PBA (N=10)	**HPN-100 (N=10)**
PBA in Plasma		
AUC_{0-24} (μg·h/mL)	739 (49.2)	540 (60.1)
$Cmax_{ss}$ (μg/mL	141 (44.3)	70.1 (64.7)
$Cmin_{ss}$ (μg/mL	0.588 (255)	2.87 (265)
PAA in Plasma		
AUC_{0-24} (μg·h/mL)	595.6 (123.9)	574.6 (168.9)
$Cmax_{ss}$ (μg/mL	53.0 (94.7)	40.5 (147.6)
$Cmin_{ss}$ (μg/mL	3.56 (194.4)	7.06 (310.7)
PAGN in Plasma		
AUC_{0-24} (μg·h/mL)	1133 (31.1)	1098 (44.2)
$Cmax_{ss}$ (μg/mL	83.3 (25.8)	71.9 (56.0)
$Cmin_{ss}$ (μg/mL	16.8 (86.1)	12.1 (134.4)

AUC_{0-24}: Area under the concentration from time 0 (pre-dose) to 24 hours, $Cmax_{ss}$: Maximum plasma concentration at steady state, $Cmin_{ss}$: Minimum plasma concentration at steady state, A_e: Amount excreted over 24 hours

[1] The mean (SD) sodium PBA dose = 12.6 (4.11) g; the mean (SD) HPN-100 dose = 12.3 (3.91) g.

[00118] Despite dissimilar PBA blood levels, overall urinary excretion of PAGN was similar for the two treatments as summarized in the table below. Importantly, and in contrast to the assumptions inherent in current treatment guidelines that all administered sodium PBA is converted to urinary PAGN, considerable inter-individual variability was observed in the percentage of administered PAA converted to PAGN, which averaged ~60% and similar both sodium PBA and HPN-100. Moreover, the 24 hour pattern of excretion appeared to differ in that urine output of PAGN reached its highest level during the 'afternoon hours' (6-12 hour urine collection) for patients treated with sodium PBA, whereas peak output of PAGN occurred overnight (12-24 hour urine collection) for patients

on HPN-100 treatment. This difference presumably reflects the slow release characteristics and longer duration of effective blood concentrations of PAA following administration of HPN-100 as compared with sodium PBA. HPN-100 was either not detectable or below the limits of quantitation in all blood samples.

Comparison of Mean PAGN Amount Excreted (μg) – sodium PBA (sodium phenylbutyrate) vs. HPN-100

Treatment	PAGN 0-6 hours	PAGN 0-12 hours	PAGN 12-24 hours	Total PAGN Excretion (CV%)
sodium PBA	2,452,838	4,859,121	4,645,447	12,153,473 (48.2)
HPN-100	2,381,371	3,027,310	5,433,033	10,784,747 (25.9)

[00119] As summarized in the table below, mean time normalized area under the curve (TN-AUC) values for venous ammonia following HPN-100 were directionally (~31%) lower than those observed with sodium PBA (26.1 vs. 38.4 μmol/L) although the differences did not achieve statistical significance (Figure 10). Likewise, peak venous ammonia concentrations following HPN-100 were directionally (~29%; not statistically significant) lower than those observed with sodium PBA (56.3 vs. 79.1 μmol/L, respectively).

[00120] The normal upper limit for venous ammonia varied among the study sites from 26 to 35 μmol/L. Examination of ammonia values (TN-AUC) for individual patients demonstrated that patients with higher ammonia levels on sodium PBA exhibited greater decreases in ammonia values following administration of HPN-100 (Figure 12). Moreover, the mean ammonia value after HPN-100 (26.1 μmol/L) was within the normal range while it was above the upper limit of normal (ULN) after sodium PBA (sodium phenylbutyrate) (38.4 μmol/L) (Figure 13). Likewise the mean percentage of normal ammonia values increased from 58% after sodium PBA treatment to 83% after HPN-100 treatment.

Venous Ammonia Pharmacodynamics Following Seven Days of Dosing With Either Sodium PBA or HPN-100 (Steady State)

	Sodium PBA			HPN-100		
Subject	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**
1001	29.0	16.47	17.5	63.0	19.8	13.1
1002	31.0	20.9	15.8	31.0	19.3	15.9
1004	85.0	46.8	99.2	106	35.1	9.16
1006	150	71.5	17.5	13.0	8.30	17.7
2001	88.0	52.1	6.57	33.0	22.7	6.71

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	Sodium PBA			HPN-100		
Subject	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC** (μmol/L)	**PBA Equivalent dose[1]**	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**
2003	31.0	17.5	11.8	74.0	21.1	12.2
3002	108	22.3	16.5	36.0	21.9	17.7
3004	115	62.9	13.1	75.0	38.4	13.1
5001	82.2	35.8	8.76	57.0	35.5	8.85
5002	72.2	37.7	8.76	75.2	39.1	8.85
N	**10**	**10**	**10**	**10**	**10**	**10**
Mean	79.1	38.4	12.6	56.3	26.1	12.3
SD	40.1	19.6	4.11	27.9	10.3	3.91
Median	83.6	36.8	12.5	60.0	22.3	12.7
Min	29.0	16.4	6.57	13.0	8.30	6.71
Max	150	71.5	17.5	106	39.1	17.7
25%	31.0	20.0	--	32.5	19.7	--
75%	110	54.8	--	75.0	36.2	--

[00121] This reduction in ammonia exposure among UCD patients reflects better overnight control among subjects receiving HPN-100, as summarized in the table below and in Figure 11. This study shows that both AUC and Cmax for ammonia were lower with HPN-100, indicating less total ammonia exposure, and especially at night, HPN-100 exhibited a significantly stronger effect. While not statistically significant due to the small population size, this demonstrates that HPN-100 is at least as effective, and apparently more so, than PBA on an equimolar basis based on the key measure, its ability to mobilize ammonia for urinary elimination. Based on preliminary results, HPN-100 also provides more stable ammonia levels, and reduces risk of hyperammonemia. In this trial, 9 of 10 subjects who experienced both HPN-100 and sodium PBA indicated a preference for HPN-100.

[00122] In addition, in this trial, no serious adverse effects (SAEs) were observed in patients taking HPN-100, while two subjects receiving PBA experienced symptomatic hyperammonemia; and the total number of adverse effects (AEs) reported among subjects taking HPN-100 (5 subjects reported a total of 15 AEs) was lower than the number of AEs among subjects taking PBA (7 subjects reported 21 AEs).

[00123] The following table summarizes overall comparative data for sodium PBA and HPN-100, administered at equimolar rates (n=10) (see tables above and figures 10-13 for additional detail).

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Parameter	Sodium PBA	HPN-100
NH_3: Total AUC	38.4 ± 19.6	26.1 ± 10.3
NH_3 Cmax	79.1 ± 40.1	56.3 ± 27.9
NH_3 exposure: DAY (hours 6-12)	37.1	32.9
NH_3 exposure: NIGHT (hours 12-24)	36.3	21.3
Adverse effects	21 reported by 7 subjects	15 reported by 5 subjects
Serious adverse effects	2 (symptomatic hyperammonemia)	0
PAGN excretion	Comparable	Comparable

[00124] While the differences between sodium PBA and HPN-100 did not reach statistical significance due to the small sample size, HPN-100 exhibited a clear trend toward being more efficacious at equimolar dosages, and it was particularly effective for improving overnight control of ammonia levels.

[00125] Figure 9a demonstrates that PBA levels in the blood are not correlated with HPN-100 dosages received. It plots the 24-hour AUC for PBA and the Cmax for PBA against HPN-100 dosage (top panel), and while the AUC and Cmax track together in each patient, they show no relationship to HPN-100 dose: both the highest and the lowest PBA exposures occurred in patients receiving high doses of HPN-100. Figure 9b shows that levels of PAA are similarly uncorrelated with HPN dosages.

[00126] Figure 10 illustrates the trend shown in the clinical testing, where HPN-100 provided better overall control of waste nitrogen.

[00127] Figure 11 illustrates that improved night time control of excess ammonia is achieved with HPN-100.

[00128] Figure 12 shows that especially for patients with higher ammonia levels when treated with sodium PBA (Na PBA), HPN-100 provides better control than sodium PBA, while in patients with lower ammonia levels (ones for whom sodium PBA seems to work relatively well), HPN-100 provides at least comparable ammonia control. Note that for patients having ammonia levels above about 40 μmol/L when treated with sodium PBA, HPN-100 at equimolar dosages provided superior control of ammonia, and consistently reduced ammonia levels to below about 40 μmol/L. Thus for patients whose ammonia levels are abnormal (e.g. above about 40 μmol/L) when treated with sodium PBA, it is

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expected that better ammonia control can be achieved with an equimolar amount of HPN-100. Based on this, dosages of HPN-100 can be determined as set forth herein. Figure 13 illustrates that ammonia levels were better controlled in this test by HPN-100 than with sodium PBA, e.g., the average ammonia levels are lower, and tend to be below the upper limit for normal.

Example 4

Relationship Between Ammonia Control and Urinary PAGN Excretion

[00129] As part of the clinical study in UCD patients described in the example above (Example 3), the relationship between plasma ammonia levels and urinary excretion of PAGN was examined. Unlike blood levels of PAA or PBA which exhibited no consistent relationship to ammonia levels (i.e. ammonia control), blood ammonia assessed as the time-normalized area under the curve exhibited an inverse curvilinear relationship to urinary PAGN. That is, plasma ammonia decreased as urinary PAGN increased. Moreover, the relationship between ammonia and urinary PAGN excretion did not differ between sodium PBA and HPN-100 suggesting that this method of dose determination is independent of product formulation. Figure 5 shows a plot of Plasma Ammonia (TN-AUC) versus Urinary PAGN Excretion.

Example 5

Experimentation With Dosing Schedule

[00130] The results of single dose PK/PD modeling observed in the examples above suggested that HPN-100 exhibits delayed release characteristics as compared with sodium PBA with a corresponding potential for increased flexibility in dosing, which was further explored in additional clinical studies described above. In one of these, HPN-100 was administered twice daily as well as in the fasted and fed state. In the other, HPN-100 was administered three times daily with meals. Both 3x daily and 2x daily dosing resulted in a similar proportion of PAGN excreted in the urine and, as demonstrated in adult UCD patients, three times daily dosing was associated with effective ammonia control.

[00131] In Example 2, a number of secondary statistical analyses comparing PK variables after fed versus fasted HPN-100 dosing and single versus multiple HPN-100 dosing were also done. There were no PK or PD differences observed when HPN-100 was administered

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after fasting (day 1) or with a meal (day 8). Accordingly, it is believed that HPN-100 can be effectively administered without the need for it to accompany a meal, while the label and package insert for sodium PBA (sodium PBA) indicate that it should be taken with meals. In addition to the lack of difference for PAA PK variables between the fasted and fed states (Days 8 vs 1), the table below also illustrates plasma accumulation of PAA that occurs with multiple dosing (Days 15 vs. 8).

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Plasma PK Variables For PAA

PK variable	Child-Pugh A (n = 8)	Child-Pugh B (n = 8)	Child-Pugh C (n = 8)	Healthy volunteers (n = 8)
AUC_{0-12} [(μg/mL)·h]				
Day 1				
Geo. mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00
CV%	53.41	64.91	109.58	79.59
Day 8				
Geo. mean (range)	39.64 (5.96–153.14)	73.44 (26.83–279.48)	86.36 (28.12–367.70)	34.07 (5.27–134.99)
CV%	78.73	85.58	92.85	80.59
Day 15				
Geo. mean (range)	117.89 (23.28–413.43)	138.95 (40.21–652.99)	184.26 (14.97–2245.51)	99.16 (30.06–394.79
CV%	76.82	99.48	170.56	88.59
AUC_{0-t} [(μg/mL)·h]				
Day1				
Geo. Mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00
CV%	53.41	64.91	109.58	79.59
Day 15*				
Geo. Mean (range)	121.57 (23.28–528.73)	153.00 (40.21–938.85)	194.17 (14.97–3415.51)	99.94 (30.06–420.32
CV%	92.27	118.54	198.42	93.08
C_{max} [μg/mL]				
Day 1				
Geo. mean (range)	9.65 (2.58–26.93)	13.52 (6.94–27.97)	10.95 (2.68–40.30)	11.81 (4.14–29.79)
CV%	63.78	57.70	82.65	68.72
Day 8				
Geo. mean (range)	10.21 (1.64–25.66)	14.78 (4.46–42.02)	16.03 (6.49–48.07)	10.03 (2.90–28.43)
CV%	62.25	74.53	72.29	66.97
Day 15†				
Geo. mean (range)	29.07 (7.29–53.48)	25.46 (10.54–65.40)	33.28 (5.03–208.80)	21.92 (7.76–61.31)
CV%	44.21	64.26	121.51	62.88
$t_{1/2}$ [h]‡				
Day 1				
Mean (SD)	0	0	2.10 (0.32)	0
Range			1.88–2.33	
Day 15				
Mean (SD)	1.80 (0.94)	2.76 (1.53)	7.70	1.91 (0.37)
Range	1.01–3.14	1.68–3.84	7.70–7.70	1.68–2.33
T_{max} [h]				
Day 1				
Median (range)	3.50 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (2.00–8.00)	6.00 (4.00–6.00)
Day 8				
Median (range)	4.00 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (4.00–8.00)	4.00 (3.00–6.00)
Day 15				
Median (range)	4.00 (2.00–6.00)	4.00 (3.00–8.00)	5.00 (0.00–8.00)	4.00 (3.00–4.00)

*p = 0.64 for group cffcct; †p = 0.72 for group cffcct
‡On day 1, n = 2 in Child-Pugh group B and n = 0 in all other groups; on day 15, n = 4 in group A, 2 in group B, 1 in group C, and 3 in group D
AUC_{0-12}, area under the plasma concentration curve from time 0 up to 12 hours after dosing; AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; geo. Mean, geometric mean; n, number of subjects; SD, standard deviation; T_{max}, time to maximum observed plasma concentration; $t_{1/2}$, half-life

Example 6

PK/PD Modeling Results

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[00132] In the case of most drugs, the fraction of an orally administered dose which is removed and metabolized by the liver prior to reaching the systemic circulation (i.e. first pass effect) is not considered bioavailable, since it does not enter the systemic circulation and therefore is not able to reach its target organ or receptor. However, this is not the case for ammonia scavenging drugs described in this invention. Since hepatocytes and possibly enterocytes contain the enzymes necessary for conversion of PBA to PAA and conversion of PAA to PAGN and since glutamine is present in the splanchnic as well as the systemic circulation, it is likely that PBA can be converted to PAGN prior to reaching the systemic circulation (i.e. "pre-systemically") and that this PBA is fully effective with respect to ammonia scavenging (Figure 6); i.e. fully active. To verify this possibility, PK/PD modeling using NONMEM VI (Icon, Ellicot City, MD.) was carried out on plasma and urinary metabolite data (over 5000 data points) from the clinical studies described above involving healthy adults, subjects with cirrhosis and UCD subjects. The results of this PK/PD modeling have validated the model depicted in Figure 3. Moreover, the modeling has verified that HPN-100 exhibits slow release characteristics as compared with sodium PBA and provided an explanation for the poor correlation between blood levels of PBA/PAA and ammonia and the importance of urinary PAGN is dose adjustment. Key conclusions resulting from the PK/PD modeling were as follows

1. PBA is more slowly absorbed (~40% as fast) from the intestine after administration of HPN-100 versus sodium PBA (absorption rate constants and absorption half-lives for HPN-100 and sodium PBA are 0.544 h^{-1} vs. 1.34 h^{-1} and 1.27 h vs. 0.52 h, respectively).

2. The lower plasma levels of PBA following administration of HPN-100, as compared with sodium PBA, reflect results indicating a fractionally greater amount of PBA (31% vs. 1%) being converted pre-systemically (to PAA and PAGN) following administration of HPN-100 than Na PBA.

3. In a dataset containing healthy, cirrhotic, and UCD individuals, diagnosis was introduced as a covariate on the estimated bioavailability of HPN-100 revealing a 32% lower estimated bioavailability of PBA in healthy adults compared to adult UCD patients. Cirrhotic and UCD patients had similar PBA bioavailability following HPN-100 treatment.

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Example 7

ADME Study In Three Cynomolgous Monkeys

[00133] To assess the preclinical handling of ammonia scavenging drugs, 600 mg/kg of either radio labeled sodium PBA or radio labeled HPN-100 was administered as a single dose to 3 cynomolgous monkeys. These monkeys were chosen because, like humans (and unlike most other species), they metabolize PAA to PAGN and thus provide a useful model for testing prodrugs of PAA. This study corroborated clinical findings summarized in Examples 1-3, including the following: (a) dosing with oral sodium PBA or oral HPN-100 did not result in 100% conversion to urinary PAGN, (b) plasma PBA and PAA blood levels did not correlate consistently with ammonia scavenging activity as reflected by urinary PAGN output, and (c) HPN-100 exhibited slow release characteristics as compared with sodium PBA.

[00134] Radio labeled PBA and PAA entered the systemic circulation rather slowly following administration of radio labeled HPN-100 [Cmax for PBA was achieved 1.5 hours post-dosing (52.2 μg/mL) and Cmax for PAA was achieved 8 hours post dosing (114 μg/mL)], corroborating the findings observed in humans (including the PK/PD modeling), and essentially no HPN-100 appeared in systemic circulation or in excretions. About 90% of radioactive material derived from HPN-100 that was excreted in urine was PAGN, accounting for 39% of the administered HPN-100. By contrast, when oral sodium PBA was administered, PAGN accounted for only 23% of the radio labeled material, and unchanged PBA accounted for 48% of the administered dosage of oral sodium PBA. Thus oral sodium PBA was utilized less efficiently than HPN-100, and an unexpectedly high amount of PBA was excreted unchanged.

Example 8

Biological and Anatomical Considerations

[00135] Unlike most drugs which act on a target organ/cell/receptor (etc.) perfused by systemic blood, ammonia scavenging drugs of the types covered by this invention do not act on a target organ, rather they act through the combination of PAA with glutamine to form PAGN (Figure 6). Since glutamine is present in the splanchnic as well as the systemic circulation and since the liver is a metabolically active organ capable of catalyzing all steps involved in the conversion of HPN-100 or PBA to PAA and then to PAGN, the data accumulated to date, including the PK/PD modeling, as well as anatomical consideration

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lead us to the conclusion that the formation of PAGN from PBA/PAA occurs to a significant degree before PBA/PAA reach the systemic circulation (e.g. within the liver). This is especially true when HPN-100 is administered as a PBA prodrug. This explains the poor correlation between plasma levels and ammonia trapping effects and leads to the conclusion that the dosing and dose adjustment of these PBA prodrugs should be based on urinary excretion of PAGN and total urinary nitrogen. Figure 6 illustrates how this occurs.

[00136] For certain clinical trials, particularly for comparing HPN-100 to PBA, HPN-100 will be administered at a dose that is equivalent (equimolar) to an amount of sodium PBA that would be considered suitable for the particular patient; and the dosage can then be adjusted by the methods described herein. For example, the HPN-100 dose range will match the PBA molar equivalent of the approved sodium PBA (sodium phenylbutyrate) (NaPBA) dose range. HPN-100 will be administered three times a day (TID) with meals. Note that the conversion of the dose of NaPBA to the dose of HPN-100 involves correction for their different chemical forms (i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium) (NaPBA [g] x 0.95 = HPN-100 [g]) as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

HPN-100 Dose Ranges Corresponding to Recommended Daily Doses of Sodium PBA

Sodium PBA	HPN-100 PBA Equivalent Dose (mg)	HPN-100 PBA Equivalent Dose (mL)
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[1] 20 g of sodium PBA contains ~17.6 g of phenylbutyric acid; 19 g of HPN-100 contains ~17.6 g of phenylbutyric acid

Example 9

Determination of a Starting Dosage and Dose Adjustment of HPN-100

[00137] A patient having a nitrogen retention state (e.g. an inherited urea cycle disorder or cirrhosis) who is currently not being treated with an ammonia scavenging agent as described in this invention is determined clinically to be in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of HE in patients with cirrhosis, elevated blood ammonia levels).

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[00138]. The starting dosage is based on clinical considerations, including the estimation of residual urea synthetic capacity (an infant with UCD presenting with hyperammonia in the first few days of life would be presumed to have no significant urea synthesis capacity) and appropriate dietary protein intake (i.e., infants with UCD require increased dietary protein to support body growth, but long-term dietary protein restriction in patients with cirrhosis is usually ineffective or counterproductive, and the methodology outlined in this invention. For example, an adult with limited residual urea synthetic capacity is treated with an initial dosage of HPN-100 of 19 g per day and placed on a protein-limited diet containing about 25 g of protein per day. The patient's daily urinary output of PAGN is monitored. The daily intake of HPN-100 amounts to 19 g of HPN-100, at a molecular weight of ~530, which is 0.0358 mol HPN-100. Each mole of HPN-100 can theoretically be converted into three moles of PAA and thus three moles of PAGN, so the 19 g daily dosage of HPN-100 could produce 0.108 mol of PAGN in vivo. If entirely converted into PAGN and all of the PAGN is excreted in the urine, the theoretical quantity of PAGN would be 28.4 g per day, which would be sufficient to mediate the waste nitrogen excretion resulting from ~41 grams of dietary protein, assuming that 16% of dietary protein is nitrogen and ~47% of dietary nitrogen is excreted as waste nitrogen (see Brusilow).

[00139] However, as demonstrated herein, HPN-100 is typically converted into urinary PAGN with an efficiency of about 60% to 75% (typically about 60% conversion was found in UCD patients; conversion in cirrhotic patients was about 75%), thus the physician would expect to observe about 17 g of urinary PAGN output per day from this dosage of HPN-100. This corresponds to ~25 grams of dietary protein – which is similar to the prescribed amount, but less than the theoretical amount (41 grams) this dosage of HPN-100 might have been expected to account for theoretically. Thus the adjustment for 60-75% efficiency significantly affects the overall treatment program, and knowing what efficiency to expect enables the treating physician to avoid putting the patient on a diet containing too much protein for the patient to manage on this dosage of HPN-100.

[00140] When monitoring the patient, if the doctor observes a higher output of urinary PAGN than expected, the dosage of HPN-100 is reduced proportionally; thus if 21 g of urinary PAGN per day is observed, the physician will reduce the dosage of HPN-100 to $(17/21)*19g = 15$ g. Similarly, if urinary PAGN output is below that expected amount, such as 12 g per day, the amount of HPN-100 would be increased: if 12 g is observed and 17 is expected, the physician could adjust the HPN-100 dosage to $(17/12) * 19g = 27$ g HPN-100

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per day, if that dosage is within a range considered safe to administer to the patient. Either the dosage of HPN-100 or dietary protein intake could be adjusted to optimize the treatment plan for this subject.

[00141] Optionally, the urinary PAGN output may be determined as a ratio of urinary PAGN concentration to urinary creatinine concentration; creatinine levels are typically stable enough for a given individual to provide a normalization factor for urine volume so that rather than determining total daily urinary PAGN, the physician can estimate total daily urinary PAGN from testing a single urine sample.

[00142] The physician may also monitor the plasma ammonia levels and dietary protein intake in the patient to ascertain whether the patient's dietary protein intake and drug treatment combined are producing the appropriate therapeutic effect. Dietary protein intake or drug dosage or both could be adjusted to attain a normal or desired plasma ammonia level, e.g., a level below about 40 umol/L. However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA to adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00143] If the 19g dose of HPN-100 is determined to be inadequate (e.g. patient requires an increase in dietary protein which would result in excretion of waste nitrogen exceeding his or her urea synthesis capacity and PAGN excretion), HPN-100 dose would be increased sufficiently to cover the necessary dietary protein and the same methodology of dose adjustment based on urinary PAGN excretion would be applied to determine that dosage of HPN-100.

[00144] In a subject having little or no urea synthesis capacity where essentially all urinary nitrogen would be accounted for by PAGN, the ammonia scavenging effect may be monitored by determination of total urinary nitrogen (TUN), rather than directly measuring PAGN levels in the urine.

[00145] Optionally, the TUN can be used as a measure of urea synthesis capacity, by subtracting the amount of nitrogen present as PAGN.

Example 10

Determination of a Dosage of HPN-100 for a Patient already on sodium PBA

[00146] A patient with a UCD already on sodium PBA who is to be transitioned to HPN-100 would undergo assessment of dietary protein and measurement of urinary PAGN excretion.

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[00147] If the patient is judged to be adequately controlled on sodium PBA , then the starting dose of HPN-100 would be the amount necessary to deliver the same amount of PAA (e.g. 19 grams of HPN-100 would correspond to 20 grams of sodium PBA). Subsequent dose adjustment would be based on repeated measurement of urinary PAGN as well as assessment of dietary protein and ammonia. , However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA either to determine the initial dosage of HPN-100 or adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00148] If the patient is determined to be inadequately controlled on sodium PBA , then the starting dose of HPN-100 would be selected to deliver an amount of PAA higher than the dose of sodium PBA provided such HPN-100 dosage is otherwise appropriate. Subsequent dose adjustment would be based on repeated measurement of urinary PAGN as well as assessment of dietary protein and plasma ammonia. However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA either to determine the initial dosage of HPN-100 or adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00149] Optionally, for example in a 'fragile' UCD patient with a history of repeated episodes of hyperammonemia, the conversion from sodium PBA to HPN-100 might occur in more than one step, whereby, at each step, the dose of sodium PBA would be reduced in an amount corresponding to the amount of PAA delivered by the incremental dose of HPN-100.

[00150] If the dose of HPN-100 is determined to be inadequate (e.g. patient requires an increase in dietary protein which would result in production of waste nitrogen exceeding his or her urea synthesis capacity and PAGN excretion), HPN-100 dose would be increased sufficiently to cover the necessary dietary protein and the same methodology of dose adjustment based on urinary PAGN excretion would be applied.

[00151] The examples set forth herein are illustrative only, and should not be viewed as limiting the invention.

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Claims

1. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100,

wherein monitoring the effect consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output;

and determining from the urinary PAGN output whether and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect.

2. The method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine.

3. The method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy or a urea cycle disorder.

4. The method of claim 1, wherein administering the effective dosage of HPN-100 to the patient produces a normal plasma ammonia level in the patient.

5. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100,

wherein monitoring the effect consists of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen.

6. A method to determine a dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a utilization efficiency for HPN-100 conversion into PAGN of about 60% to about 75%.

7. The method of claim 6, wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake.

8. The method of claim 7, wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity.

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9. A method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to produce the target amount of urinary PAGN,

wherein about 60% to about 75% of the PAA prodrug is converted into urinary PAGN.

10. The method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof.

11. The method of claim 9, wherein the PAA prodrug is HPN-100.

12. A method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN; and

e) administering to the patient the suitable dosage of the PAA prodrug.

13. The method of claim 12, wherein the PAA prodrug is phenylbutyrate or a pharmaceutically acceptable salt thereof, or HPN-100.

14. The method of claim 12, wherein the PAA prodrug is HPN-100, the patient is a patient with clinically significant residual urea synthetic capacity, and the HPN-100 is administered in two or three doses per day.

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15. A method to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, comprising:

determining a replacement amount of HPN-100 to replace at least a portion of the phenylacetate or phenylbutyrate;

substituting the replacement amount of the HPN-100 for the phenylacetate or phenylbutyrate; and

monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the HPN-100.

16. The method of claim 15, wherein an increase in the amount of urinary PAGN caused by the transition indicates that the amount of HPN-100 can be reduced.

17. A method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted urinary PAGN for the subject; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

18. A method to initiate treatment with phenylacetate, phenylbutyrate or a HPN-100 in a step-wise fashion, comprising:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion based upon diet and urea synthetic capacity; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as urinary PAGN taking into account an estimated 60% to 75% conversion of the administered drug into PAGN; and

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d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

19. A method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA when the patient receives the PBA prodrug, when compared to the AUC and Cmax observed when the patient receives an equimolar amount of PBA.

20. The method of claim 19, wherein the PBA prodrug is HPN-100.

21. The method of claim 20, wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20%.

22. The method of claim 20, wherein the exposure to PBA upon treatment with the prodrug is lower by at least about 30% compared to treatment with PBA.

23. A method to determine a suitable dietary protein level for a patient having a nitrogen retention disorder, comprising:

determining the patient's endogenous nitrogen elimination capacity;

calculating from the endogenous nitrogen elimination capacity an amount of dietary protein the patient can process without the aid of a nitrogen scavenging drug;

then adding an amount of protein that the patient should be able to process with the assistance of selected dosage of a nitrogen scavenging drug to arrive at an amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, taking into account the of protein required for health and body growth.

24. The method of claim 23, wherein the nitrogen scavenging drug is HPN-100.

25. The method of claim 24, wherein the selected dosage of HPN-100 is up to about 19 grams per day, and wherein the amount of dietary protein the patient should be able to

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process with the assistance of this amount of HPN-100 is about 1 g of protein per gram of HPN-100.

26. A method to treat a patient with a PBA prodrug, comprising administering HPN-100 at a daily dose in excess of 19 g per day to a subject having HE or UCD.

27. The method of claim 26, wherein the daily dose of HPN-100 is between about 19g and about 57 g.

28. A method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered.

29. The method of claim 28, wherein the subject's plasma ammonia levels are on average normal when treated with HPN-100.

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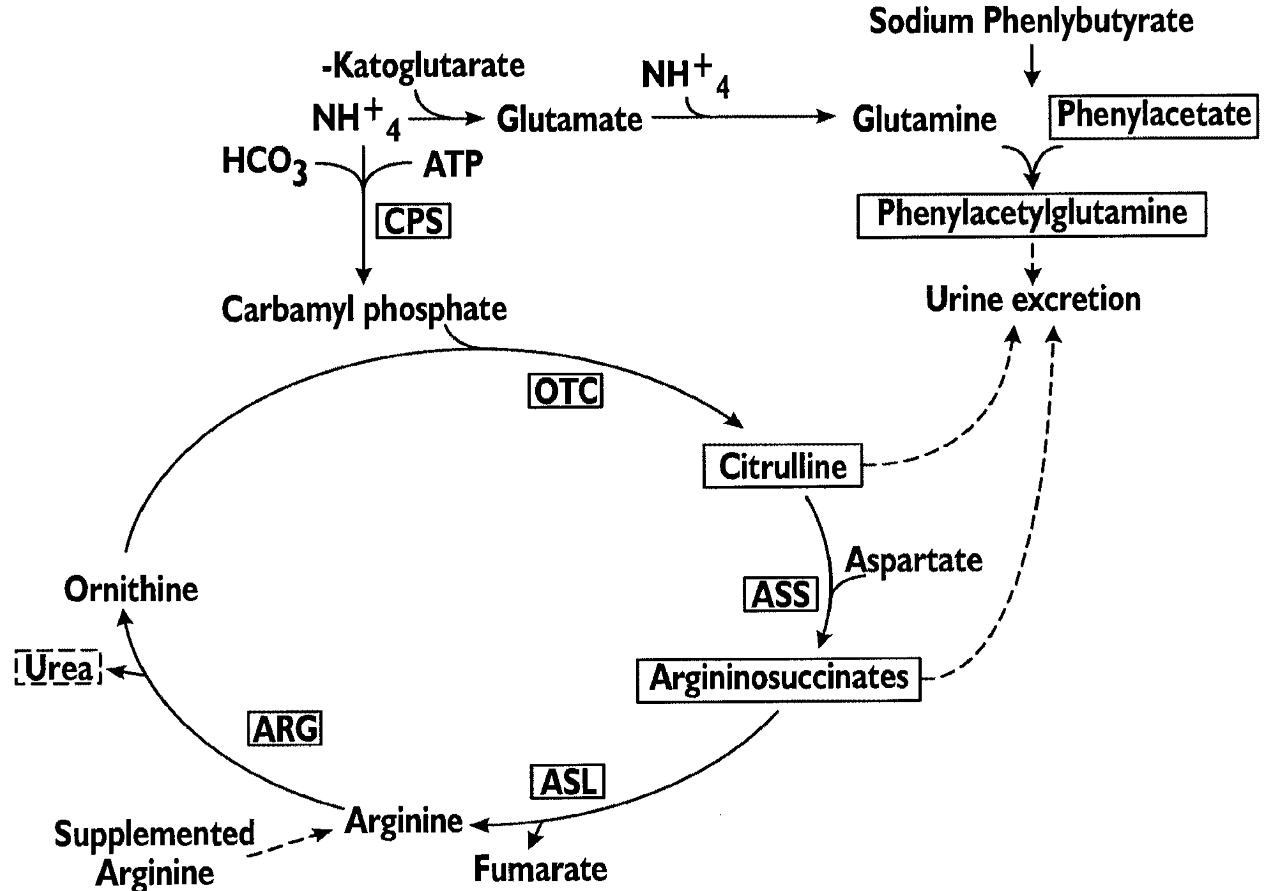

Figure 1

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Figure 2

A conventional clinical pharmacology model in which only drug reaching the central (systemic) circulation is assumed to be active.

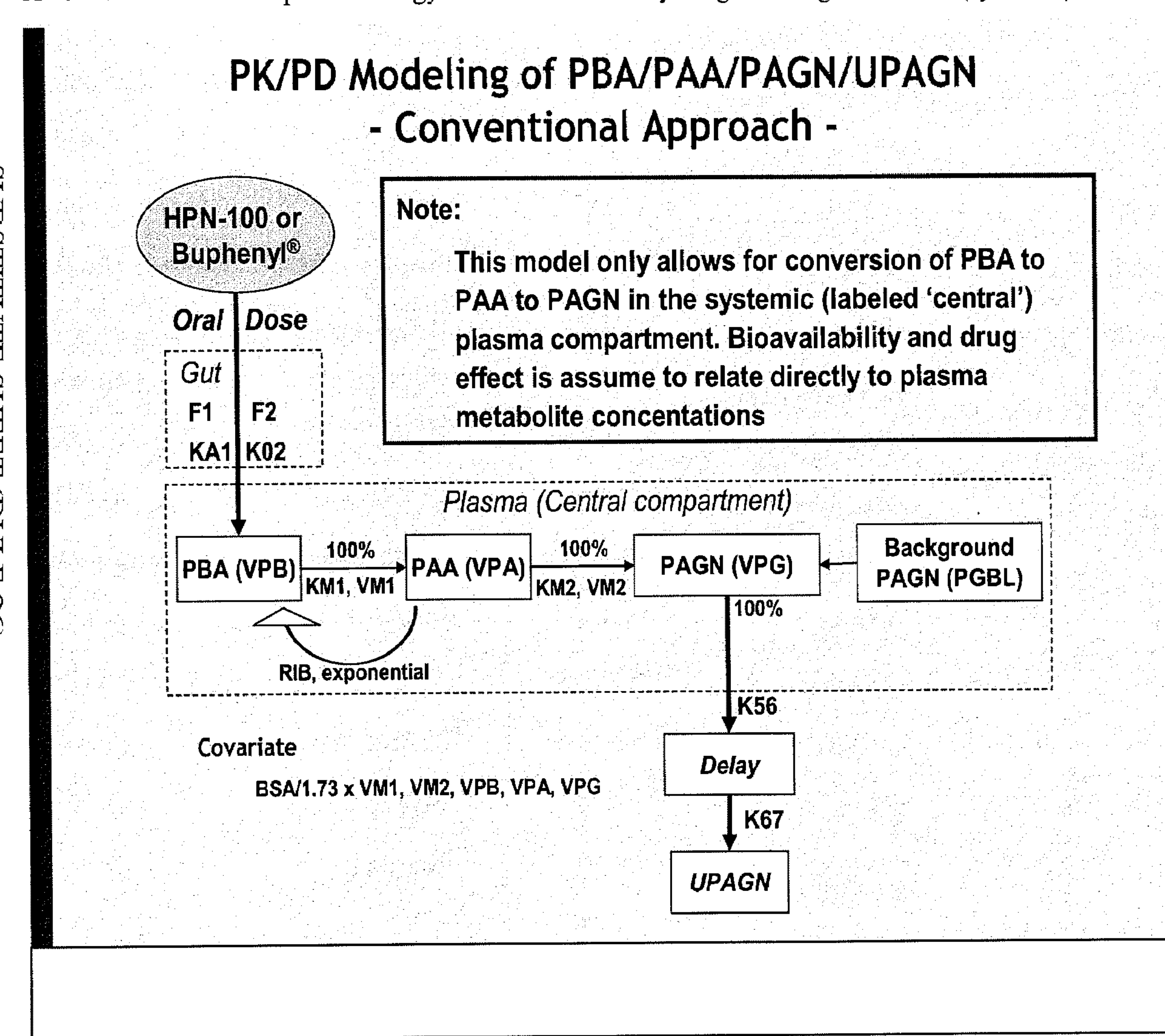

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Figure 3

A modified clinical pharmacology model as described in this application in which an ammonia scavenging agent converted into PAGN prior to reaching the systemic circulation is fully active with respect to excretion of waste nitrogen. As a corollary, concentrations of metabolites in the systemic circulation do not correlate consistently with drug effect.

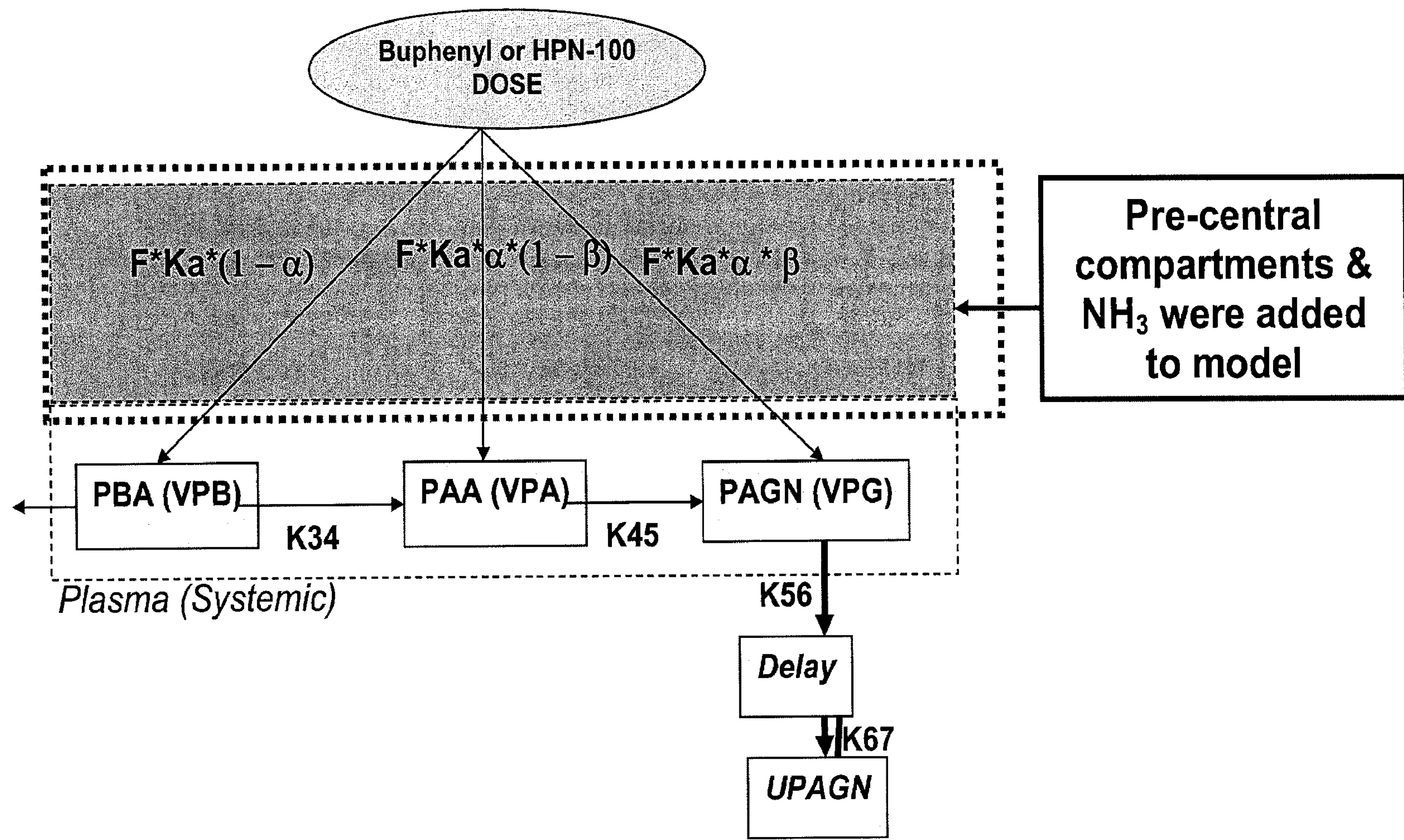

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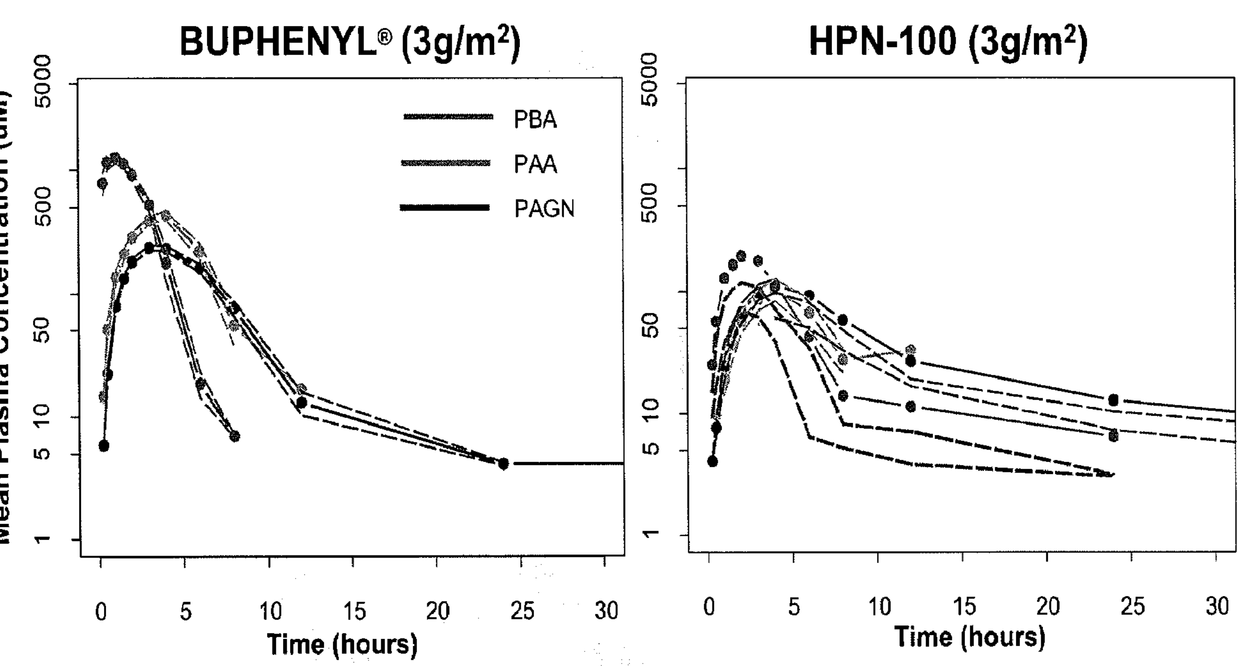
FIGURE 4



In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving BUPHENYL® (sodium phenylbutyrate) (sodium PBA) at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100. In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately12 hours, and fall generally close to the PAGN curves up to that time.

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Figure 5

Relationship between blood ammonia levels (partial time-normalized area under the curve [partial AUC]) and urinary output of PAGN in 10 subjects during steady state treatment with HPN-100 or sodium PBA. Partial AUCs are plotted against the corresponding time of the urine collection, which ranged from 6 to 12 hours.

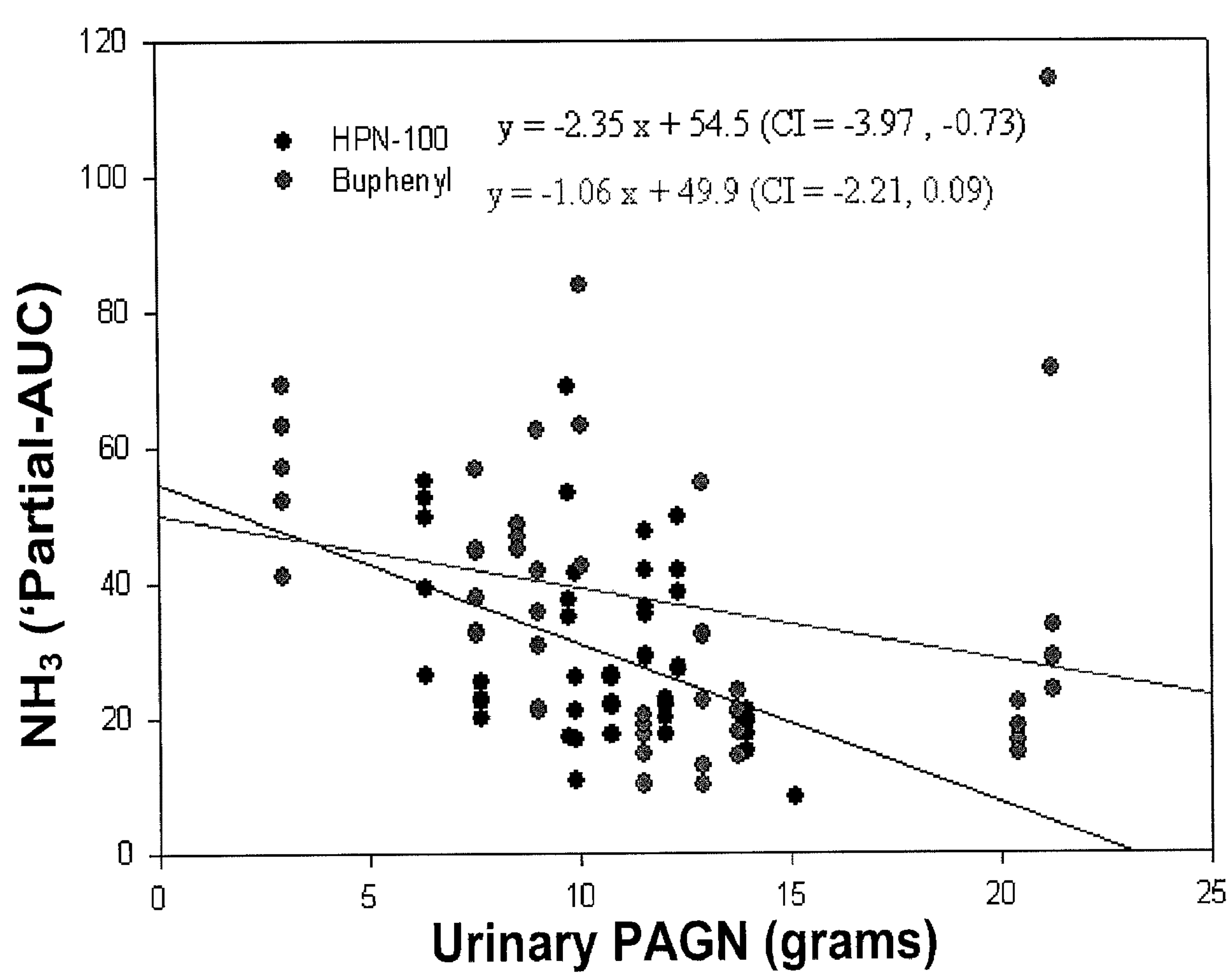

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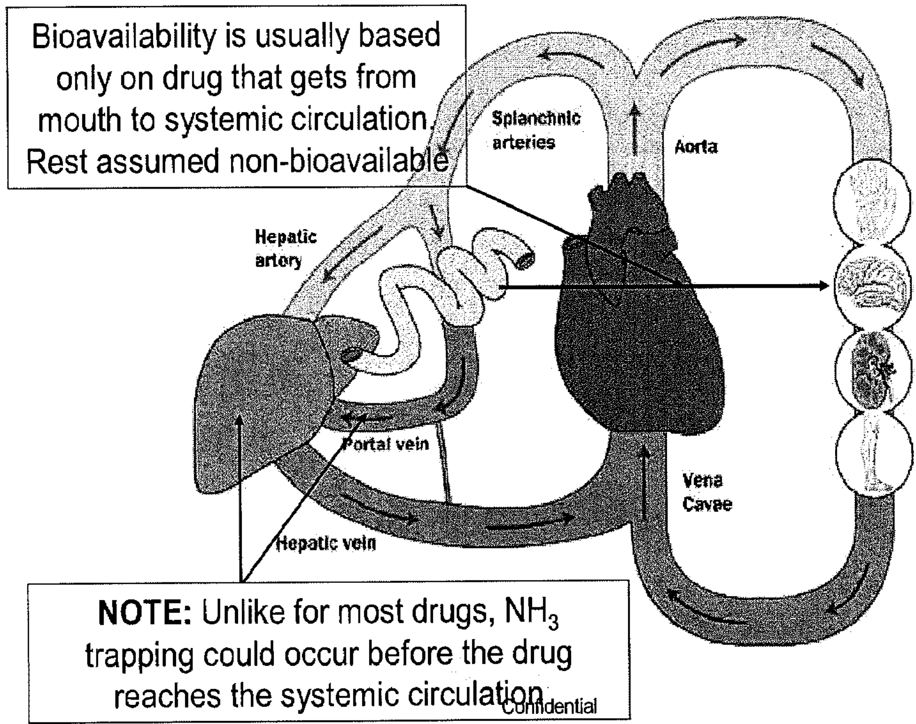
Figure 6



Schematic anatomic depiction of the systemic and presystemic (represented by the portal vein) compartments. Unlike the case for most drugs which need to pass through the liver to the systemic circulation to exert an effect, PAA converted to PAGN prior to reaching the systemic circulation (e.g. in the liver) is still effective in clearing ammonia from the body.

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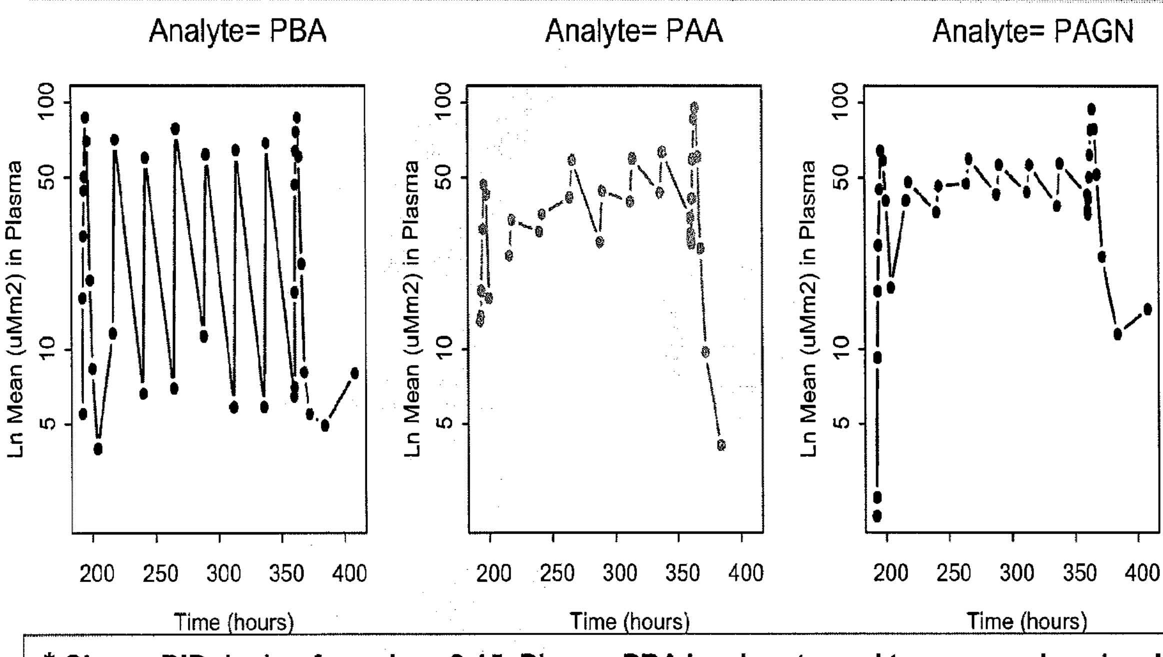
Figure 7

UP1204-002: Blood Metabolite Concentrations Vs. Time In Healthy Adults*



*** Shows BID dosing from days 8-15. Plasma PBA levels returned to near predose level between doses on each day during multiple dosing for healthy individuals. PAA levels increase, but reach a steady-state after 3 days of BID dosing**

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Figure 8

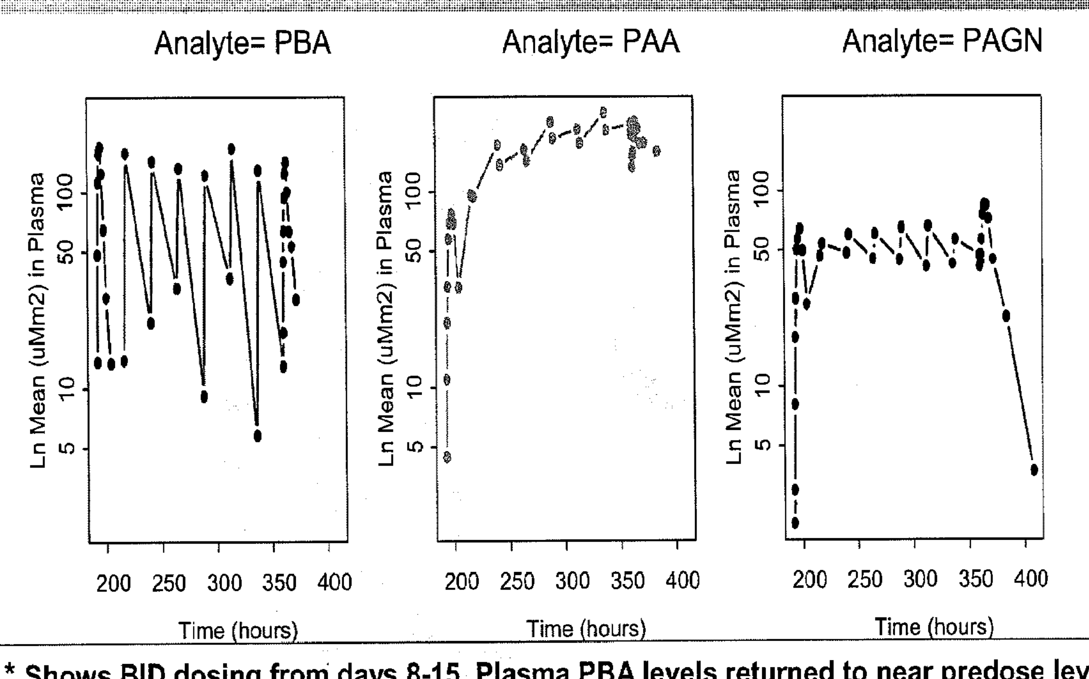

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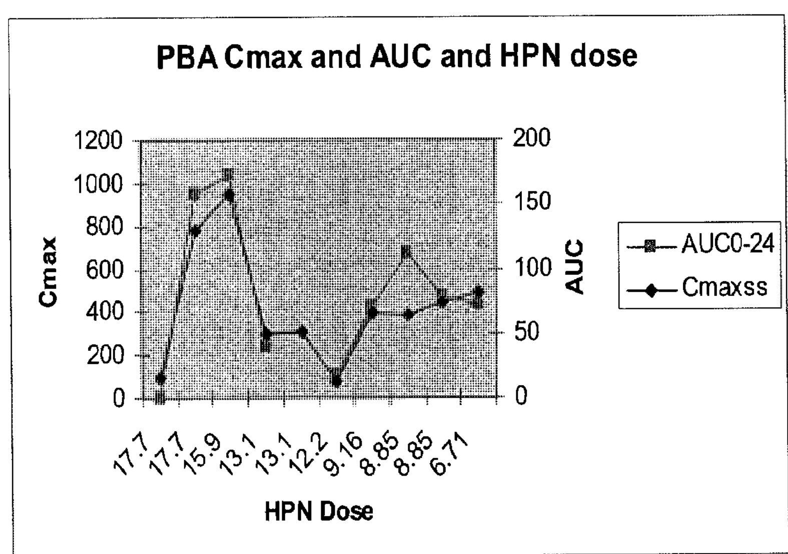
Figure 9a

Figure 9 depicts the lack of correlation between drug dose and plasma PBA (9a) and plasma PAA (9b), as compared with a significant correlation with urinary output of PAGN (9c).



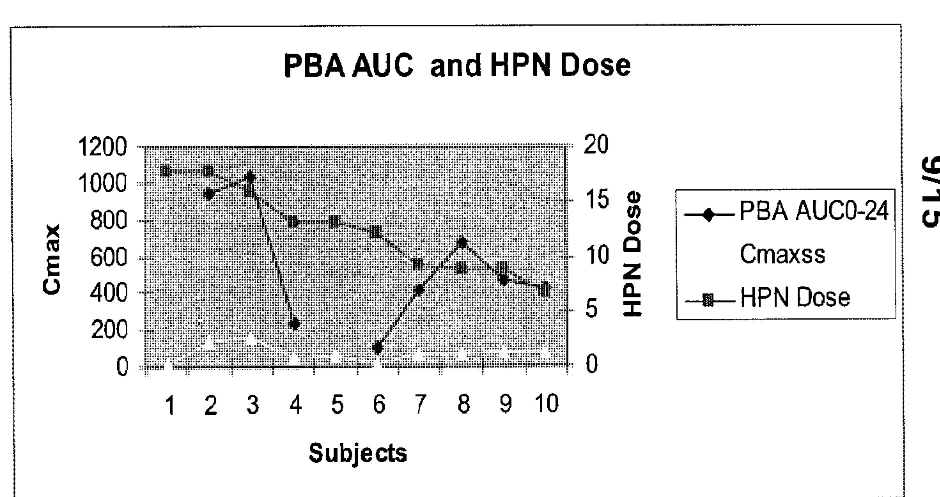


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Figure 9b

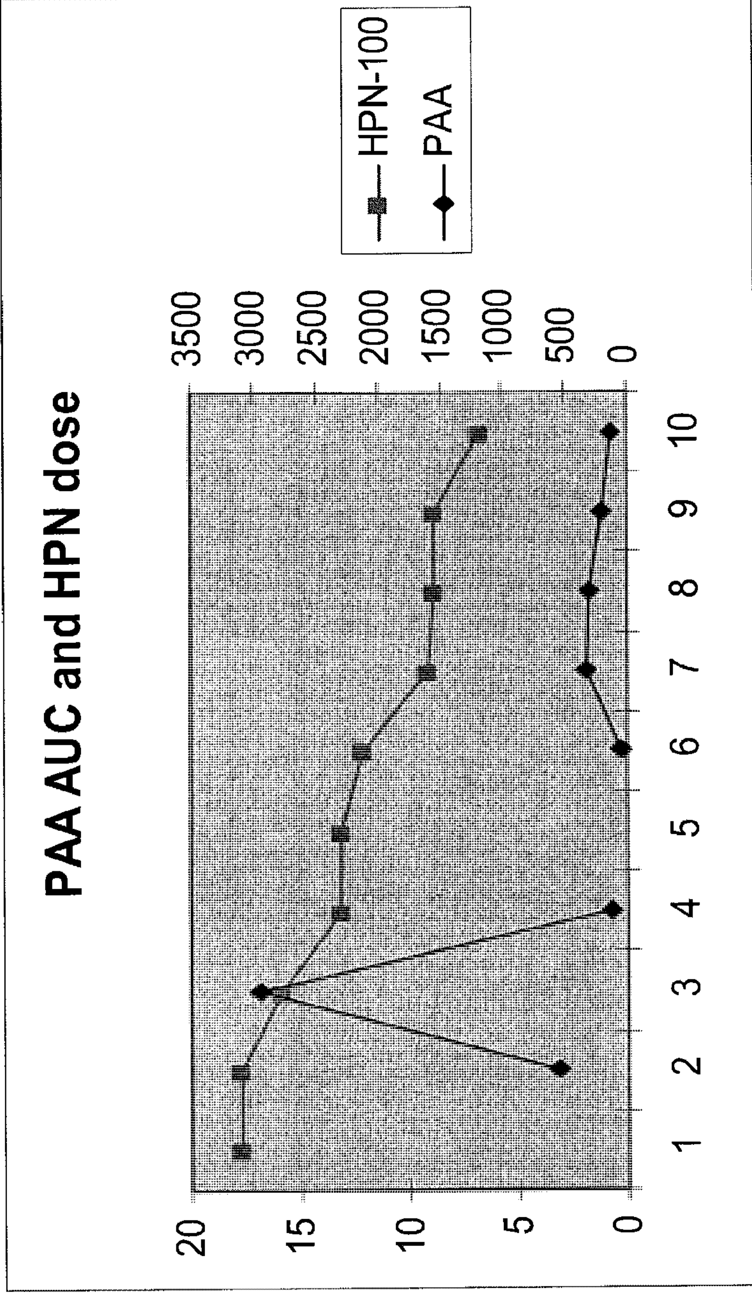

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Figure 9c

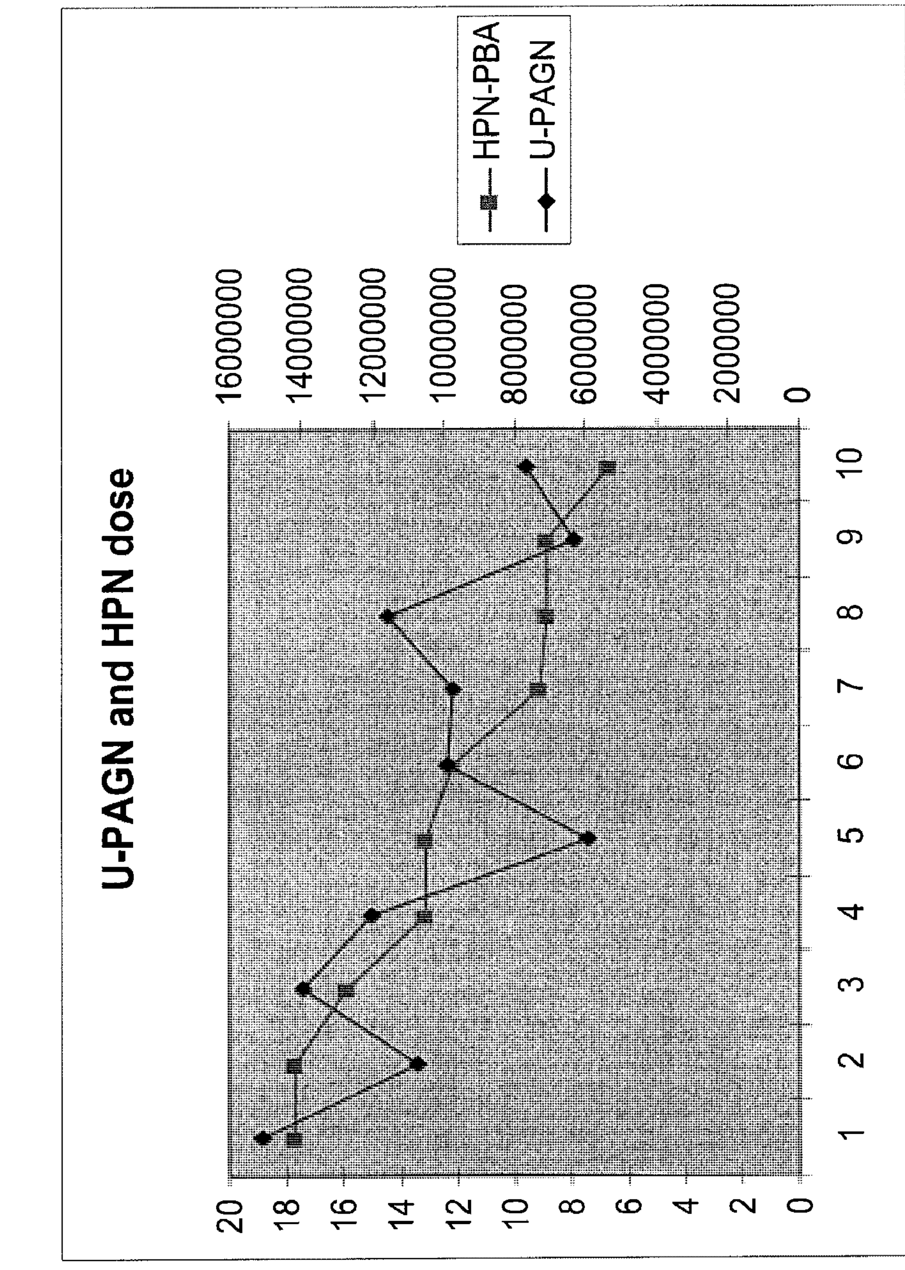

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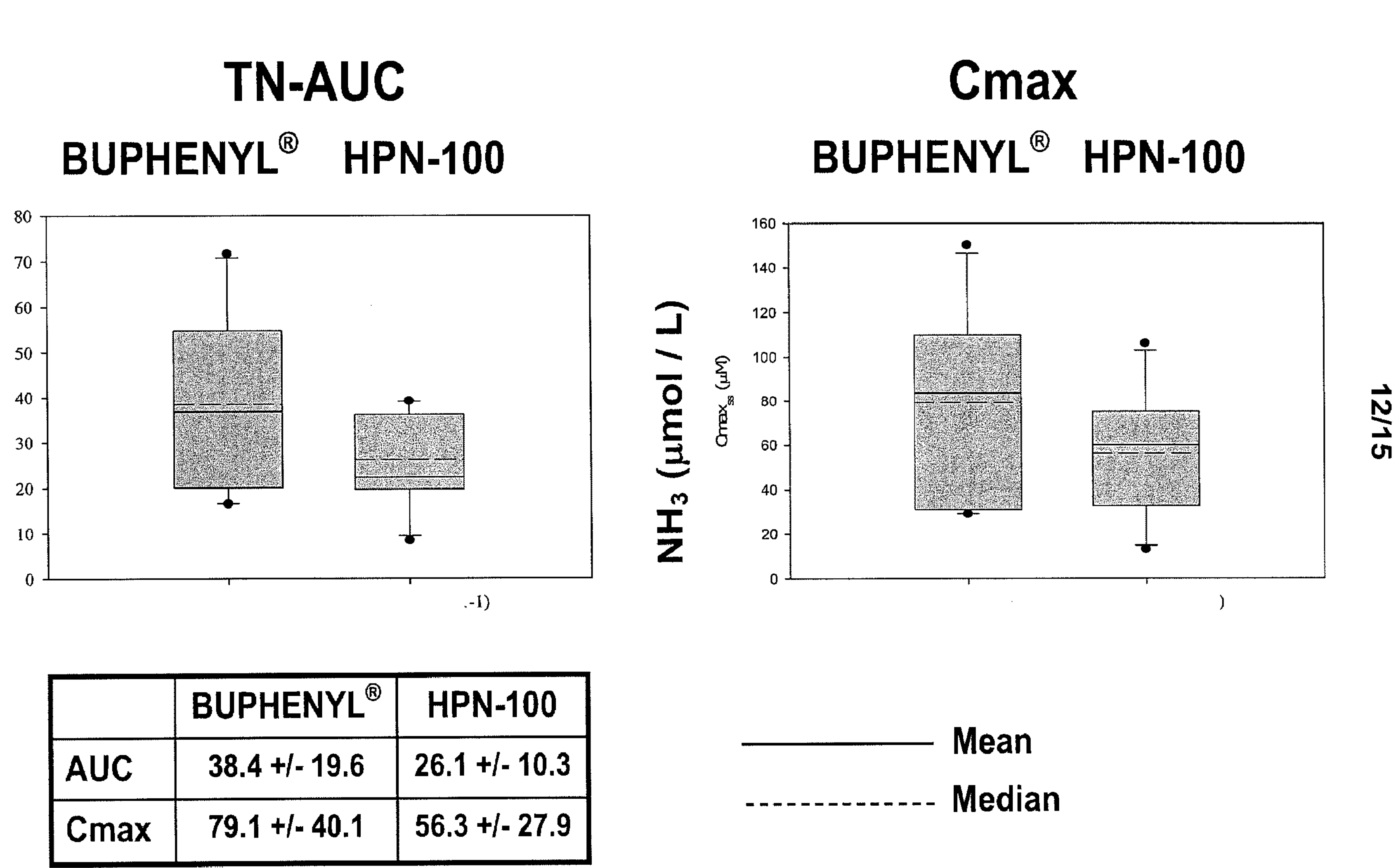
Figure 10



	BUPHENYL®	HPN-100
AUC	38.4 +/- 19.6	26.1 +/- 10.3
Cmax	79.1 +/- 40.1	56.3 +/- 27.9

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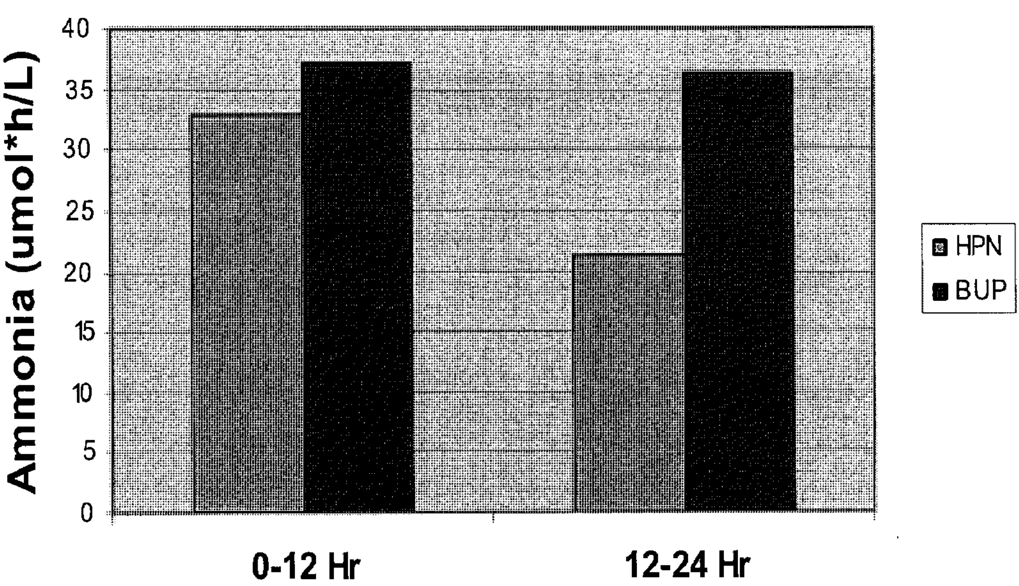
Figure 11



Plasma ammonia levels (time-normalized area under the curve [TN-AUC or AUC]) during the day and night in 10 UCD patients treated for seven days with either sodium PBA (BUP) or a PBA equimolar dose of HPN-10.

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Plasma ammonia levels (time-normalized area under the curve TN-AUC)
in 10 UCD patients treated for seven days
with sodium PBA (BUP) followed by seven days with a PBA equimolar dose of HPN-100.

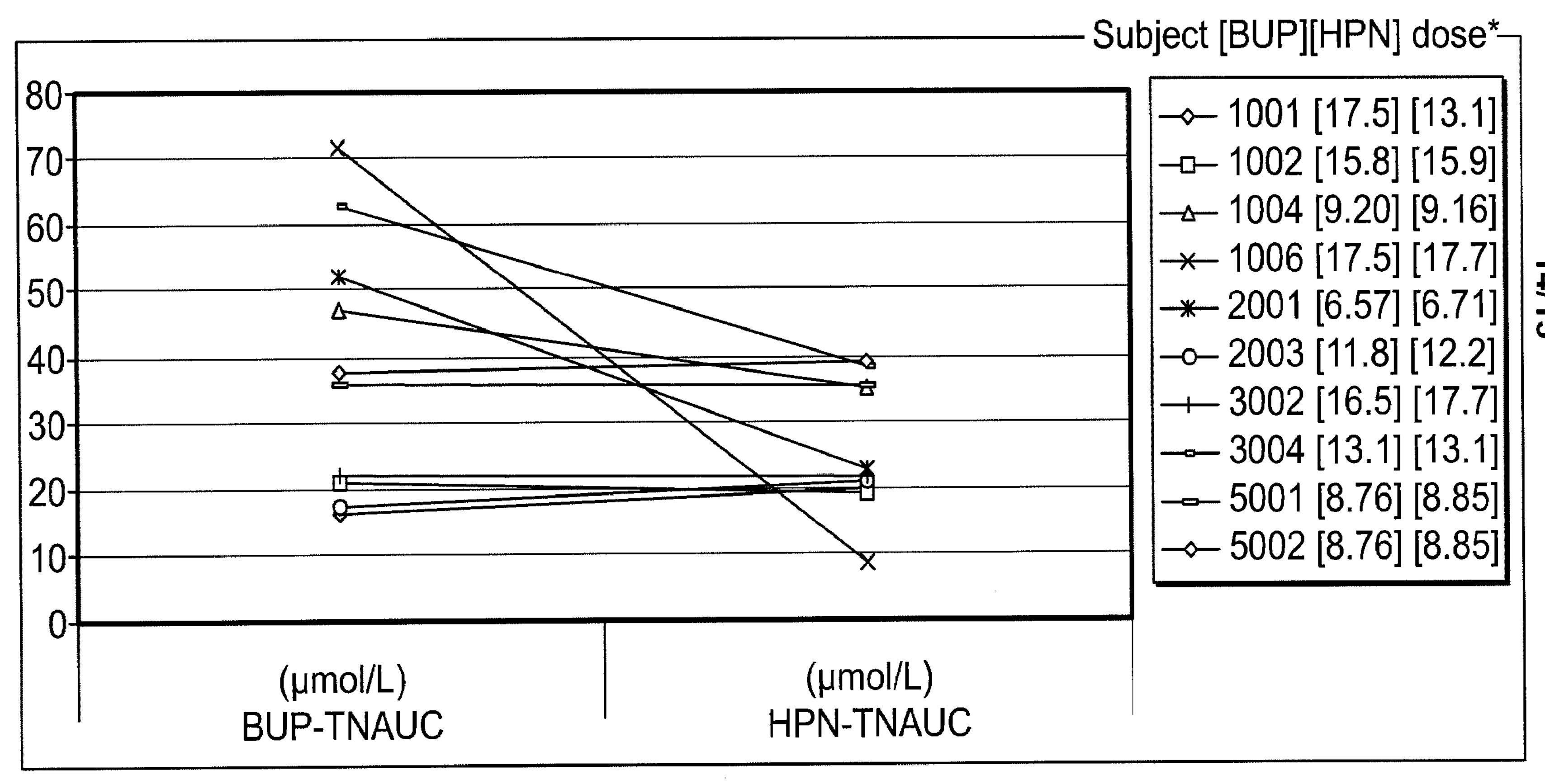

Figure 12

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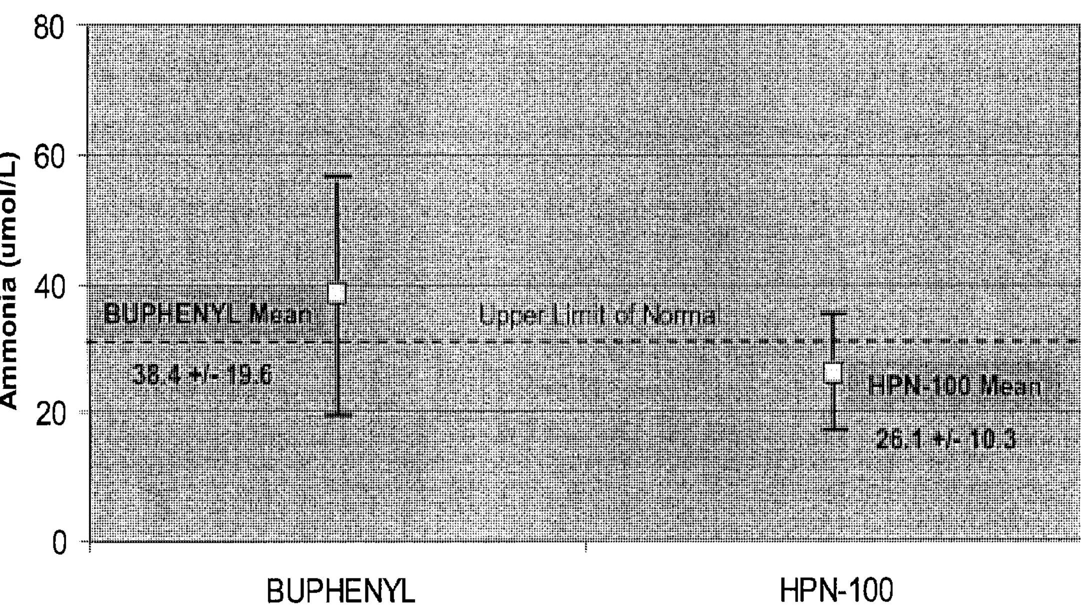
Figure 13



Mean plasma ammonia levels (time-normalized area under the curve [TN-AUC]) in 10 UCD patients treated for seven days with sodium PBA followed by seven days with a PBA equimolar dose of HPN-100.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/30362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
US WEST(PGPB,USPT,EPAB,JPAB), Google Scholar, Dialog PRO (Engineering)
ammonia scavenging, accumulation, retention, hepatic encephalopathy, urea cycle disorder, phenylacetyl glutamine, PAGN, HPN-100, phenyl butyrate, glyceryl tri-(4-phenyl butyrate)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0229948 A1 (SUMMAR, et al.) 18 November 2004 (18.11.2004), para [0022], [0029], [0035]	1-11, 19-22, 28, 29
Y	US 4,284,647 A (BRUSILOW, et al.) 18 August 1981 (18.08.1981) col 2, ln 26-32; Fig. 3; col 4, ln 35-46.	1-5, 9-18, 23-27, 29
Y	US 5,968,979 A (BRUSILOW) 19 October 1999 (19.10.1999), col 1, ln 27-34; col 1, ln 41-45; col 2, ln 25-34; col 3, ln 3-7; col 3, ln 42-59; col 4, ln 1-26; col 4, ln 54-58; col 5, ln 3-15; ln 29-35	6-29

☐ Further documents are listed in the continuation of Box C. ☐

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"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2009 (24.02.2009)	02 MAR 2009

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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[Continued on next page]

(54) **Title**: DOSING AND MONITORING PATIENTS ON NITROGEN-SCAVENGING DRUGS

Figure 1a
Nitrogen Retention States

Human Nitrogen Retention States: Hereditary (UCDs) And Acquired (Cirrhosis) Liver Disease And Chronic Renal Failure (CRF)

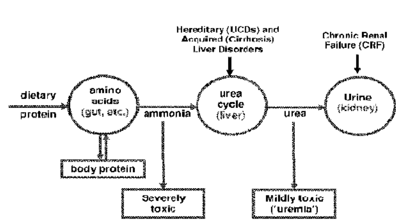
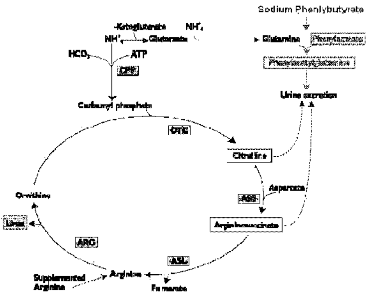


Figure 1b
The Urea Cycle



(57) **Abstract**: The invention provides a method for determining a dose and dosing schedule, and making dose adjustments of patients taking PBA prodrugs as nitrogen scavengers to treat nitrogen retention states, including ammonia accumulation disorders as well as chronic renal failure, by measuring urinary excretion of phenylacetylglutamine and/or total urinary nitrogen. The invention provides methods to select an appropriate dosage of a PBA prodrug based on the patient's dietary protein intake, or based on previous treatments administered to the patient. The methods are applicable to selecting or modifying a dosing regimen for a subject receiving an orally administered waste nitrogen scavenging drug, and to monitoring patients receiving such drugs.

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ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

DOSING AND MONITORING PATIENTS ON NITROGEN-SCAVENGING DRUGS

Cross-Reference to Related Applications

[0001] This application is a continuation in part of U.S. Nonprovisional Patent Application Serial No. 12/350,111, filed January 7, 2009 which is pending, and a continuation in part of International Application No. PCT/US08/30362, filed January 9, 2009, each of which claims benefit of priority to U.S. Provisional Application Serial Number 61/093,234, filed August 29, 2008, each of which is incorporated herein by reference in its entirety. This application is also related to the U.S. provisional patent application entitled "Treating special populations having liver disease with nitrogen-scavenging compounds," naming Sharron Gargosky as inventor, serial number 61/048,830, filed on April 29, 2008.

Technical Field

[0002] This invention relates to treatment of patients with nitrogen retention states, including urea cycle disorders (UCDs), cirrhosis complicated by hepatic encephalopathy (HE) and chronic renal failure (CRF), using administered compounds that assist in elimination of waste nitrogen from the body. The compounds can be orally administered small-molecule drugs, and the invention provides methods for delivering such compounds and selecting suitable dosages for a patient as well as adjusting dosages and monitoring effectiveness of a treatment. As depicted in Figure 1a, inherited disorders (e.g., UCDs) and acquired disorders (e.g. cirrhosis, typically with portal systemic shunting, complicated by HE) involving the liver which impair the normally efficient clearance of ammonia from the portal circulation and conversion to urea via the urea cycle, depicted in Figure 1b, result in elevated levels in the blood of ammonia, a potent neurotoxin. CRF, while associated in some instances with mildly elevated levels of ammonia, (Deferrari, Kid Int. 1980; 20:505), results in retention of other nitrogenous waste products normally excreted in the urine, in particular urea, the blood levels of which are commonly used to assess renal function.

[0003] Restriction of dietary protein (i.e. intake of dietary nitrogen) is commonly used in the management of each of these nitrogen retention states, to avoid accumulation of ammonia or metabolic products containing ammonia, e.g., urea. References herein to ammonia and ammonia

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scavenging refer primarily to treating UCDs and HE and conditions that emulate UCDs, although the terms ammonia scavenging and waste nitrogen scavenging are used interchangeably.

Background Art

[0004] Drug dosing is usually based upon measurement of blood levels of the active drug species in conjunction with clinical assessment of treatment response. However, the present invention is based on evidence that for certain prodrugs of phenylacetic acid (PAA), measuring the blood level of the prodrug (e.g. PBA) or of PAA formed from it is unreliable in assessing drug effect: drug levels in the blood do not correlate with efficacy in this case. In addition, assessment of treatment effect by measuring levels of ammonia in the blood in UCD patients is also potentially unreliable. Individual ammonia level measurements vary several-fold over the course of a day for a given patient, and withdrawing multiple blood samples under carefully controlled conditions over an extended period of time is clinically impractical as a way to monitor a treated patient. The variability in blood ammonia levels reflects the fact that ammonia levels in UCD patients are affected by various factors including dietary protein and timing in relation to meals, such that any individual value fails to provide a reliable measure of how much ammonia the drug is mobilizing for elimination; i.e. drug effect. The invention demonstrates that prodrugs of phenylbutyric acid (PBA) behave similarly to sodium PBA, in that measuring PBA levels is unreliable for assessing their effectiveness. This invention provides a novel method for dosing in patients with nitrogen retention states, in particular patients with liver disease and clinical manifestations of hepatic encephalopathy and patients with UCDs. It is particularly applicable to prodrugs that liberate or are metabolized to form phenylacetic acid, i.e., prodrugs of PAA, and those prodrugs that are metabolized to form PBA.

[0005] Hepatic encephalopathy (HE) refers to a reversible spectrum of neurologic signs and symptoms which frequently occur in patients with cirrhosis or certain other types of liver disease.

[0006] Urea cycle disorders (UCDs) comprise several inherited deficiencies of enzymes or transporters necessary for the synthesis of urea from ammonia. The urea cycle is depicted in Figure 1b, which also illustrates how certain ammonia-scavenging drugs act to assist in elimination of excessive ammonia. UCDs include inherited conditions associated with insufficient function of any one of several ammonia-processing enzymes. Individuals born with no meaningful residual urea synthetic capacity typically present in the first few days of life (neonatal presentation). Individuals with residual function typically present later in childhood or even in adulthood, and

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symptoms may be precipitated by increased dietary protein or physiological stress (e.g. intercurrent illness.) Some enzymes whose deficient functioning causes UCDs include the following:

- Carbamyl phosphate synthetase (CPS),
- ornithine transcarbamylase (OTC),
- argininosuccinate synthetase (ASS),
- argininosuccinate lyase (ASL),
- arginase (ARG; EC Number 3.5.3.1; autosomal recessive), (ARG) and
- N-acetyl glutamine synthetase (NAGS)

[0007] Mitochondrial transporter deficiency states which mimic many features of urea cycle enzyme deficiencies, and thus emulate UCDs and are treatable by the methods described herein for treating UCDs, include the following:

- Ornithine translocase deficiency (hyperornithinemia, hyperammonemia, homocitrullinuria or HHH Syndrome)
- Citrin (aspartate glutamate transporter) deficiency

[0008] The common feature of UCDs and similar conditions and hepatic encephalopathy that render them treatable by methods of the invention is an accumulation of excess waste nitrogen in the body, and hyperammonemia. CRF is similarly characterized by build-up of excessive waste nitrogen in the blood in the form urea, and the ammonia scavenging drugs described herein are likewise effective to prevent accumulation of excess levels of urea. In normal individuals, the body's intrinsic capacity for waste nitrogen excretion is greater than the body's waste nitrogen production, so waste nitrogen does not accumulate and ammonia does not build up to harmful levels. For patients with nitrogen retention states such as UCD or HE, the body's intrinsic capacity for waste nitrogen excretion is less than the body's waste nitrogen production based on a normal diet that contains significant amounts of protein. As a result, waste nitrogen builds up in the body of a patient having a nitrogen retention disorder, which usually results in excess ammonia in the blood. This has various toxic effects; drugs that help eliminate the excess ammonia are an important part of an overall management strategy for such disorders.

[0009] To avoid build-up of ammonia to toxic levels in patients with nitrogen retention states, dietary intake of protein (a primary source of exogenous waste nitrogen) must be balanced by the patient's ability to eliminate excess ammonia. Dietary protein can be limited, but a healthy diet requires sufficient protein to support normal growth (i.e. in growing children) and repair; thus in

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addition to controlling dietary protein intake, drugs that assist with elimination of nitrogen are used to reduce ammonia build-up (hyperammonemia). The capacity to eliminate excess ammonia in treated patients can be considered the sum of the patient's endogenous capacity for nitrogen elimination (if any) plus the amount of additional nitrogen-elimination capacity that is provided by a nitrogen scavenging drug. The methods of the invention use a variety of different drugs that reduce excess waste nitrogen and ammonia by converting it to readily-excreted forms, such as phenylacetyl glutamine (PAGN). In some embodiments, the invention relates to methods for determining or adjusting a dosage of an oral drug that forms PAA *in vivo*, which is converted into PAGN, which is then excreted in urine and thus helps eliminate excess nitrogen.

[0010] Based on prior studies in individual UCD patients (e.g. Brusilow, Pediatric Research, vol. 29, 147-50 (1991); Brusilow and Finkelstien, J. Metabolism, vol. 42, 1336-39 (1993)) in which 80-90% of the nitrogen scavenger sodium phenylbutyrate (a PAA prodrug) was reportedly excreted in the urine as PAGN, current treatment guidelines typically either assume complete conversion of sodium phenylbutyrate or other PAA prodrugs to PAGN (e.g. Berry et al., J. Pediatrics, vol. 138, S56-S61 (2001)) or do not comment on the implications of incomplete conversion for dosing (e.g. Singh, Urea Cycle Disorders Conference Group '*Consensus Statement from a Conference for the Management of Patients with Urea Cycle Disorders*', Suppl to J Pediatrics, vol. 138(1), S1-S5 (2001)). Based on what is known, one expects essentially complete conversion of these drugs into urinary PAGN.

[0011] PBA is currently the preferred nitrogen scavenging drug for UCD patients in need of substantial nitrogen elimination capacity. Current treatment guidelines recommend 4 times per day dosing with PBA, based on the fact that PBA is absorbed rapidly from the intestine when administered in the form of sodium PBA and exhibits a short half life in the bloodstream (Urea Cycle Disorders Conference Group 'Consensus Statement' 2001). Current recommendations for sodium phenylbutyrate dosing in UCD patients indicate that dosage should not exceed 600 mg/kg (for patients weighing up to 20 kg) or in any case 20 grams total per day. Frequent dosing helps minimize the peak levels of ammonia, which can be very harmful, and it minimizes buildup of high concentrations of PAA as well.

[0012] CRF (chronic renal failure) resulting from a variety of causes (e.g. diabetes, hypertension, glomerular disease, etc.) is associated with diminished excretion from the body of water soluble waste products normally present in the urine, including nitrogenous waste such as urea. While the contribution of increased blood levels of urea, per se, to the clinical manifestations

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of CRF and end-stage renal disease (ESRD) known as uremia is uncertain, urea levels in the blood are commonly used as one measure of renal function and the need for and frequency of renal replacement therapy such as dialysis. As a corollary of the findings noted above in UCD patients (e.g. Brusilow, *Pediatric Research*, vol. 29, 147-50 (1991); Brusilow and Finkelstien, J. Metabolism, vol. 42, 1336-39 (1993)), increased waste nitrogen excretion in the form of PAGN resulting from administration of PAA prodrugs decreases urea synthesis and therefore can serve as an alternative to urea excretion. Consistent with this, Brusilow (US Patent # 4,284,647) has demonstrated that administration of sodium benzoate, which increases waste nitrogen excretion in the form of hippuric acid, lowered blood urea levels in a patient with renal failure (Figure 14). Accordingly, PAA prodrugs, including PBA and HPN-100 can be used to treat CRF as well as UCDs and HE, and methods for determining and adjusting dosage of these PAA prodrugs and monitoring treatment efficacy are among the inventions disclosed herein. In general, and without being limited by theory, prodrugs of PAA which do not contain sodium would be preferred for treatment of treatment of those nitrogen retention states, including CRF as well as cirrhosis and HE, which are also known to be associated with sodium and fluid retention manifested, for example, as ascites and or peripheral edema. HPN-100 is one such sodium-free PAA prodrug.

Disclosure of Embodiments of the Invention

[0013] The invention provides a novel approach for determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs, including sodium phenylbutyrate and glyceryl tri-[4-phenylbutyrate] (HPN-100), based upon the urinary excretion of the drug metabolite phenylacetylglutamine (PAGN) and/or total urinary nitrogen. It is based in part on the discovery that bioavailability of these drugs as conventionally assessed based on systemic blood levels of the drugs themselves or of the active species produced *in vivo* from these drugs does not accurately predict removal of waste nitrogen or reduction of plasma ammonia in healthy human volunteers, adults with liver disease, or patients with UCDs receiving ammonia scavenging drugs as defined below. Conversion of orally administered sodium phenylbutyrate (NaPBA, or sodium PBA) to urinary PAGN (uPAGN) is now shown to be incomplete: conversion is typically about 40-70%, or about 54% on average. (A preliminary analysis suggested the range would be around 60-75%, but final analysis shows the average is about 54%.) The average value of about 54% conversion was determined experimentally for orally administered HPN-100 or PBA converting into urinary PAGN, and a range of about 40-70% represents the average plus or minus approximately one

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standard deviation for this data set. By comparison, correlating urinary PAGN with drug dosage using information available in the art would have provided substantially different results, since the prior art suggests a much higher conversion, e.g., 90% or more. As used in this context, "about 54%" refers to a value between 50% and 60%, and the urinary PAGN output refers to a measure of urinary PAGN output for a subject receiving ongoing stable daily dosages of the nitrogen scavenging drug.

[0014] Urinary PAGN can be measured in various ways; in some embodiments, as described herein, it is a 24-hour measurement, which means measurement of total urinary PAGN output for a period of 24 hours following the first dose of the day of a nitrogen scavenging drug. In other embodiments, a 12-24 hour urinary PAGN level is used, which is the total amount of urinary PAGN excreted over the time period 12-24 hours after the first dose of the day. As an alternative, as described herein, spot testing of urinary PAGN levels can be used, by normalizing the value as a ratio to urinary creatinine output. Daily creatinine output is relatively stable for most subjects, and this has been found to be true even in the UCD, HE, and CRF patients receiving the nitrogen scavenging drugs described herein. Because creatinine output is relatively stable, it can be used to normalize urinary PAGN output levels: from a 'spot test' of a partial sample, the ratio of uPAGN to urinary creatinine can be used to estimate a total daily urinary PAGN output. These values may be used in calculations of dosages or protein intake based on urinary PAGN output as well as for determining initial drug dosage for a patient taking a given amount of protein.

[0015] The invention further provides methods to easily monitor treated patients to determine from urinary PAGN output whether their overall treatment program (diet and medication) is working, and when the patient needs a modified treatment program or adjusted drug dosage. These methods comprise monitoring urinary PAGN output, either as a 24 hour output, or as a 12-24 hour total urinary PAGN output, or as an estimated value from a spot test, where the urinary output is normalized to urinary creatinine and converted to an estimated 24-hour (or 12-24 hour) output. In one embodiment, the method comprises comparing that value for urinary PAGN to a cut-off value that distinguishes patients likely to have normal ammonia levels from patients likely to have high ammonia levels.

[0016] Prodrugs of phenylbutyrate (PBA, the active ingredient in BUPHENYL®(sodium phenylbutyrate), which is the sodium salt of PBA along with small amounts of inert ingredients), which is itself a prodrug of phenylacetic acid (PAA), are especially subject to the effects described herein.

phenylbutyrate

Phenylacetic acid

Phenylacetylglutamine

[0017] As used herein "ammonia scavenging drugs" is defined to include all orally administered drugs in the class which contain or are metabolized to phenylacetate. Thus, the term includes at least phenylbutyrate, BUPHENYL® (sodium phenylbutyrate), AMMONAPS®, butyroyloxymethyl-4-phenylbutyrate, glyceryl tri-[4-phenylbutyrate] (HPN-100), esters, ethers, and acceptable salts, acids and derivatives thereof. These drugs reduce high levels of endogenous ammonia by providing phenylacetic acid in vivo, which is metabolized efficiently to form phenylacetyl glutamine (PAGN). PAGN is efficiently excreted in urine, carrying away two equivalents of nitrogen per mole of PAA converted to PAGN. References herein to sodium phenylbutyrate are understood to include reference to the drug product BUPHENYL®, and BUPHENYL® was used for the Examples herein wherever test subjects were treated with sodium phenylbutyrate. Thus the sodium PBA dosages used in the Examples generally refer to a dosage of BUPHENYL®, and the amounts of sodium phenylbutyrate in those Examples should be interpreted accordingly. Note that the terms 'ammonia scavenger' and 'nitrogen scavenger' are used interchangeably in this invention, reflecting the fact that the drugs described herein lower blood ammonia and/or urea levels through elimination of waste nitrogen in the form of PAGN.

[0018] In some embodiments, the invention uses prodrugs that can be converted into PAA within the body. Sodium phenylbutyrate (sodium PBA) is one such drug; it is converted by oxidative mechanisms into PAA in the body. HPN-100 is another such drug: it can be hydrolyzed to release PBA, which in turn can be converted to PAA. Thus, HPN-100 is a prodrug of PBA, and also a pre-prodrug of PAA. Clinical evidence demonstrates that HPN-100 is converted into PAA

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in the body as expected, and that PAA is then linked to a molecule of glutamine and converted into PAGN, which is eliminated in the urine as predicted. This process can be summarized as follows:

HPN-100 → 3 PBA → 3 PAA

PAA + glutamine → PAGN.

[0019] PAGN is mainly excreted in the subject's urine, and removes two molecules of ammonia per molecule of excreted PAGN. Each HPN-100 molecule forms three PAA molecules, so each molecule of HPN-100 can promote excretion of six molecules of ammonia. The clinical results suggest that conversion of HPN-100 into PBA and PAA is efficient, in that HPN-100 is generally not detectable in blood, but surprisingly suggest that some PBA derived from HPN-100 is converted to PAGN before the HPN-100 (or PBA, or PAA derived from PBA) enters systemic circulation. As a result, systemic levels of PAA or PBA are not reliably correlated with the efficacy of HPN-100 as an ammonia scavenger.

[0020] In some embodiments, the invention uses a prodrug of PBA, including HPN-100 and other esters of phenylbutyrate. The PBA prodrug is thus a prodrug of a prodrug, since PBA acts to scavenge ammonia after it is converted to PAA and is thus considered a prodrug of PAA. In some embodiments, the PBA prodrug is an ester of phenylbutyrate, such as those described below; a preferred PBA prodrug for use in the invention is HPN-100. These compounds can be made and used by methods disclosed in U.S. Patent No. 5,968,979, which is incorporated herein by reference for its description of these compounds and methods for their administration.

[0021] Where an 'equal molar' or 'equimolar' amount of a second drug is to be used along with or instead of a certain amount of a first drug, the amount of each drug is calculated on a molar basis, and the equimolar amount of the second drug is the amount that produces an equal molar amount of active drug *in vivo*. Where one of the drugs is a prodrug, the amount of prodrug will typically refer to the molar amount of the active species formed from that prodrug. That active species is usually PAA for the prodrugs described herein, and the molar amount of a prodrug corresponds to the amount of PAA that would form in the body from that amount of the prodrug, assuming complete conversion into PAA occurs *in vivo*. Thus, for example, a molecule of HPN-100 can be metabolized by ester hydrolysis followed by oxidation to form three molecules of PAA, so a mole of HPN-100 would be considered equimolar to three moles of PAA. Similarly, since HPN-100 hydrolyzes to form three molecules of PBA (and one molecule of glycerol), an equimolar amount of HPN-100 would be one-third of the molar amount of PBA.

[0022] The following Table sets forth amounts of HPN-100 that correspond to equimolar amounts of certain relevant doses of BUPHENYL® (sodium phenylbutyrate). Note that the conversion of the dose of sodium PBA to the dose of HPN-100 involves correction for their different chemical forms [i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium; (sodium PBA [g] x 0.95 = HPN-100 [g])] as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

Table 1. Conversion Factors.

BUPHENYL® (sodium PBA)	**HPN-100 PBA Equivalent Dose (mg)**	**HPN-100 PBA Equivalent Dose (mL)**
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[0023] The present invention can use prodrugs of the formula (I):

(I)

wherein R_1, R_2, and R_3 are independently, H,

or

and n is zero or an even number, m is an even number and at least one of R_1, R_2, and R_3 is not H. For each R_1, R_2, or R_3, n or m is independently selected, so the R_1, R_2, and R_3 groups in a compound of formula I do not have to be identical. The preferred compounds are those wherein none of R_1, R_2, and R_3 is H, and frequently each n or m for a particular embodiment is the same, i.e., R_1, R_2, and R_3 are all the same. The advantage over the prior art of decreased

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dosage is greater with such triesters, and having all three acyl groups the same reduces issues related to mixtures of isomers. Moreover, the triol backbone liberated by hydrolysis of the esters is glycerol, a normal constituent of dietary triglyceride which is non-toxic.

[0024] The present invention also utilizes phenylbutyrate and phenylacetate prodrugs of the formula II:

$$R-O-C(=O)-R_4 \quad \text{(II)}$$

wherein R is a C_1-C_{10} alkyl group,

R_4 is

$-(CH_2)_n-CH_2-C_6H_5$ or $-(C_mH_{2m-2})-CH_2-C_6H_5$

and n is zero or an even number, and m is an even number.

[0025] In Formula II, R can be, for example, ethyl, propyl, isopropyl, n-butyl, and the like.

[0026] The compounds of the invention are esters of the congeners of phenylalkanoic and phenylalkenoic acids having an even number of carbon atoms in the alkanoic acid portion, which include phenylacetic acid esters and those of phenylbutyric acid, etc., which can be converted by efficient beta-oxidation processes to phenylacetic acid in the body. They are thus prodrugs for phenylacetic acid. Where n is 2 or 4, the esters are also prodrugs for phenylbutyric acid. Preferably the alkylene or alkenylene carboxylate group contains 24 or fewer carbon atoms, so n or m is less than 24. In some embodiments, n and m are 0, 2, 4 or 6, and in some preferred embodiments n or m is 2.

[0027] Certain preferred embodiments of the invention use HPN-100 (Formula III):

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(III)

[0028] Total daily dosage of prodrugs like sodium PBA can often be selected according to the amount needed to provide an appropriate amount of the active species, if that amount is known or can be determined. PBA is a prodrug for PAA; therefore, an initial dose of PBA could be selected if an effective dosage of PAA were known, taking into account the fraction of PBA that is converted into PAA and ultimately into PAGN. If a subject has been treated with PAA or a prodrug that forms PAA in the body, the amount of the previously used drug that was effective provides a possible starting point for selecting a dosage of a new prodrug of PAA. In this same patient, after the new prodrug is administered at the expected PAA dose equivalence, the PAA levels in the subject could be monitored and the dose of the prodrug adjusted until the same plasma level of PAA that was effective with the previous treatment is achieved. However, the current invention is based in part on finding that plasma PAA and PBA levels are not well correlated with the dose of a PBA prodrug administered or with ammonia elimination; for monitoring a dosing level of a PBA prodrug, one should not rely upon these parameters to assess the effectiveness of the prodrug. While not bound by the underlying theory, explanations for this effect (i.e. the inconsistent relationship between ammonia scavenging and PBA and/or PAA blood levels) are provided herein.

[0029] The following Tables provides data from three clinical test groups showing the inconsistent relationship between plasma PAA and PBA levels among healthy volunteers, patients with cirrhosis and UCD patients, despite that fact that, as described in detail below, all groups exhibited similar ammonia scavenging activity based on urinary excretion of PAGN. Note in Table

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2, for example, that plasma PAA and PBA, measured as AUC_{24} were both about 4-fold lower following single dose administration of HPN-100 as compared with sodium PBA to healthy volunteers (see also Figure 4), despite similar ammonia scavenging as determined by urinary output of PAGN. Similarly, healthy volunteers and cirrhotic subjects exhibited no differences in urinary PAGN output, yet PAA blood levels tended to be higher in Child-Pugh C cirrhotics.

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Table 2. Plasma Pharmacokinetics of PBA, PAA, and PAGN Comparison across Studies

Analyte	Treatment	C_{max} (μg/mL)	T_{max} (h)	T½ (h)	AUC_{24} (μg·h/mL)
Healthy Volunteers (Single Dose – 3 g/m²/day PBA Mole Equivalent)					
PBA	Sodium PBA	221.0	0.9	0.7	542.6
	HPN-100	37.0	2.4	1.9	137.2
PAA	Sodium PBA	58.8	3.9	1.2	279.8
	HPN-100	14.9	4.0	NC	70.9
PAGN	Sodium PBA	63.1	3.2	1.7	395.1
	HPN-100	30.2	4.0	NC	262.1
Healthy Volunteers and Cirrhotic Patients (Multiple day dosing of HPN-100 at 100 mg/kg BID) [1]					
PBA	Child-Pugh A	42.8	2.3	1.2	131.7
	Child-Pugh B	41.8	2.9	3.4	189.5
	Child-Pugh C	44.3	3.1	1.9	192.1
	Volunteers	29.8	3.0	2.1	132.7
PAA	Child-Pugh A	33.2	3.8	1.8	168.8
	Child-Pugh B	30.8	4.5	2.8	252.4
	Child-Pugh C	53.1	4.8	7.7	579.9
	Volunteers	25.5	3.6	1.9	130.5
PAGN	Child-Pugh A	37.7	3.9	5.0	335.1
	Child-Pugh B	38.1	4.0	7.5	466.99
	Child-Pugh C	43.1	5.3	4.0	578.4
	Volunteers	46.3	4.3	7.2	550.9
UCD Subjects (Multiple Dose – PBA Mole Equivalent)					
PBA	Sodium PBA	141.0	2.1	NC	739.0
	HPN-100	70.1	6.1	NC	540.0
PAA	Sodium PBA	53.0	8.1	NC	595.6
	HPN-100	40.5	8.0	NC	574.6
PAGN	Sodium PBA	83.3	7.2	3.9	1133.0
	HPN-100	71.9	8.0	4.8	1098.0

C_{max}= maximum plasma concentration; T_{max} = time of maximum plasma concentration; AUC_{24} = AUC from time 0 to 24 hours; NC = not calculated

[1]Study did not include a sodium phenylbutyrate comparator arm, values represent HPN-100 dosing only. AUC values represent the AUC from time 0 to the last measurable plasma concentration.

[0030] The plasma and urinary pharmacokinetic parameters for 10 UCD patients are summarized at the bottom of Table 2 and in more detail in the following Table 3. Note that urinary PAGN output was very similar for sodium PBA (as BUPHENYL) and HPN-100 in UCD patients at steady state following multiple day dosing. However, in this case, plasma PBA levels measured as AUC_{24} following HPN-100 were about 27% lower following HPN-100 administration, as compared with sodium PBA administration, whereas PAA blood levels were similar for subjects

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on both drugs. Notably, peak urinary output of PAGN occurred during hours 6-12 after the first dose of PBA, but during hours 12-24 following HPN-100 dosing, again demonstrating the slow-release characteristics of HPN-100. Collectively, these findings demonstrate that the blood levels of PBA, PAA and PAGN do not correlate consistently with urinary PAGN output, which is stoichiometrically related to ammonia scavenging, and suggest that urinary PAGN output provides a convenient method for monitoring ammonia elimination induced by the administered drug.

Table 3. PK Parameters and Ammonia Following NaPBA and HPN-100 Administration

PK Parameters	Arithmetic Mean (CV%)	
	HPN-100 (n=10)	**NaPBA (n=10)**
PBA in Plasma		
AUC_{0-24} (µg•h/mL)	540 (60.2)*	739 (49.1) *
$Cmax_{ss}$ (µg/mL)	70.1 (64.7)	141 (44.5)
$Cmin_{ss}$ (µg/mL)	2.87 (265)	0.588 (255)
PAA in Plasma		
AUC_{0-24} (µg•h/mL)	575 (169) *	596 (124) *
$Cmax_{ss}$ (µg/mL)	40.5 (148)	53.0 (94.7)
$Cmin_{ss}$ (µg/mL)	7.06 (311)	3.56 (194)
PAGN in Plasma		
AUC_{0-24} (µg•h/mL)	1098 (44.2) *	1133 (31.0)**
$Cmax_{ss}$ (µg/mL)	71.9 (56.0)	83.3 (25.8)
$Cmin_{ss}$ (µg/mL)	12.1 (134)	16.8 (86.1)
PAGN in Urine*		
Total excreted 0-24 hr (µg)	10 784 747 (25.9)	12 153 473 (48.2)
0-6 hr (µg)	2381371 (61.3)	2452838 (41.6)
6-12 hr (µg)	3027310 (44.9)	4859121 (54.7)
12-24 hr (µg)	5433033 (50.4)	4645447 (59.8)
Recovery of PBA as PAGN (%)	54 (15)	54 (16)
Total Urinary Nitrogen in 24 hr Mean (SD) g	8.9 (3.0)**	9.6 (3.9)**
Ammonia		
TNAUC (µmol/L)	26.2 (39.2)	38.4 (51.0)
$Cmax_{ss}$ (µmol/L)	56.3 (49.5)	79.1 (50.6)
% normal ammonia values *	57.98 (33.37)	72.22 (27.23)
Mean Ammonia Ratio (glycerol phenylbutyrate /NaPBA)	0.71	
95% CI of ratio	0.44-1.15	

AUC_{0-24}: Area under the concentration from time 0 (pre-dose) to 24 hours, $Cmax_{ss}$: Maximum plasma concentration at steady state, $Cmin_{ss}$: Minimum plasma concentration at steady state,, * n=8, **n=9
TNAUC: Time-normalized area under the curve;
*% normal ammonia values are presented as mean (SD)

[0031] The following Table further demonstrates the utility of urinary PAGN as a measure of drug effect, assessed by examining correlates of blood ammonia measured over 24 hours and expressed as time-normalized area under the curve (TNAUC). These measurements were made following administration of either sodium PBA or HPN-100, and correlation coefficients are

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shown as 'r' values and tests of statistical significance by 'p' values. As the table shows, there was no statistical correlation between ammonia levels and PAA or PBA plasma levels and a barely significant correlation with plasma PAGN levels (p = 0.04). As expected, there is a correlation with drug dose; and surprisingly, there is a very strong (p <0.0001) inverse correlation with 24 hour urinary PAGN output (see also Figure 13), as well as with the 12-24 hour urinary PAGN output (p **<0.001**). This data is related to the testing described in Example 4 herein.

Table 4. Correlation Between Ammonia and Plasma PAA, PBA and PAGN, and Urinary PAGN (UPAGN) [1, 2]

	Plasma PAA	Plasma PBA	Plasma PAGN	UPAGN	Dose	U-PAGN 12-24
N	15	15	16	18	18	16
r	-0.23	0.08	-0.52	-0.80	**-0.55**	**-0.75**
p	NS	NS	0.04	<0.0001	**0.02**	**<0.001**

[1] Ammonia was measured as time-normalized area under the curve (TNAUC) based on up to 11 samples drawn over 24 hours
[2] Spearman Rank-Order Correlation
Data from both NaPBA and HPN-100 treated subjects were included in the analysis
Data from one subject with more than 50% missing data on HPN-100 were excluded.
NS = not significant at α=0.05.

[0032] As the data in Table 4 demonstrate, the 12-24 hour urinary PAGN output level for these subjects correlates well with plasma ammonia levels, too; thus where a 24 hour urinary PAGN output is used herein, a 12-24 hour urinary PAGN output can be used instead of the 24-hour output. The 12-24 hour sample collection is typically more convenient than interrupting daily activities to collect a 24-hour sample, and it was particularly surprising that 12-24 hour uPAGN output would correlate with ammonia control well enough to be useful.

[0033] One embodiment of the invention is a method for determining and/or adjusting the dose of ammonia scavenging drugs in patients who have UCDs, whereby dose would be based on the amount of dietary protein the patient is consuming, the anticipated percentage conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. Dose adjustments, if necessary, would be based on the observed urinary excretion of PAGN and/or total urinary nitrogen (TUN), the difference between the two reflecting the patient's endogenous capacity for waste nitrogen excretion. This endogenous capacity may be absent in certain patients having innate urea cycle disorders due to inborn metabolic deficiencies, but patients with later-onset nitrogen accumulation disorders generally have some endogenous capacity, referred to sometimes

as their residual urea synthesis capacity. See Brusilow, PROGRESS IN LIVER DISEASES, Ch. 12, pp. 293-309 (1995).

[0034] The subject's plasma ammonia level may also be determined; this is a critical parameter for tracking effectiveness of an overall treatment program, but any given measurement of ammonia levels reflects a variety of factors such as dietary protein and physiological stress, as well as the effect of a drug used to promote nitrogen excretion. Moreover, because ammonia values vary widely during the day for these patients and because repeated monitoring over the course of an entire day is impractical, blood ammonia levels are neither practical nor reliable for routine patient monitoring. The present invention provides practical alternative methods based on urinary PAGN output. A preferred embodiment of the invention provides methods for monitoring plasma ammonia or efficacy of a PAA prodrug treatment without routinely measuring blood levels of ammonia.

[0035] Once the patient's residual endogenous capacity for waste nitrogen excretion has been determined, either as the difference between PAGN output and total nitrogen output or as total urinary nitrogen output in the absence of an ammonia scavenging drug, the tolerable amount of dietary protein can be calculated for that patient according to the dosage of the ammonia scavenging drug being administered, or the dosage of the ammonia scavenging drug can be adjusted or calculated to compensate for an estimated protein intake. The average nitrogen content of dietary protein is well known, as is the stoichiometry for its conversion into PAGN for excretion. The prior art has indicated or assumed that all of the PAA prodrug would be converted into uPAGN. As demonstrated herein, that is not the case, and particularly for HPN-100, improved methods for correlating dietary protein, residual endogenous capacity for waste nitrogen excretion, and drug dosage with urinary PAGN excretion are provided.

[0036] Another embodiment is a method for determining and adjusting the dose of an ammonia scavenging drug to be administered to a patient with liver disease, including hepatic encephalopathy, whereby the starting dose would be based on the amount of dietary protein the patient is consuming, the anticipated conversion of the drug to PAGN, and the patient's residual urea synthetic capacity, if any. While the urea synthetic capacity in patients with liver disease would generally be greater than for patients with UCDs, considerable patient to patient variability would be expected among both groups depending, respectively, on the severity of their liver disease and the severity of their inherited enzymatic defect. Dose adjustments based on the

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observed urinary excretion of PAGN and total waste nitrogen would adjust for these individual patient characteristics.

[0037] Another embodiment is a method for determining or adjusting allowable dietary protein in the diet of a patient with UCD or with hepatic encephalopathy, who is being treated with an oral PAA-forming ammonia scavenging drug, whereby the amount of allowable protein would be determined by the amount of PAGN and total nitrogen in the urine. The difference between total waste nitrogen in the urine and the amount of PAGN excreted is indicative of the patient's endogenous waste nitrogen processing capacity. Once the patient's endogenous nitrogen processing capacity is known, the patient's endogenous nitrogen processing capacity can be used to adjust dietary protein intake while administering a fixed dosage of an ammonia scavenging drug, or the dosage of the ammonia scavenging drug can be determined according to the amount needed to facilitate elimination of the waste nitrogen from the patient's dietary protein. Dietary protein intake should be determined or adjusted according to how much nitrogen the subject can eliminate above the amount that is eliminated as PAGN, which results from the PAA-forming ammonia scavenging drug being administered. When making these calculations or adjustments, it is suitable to assume that about 47% of nitrogen in protein will become waste nitrogen that needs to be excreted in the urine (the amount may be less for very young growing patients, who retain a greater fraction of ingested nitrogen to support body growth), and that about 16% of protein, on average, is nitrogen (see Brusilow 1991).

[0038] It has generally been assumed for such determinations that an orally administered prodrug would be converted with 100% efficiency into PAGN for elimination [see, e.g., Berry et al., J. Pediatrics 138(1), S56-S61 (2001) where Figure 1 assumes 100% conversion]; and one report found that about 80-90% of PAA or PBA was excreted from a specific individual as PAGN. Brusilow, Pediatric Research 29(2), 147-150 (1991). The label for BUPHENYL® is consistent with this, indicating conversion of 80-100% of PBA into urinary PAGN within 24 hours of administration.

[0039] It has now been found that HPN-100 and phenylbutyrate are both converted into urinary PAGN at an overall conversion of about 40% to about 70% on average in 24 hours following drug administration. About 60% conversion efficiency was seen in UCD patients and up to 75% was seen in cirrhotic patients in a preliminary assessment of the data; however, some data used for that assessment was misleading. Upon completion of the study and more detailed analysis of the data, an overall average conversion of about 54% was found, with a standard deviation of about 15%.

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Results are shown in Table 3, which shows the average urinary PAGN output in a 24 hour period was 54±15% for subjects receiving HPN-100, and 54±16% for subjects receiving PBA. Based on this, a conversion efficiency in a range of about 40-70% is expected for both HPN-100 and PBA, with an average conversion efficiency of about 54%; consequently, this efficiency factor can be used to more accurately calculate or determine initial dosing levels for these drugs, or dietary protein levels acceptable for patients who use these drugs. Given this conversion rate, each gram of HPN-100 can facilitate elimination of waste nitrogen from about a gram (~1.3 grams) of dietary protein per day. Note that PAGN carries away two molecules of ammonia per molecule of PAGN. Examples of calculations based on these parameters are provided in Examples 9 and 10 herein. Note that reliance upon the previously used assumptions of near quantitative conversion would result in calculating dosages that would be nearly two-fold different from those calculated using the conversion efficiency measured herein for a relevant UCD patient population, resulting in potentially serious treatment errors. The improved methods herein thus reduce likelihood of administering incorrect drug dosages to a highly sensitive patient population, and should reduce occurrence of hyperammonemia or overmedication.

[0040] In one aspect, the invention provides a method for transitioning a UCD patient from phenylacetate or phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate, and is adjusted according to the levels of excreted PAGN that result when the prodrug is administered.

[0041] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of ammonia scavenging during the transition (e.g. for clinically 'fragile' patients with a propensity for frequent hyperammonemia). The methods can use two, three, four, five, or more than five steps as judged clinically prudent. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate, amount (i.e. the amount necessary to deliver an equimolar amount of PBA) of HPN-100 or other prodrug of phenylbutyrate, e.g., if the transition is to be done in three steps, about one-third of the phenylbutyrate would be replaced with a prodrug at each step.

[0042] Another embodiment of the invention would be the administration of a PAA prodrug, for example a non-sodium containing prodrug such as HPN-100, to a patient with CRF. The enhanced excretion of waste nitrogen in the form of PAGN would be expected to lower urea

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synthesis and levels of urea in the blood, as well as potentially other nitrogenous waste products for which urea accumulation serves as a marker. The dosing principles noted above, including the percent conversion to PAGN for initial dosage selection and for determination of dietary protein would pertain to CRF, as would the utility of urinary PAGN for dose adjustment and patient monitoring.

[0043] Another embodiment of the invention is based on observations that delivery of PBA in the form of a glyceryl tri-ester or other prodrug imparts slow release characteristics that allow greater flexibility in dosing schedule. Sodium phenylbutyrate (sodium PBA), for example, is typically dosed every 4 to 8 hours, or even more frequently, in order to maintain a suitable plasma level of PAA. This regimen reflects the rapid absorption of phenylbutyrate from the gastrointestinal tract and quick metabolic conversion to PAA. HPN-100, by contrast, which is a glyceryl tri-ester of phenylbutyrate, has been found to be absorbed only 40% as rapidly as sodium PBA, enabling dosing three times daily, such as with meals, or even twice daily, such as morning and evening. This dosing flexibility is further enhanced by the fact that the pharmacokinetic (PK) and pharmacodynamic (PD) properties of HPN-100 are indistinguishable in the fed or fasted states. It is thus not critical for the frequency of administration to be rigidly maintained with the PBA prodrugs in the form of an ester; the number of doses per day can be reduced for greater convenience, and the dosages do not have to be linked to meal schedules as is recommended in the label for sodium PBA. Indeed, pharmacokinetics for utilization of HPN-100 were very similar when HPN-100 was taken with food or without food, after a day of fasting, so HPN-100 can be taken with food or without food. This translates into a more convenient treatment protocol and potentially higher patient compliance upon substituting HPN-100 for phenylbutyrate or phenylacetate. Surprisingly, even though HPN-100 and sodium PBA are both prodrugs of PAA, HPN-100 is effective when administered less frequently than sodium PBA. While it is typically necessary to administer smaller doses of sodium PBA 3-6 times per day to maintain a stable level of plasma ammonia, similar results can be achieved with only 2-3 doses of HPN-100 per day. In some embodiments discussed in greater detail below, HPN-100 is administered in two doses per day (BID), and in some embodiments it is administered in three doses per day (TID).

[0044] It has also been found that because of the slow-release characteristics of HPN-100, a patient taking HPN-100 has more stable and often lower plasma levels of PBA than a patient taking sodium PBA itself. For example, systemic exposure to PBA for a subject treated with HPN-100 was 27% lower than that observed for a subject treated with sodium phenylbutyrate. The

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subjects receiving HPN-100 had PBA exposure, measured as a 24 hour AUC, of 540 µg-hr/mL, compared to 739 µg-hr/mL for subjects treated with PBA. This is believed to result from the slow release characteristics of HPN-100 and greater fractional conversion of PBA to PAA/PAGN prior to reaching the systemic circulation following administration of PBA in the form of HPN-100 as compared with sodium PBA (e.g. see Figures 2 and 3), characteristics which offer greater flexibility in dosing that is discussed in more detail elsewhere in this application (plasma levels of PBA rise and fall more quickly after administration of sodium PBA than after administration of HPN-100, and peak plasma levels of PBA are higher as a result). In contrast, levels of PAA in the blood were essentially the same in both groups (1098 µg-hr/mL for subjects receiving HPN-100, and 1133 µg-hr/mL for subjects receiving PBA).

[0045] Other aspects of this invention relate to the observation that there is apparently no saturation in the ability of the body to convert sodium PBA or HPN-100 to urinary PAGN over a several-fold dose range up to and including, the maximum doses of sodium PBA recommended to date. This should enable a patient to take a higher dose of HPN-100 than an equimolar amount compared to the patient's dosage of PBA. It suggests a patient can receive a higher dosage of HPN-100 than those dosages of sodium PBA that have been recommended to date, which is especially useful for patients whose ammonia levels were not adequately controlled by the highest labeled dosages of sodium PBA. Such patients can receive doses of HPN-100 that are higher than previously recommended sodium PBA dosages.

[0046] Other aspects of the invention will be apparent from the following detailed description and the examples provided herein.

[0047] For convenience, the amounts of PAA (phenylacetic acid), PBA (phenyl butyric acid), or HPN-100 to be administered to a subject as discussed herein refer to a total daily dosage. Because these compounds are used in relatively large daily amounts, the total daily dosage may be taken in two, three, four, five, or six, or more than six daily doses, and different drugs may be administered on different schedules. Thus the total daily dosage better describes a treatment regimen with one drug for comparison to treatments with related drugs.

Brief Description of the Drawings

[0048] Figure 1a depicts human nitrogen retention states including urea cycle disorders (UCDs), cirrhosis (e.g. accompanied by portal systemic shunting and hepatic encephalopathy (HE), and chronic renal failure (CRF).

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[0049] Figure 1b shows waste nitrogen disposal via the urea cycle and by the auxiliary pathway involving PAGN.

[0050] Figure 2 depicts a conventional model to describe pharmacokinetic (PK) behavior of a prodrug, which, in the case of phenylbutyrate, assumes that PBA and PAA must reach the systemic circulation in order to be active; i.e., in order to be converted to PAGN and effect ammonia scavenging.

[0051] Figure 3 depicts an adapted model to describe PK behavior of sodium PBA or other drugs such as HPN-100 that can be converted to PBA and PAA, informed by the observations described herein showing that metabolism of HPN-100 results in lower plasma levels of PAA and PBA while providing equivalent pharmacological effect. Unlike the conventional model, this model allows for 'pre-systemic' conversion of PBA/PAA to PAGN and explains inconsistent relationship between blood levels of these metabolites and PAGN-mediated excretion of waste nitrogen

[0052] Figure 4 shows how plasma levels of PAA, PBA, and PAGN change over time following administration of a single dose of either PBA or HPN-100. It shows that the peak level of PAA is lower when the PBA prodrug, HPN-100, is used, and the PAA level at 24 hours post-administration is higher with the prodrug. Thus the prodrug provides a more sustained level of plasma PAA.

[0053] Figure 5 presents an anatomic explanation for the observations that the prodrug (PBA) can be converted to PAGN prior to reaching the systemic circulation (corresponds to the model depicted in Figure 3).

[0054] Figure 6 shows that PBA levels fluctuate relatively rapidly after dosing in healthy adults, while PAA and PAGN levels reach a fairly stable steady state after a few days of treatment with sodium phenylbutyrate.

[0055] Figure 7 shows that PBA, PAA and PAGN levels reach steady states at different times in healthy adults and that PAA takes longer to reach a steady state level in cirrhotics

[0056] Figures 8a, 8b, and 8c show that in subjects treated with HPN-100, there is little or no correlation between the dose of HPN-100 and plasma levels of either PBA or PAA in the subject. However, it also shows that urinary excretion of PAGN correlates well with dosage of HPN-100.

[0057] Figure 9 shows plasma ammonia levels [time-normalized area under the curve, or TN-AUC or Area under the curve (AUC)] during the day and night for 10 UCD patients treated for seven days with sodium PBA, or with an equimolar dosage of HPN-100, and illustrates that HPN-

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100 provided better control of ammonia levels than PBA: both the AUC (area under the curve), which is an index of total ammonia exposure, and Cmax, which measures the peak concentration of ammonia, were lower in subjects receiving HPN-100 than in subjects receiving an equimolar dosage of PBA.

[0058] Figure 10 shows that HPN-100 did a better job than PBA of managing plasma levels of ammonia overnight.

[0059] Figure 11 demonstrates that in patients whose ammonia levels were well controlled on sodium PBA, HPN-100 maintained control. By contrast, patients whose ammonia levels were elevated despite treatment with sodium PBA exhibited the greatest benefit in terms of improved ammonia control from HPN-100.

[0060] Figure 12 summarizes the data from Figure 11 and provides a statistical comparison of ammonia levels for patients on sodium PBA and those on HPN-100. It also shows the normal range for each set of patients.

[0061] Figure 13 depicts the strong negative correlation between urinary PAGN output and blood ammonia, measured over 24 hours and expressed as time-normalized area under the curve, in patients with urea cycle disorders. Blood ammonia assessed as TNAUC (Y-axis) correlated inversely ($r = -0.80$; $p < 0.001$) with urinary PAGN output (X-axis).

[0062] Figure 14 depicts the effect on blood urea of administered sodium benzoate to a patient with chronic renal failure.

Modes of Carrying Out the Invention

[0063] In one aspect, the invention is reduced to practice in determining the dose, dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy. The starting dose and schedule for administration of an ammonia scavenging drug would be based upon the theoretical considerations including the estimated percentage conversion of the drug to PAGN, the waste nitrogen resulting from the patient's dietary protein, and the percentage of drug converted to and excreted as PAGN. Following initiation of treatment, further dose adjustments would then be made if necessary, based upon the actual measurement of urinary PAGN output, or a well-correlated parameter like total urinary ammonia or the ratio of PAGN to creatinine.

[0064] In another aspect, the invention provides a method to transition a patient having a nitrogen retention state from phenylbutyrate or phenylacetate to a prodrug of phenylbutyrate

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(which is a prodrug of PAA), such as HPN-100, or other ester or prodrugs such as compounds of Formula I and II as shown herein.

[0065] For a number of reasons, HPN-100 is considered a more desirable drug than sodium PBA for many patients who have high ammonia levels and require treatment with an ammonia scavenging drug. In particular, it avoids the unpleasant taste and small associated with sodium PBA, and it reduces potentially harmful sodium intake, since phenylbutyrate is administered as a sodium salt, and daily doses are quite large--up to 20 grams of PBA can be administered per day. This amount of the drug product BUPHENYL® would include over 2 g of sodium, which equals the entire recommended daily sodium intake for a healthy adult. A large majority of patients (nine out of ten UCD patients who participated in the clinical study described in Example 3 herein) preferred HPN-100 over sodium PBA in clinical testing. Thus many patients who have been treated with phenylbutyrate as an ammonia scavenging drug may want to transition from it to HPN-100.

[0066] It would seem logical for a physician to transition a patient from phenylbutyrate to a prodrug of phenylbutyrate by calculating the amount of the prodrug that would produce an amount of PBA that corresponds to the dosage of phenylbutyrate previously administered to the patient. This would be expected to produce about the same blood plasma level of the active ingredient, PBA. Efficacy of the new treatment with the prodrug could then be assessed by monitoring levels of phenylbutyrate in the blood, to establish the same levels achieved when PBA was administered. As summarized above and discussed in more detail below, however, that approach is not appropriate because, surprisingly, plasma levels of PBA do not correlate well with administered dosages of HPN-100 or with the effectiveness of a dose of HPN-100 or sodium PBA. (Note that sodium PBA is the acid form of phenylbutyrate, which is the common name for the drug BUPHENYL®, and is typically administered as BUPHENYL®, which is a sodium salt of PBA. References to treatment with PBA herein encompass administration of the phenylbutyrate neutral compound or a salt of phenylbutyrate. Typically, and in all of the working examples herein, PBA is administered as the sodium salt, in the form of the drug BUPHENYL®.)

[0067] Alternatively, since PBA is a prodrug for PAA, the dosage of a phenylbutyrate prodrug could be calculated according to the theoretically formed amount of PAA, which should be the same amount as what would be calculated from the PBA dosage, since one molecule of PBA is expected to produce one molecule of PAA. The molecular weight of sodium PBA, the registered drug form of PBA (the sodium salt of PBA), is 186; the molecular weight of HPN-100 is 530, and

of course HPN-100 provides three equivalents of PBA per molecule, so only one-third as many moles of HPN-100 would be needed to replace a molar quantity of either PBA or PAA. Thus each gram of sodium PBA could be replaced by 0.95 grams of HPN-100; and since HPN-100 is a liquid having a density of 1.1 g/mL, each gram of sodium PBA would be replaced by 0.87 mL of HPN-100, assuming HPN-100 is used as an undiluted liquid. This can be used to select a starting dosage of HPN-100 for patients being transitioned from sodium PBA to HPN-100. Alternatively, a starting dose of HPN-100 in a patient not already taking BUPHENYL® (sodium phenylbutyrate) would need to take into account the surprising observation described in more detail below (see examples 2 and 3) that conversion of the PBA, when administered as HPN-100, into urinary PAGN is incomplete and averages about 54%, and over a range of about 40-70%.

[0068] Alternatively, the physician could measure plasma levels of either PBA or PAA in a subject receiving an effective amount of PBA, and determine a dosage of a PBA prodrug by administering enough of the prodrug to produce the same plasma levels of PBA or PAA. The physician could then monitor the amount of either PBA or PAA in the blood to ensure that the appropriate amount of active drug was being produced in the body. It might be expected that a prodrug of phenylbutyrate would provide a slightly lower blood plasma concentration of PAA or PBA than phenylbutyrate, and thus a lower nitrogen-scavenging effect, since conversion of the prodrug to the active drug might be less than 100% efficient. Thus monitoring PAA or PBA plasma levels and increasing the prodrug dosage to bring levels up to those obtained by administering phenylbutyrate might be expected to produce the same physiological effect as the phenylbutyrate dosage. However, it was found that it is not necessary for the plasma level of PAA or PBA observed upon administration of a prodrug of phenylbutyrate to match that produced by an effective amount of phenylbutyrate, in order to achieve the same ammonia-scavenging effect. Rather, efficacy of the prodrug HPN-100 correlates with urinary PAGN levels, not with plasma levels of PAA or PBA.

[0069] Models have been developed to describe how ammonia-scavenging drugs or prodrugs are expected to behave *in vivo*. One model, shown in Figure 2, reflects conventional approaches to assessing drug effectiveness as applied to HPN-100 based on blood levels of PAA or PBA. Clinical testing has shown that HPN-100 does not produce the plasma levels of PAA and PBA that might be expected from this model, though, even though it is at least as effective on an equimolar basis as PBA for controlling blood ammonia levels, and for eliminating ammonia as PAGN via the urine. Thus the conventional model fails to account for some important pharmacokinetic

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differences between PBA and HPN-100. It was hypothesized that, as compared with sodium PBA, a greater percentage of PBA derived from HPN-100 is converted into PAGN for elimination (or PAA or PBA derived from it) *before entering the systemic circulation* (the "central compartment" in Figure 2). Recognition of this important and unexpected difference underlies certain aspects of the present invention.

[0070] A refined working model based upon the observations described herein and as outlined in this disclosure is depicted in Figure 3. It supports the conclusion that PBA derived from HPN-100 as well as from sodium PBA can be converted into PAGN without entering into systemic circulation; presumably, HPN-100 or its initial metabolic products (*e.g.*, a compound of formula I wherein one or two of R_1-R_3 represent phenylbutyryl groups, and the remaining one or two of R_1-R_3 represent H—the expected products of partial hydrolysis of HPN-100) may reach the liver and be converted into PAGN there, prior to reaching the systemic circulation. Moreover, the fractional conversion of PBA derived from HPN-100 is greater than for PBA absorbed when PBA is administered as the salt, an observation which explains the lower blood levels of PBA following administration of HPN-100 as compared with sodium PBA despite equivalent or potentially superior ammonia scavenging activity. This observation led to the recognition that plasma levels of PAA or PBA are not reliable indicators of the effectiveness of a PBA prodrug like HPN-100, and should not be relied upon to set or adjust dosages of such PBA prodrug compounds. Data presented herein, e.g. as summarized in Figure 8, demonstrate this effect. Alternative methods for monitoring a subject treated with HPN-100 are needed, and are provided herein.

[0071] In addition, PK/PD modeling demonstrates that HPN-100 is absorbed only about 40% as rapidly as PBA when dosed orally. As a result, HPN-100 provides a slow-release delivery effect, even though it appears to metabolize to PBA rapidly once absorbed. This provides greatly enhanced flexibility in dosing and explains why HPN-100 can be dosed, e.g., three times per day or even twice per day to provide stable ammonia levels that require four or more doses of PBA to achieve. This slower release, in conjunction with pharmacokinetic and anatomic considerations as depicted in figures 3 and 5, respectively, explain the greater conversion of PBA to PAA/PAGN following administration in the form of HPN-100 as compared with sodium PBA.

[0072] In view of these observations of unexpected pharmacokinetic behavior, neither plasma PAA nor PBA levels should not be used to evaluate or monitor treatment of a subject with HPN-100 or sodium PBA. Alternative methods are needed, and are provided herein, for monitoring a subject treated with HPN-100. For one, it has been found that between 40 and 70% of HPN-100 is

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converted into urinary PAGN, typically about 50-60%. This elimination efficiency for HPN-100 and sodium PBA in UCD patients averages 54%, which is surprisingly low in light of previous references that have generally assumed the conversion of sodium PBA into urinary PAGN to be about 100%. Because the conversion of HPN-100 is much lower than expected, it is important to appropriately adjust for this conversion efficiency before interpreting urinary PAGN output. Failure to take into account this conversion efficiency could lead to substantial misjudgments in adjusting therapeutic dosing of a nitrogen scavenging drug; for example a doctor might increase dosage of an ammonia scavenging drug upon finding that urinary PAGN output was only about half of that expected, resulting in an unnecessary overdose of the drug.

[0073] Urinary PAGN has also been shown to be inversely correlated with levels of waste nitrogen, e.g. ammonia, in the blood, thus efficacy of HPN-100 can be evaluated by measuring urinary PAGN. This is particularly valuable because it is very difficult to rely on monitoring of ammonia levels for routine patient maintenance, and surprisingly, none of the other parameters associated with HPN-100 treatment, such as plasma levels of PAA or PBA or PAGN or HPN-100, correlated with ammonia levels well enough to be useful for guiding therapy.

[0074] In one embodiment, the invention thus provides a method to determine an effective dosage of a nitrogen scavenging drug, particularly HPN-100, for a patient in need of treatment for a nitrogen retention disorder. The patient may be one having a UCD or similar condition, HE, or CRF, for example. The method comprises monitoring the effect of an initial dosage of the drug on a subject by determining the subject's urinary PAGN output. While plasma ammonia levels can also be tested, particularly for a treatment naïve patient, it is demonstrated herein that the PAGN level can be used much more conveniently for out-patient monitoring, for example, and correlates well with effectiveness of the drug dosage. Thus in a preferred embodiment, the method does not involve use of ammonia level information for monitoring the effectiveness of a treatment with HPN-100, or for adjusting an initial dosage of HPN-100; rather, the method relies upon urinary PAGN output levels to monitor the effect of an initial dosage, and to determine whether and/or how to adjust the initial dosage of HPN-100 to provide a desired ammonia scavenging effect, such as adequate ammonia control to produce an average ammonia level that is normal, or is below the upper limit of normal for the testing methods and facility. From urinary PAGN output, the effectiveness of an initial dose of HPN-100 can be determined readily as further discussed herein. Preferably, the initial dosage is a dosage amount that is administered daily to the subject, and

typically the subject will receive the initial daily dosage for at least several days, preferably at least a week, before this method is applied so a relatively steady state is achieved.

[0075] Optionally, the method further comprises determining from the initial dosage whether a dose adjustment is needed. This can be based on comparing the subject's urinary PAGN output to the subject's daily protein intake, to ascertain whether the expected amount of waste nitrogen is being excreted. Methods for calculating how much nitrogen excretion is needed are known in the art—see Brusilow, et al. Alternatively, as described below, a target level of urinary PAGN can be determined for the subject's population, and the subject's uPAGN can be compared to that target value. For example, as set forth below, for an adult UCD patient receiving daily dosages of HPN-100 or PBA, a cut-off level of about 10 g uPAGN per day distinguishes those who achieve normal plasma ammonia levels (ones having 10g or higher daily uPAGN output) from those having ammonia levels above normal. The subjects for these methods can be patients having HE, CRF, or UCD conditions and needing treatment with a nitrogen scavenging drug. uPAGN levels can be determined either as total urinary PAGN over a 24 hour period following the first dose of HPN-100 in a day, or as the 12-24 hour uPAGN output, measured for the period 12-24 hours after the first dose of HPN-100 of the day. Alternatively, as set forth herein, uPAGN can be determined as a ratio with urinary creatinine in a sample of the subject's urine, and this measurement can be used to estimate a 24-hour or a 12-24 hour uPAGN output for the patient.

[0076] In each case, if urinary PAGN output indicates an adjustment is needed, an adjusted dosage can be determined from the urinary PAGN output in view of the information herein, which demonstrates that a daily dosage of HPN-100 or PBA is converted to a 24-hour urinary PAGN output that corresponds to about 54% of the administered dosage of the drug. If the subject's urinary PAGN output indicates a need to increase or decrease drug dosage, an adjusted dosage can be determined as the amount of additional drug needed to produce the desired uPAGN level, in view of the conversion efficiency of about 54%. The desired uPAGN level would be that corresponding to the amount of waste nitrogen to be removed, as calculated from the subject's daily protein intake, or as the difference between the observed uPAGN level and a target uPAGN level for the subject's population as discussed further below. The subject's daily protein intake can also be adjusted, if appropriate, for the subject's residual urea synthesis capacity, if any. In many UCD subjects having inborn severe enzyme function deficiencies in the urea cycle or transporter enzymes mentioned above, minimal residual urea synthesis capacity is likely to be present, and no adjustment may be needed.

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[0077] Plasma levels of ammonia are difficult to measure—a blood sample is needed and must receive special handling to ensure accurate measurement. Moreover, ammonia levels vary widely, depending on time of day and relationship to meals, especially in the population having UCDs. Among the patients tested for the study described herein, measured ammonia levels varied from 2 to 150 μmol/L among patients receiving sodium phenylbutyrate, and from 2 to 106 μmol/L among patients taking HPN-100. Even for a single patient, ammonia levels varied over a range of 2.4 to 54-fold within a single day among the subjects receiving NaPBA, and over a range of 2.4 to 12.3-fold among subjects receiving HPN-100. Even among subjects being managed in a controlled clinical setting, ammonia levels for individuals varied over a 7-fold range in a single day. Individual plasma levels of ammonia are thus highly variable, and repeating monitoring is impractical in the usual clinical setting, making blood levels of ammonia a poor choice for monitoring effectiveness of a drug regimen—particularly on an outpatient basis. A more reliable measure of the effectiveness of ammonia scavenging drugs is thus needed, and it is shown herein that urinary PAGN provides such a measure: it uses the far more practical collection of urine rather than blood samples, and measures a parameter that does not require particularly careful sample storage and handling. Moreover, the measured urinary PAGN level correlates well with ammonia control, while being less prone to fluctuations caused by timing of the sample collection.

[0078] In another aspect, the invention provides a method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder, comprising:

a) determining the patient's dietary protein intake;

b) determining the patient's residual urea synthesis capacity, if any;

c) estimating from a) and b) the amount of excess waste nitrogen the patient needs to excrete to remove the waste nitrogen associated with the dietary protein intake that is not excreted as urea; and

d) determining an amount of the PAA prodrug needed to eliminate the estimated amount of excess waste nitrogen as urinary PAGN,

wherein the amount of PAA prodrug needed is determined based on a conversion factor whereby about 40% to about 70% of the PAA prodrug is converted into urinary PAGN.

[0079] The PAA prodrug can be HPN-100 or PBA in some embodiments.

In some embodiments, the conversion factor is about 54%. The subject (patient) for these methods can be one having a UCD, HE, or CRF and needing treatment with a nitrogen scavenging drug. The patient's urinary PAGN output can be measured as a 24 hour, or a 12-24

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hour total output, or it can be estimated from the ratio of PAGN to creatinine in a spot sample of the subject's urine.

[0080] In addition, it has been found that among adult patients with nitrogen retention disorders, ammonia levels in blood, measured as time normalized area under the curve (TN-AUC) exceeded 30 μmol/L in 7 of 9 patients whose 24-hour urinary PAGN output was under 10 g; while ammonia levels were under 30 μmol/L in 7 of 9 subjects whose 24-hour urinary PAGN output exceeded 10 g. (The threshold level for this analysis, 30 μmol/L, was the average 'upper limit' for normal ammonia levels among the study sites.) Thus a single parameter that can be readily measured, a urinary PAGN output cut-off value of 10 g per 24 hour period, was found to be surprisingly useful to predict effective ammonia level management in a group of adult UCD patients receiving HPN-100 or NaPBA, in spite of the high variability of direct measurements of ammonia levels. These findings further demonstrate the utility of urinary PAGN in UCD patients, and indicate that a target urinary PAGN output level can be a useful indicator of the effectiveness of a nitrogen scavenging drug treatment that provides adequate control of blood ammonia level control. Output above the cut-off value was highly correlated with achieving normal ammonia levels, while output below the cut-off value was highly correlated with failure to achieve normal ammonia levels.

[0081] While the measured cut-off level in this patient population was 10 g per day, different populations are expected to exhibit different cut-off values; however, based on the surprising observation that a single easily-measured parameter readily predicts whether adequate ammonia control is achieved, determining the correct cut-off value for a particular patient population taking HPN-100 or PBA for ammonia scavenging can be done without undue experimentation. It is only necessary to measure urinary PAGN output for subjects in the population of interest, and assess their ammonia levels, then correlate the ammonia control with urinary PAGN to determine the cut-off value for that population. For a different population, the cut-off level may be about 5g, 6g, 7 g, 8g, 9g, 10g, 11g, 12g, 13g, 14g, or 15g of urinary PAGN per day.

[0082] Thus in another aspect, the invention provides a method to identify a subject in need of close monitoring, or a need to modify a treatment plan for a subject, where the subject is a person treated with an ammonia scavenging drug, particularly HPN-100 or PBA. The method comprises comparing the 24-hour urinary PAGN output for the subject to a cut-off value for the population in which the subject fits. Routine experimentation enables identifying the level of urinary PAGN that correlates with successful ammonia control in the patient's population, as a cut-off level that can be

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used to distinguish subjects likely to achieve normal ammonia levels from those unlikely to achieve normal ammonia levels, or to identify subjects who need a modified ammonia control treatment program.

[0083] Based on the data herein, a cut-off level of urinary PAGN output of approximately 10 g per day distinguishes adult UCD patients into groups who generally had normal ammonia levels (those producing over 10 g urinary PAGN per day) and those who failed to achieve normal ammonia levels (those producing less than 10 g urinary PAGN per day). The method can also comprise testing a subject by determining the subject's 24-hour urinary PAGN output, and classifying the subject as one likely to have acceptable ammonia control on a current treatment program (normal ammonia levels) based on a urinary PAGN level above the cutoff, or as one likely to have insufficient ammonia control (excessive ammonia levels) on a current treatment program, based on having a urinary PAGN output below the cutoff. Subjects having a urinary PAGN output below the cut-off (e.g., an adult UCD patient with a 24-hour urinary PAGN output under 10 g) would be recommended to undergo further testing, and possibly an adjusted treatment regimen involving lower protein intake or increased dosage of an ammonia scavenging drug such as HPN-100 would be needed. Optionally, the method can also comprise determining an adjusted dosage of the ammonia scavenging drug for a subject whose urinary output is less than the cut-off level. Determining the cut-off for a given population is a matter of routine experimentation in view of the surprising observation herein showing that this parameter, urinary PAGN, can serve that function. If the drug is HPN-100 or PBA, the conversion efficiency factor of about 54% of administered drug being excreted as urinary PAGN can be used to determine how much additional drug to administer, subject to the limitations on recommended daily dosing of the drug for that subject.

[0084] It has also been found that HPN-100 has little to no effect on urinary creatinine output, and that urinary creatinine levels were about the same for subjects on BUPHENYL® and HPN-100. The average total 24-hour creatinine excretion for a subject receiving PBA was 1.08 (0.43) grams, and for a subject receiving HPN-100, it was 1.03 (0.38) grams. Moreover, because daily urinary creatinine outputs in healthy adults and patients with nitrogen retention states are typically rather stable, either measuring PAGN output in urine over time, or measuring the ratio of the concentrations of PAGN to creatinine, which can be conveniently done in spot testing, provides a way to monitor HPN-100's effectiveness. In one aspect, the invention thus provides a method to assess the effectiveness of a treatment with HPN-100, comprising determining the ratio of PAGN

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to creatinine in a 'spot urine' test. Clinical studies show that urinary excretion of PAGN, and the ratio of PAGN to creatinine in urine, correlate well with blood ammonia levels: an increase of PAGN or of the PAGN / creatinine ratio correlates with decreasing plasma ammonia levels. Accordingly, in one method, HPN-100 treated patients are monitored by measuring urinary PAGN output, or by measuring the ratio of PAGN to creatinine in spot urine testing. The ratio from spot testing can be used to estimate a 24-hour uPAGN output level for the subject or a 12-24 hour uPAGN output level. This method can be used to monitor treatment of a treatment-naïve patient, or of a patient being transitioned from PBA to HPN-100, or a patient being treated with HPN-100. Increasing levels of urinary PAGN output, or an increase in the ratio of PAGN to creatinine in spot testing provides a way to determine whether a dosing regimen that utilizes HPN-100 or another PBA prodrug is promoting elimination of excess ammonia to an adequate degree, and to compare two treatment methods to determine which is more effective for the particular subject, and to monitor a subject receiving such treatment or being transitioned from PBA to HPN-100 treatment.

[0085] While plasma ammonia levels have traditionally been used to assess disease control in UCD patients, ammonia levels have critical limitations as pertains to use in dosing of HPN-100. For example, individual plasma ammonia levels are affected by many factors including diet, vary widely throughout the course of the day even under controlled conditions, and might be elevated regardless of how well a drug treatment works. Thus to meaningfully reflect drug effect, the plasma ammonia levels need to be monitored over time by repeated blood samplings, which is not practical for routine clinical monitoring patients and which does not provide direct information about whether an ammonia scavenging drug is working. Measurements of urinary PAGN, on the other hand, can be done more conveniently as a routine monitoring method because they do not require medical assistance to collect the samples for testing. Moreover, urinary PAGN specifically measures the waste nitrogen clearance provided by the scavenging agent, while many other factors affecting ammonia levels may cause ammonia control to be misleading with regard to the actual effect of the nitrogen scavenging drug. Thus, even though in theory a number of different parameters could be measured to assess effectiveness of a dosage of HPN-100, only measurements based on urinary PAGN are both convenient and reliable as a direct measurement of the nitrogen scavenging drug's effect.

[0086] Thus in one embodiment, the invention provides a method to monitor the effectiveness of treatment of a UCD patient with HPN-100, where monitoring consists essentially of monitoring the patient's urinary PAGN excretion. Urinary PAGN levels comparable to those achieved with a

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previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level of less than about 40 μmol/L, or of not greater than 35 μmol/L would indicate the treatment was effective; however, the method can be practiced without measuring plasma ammonia levels, and in some embodiments, ammonia levels are not used in the determination of efficacy, or they are not used in adjusting a treatment plan, dosage, or protein intake level. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test, to estimate the 24 hour (or 12-24 hour) uPAGN level.

[0087] In another aspect, the invention provides a utilization efficiency factor for HPN-100 or for sodium PBA of about 40% to about 70%, with an average value of about 54%, which can be used to more accurately determine an initial starting dose of either drug and/or correlate dietary protein intake with projected urinary PAGN.

[0088] In one aspect, the invention provides a method for transitioning a patient from phenylbutyrate to HPN-100 or other esters or prodrugs of phenylbutyrate. The method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylbutyrate. For example, the amount of HPN-100 needed to provide an equal molar amount of PBA would be calculated (an equimolar amount), and this equimolar amount would be administered to the patient. Urinary excretion of PAGN or plasma ammonia levels would be monitored, and the dosage of HPN would be increased or decreased as needed to establish a level of PAGN excretion that is about the same as that provided by a previously used effective amount of phenylbutyrate or another nitrogen scavenging drug. Typically, a subject being transitioned from PAA or another PAA prodrug onto HPN-100 using this method would be tested for urinary PAGN output prior to the transition and afterwards, and the dosage of HPN-100 would be adjusted as needed to match the urinary PAGN output from this patient when treated with the previous PAA drug or prodrug, assuming the previous PAA prodrug treatment was considered effective. This provides a safer and more effective transition to the new prodrug than methods that rely upon using an equimolar amount without monitoring the *in vivo* effects of that amount of the new drug. It also avoids the risk of inaccurate dosing and potential overtreatment that could result if one monitored PAA or PBA and tried to adjust the prodrug (i.e. HPN-100) dosage to match the PAA or PBA level to the corresponding level provided by administering sodium phenylbutyrate itself.

[0089] In some embodiments, the transition from phenylbutyrate might be undertaken in more than a single step and urinary excretion of PAGN and total nitrogen would allow monitoring of

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ammonia scavenging during the transition. In some embodiments, a patient taking an initial dosage of phenylbutyrate is transitioned from phenylbutyrate to a prodrug of phenylbutyrate in steps. The methods can use two, three, four, five, or more than five steps. At each step, a fraction of the initial dosage of phenylbutyrate corresponding to the number of steps used for the transition is replaced by an appropriate amount of HPN-100 or other prodrug of phenylbutyrate. The appropriate amount for each step can be approximately an amount sufficient to provide an equal molar amount of PBA if it is assumed that the prodrug is quantitatively converted into PBA. Note, too, that BUPHENYL® (sodium phenylbutyrate) contains about 6% inactive ingredients, so it is appropriate to base calculations upon the PBA content of the drug rather than on the weight of the formulated drug. The patient is then monitored to determine how much ammonia scavenging effect has been provided. The amount of HPN-100 (or prodrug) can then be adjusted to produce about the same amount of ammonia excretion in the form of excreted PAGN that was achieved by the initial dosage of phenylbutyrate, if the patient was well controlled.

[0090] A physician who is switching a patient from PBA to HPN-100 or another ester of phenylbutyrate should be aware that an effective amount of HPN-100 does not necessarily produce a PAA or PBA level that is as high as those seen when sodium phenylbutyrate is administered. It is reported that PAA exhibits some toxicity at high plasma concentrations. Thibault, et al., *Cancer Research*, 54(7):1690-94 (1994) and *Cancer*, 75(12):2932-38 (1005). Given this, and given the unique properties of HPN-100 described above, it is particularly important that a physician not use plasma levels of PAA or PBA to measure the efficacy of HPN-100. If one administers HPN-100 in amounts sufficient to match the plasma PBA or PAA levels provided by administering phenylbutyrate, for example, the dose of HPN-100 may be unnecessarily high.

[0091] The treatment-naïve patient is one not presently receiving an ammonia-scavenging drug treatment to manage nitrogen levels. While there are recommended dosage levels for the nitrogen scavenging drugs in many cases, the right dosage for a naïve patient may be lower than those ranges, for example, and, less commonly, it may be above an equimolar amount when compared to the dosages recommended for sodium PBA. The initial dosage of PAA or a PAA prodrug can be calculated by methods known in the art once a patient's dietary intake of protein is known, and assuming the patient has a relatively normal liver function. Saul W Brusilow, "Phenylacetylglutamine may replace urea as a vehicle for waste nitrogen excretion," *Pediatric Research* 29:147-150, (1991). Methods are also know for measuring the total amount of nitrogen

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excreted in the urine; in the case of a subject taking a drug that acts by providing PAA, the total waste nitrogen will include PAGN excreted.

[0092] It is estimated that about 47% of nitrogen in proteins consumed will be converted into waste nitrogen, and that about 16% of protein on average is nitrogen. Using these figures, and assuming HPN-100 is efficiently converted to PAGN, a daily dosage of about 19 g of HPN-100 would provide a vehicle to excrete the waste nitrogen from about 43 g of dietary protein; each gram of HPN-100 would thus be able to carry away waste nitrogen from about 2 g of dietary protein. In addition, if it is found that HPN-100 utilization efficiency is between about 40% and 70% in various individual patients (as disclosed herein, it has been found that about 40-70% of HPN-100 is converted into urinary PAGN in the tested UCD patients, with an average value of 54%), which is consistent with clinical observations to date, and the average value of about 54% can be used to further refine the relationship between dietary protein intake and HPN-100 dosing levels for a given subject. With this refinement, each gram of HPN-100 would assist with removal of waste nitrogen for about 1 gram (~ 1.3 grams) of dietary protein. This factor can be used to calculate a suitable dosage of HPN-100 if dietary protein intake is known or controlled, and it can be used to calculate a tolerable dietary protein intake for subject receiving a set dosage of HPN-100.

[0093] This method can also be used to establish a recommended daily dietary protein intake for a patient, by determining the patient's endogenous nitrogen elimination capacity, calculating an amount of dietary protein that this endogenous capacity permits the patient to process without assistance from a nitrogen scavenging drug, and adding to the amount of dietary protein the patient can process on his/her own an amount of protein that the patient would be able to process when using a particular dosage of PBA or a PBA prodrug like HPN-100. Using HPN-100 as an example, a daily dosage of about 18 grams of HPN-100, utilized at an estimated efficiency of 54%, would enable the treated patient to eliminate waste nitrogen corresponding to about 25 g of dietary protein. Thus the invention provides a method to establish a suitable dietary protein level for a patient having a urea cycle disorder or HE, by adding this amount of protein to the amount the patient's endogenous nitrogen elimination capacity can handle.

[0094] In some embodiments, it is also useful to measure PAGN excretion, which accounts for some of the total waste nitrogen excreted when PAA or a PAA prodrug is working. The total waste nitrogen excreted minus the amount of PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via the urea cycle or other mechanisms, and is helpful in

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determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific in some cases, while some patients may be known based on their condition or history to have little or no residual endogenous capacity to eliminate waste nitrogen. Dosage of HPN-100 can then be established by determining the subject's endogenous capacity to eliminate waste nitrogen; subtracting the amount of dietary protein corresponding to the subject's endogenous nitrogen elimination capacity; and providing a dosage of HPN-100 sufficient to permit the subject to handle the balance of waste nitrogen, based on the subject's dietary protein intake. Here again, the amount of urinary PAGN expected from the dosage of HPN-100 can be determined from the average conversion (54%), and this can be used to determine how much urinary PAGN to expect, and the dosage can be adjusted if necessary based on monitoring urinary PAGN output.

[0095] The plasma or blood level of ammonia is optionally also determined, at least periodically if not on an ongoing basis, in addition to measuring urinary PAGN, to assess the effectiveness of the overall drug and dietary regimen for a particular patient. If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug may need to be increased if that can be done, or the patient's dietary protein intake can be decreased if that is feasible.

[0096] In some instances, the dosage of HPN-100 may be limited to dosages that do not exceed recommended dosing levels for phenylbutyrate, adjusting for the fact that each mole of HPN-100 can produces three moles of phenylbutyrate. The label for the use of sodium PBA for the chronic treatment of UCDs recommends a daily dosage not to exceed 20 g; a daily dosage in a range of 9.9-13.0 g/m^2 set according to the subject's size for subjects over 20 kg in weight; and a dosage within a range of 450-600 mg/kg for subjects weighing less than or equal to 20 kg is indicated. While lower doses of HPN-100 may provide comparable ammonia scavenging to PBA on a molar equivalent basis, it may be suitable to select a higher dosage of HPN-100 to achieve adequate ammonia control for certain subjects. Typically, that dose will not exceed the recommended ranges for dosages of phenylbutyrate for a given indication. Thus it may be appropriate to administer HPN-100 at a daily dosage not to exceed an amount of HPN-100 that corresponds to the molar amounts of phenylbutyrate described above (and correcting for the fact that HPN-100 can provide three molecules of PBA). For a subject weighing more than 20 kg, a dosage range for HPN-100 would be between 8.6 and 11.2 mL/m^2. For a subject weighing less than 20 kg, a dosage range of about 390 to 520 μL/kg per day of HPN-100 would be appropriate, based on the use of an

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equimolar amount compared to the recommended doses of HPN-100. There is no evidence to suggest that HPN-100 would produce adverse effects at a rate in excess of that from an equimolar amount of sodium PBA, so the daily recommended upper limit of 20 g per day of sodium PBA suggests that a daily dose limit of HPN-100 based on the recommendations for sodium PBA would correspond to an equimolar amount of HPN-100, or about 19 g or 17.4 mL.

[0097] Thus in one embodiment, the invention provides a method to monitor the effectiveness of a treatment of a UCD patient with HPN-100, where monitoring consists of, or consists essentially of, monitoring the patient's urinary PAGN excretion and/or plasma ammonia levels. Urinary PAGN levels comparable to those achieved with a previous PBA dosing regimen would be considered evidence that the HPN-100 treatment was equally effective as the PBA treatment it replaced. Alternatively, a plasma ammonia level that was normal, e.g., a level of less than about 40 μmol/L, or of not greater than 35 μmol/L, or less than about 30 μmol/L, would indicate the treatment was effective. In some embodiments, rather than using urinary PAGN output measured over time, one can use the ratio of PAGN to creatinine in the urine, in a spot test. Because urinary creatinine is relatively constant, the ratio of PAGN to creatinine can be used to estimate daily urinary PAGN output without requiring collection of a full 24-hour urine sample.

[0098] However, it has also been found that HPN-100 exhibits no indications of toxicity at equimolar doses when compared to the approved PBA dosage of 20 g / day and a dose 2-3 times the equivalent of 20 grams of PBA is unlikely to produce PAA blood levels leading to AEs. Moreover, tolerability of taking HPN-100 is much higher than for PBA and a linear relationship has been observed between HPN-100 dose and PAGN output up to doses of 17.4 mL. In some patients or clinical settings, HPN-100 doses well above the approved PBA dosage are expected to be beneficial; for example, in UCD patients who exhibit recurrent hyperammonemia even on maximal doses of sodium PBA, in UCD patients who need increased dietary protein to support body requirement, or in patients with other nitrogen retaining states.

[0099] Thus in another embodiment, the invention provides methods to treat a subject having HE or UCD, with a dosage of HPN-100 that corresponds to between 100 and 300% of the equimolar amount of the recommended highest dose of PBA. In some embodiments, the suitable dosage will be between about 120% and 180% of the highest recommended dose of PBA; in other embodiments it will be between 120-140% or from 140-160% or from 160-180% of the equimolar amount of the recommended highest dosage of PBA. In accordance with this aspect, the daily

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dosage of HPN-100 could be as much as 57 g, or up to about 38 g, or up to about 33 g, or up to about 30g, or up to about 25g.

[00100] In one aspect, the invention provides a method to identify the starting dose or dose range and to individually adjust the dose or dose range of a nitrogen scavenging drug comprising PAA or a PAA prodrug (including HPN-100) used for the management of a treatment-naïve patient, which method comprises the steps of:

a) administering an initial dosage of the drug estimated according to the patient's dietary protein load, taking into account the expected percentage conversion to PAGN

b) measuring the amount of total waste nitrogen excreted following administration of the nitrogen scavenging drug comprising PAA or a PAA prodrug;

c) measuring blood ammonia to determine if the increase in urinary excretion of total waste nitrogen is sufficient to control blood ammonia levels; and

d) adjusting the initial dosage to provide an adjusted dosage of the nitrogen scavenging drug comprising PAA or a PAA prodrug based upon ammonia control, dietary protein, and the amount of total waste nitrogen excreted by the patient, or the amount of waste PAGN excreted. Either or each of these parameters can be monitored to assess the dosage of HPN-100 or other nitrogen scavenging drug being administered. Optionally, the method also includes determining the subject's endogenous nitrogen eliminating capacity (residual urea synthesis capacity) to further help determine an initial dose of HPN-100.

[00101] The initial dosage of the HPN-100 for a treatment naïve patient can be calculated as the amount of waste nitrogen that needs to be eliminated based on the patient's dietary protein intake. This amount can be reduced by an amount equivalent to the waste nitrogen the patient can eliminate using the patient's endogenous waste nitrogen elimination capacity, which can be measured as described herein. The suitable starting dose of HPN-100 can be calculated by estimating dietary protein intake that needs to be managed via the nitrogen scavenging drug, and providing a dose of drug amounting to about 1 g of HPN-100 per 1-2 grams of dietary protein in excess of the amount the patient's endogenous nitrogen elimination capacity can handle, taking into account the expected percentage conversion of the administered PBA to urinary PAGN. The method optionally further includes assessing urinary PAGN output to see if it accounts for the expected amount of waste nitrogen, and optionally may include measuring plasma levels of ammonia in the subject to ensure that an acceptable level of ammonia has been achieved.

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Checking the patient's plasma ammonia levels provides a measure of the effectiveness of the overall treatment program, including diet and drug dosing.

[00102] The table below summarizes the amount of dietary protein that doses of HPN-100 below (dose 1), within (dose 2) and above (dose 3) those corresponding to the recommended dosages of sodium PBA would be expected to 'cover' (i.e. mediate resulting waste nitrogen excretion), given the following assumptions: 1 gram of PAA mediates the excretion of ~0.18 grams of waste nitrogen if completely converted to PAGN; 54% of the PAA delivered as the PBA prodrug released from HPN-100 is converted to PAGN; 47% of dietary protein is excreted as waste nitrogen, and 16% of dietary protein consists of nitrogen (Brusilow 1991; Calloway 1971). These factors can be used when relating dietary protein intake, drug dosing and waste nitrogen elimination for purposes of the present invention.

Table 5. HPN-100 Doses and Expected Waste Nitrogen Excretion Based on Dietary Protein

Dose 1	3 mL BID	Corresponds to ~0.47x the dose administered in Example 2, for a 70 kg adult and ~0.35x the amount of PBA (~6.1 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~8 g of dietary protein
Dose 2	9 mL BID	Corresponds to ~1.42x the dose administered in Example 2, for a 70 kg adult and ~01.1x the amount of PBA (~18.2 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~25 g of dietary protein
Dose 3	15 mL BID	Corresponds to ~2.36x the dose administered in Example 2, for a 70 kg adult and ~1.73 x the amount of PBA (~30.3 g) delivered in the maximum approved dose of sodium PBA of 20 g Expected to mediate excretion of waste nitrogen associated with ~40 g of dietary protein

[00103] Note that this also indicates that HPN-100 can be administered in two doses per day, while PBA typically is administered in four doses per day. This is likely associated with the slow-release characteristics of HPN-100 described herein, and is expected to improve quality of life and compliance with the treatment program for subjects receiving HPN-100 rather than PBA.

[00104] As used herein, plasma levels of ammonia are acceptable when they are at or below a level considered normal for the subject, and commonly this would mean plasma ammonia level is below about 40 μmol/L. In certain clinical tests described herein the upper limit of normal for the

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subjects was between 26 and 35 μmol/L, (e.g., in some of the tests described herein the site average for normal ammonia levels was about 30 μmol/L), and it is recognized in the art that a normal ammonia level will vary depending upon exactly how it is measured; thus as used to describe ammonia levels herein, 'about' means the value is approximate, and typically is within ± 10% of the stated numeric value, and 'normal' is determined according to the particular testing methods.

[00105] In other aspects, the invention provides a method to identify a suitable starting dose or dose range for a UCD or HE patient and to individually adjust the dose or dose range of a new nitrogen scavenging drug used for the management of a patient already treated with a previous nitrogen scavenging drug, which method comprises the steps of:

a) administering an initial dosage of the new nitrogen scavenging drug (which can be estimated according to the patient's dietary protein load and/or the dose of the new drug expected to yield the same amount of urinary PAGN excretion as a previously used nitrogen scavenging drug);

b) measuring the amount of total waste nitrogen and/or of PAGN excreted following administration of the new drug;

c) optionally measuring blood ammonia to determine if the initial dosage is sufficient to control blood ammonia levels, or to establish a suitable average ammonia level; and

d) adjusting the initial dosage of the new drug as needed to provide an adjusted dosage based upon ammonia control, dietary protein, and the amount of total waste nitrogen or urinary PAGN excreted by the patient. The adjusting of the initial dosage is done based on the amount of urinary PAGN, without relying upon plasma levels of PAA, PBA, or PAGN, and preferably without relying upon plasma levels of ammonia.

[00106] Where the patient has previously been treated with PAA or a PAA prodrug, the treating physician may rely, wholly or in part, upon the previous treatment to set a dosage for a new PAA prodrug, or a PBA prodrug, to be administered to the same patient. If the previous drug was reasonably effective for managing the patient's condition, the physician may set the dosage for a new PAA or PBA prodrug by reference to the previous one, so that the new drug is administered at a dosage that provides the same dosage of PAA to the patient, assuming complete conversion of each prodrug into PAA.

[00107] Again, as discussed above, it is sometimes desirable to measure PAGN excreted in addition to total waste nitrogen excreted. The total waste nitrogen excreted minus the amount of

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PAGN excreted represents the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms, and is helpful in determining how much protein intake the patient can manage at a given drug dosage, and also for understanding whether the patient requires extremely close monitoring. The endogenous capacity to excrete nitrogen wastes will be very patient-specific.

[00108] In another aspect, the invention provides a method to identify the amount of dietary protein that could be safely ingested by a subject with a nitrogen accumulation disorder, including hepatic encephalopathy and UCD, where the patient is taking an ammonia-scavenging drug that comprises PAA or a PAA prodrug, which method comprises the steps of:

a) measuring the amount of total waste nitrogen excreted following administration of the drug,

b) determining the amount of dietary protein calculated to yield an amount of waste nitrogen less than or equal to urinary waste nitrogen; and

c) adjusting dietary protein and/or drug dosage as appropriate based upon measurement of blood ammonia and total waste nitrogen excretion or urinary PAGN output.

[00109] Where the subject is receiving treatment with a nitrogen-scavenging drug, it may be necessary to reassess the patient's dietary intake of protein periodically, since many factors will affect the balance between nitrogen intake, nitrogen excretion, and dosage of a nitrogen scavenging drug. The invention provides methods to determine how much dietary protein a patient can handle, based on measuring the patient's nitrogen excretion levels. It may further be useful to measure the patient's PAGN output level as discussed above, to help determine the patient's endogenous capacity for excreting nitrogen wastes via urea cycle or other mechanisms.

[00110] In the above methods, the patient may be one having a urea cycle disorder, or other nitrogen accumulation disorders. In many embodiments, the methods are applicable to patient's having a urea cycle disorder, but relatively normal liver function.

[00111] The above methods can be practiced with a variety of prodrugs of PAA or PBA. In some embodiments, HPN-100 is the PBA prodrug of choice for these methods.

[00112] In another aspect, the invention provides a method to transition a patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

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a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the portion of phenylacetate or phenylbutyrate; and

c) monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the prodrug.

[00113] Optionally, this method comprises adjusting the amount of the prodrug and administering an adjusted amount of the prodrug, then further monitoring PAGN excretion to assess the effectiveness of the adjusted amount of the prodrug. The replacement amount of the PBA prodrug can be about an equimolar amount to the amount of PBA being replaced.

[00114] For reasons discussed extensively herein, it is misleading to rely upon PAA levels when moving a patient to a prodrug (or a new prodrug) of PAA or PBA. The availability of liver-based mechanisms for rapid conversion of a prodrug into PAGN without necessarily entering the systemic system renders plasma levels of PAA and PBA insufficient as predictors of efficacy, so the method relies upon the excreted PAGN for assessing and monitoring treatment with a PAA or PBA prodrug that is to be given to the patient.

[00115] In many cases, it will be possible to transition a patient directly from, e.g., phenylbutyrate to HPN-100 or another PBA prodrug in a single stage, rather than in incremental steps. Thus all of the previously used PAA or PAA prodrug may be replaced with a suitable substitution amount of the new drug (PBA prodrug). However, in some situations (e.g. 'fragile patients', patients taking dosages at or near the recommended limits of PAA or PAA prodrug, and for patients having very limited endogenous capacity for excreting nitrogen wastes, or in situations where the ability of the patient to metabolize or excrete the drug is uncertain), it may be preferable to transition from the initial drug to a new PBA prodrug like HPN-100 in two or more stages or steps. Thus the transition may be made in 2, 3, 4 or 5 steps, and at each step a fraction of the original drug (e.g., about half for a two-step transition, about a third for a three-step transition, etc.) is replaced by the new PBA prodrug to be administered. This approach might be appropriate for a 'fragile' UCD patient known to be susceptible to repeated episodes of hyperammonemia while receiving treatment or while taking a large amount of drug that promotes nitrogen elimination.

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[00116] Thus in another aspect, the invention provides a method to transition a UCD patient from treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of a PBA prodrug, comprising:

a) determining a replacement amount of a PBA prodrug to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the prodrug for the phenylacetate or phenylbutyrate; and

c) monitoring plasma level of ammonia in the patient to assess the effectiveness of the replacement amount of the prodrug. Optionally, this may be done by monitoring urinary PAGN output.

[00117] In some embodiments, the replacement amount of the prodrug is an equimolar amount compared to the amount of PBA being replaced

[00118] During the monitoring step, the patient is being treated with a mixture of phenylacetate or phenylbutyrate plus the new prodrug. The proportion depends upon what step of the transition the patient is in. The physician can also use information about the effects of a first step in setting the replacement amount of the prodrug for use in subsequent steps; thus if the prodrug is significantly more effective than predicted when the estimated amount used as a replacement amount is administered in a first step, the replacement amount used in a subsequent step of the transition can be proportionally reduced.

[00119] In another aspect, the invention provides a method to initiate treatment with phenylacetate, phenylbutyrate or a PBA prodrug in a step-wise fashion, as might be appropriate for a 'fragile patient' (a UCD patient with a history of frequent symptomatic hyperammonemia and/or neonatal onset disease who presumably has no urea synthetic capacity, or a patient with severely compromised liver function whose ability to metabolize the drug may be uncertain). This process may be more complex, since the prodrug will rely upon liver function to be activated and to function; thus the method is preferably done in a stepwise fashion, exemplified by the following steps:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion; then

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c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as excreted PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

Monitoring of the subject may be done by testing ammonia levels, or by monitoring urinary PAGN output. In one embodiment, the subject's urinary output of PAGN is monitored to ensure that the output exceeds a cut-off value that correlates well with achieving normal ammonia levels, such as a level of about 10 g urinary PAGN per day in adult UCD patients.

[00120] The methods also include optionally measuring total urinary nitrogen and urinary PAGN after at least 3 days of drug administration, at which point a steady state has been achieved. It also can include calculating the amount of drug converted to PAGN, which would be expected to be at least 50%, to determine if the drug is having the desired effect. A suitable dosage of the drug would be identified as one where the amount of excreted PAGN is sufficient to clear the expected amount of waste nitrogen from the dietary intake of protein, which can be adjusted to account for the patient's endogenous nitrogen elimination capacity.

[00121] The fraction of nitrogen waste to be cleared in a single step can be selected with due regard to the severity of the patient's condition (nitrogen accumulation disorder). In some embodiments, it will be appropriate to target removal of about 50% of the waste nitrogen for which clearance assistance is needed. In some embodiments, the method will target removal of about 100% of the waste nitrogen.

[00122] In another aspect, the invention provides a method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

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c) determining the level of excreted urinary PAGN for the subject to make sure it has not decreased; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

[00123] If it is found that the amount of excreted PAGN decreases, additional HPN-100 or additional PBA would be administered to reestablish a level of PAGN excretion that is suitable for the patient, and the replacement steps would then be continued until all of the PBA was replaced by HPN-100.

[00124] Here again, the portion of phenylbutyrate to be replaced in an initial step can be 100%, about 1/2, about 1/3, or about ¼, or some value between these. During a stepwise process, where less than all of the phenylbutyrate is replaced in a first step, the patient will receive both HPN-100 and phenylbutyrate. As demonstrated herein, the appropriate method for determining a suitable dose of HPN-100 will take account of the excreted PAGN, rather than being based only on less reliable criteria for evaluating the orally delivered PBA prodrug.

[00125] In another embodiment, the invention provides a method to administer a phenylbutyrate prodrug to a patient, comprising determining the rate of PAGN excretion for the subject following administration of at least one phenylbutyrate prodrug, and selecting or adjusting a dose administration schedule based on the PAGN excretion rate. The compound can be a compound of Formula I, Formula II or Formula III as described above. Advantageously, the compounds used herein as prodrugs of PBA achieve nitrogen scavenging comparable to that of PBA but exhibit a slow-release kinetic profile that produces a more stable ammonia level in the treated subject. In some embodiments, the methods of the invention include administering a prodrug as described herein to a subject at a dosage that provides comparable ammonia level control to that achieved by PBA, but with significantly lower exposure of the subject to systemic PBA. In some embodiments, the subject experiences pharmacokinetic parameters for PBA that demonstrate lower exposure to PBA, including a lower AUC and Cmax for PBA, while maintaining a plasma ammonia level comparable to or better than that provided by treatment with a dosage of PBA within the normal dosing range. When HPN-100 and PBA were administered to UCD patients at equimolar dosages, the patient receiving HPN-100 had overall lower plasma ammonia levels, and also lower PBA exposure:

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	AUC (NH_3) µg-hr/mL	C_{max} (NH_3) µg-hr/mL	AUC (PBA) µg-hr/mL	C_{max} (PBA) µg-hr/mL
PBA	38.4(20)	79.1(40)	739(49)	141(44)
HPN-100	26.1(10)	56.3(28)	540(60)	70(65)

[00126] While a larger data set is needed to demonstrate statistical significance, limited amounts of data are available in part due to the rarity of these conditions. Nevertheless, the data indicates that PBA treatment resulted in less effective ammonia level control and greater exposure to PBA, while the PBA prodrug HPN-100 at equimolar dosing provided better ammonia level control and lower PBA exposure levels. Accordingly, in one aspect the invention provides a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces better ammonia level control than PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA, when compared to treatment with an equimolar amount of PBA. In some embodiments, the treatment uses HPN-100 as the prodrug, and in some embodiments the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20%; or the exposure to PBA upon treatment with the prodrug is lower by at least about 27%% compared to treatment with PBA; or both of these conditions are met to demonstrate reduced exposure to PBA. In some embodiments, the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the prodrug is administered. Preferably, the prodrug provides plasma ammonia levels that average less than about 40 µmol/L or not more than 35 µmol/L, or not more than about 30 µmol/L.

[00127] The advantageous slow-release kinetic profile of compounds used herein as prodrugs of PBA permits less frequent and more flexible dosing in selected patients as compared with sodium PBA. While all patients with UCDs and a propensity for elevated ammonia levels should in principle be able to benefit from the ammonia scavenging activity of HPN-100, UCD patients with substantial residual urea synthetic capacity (e.g., UCD whose first manifestations occur at several years of age or older; i.e. patients who do not exhibit neonatal onset) would be the best candidates for three times daily or even twice daily dosing with PBA prodrugs such as HPN-100. Patients with cirrhosis and HE would also be candidates for less frequent dosing, as even patients with severe liver disease have significant residual urea synthetic capacity (Rudman et al., J. Clin. Invest. 1973).

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[00128] Specific embodiments of the invention include the following:

A. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100, wherein monitoring the effect consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output.

In this method, the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and urinary PAGN output can be determined as a ratio of urinary PAGN to urinary creatinine, since it has been demonstrated by others that creatinine, the daily excretion of which tends to be constant for a given individual, can be used as a means to normalize measures of urinary parameters while correcting for variations in urinary volume. In these methods, the nitrogen retention disorder can be chronic hepatic encephalopathy or a urea cycle disorder. Plasma ammonia levels (or urea levels in the case of CRF) may also be monitored to adjust the overall treatment program and dietary protein intake, but as discussed above, urinary PAGN provides a preferred way to assess the drug's role in waste nitrogen elimination.

B. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100, wherein the initial dose for a treatment-naïve patient would take into account the expected percentage conversion of the administered PBA to urinary PAGN, and wherein monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. In these methods, administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level in the patient. This can be a level of about 35 or about 40 µmol/L.

C. A method to determine a starting dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a utilization efficiency of about 40% to about 70%. In such methods, the dosage of HPN-100 can be calculated from the patient's dietary protein intake, or it can be estimated from the patient's body weight and approximate growth rate. In such methods, the dosage of HPN-100 is sometimes reduced to account for the patient's residual urea synthesis capacity, by adjusting the amount of HPN-100 to reflect the amount of ammonia scavenging needed in view of the patient's endogenous capacity for nitrogen elimination.

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D. A method to determine a dosage of a PAA prodrug for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 40% to about 70% conversion (preferably about 54%) of the PAA prodrug into urinary PAGN.

In these methods, the PAA prodrug can be phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof, or it can be HPN-100.

E. A method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 40% to about 70% conversion (preferably about 54%) of the PAA prodrug into urinary PAGN; and

e) administering to the patient the suitable dosage of the PAA prodrug.

In these methods, the PAA prodrug is often phenylbutyrate or a pharmaceutically acceptable salt thereof, or HPN-100.

G. A method to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, comprising:

a) determining a replacement amount of HPN-100 to replace at least a portion of the phenylacetate or phenylbutyrate;

b) substituting the replacement amount of the HPN-100 for the phenylacetate or phenylbutyrate; and

c) monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the HPN-100.

In these methods, an increase the amount of urinary PAGN may indicate that the amount of HPN-100 can be reduced, and a decrease in urinary PAGN may indicate the amount of HPN-100 needs to be increased. Alternatively, a urinary PAGN level above the level that correlates with

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achieving normal ammonia levels (e.g., about 10 g urinary PAGN per day in adult UCD patients) may be used as an indicator that the dosing level is adequate.

II. A method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted urinary PAGN for the subject; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

I. A method to initiate treatment with phenylacetate, phenylbutyrate or a HPN-100 in a step-wise fashion, comprising:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion based upon diet and urea synthetic capacity; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as urinary PAGN taking into account the expected percentage conversion of the administered PBA to urinary PAGN; and

d) increasing the dose of drug as appropriate, and repeating the steps above, to reach a maintenance dose of the drug.

J. A method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA when the patient receives the PBA prodrug, when compared to the AUC and Cmax observed when the patient receives an equimolar amount of PBA.

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[00129] In the foregoing embodiments and methods, the PBA prodrug is often HPN-100. Urinary PAGN output is typically measured either as a total output over the 24 hour period following the first dose of the PBA prodrug of the day, or as a total urinary PAGN output for the period 12-24 hours following the first dose of the PBA prodrug of the day. These values may be measured directly, or they may be estimated from the ratio of PAGN to creatinine in a sample of the patient's urine taken while the subject is on a stable drug dosage, optionally during the 12-24 hour period following the first dose of the drug of the day.

[00130] The methods include a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA exposure can be lower with the prodrug than with PBA by at least about 20%, or by at least about 27% compared to treatment with PBA. This is believed to be related to the slow absorption or uptake characteristics of HPN-100, which provide a more stable level of PBA exposure and provide an unexpected advantage of HPN-100 to be effective with less frequent dosing when compared to sodium phenylbutyrate.

K. A method to determine a suitable dietary protein level for a patient having a nitrogen retention disorder, comprising:

a) determining the patient's endogenous nitrogen elimination capacity;
b) calculating from the endogenous nitrogen elimination capacity an amount of dietary protein the patient can process without the aid of a nitrogen scavenging drug;
c) then adding an amount of protein that the patient should be able to process with the assistance of selected dosage of a nitrogen scavenging drug to arrive at an amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, taking into account the amount of protein required for health and body growth.

[00131] In this method, the nitrogen scavenging drug can be HPN-100. Commonly, the selected dosage of HPN-100 is not more than about 19 grams per day, and the amount of dietary protein the patient should be able to process with the assistance of this amount of HPN-100 is about 1 grams (~1.3 g) of protein per gram of HPN-100.

L. A method to treat a patient with a PBA prodrug, comprising administering HPN-100 at a daily dose in excess of 19 g per day to a subject having HE or UCD. Optionally, the daily dose of HPN-100 is between about 20 g and about 57 g.

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M. A method for determining the dosing schedule of a PBA prodrug wherein the patient retains substantial residual urea synthetic capacity, as would be the case for most patients with cirrhosis and HE or most UCD patients who do not exhibit symptoms within the first two years of life.

N. A method for determining the dosing schedule of a PBA prodrug (preferably sodium free) in a patient with CRF based on the anticipated conversion to PAGN and urinary PAGN excretion. The method comprises using a conversion efficiency factor of 54% when dosing with HPN-100, in order to determine a recommended daily dose of the prodrug. The method can also comprise monitoring efficacy of a treatment of a CRF patient with HPN-100 by measuring urinary PAGN output.

[00132] In the foregoing methods that utilize HPN-100, the exposure to PBA upon treatment with the prodrug HPN-100 is lower by at least about 27% compared to treatment with PBA. Also, commonly the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the prodrug is administered. Also, in the foregoing methods, when the subject is treated with the prodrug, which can be HPN-100, the subject will typically achieve and maintain normal plasma ammonia levels.

[00133] Some additional enumerated embodiments of the invention include the following:

Embodiment 1. A method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder, comprising:

a) determining the patient's dietary protein intake;

b) determining the patient's residual urea synthesis capacity, if any;

c) estimating from a) and b) the amount of excess waste nitrogen the patient needs to excrete to remove the waste nitrogen associated with the dietary protein intake that is not excreted as urea; and

d) determining an amount of the PAA prodrug needed to eliminate the estimated amount of excess waste nitrogen as urinary PAGN,

wherein the amount of PAA prodrug needed is determined based on a conversion factor whereby about 40% to about 70% of the PAA prodrug is converted into urinary PAGN.

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2. The method of embodiment 1, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof.

3. The method of embodiment 1, wherein the PAA prodrug is HPN-100.

4. The method of embodiment 2 or 3, wherein the conversion factor is about 54%.

5. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100,

wherein monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output;

and determining from the patient's urinary PAGN output whether and/or how to adjust the initial dosage of HPN-100 to provide a desired ammonia scavenging effect.

6. The method of embodiment 5, wherein determining from the patient's urinary PAGN output whether to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect comprises comparing the patient's urinary PAGN output to a cut-off level of urinary PAGN output that correlates with achieving effective ammonia control for comparable patients.

7. The method of embodiment 6, wherein the patient is an adult UCD patient, and the cut-off level is about 10 g/day of urinary PAGN.

8. The method of any one of embodiments 5-7, wherein determining from the patient's urinary PAGN output how to adjust the initial dosage of HPN-100 comprises calculating an adjusted dosage based on a conversion efficiency of about 40-70% for conversion of HPN-100 to urinary PAGN.

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9. The method of embodiment 8, wherein the conversion efficiency is about 54%.

10. The method of any of the preceding embodiments, wherein the nitrogen retention disorder is hepatic encephalopathy (HE), a urea cycle disorder (UCD), or chronic renal failure (CRF).

11. The method of any of the preceding embodiments, wherein the patient's urinary PAGN output is determined from the ratio of PAGN to creatinine in a sample of the subject's urine.

12. The method of any one of the preceding embodiments, wherein the patient's urinary PAGN output is the patient's total urinary PAGN for 24 hours following the first dose of HPN-100 of the day, or the patient's total urinary PAGN for the period 12-24 hours following the first dose of HPN-100 of the day.

13. A method to determine a dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a conversion efficiency for HPN-100 conversion into urinary PAGN of about 54%.

14. The method of embodiment 13, wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake.

15. The method of embodiment 14, wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity.

16. Use of a PBA prodrug in the treatment of HE or UCD or CRF, comprising administering HPN-100 at a daily dose in excess of 19 g per day. .

17. Use of a PBA prodrug in the manufacture of a medicament for the treatment of HE or UCD, wherein the PBA prodrug is HPN-100 in a form for administration at a daily dose in excess of 19 g per day.

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18. The method of embodiment 16 or claim 17, wherein the daily dose of HPN-100 is between about 19g and about 57 g.

19. Use of the PBA prodrug HPN-100 to treat a nitrogen retention disorder, , wherein the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered.

20. Use of the PBA prodrug HPN-100 in the manufacture of a medicament for use to treat a nitrogen retention disorder, wherein the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered.

21. The use of embodiment 19 or embodiment 20, wherein a subject's plasma ammonia levels are on average normal when treated with HPN-100.

22. A method to determine whether a dosage of HPN-100 being administered to a subject having a nitrogen retention disorder is providing adequate ammonia control for the subject , or if the dosage needs to be modified,, wherein the method comprises measuring the subject's urinary PAGN output level, and comparing this urinary PAGN output level to a cut-off value for urinary PAGN output determined for comparable subjects,

wherein the dosage is adequate if the subject's urinary PAGN output equals or exceeds the cut-off value, and the dosage needs to be modified if the subject's urinary PAGN output is less than the cut-off value.

23. The method of embodiment 22, wherein the subject is an adult UCD patient, and the cut-off value is about 10 g of urinary PAGN per day.

24. The method of embodiment 22, wherein the nitrogen retention disorder is a UCD, HE, or CRF.

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25. The method of embodiment 22, further comprising modifying the dosage if the subject's urinary PAGN output is less than the cut-off value to determine an adjusted dosage.

26. The method of embodiment 25, wherein the adjusted dosage is calculated by subtracting the subject's urinary PAGN output from the cut-off value to determine an amount by which the subject's urinary PAGN output needs to be increased, and calculating an amount of HPN-100 that corresponds to the amount by which the subject's urinary PAGN output needs to be increased while taking into account that the conversion of HPN-100 into urinary PAGN is about 54%.

[00134] In the foregoing embodiments and methods, the PBA prodrug is often HPN-100. Urinary PAGN output is typically measured either as a total output over the 24 hour period following the first dose of the PBA prodrug of the day, or as a total urinary PAGN output for the period 12-24 hours following the first dose of the PBA prodrug of the day. These values may be measured directly, or they may be estimated from the ratio of PAGN to creatinine in a sample of the patient's urine taken while the subject is on a stable drug dosage, optionally during the 12-24 hour period following the first dose of the drug of the day.

[00135] The methods include a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA exposure can be lower with the prodrug than with PBA by at least about 20%, or by at least about 27% compared to treatment with PBA. This is believed to be related to the slow absorption or uptake characteristics of HPN-100, which provide a more stable level of PBA exposure and provide an unexpected advantage of HPN-100 to be effective with less frequent dosing when compared to sodium phenylbutyrate.

[00136] The following examples are offered to illustrate but not to limit the invention.

[00137] The data below from three human studies and one preclinical study illustrate that the conventional approach of assessing drug exposure and effect by measuring blood levels does not correlate with nitrogen scavenging as assessed by urinary excretion of PAGN or by reduction of plasma ammonia. These data demonstrate that, surprisingly, the plasma level of PBA or PAA seen with an effective amount of a prodrug can be far less the plasma level of PBA or PAA seen with a similarly effective amount of phenylbutyrate. Moreover, they demonstrate the need to allow for incomplete conversion of sodium PBA or HPN-100 into PAGN in selecting starting dosage, the

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delayed release behavior and implications for dosing schedule of delivering PBA as a triglyceride rather than as a salt, and the possibility of administering HPN-100 in doses greater than those currently recommended for sodium PBA. These are followed by a biological explanation for the findings.

Example 1

Single dose safety and PK in healthy adults

[00138] To assess its pharmacokinetic (PK) and pharmacodynamic (PD) profile, HPN-100 was administered as a single dose to 24 healthy adults. Pharmacokinetic samples were taken pre-dose and at 15 and 30 minutes post-dose and 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours post-dose. As discussed below, plasma levels of the major HPN-100 metabolites PBA, PAA and PAGN were many fold lower after administration of HPN-100 than after sodium PBA. By contrast, urinary excretion of PAGN was similar between the two groups (4905 +/- 1414 mg following sodium PBA and 4130 +/- 925 mg following HPN-100) and the differences that were observed were determined to be largely an artifact of incomplete collection due to stopping urine collection at 24 hours (note that PAGN excretion following administration of sodium PBA was largely complete at 24 hours but continued beyond 24 hours following administration of HPN-100). Thus, the plasma metabolite concentrations did not accurately reflect the comparative ammonia scavenging activity of sodium PBA and HPN-100.

[00139] Three healthy adult volunteers were treated with a single dose of either sodium PBA or HPN-100 at a dosage of 3 g/m^2. Plasma levels of PAA, PBA, and PAGN were monitored periodically for 12-24 hours by known methods. Results of this are shown in Figure 4, which shows a curve for each subject (note the log scale).

[00140] In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving sodium PBA at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100.

[00141] In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately 12 hours, and were generally close to the PAGN curves up to that time.

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Example 2

Administration of HPN-100 to patients with liver disease

[00142] To determine its pharmacokinetic (PK) and pharmacodynamic (PD) profile in patients with liver disease, clinical testing was conducted in which HPN-100 was administered orally as a single dose (100 mg/kg/day on day 1), and twice daily for 7 consecutive days (200 mg/kg/day on days 8 through 14, in two doses of 100 mg/kg per dose), to subjects with hepatic impairment with cirrhosis (Child-Pugh scores of A, B, or C) and to a gender and age-matched control group of healthy adults with normal hepatic function. Steady state levels of these metabolites in blood are reached by this time—see Figures 6 and 7. On day 15, subjects received a single dose of HPN-100 (100 mg/kg). PK blood samples were taken pre-dose, at 15 and 30 minutes post-dose, and at 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours post-dose on days 1, 8, and 15, and at 48 hours after dosing on days 1 and 15. On days 9–14, blood samples were taken pre-morning dose and at 2 hours post-morning dose. Urine was collected 0–4, 4–8, 8–12, and 12–24 hours post-dose on days 1, 8, and 15, and at 24–48 hours post-dose on days 1 and 15.

[00143] HPN-100 was metabolized via the predominant pathway in all subject groups, and the alternative HPN-100 metabolites PAG (phenylacetyl glycine), PBG (phenylbutyryl glycine), and PBGN (phenylbutyryl glutamine) were below the limit of quantification in all plasma samples. Both the extent of systemic exposure (AUC_{0-t}) and C_{max} for PBA and PAA tended to be higher in Child-Pugh group B or C than in Child-Pugh group A or the healthy volunteer group, although there were no significant differences in these variables on day 15. As described below, plasma PAA levels did correlate with Childs-Pugh classification (i.e. were higher in patients with more severe liver disease). However, the average conversion of HPN-100 to PAGN was ~ 40-50% after single dose administration, and no difference were seen between patients with cirrhosis and normal healthy volunteers, demonstrating that hepatic impairment did not affect the subjects' ability to activate the PBA prodrug HPN-100 or to utilize it for elimination of excess ammonia. Thus, as summarized in more detail below, plasma metabolite levels did not correlate well with the HPN-100 dosage and, just as for healthy adults, plasma metabolite levels did not accurately reflect the nitrogen scavenging effect of HPN-100.

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Analyte	Subject group	Geometric mean ratio	90% CI	P value for group effect
PBA	**AUC_{0-t}**			0.40
	Child-Pugh A	0.92	0.58–1.43	
	Child-Pugh B	1.26	0.80–1.97	
	Child-Pugh C	1.37	0.87–2.14	
PBA	**C_{max}**			0.52
	Child-Pugh A	1.42	0.87–2.31	
	Child-Pugh B	1.35	0.83–2.21	
	Child-Pugh C	1.50	0.92–2.45	
PAA	**AUC_{0-t}**			0.64
	Child-Pugh A	1.22	0.48–3.06	
	Child-Pugh B	1.53	0.61–3.85	
	Child-Pugh C	1.94	0.77–4.88	
PAA	**C_{max}**			0.72
	Child-Pugh A	1.33	0.70–2.52	
	Child-Pugh B	1.16	0.61–2.20	
	Child-Pugh C	1.52	0.80–2.88	

AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; CI, confidence interval; C_{max}, maximum observed plasma concentration; PAA, phenylacetic acid; PBA, phenylbutyric acid.

[00144] During multiple dosing (days 8–15), there was a trend for higher systemic concentrations of PBA and PAA in subjects with greater hepatic impairment (Child-Pugh B or C) compared with Child-Pugh group A and the healthy volunteers. Unlike PBA, PAA did accumulate significantly in plasma during multiday dosing. Differences between single (day 8) and multiple dosing (day 15: steady state—see Figures 6 and 7) were significant for AUC_{0-12} and C_{max} of PAA for all subjects combined ($p < 0.001$), but not for PBA. After dosing on day 15, extent of exposure to PAA, but not PBA, significantly correlated with hepatic impairment.

[00145] The clinical efficacy of HPN-100 is dependent on its ammonia scavenging capabilities, through conjugation of glutamine with PAA to form PAGN. After dosing on each day, PAGN was the major metabolite excreted: 42–49% of the HPN-100 dose administered was excreted as PAGN on day 1, 25–45% on day 8, and 58–85% on day 15. Very low amounts of PBA and PAA were

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excreted in the urine (≤ 0.05% of the total HPN-100 dose). There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. Urinary PAGN excretion is also an indication of the ammonia-scavenging capacity of HPN-100, as 2 moles of ammonia combine with 1 mole of PAA to produce PAGN. Hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study. There were no significant differences in the amount of PAGN excreted between any of the Child-Pugh groups and the healthy volunteers. The observations that hepatic impairment had no significant effect on the ammonia-scavenging ability of HPN-100 in this study but was associated with accumulation of PAA in plasma underscores the importance of utilizing urinary PAGN rather than metabolite blood levels to guide drug effect and, as a corollary, the importance of the invention.

Urinary Output of Phenylacetylglutamine (PAGN)—Healthy Subjects and Cirrhotic Subjects

Study UP 1204-002[1]: Healthy Volunteers and Cirrhotic Patients (100 mg/kg HPN-100 BID)

	Child-Pugh A (n=8)	Child-Pugh B (n=8)	Child-Pugh C (n=8)	Healthy Volunteers (n=8)
PAGN after dosing on day 1 (0-24 hours)				
Amount Excreted (μmol)	15553 (4201)	16847 (4326)	19291 (12054)	14903 (4292)
Proportion excreted post-24 hours (%)[2]	15.5 (7.5)	14.5 (13.3)	8.8 (6.0)	16.9 (10.5)
PAGN after dosing on day 1 (0-48 hours)				
Amount Excreted (μmol)	18386 (4961)	19902 (4505)	20854 (15281)	17869 (4312)
Mole % of dose excreted	47.1 (10.4)	44.9 (9.7)	48.5 (29.4)	42.2 (11.4)
PAGN after dosing on day 8 (0-12 hours)[3]				
Amount Excreted (μmol)	16068 (7397)	13179 (5786)	15428 (6519)	10195 (4189)
Mole % of dose excreted	40.6 (16.4)	29.9 (13.0)	44.6 (24.2)	24.5 (11.4)
PAGN after dosing on day 15 (0-48 hours)[3]				
Amount Excreted (μmol)	31431 (15291)	25152 (11426)	30752 (20860)	28716 (8223)
Mole % of dose excreted	79.6 (30.5)	58.2 (29.2)	85.0 (65.1)	68.6 (21.9)

[1]HPN-100c value is corrected for approximately 15% under collection of urine. PAGN was detectable in plasma samples of subjects receiving HPN-100 but not NaPBA after the 24 hour time point indicating that urinary collection of PAGN was incomplete at 24 hours following HPN-100 dosing.
[2]Values are not %dose; instead, they represent the amount of PAGN yet to be eliminated in urine expressed as a percentage of total PAGN eliminated over 48 hours. For example, 15553/18386*100=15.5% for Child-Pugh A patients.
[3]Urine collection was after the first HPN-100 dose (100 mg/kg) in the morning.
[4]Calculated as a percentage of the single dose administered on the morning of day 15
PAGN=phenylacetylglutamine

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[00146] Of particular note, there was no relationship between the plasma levels of PBA and PAA, which exhibited a non-statistically significant directional change toward higher plasma levels in patients with liver disease than healthy adults, and urinary excretion of PAGN.

EXAMPLE 3

Administration of HPN-100 To Adults With UCDs

[00147] To further explore its pharmacokinetic (PK) and pharmacodynamic (PD) profile in clinical states associated with nitrogen retention, 10 adult UCD patients were switched from sodium PBA to a PBA equimolar dose of HPN-100. Subjects were required to be on a stable dose of sodium PBA before enrolment. Upon enrolment, all subjects received sodium PBA for 7 days and were then admitted to a study unit (Visit 2-1) for overnight observation and 24-hour PK and ammonia measurements and urine collections. Subjects were then converted to the PBA equimolar dose of HPN-100, either in a single step or in multiple steps depending on the total dose of sodium PBA; 9 out of 10 patients converted in a single step. Subjects stayed on the 100% HPN-100 dose for one week and were then re-admitted to the study unit for repeated PK (Visit 11-1), ammonia and urine collections.

[00148] The findings from this study, summarized in detail below, demonstrate that, just as in healthy adults and patients with liver disease, plasma metabolite levels do not correlate well with ammonia scavenging activity as reflected by urinary PAGN excretion and corroborated by plasma ammonia results. Moreover, the findings demonstrate considerable inter-individual variability in the percentage of both sodium PBA and HPN-100 that is converted to urinary PAGN.

[00149] Pharmacokinetic, ammonia and safety analyses: As summarized in the table below, 7 days of HPN-100 administration resulted in comparable PAA and plasma PAGN levels but slightly lower PBA levels compared to the PBA molar equivalent dose of sodium PBA.

Comparison of Pharmacokinetic Parameters at Steady State – sodium PBA vs. HPN-100

PK Parameter	Arithmetic Mean (CV%)	
	Sodium PBA (N=10)	**HPN-100 (N=10)**
PBA in Plasma		
AUC_{0-24} (μg·h/mL)	739 (49.2)	540 (60.1)
$Cmax_{ss}$ (μg/mL	141 (44.3)	70.1 (64.7)
$Cmin_{ss}$ (μg/mL	0.588 (255)	2.87 (265)
PAA in Plasma		
AUC_{0-24} (μg·h/mL)	595.6 (123.9)	574.6 (168.9)

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PK Parameter	Arithmetic Mean (CV %)	
	Sodium PBA (N=10)	HPN-100 (N=10)
$Cmax_{ss}$ (μg/mL	53.0 (94.7)	40.5 (147.6)
$Cmin_{ss}$ (μg/mL	3.56 (194.4)	7.06 (310.7)
PAGN in Plasma		
AUC_{0-24} (μg·h/mL)	1133 (31.1)	1098 (44.2)
$Cmax_{ss}$ (μg/mL	83.3 (25.8)	71.9 (56.0)
$Cmin_{ss}$ (μg/mL	16.8 (86.1)	12.1 (134.4)

AUC_{0-24}: Area under the concentration from time 0 (pre-dose) to 24 hours, $Cmax_{ss}$: Maximum plasma concentration at steady state, $Cmin_{ss}$: Minimum plasma concentration at steady state, A_e: Amount excreted over 24 hours

[1] The mean (SD) sodium PBA dose = 12.6 (4.11) g; the mean (SD) HPN-100 dose = 12.3 (3.91) g.

[00150] Despite dissimilar PBA blood levels, overall urinary excretion of PAGN was similar for the two treatments as summarized in the table below. Importantly, and in contrast to the assumptions inherent in current treatment guidelines that all administered sodium PBA is converted to urinary PAGN, considerable inter-individual variability was observed in the percentage of administered PAA converted to PAGN, which averaged ~54% and was typically similar for both sodium PBA and HPN-100. Moreover, the 24 hour pattern of excretion appeared to differ in that urine output of PAGN reached its highest level during the 'afternoon hours' (6-12 hour urine collection) for patients treated with sodium PBA, whereas peak output of PAGN occurred overnight (12-24 hour urine collection) for patients on HPN-100 treatment. This difference presumably reflects the slow release characteristics and longer duration of effective blood concentrations of PAA following administration of HPN-100 as compared with sodium PBA. HPN-100 was either not detectable or below the limits of quantitation in all blood samples.

Comparison of Mean PAGN Amount Excreted (μg) – and Percent Conversion (%) Following Administration of Sodium PBA (sodium phenylbutyrate) vs. HPN-100

PAGN in Urine*	HPN-100 Recipients	BUPHENYL® Recipients
Total excreted 0-24 hr (μg)	10 784 747 (25.9)	12 153 473 (48.2)
0-6 hr (μg)	2381371 (61.3)	2452838 (41.6)
6-12 hr (μg)	3027310 (44.9)	4859121 (54.7)
12-24 hr (μg)	5433033 (50.4)	4645447 (59.8)
Recovery of PBA as PAGN (%)	54 (15)	54 (16)

[00151] As summarized in the table below, mean time normalized area under the curve (TN-AUC) values for venous ammonia following HPN-100 were directionally (~31%) lower than those

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observed with sodium PBA (26.1 vs. 38.4 μmol/L) although the differences did not achieve statistical significance (Figure 9). Likewise, peak venous ammonia concentrations following HPN-100 were directionally (~29%; not statistically significant) lower than those observed with sodium PBA (56.3 vs. 79.1 μmol/L, respectively).

[00152] The normal upper limit for venous ammonia varied among the study sites from 26 to 35 μmol/L. Examination of ammonia values (TN-AUC) for individual patients demonstrated that patients with higher ammonia levels on sodium PBA exhibited greater decreases in ammonia values following administration of HPN-100 (Figure 11). Moreover, the mean ammonia value after HPN-100 (26.1 μmol/L) was within the normal range while it was above the upper limit of normal (ULN) for subjects receiving sodium PBA (sodium phenylbutyrate) (38.4 μmol/L) (Figure 12). Likewise the mean percentage of normal ammonia values increased from 58% after sodium PBA treatment to 72% after HPN-100 treatment.

Venous Ammonia Pharmacodynamics Following Seven Days of Dosing With Either Sodium PBA or HPN-100 (Steady State)

	Sodium PBA			HPN-100		
Subject	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**	**$Cmax_{ss}$ (μmol/L)**	**TN-AUC (μmol/L)**	**PBA Equivalent dose[1]**
1001	29.0	16.47	17.5	63.0	19.8	13.1
1002	31.0	20.9	15.8	31.0	19.3	15.9
1004	85.0	46.8	99.2	106	35.1	9.16
1006	150	71.5	17.5	13.0	8.30	17.7
2001	88.0	52.1	6.57	33.0	22.7	6.71
2003	31.0	17.5	11.8	74.0	21.1	12.2
3002	108	22.3	16.5	36.0	21.9	17.7
3004	115	62.9	13.1	75.0	38.4	13.1
5001	82.2	35.8	8.76	57.0	35.5	8.85
5002	72.2	37.7	8.76	75.2	39.1	8.85
N	**10**	**10**	**10**	**10**	**10**	**10**
Mean	79.1	38.4	12.6	56.3	26.1	12.3
SD	40.1	19.6	4.11	27.9	10.3	3.91
Median	83.6	36.8	12.5	60.0	22.3	12.7
Min	29.0	16.4	6.57	13.0	8.30	6.71
Max	150	71.5	17.5	106	39.1	17.7
25%	31.0	20.0	--	32.5	19.7	--
75%	110	54.8	--	75.0	36.2	--

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[00153] This reduction in ammonia exposure among UCD patients reflects better overnight control among subjects receiving HPN-100, as summarized in the table below and in Figure 10. This study shows that both AUC and Cmax for ammonia were lower with HPN-100, indicating less total ammonia exposure, and especially at night, HPN-100 exhibited a significantly stronger effect. While not statistically significant due to the small population size, this demonstrates that HPN-100 is at least as effective, and apparently more so, than PBA on an equimolar basis based on the key measure, its ability to mobilize ammonia for urinary elimination. Based on preliminary results, HPN-100 also provides more stable ammonia levels, and reduces risk of hyperammonemia. In this trial, 9 of 10 subjects who experienced both HPN-100 and sodium PBA indicated a preference for HPN-100.

[00154] In addition, in this trial, no serious adverse effects (SAEs) were observed in patients taking HPN-100, while two subjects receiving PBA experienced symptomatic hyperammonemia; and the total number of adverse effects (AEs) reported among subjects taking HPN-100 (5 subjects reported a total of 15 AEs) was lower than the number of AEs among subjects taking PBA (7 subjects reported 21 AEs).

[00155] The following table summarizes overall comparative data for sodium PBA and HPN-100, administered at equimolar rates (n=10) (see tables above and Figures 10-13 for additional detail).

Parameter	Sodium PBA	HPN-100
NH_3: Total AUC	38.4 ± 19.6	26.1 ± 10.3
NH_3 Cmax	79.1 ± 40.1	56.3 ± 27.9
NH_3 exposure: DAY (hours 6-12)	37.1	32.9
NH_3 exposure: NIGHT (hours 12-24)	36.3	21.3
Adverse effects	21 reported by 7 subjects	15 reported by 5 subjects
Serious adverse effects	2 (symptomatic hyperammonemia)	0
PAGN excretion	Comparable	Comparable

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[00156] While the differences between sodium PBA and HPN-100 did not reach statistical significance due to the small sample size, HPN-100 exhibited a clear trend toward being more efficacious at equimolar dosages, and it was particularly effective for improving overnight control of ammonia levels.

[00157] Figure 8a demonstrates that PBA levels in the blood are not correlated with HPN-100 dosages received. It plots the 24-hour AUC for PBA and the Cmax for PBA against HPN-100 dosage (top panel), and while the AUC and Cmax track together in each patient, they show no relationship to HPN-100 dose: both the highest and the lowest PBA exposures occurred in patients receiving high doses of HPN-100. Figure 8b shows that levels of PAA are similarly uncorrelated with HPN dosages.

[00158] Figure 9 illustrates the trend shown in the clinical testing, where HPN-100 provided better overall control of waste nitrogen.

[00159] Figure 10 illustrates that improved night time control of excess ammonia is achieved with HPN-100.

[00160] Figure 11 shows that especially for patients with higher ammonia levels when treated with sodium PBA (Na PBA), HPN-100 provides better control than sodium PBA, while in patients with lower ammonia levels (ones for whom sodium PBA seems to work relatively well), HPN-100 provides at least comparable ammonia control. Note that for patients having ammonia levels above about 40 μmol/L when treated with sodium PBA, HPN-100 at equimolar dosages provided superior control of ammonia, and consistently reduced ammonia levels to below about 40 μmol/L. Thus for patients whose ammonia levels are abnormal (e.g. above about 40 μmol/L) when treated with sodium PBA, it is expected that better ammonia control can be achieved with an equimolar amount of HPN-100. Based on this, dosages of HPN-100 can be determined as set forth herein. Figure 12 illustrates that ammonia levels were better controlled in this test by HPN-100 than with sodium PBA, e.g., the average ammonia levels are lower, and tend to be below the upper limit for normal.

Example 4

Relationship Between Ammonia Control and Urinary PAGN Excretion

[00161] As part of the clinical study in UCD patients described in the example above (Example 3), the relationship between plasma ammonia levels and urinary excretion of PAGN was examined. Unlike blood levels of PAA or PBA which exhibited no consistent relationship to ammonia levels

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(i.e. ammonia control), blood ammonia assessed as the time-normalized area under the curve exhibited an inverse curvilinear relationship to urinary PAGN. That is, plasma ammonia decreased as urinary PAGN increased. Moreover, the relationship between ammonia and urinary PAGN excretion did not differ between sodium PBA and HPN-100 suggesting that this method of dose determination is independent of product formulation. The following Table summarizes the statistical correlations observed in this study between various parameters and ammonia levels in the subject's blood.

Correlation Between Ammonia and Plasma PAA, PBA and PAGN, and Urinary PAGN (UPAGN) [1, 2]

	PAA	PBA	Plasma PAGN	UPAGN	Dose	U-PAGN 12-24
N	15	15	16	18	18	16
r	-0.23	0.08	-0.52	-0.80	**-0.55**	**-0.75**
p	NS	NS	0.04	<0.0001	**0.02**	**<0.001**

[1] Ammonia was measured as time-normalized area under the curve (TNAUC)
[2] Spearman Rank-Order Correlation
Data from both NaPBA and HPN-100 were included in the analysis
Data from one subject with more than 50% missing data on HPN-100 were excluded.
NS = not significant at α=0.05.

[00162] Figure 13 shows a plot of Plasma Ammonia (TN-AUC) versus Urinary PAGN Excretion, and demonstrates the strong correlation between ammonia levels and urinary PAGN. 'Normal' ammonia level varies for different subjects and different testing sites, and the four sites for the studies herein reported different upper levels of normal (ULNs) for ammonia, ranging from 26-35 μmol/L. Across all sites involved, subjects treated with HPN-100 exhibited average ammonia levels that were 'normal', i.e., below the ULN (upper level of normal), 73% of the time, while subjects treated with sodium PBA had 'normal' ammonia levels, i.e., below the ULN, 60% of the time.

Example 5

Experimentation With Dosing Schedule

[00163] The results of single dose PK/PD modeling observed in the examples above suggested that HPN-100 exhibits delayed release characteristics as compared with sodium PBA with a

corresponding potential for increased flexibility in dosing, which was further explored in additional clinical studies described above. In one of these, HPN-100 was administered twice daily as well as in the fasted and fed state. In the other, HPN-100 was administered three times daily with meals. Both 3x daily and 2x daily dosing resulted in a similar proportion of PAGN excreted in the urine and, as demonstrated in adult UCD patients, three times daily dosing was associated with effective ammonia control.

[00164] In Example 2, a number of secondary statistical analyses comparing PK variables after fed versus fasted HPN-100 dosing and single versus multiple HPN-100 dosing were also done. There were no PK or PD differences observed when HPN-100 was administered after fasting (day 1) or with a meal (day 8). Accordingly, it is believed that HPN-100 can be effectively administered without the need for it to accompany a meal, while the label and package insert for sodium PBA (sodium PBA) indicate that it should be taken with meals. In addition to the lack of difference for PAA PK variables between the fasted and fed states (Days 8 vs. 1), the table below also illustrates plasma accumulation of PAA that occurs with multiple dosing (Days 15 vs. 8).

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Plasma PK Variables For PAA

PK variable	Child-Pugh A (n = 8)	Child-Pugh B (n = 8)	Child-Pugh C (n = 8)	Healthy volunteers (n = 8)
AUC_{0-12} [(μg/mL)·h]				
Day 1				
Geo. mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00)
CV%	53.41	64.91	109.58	79.59
Day 8				
Geo. mean (range)	39.64 (5.96–153.14)	73.44 (26.83–279.48)	86.36 (28.12–367.70)	34.07 (5.27–134.99)
CV%	78.73	85.58	92.85	80.59
Day 15				
Geo. mean (range)	117.89 (23.28–413.43)	138.95 (40.21–652.99)	184.26 (14.97–2245.51)	99.16 (30.06–394.79)
CV%	76.82	99.48	170.56	88.59
AUC_{0-t} [(μg/mL)·h]				
Day1				
Geo. Mean (range)	37.33 (7.29–78.42)	72.20 (23.38–174.73)	48.59 (4.75–312.43)	50.63 (14.27–150.00)
CV%	53.41	64.91	109.58	79.59
Day 15*				
Geo. Mean (range)	121.57 (23.28–528.73)	153.00 (40.21–938.85)	194.17 (14.97–3415.51)	99.94 (30.06–420.32)
CV%	92.27	118.54	198.42	93.08
C_{max} [μg/mL]				
Day 1				
Geo. mean (range)	9.65 (2.58–26.93)	13.52 (6.94–27.97)	10.95 (2.68–40.30)	11.81 (4.14–29.79)
CV%	63.78	57.70	82.65	68.72
Day 8				
Geo. mean (range)	10.21 (1.64–25.66)	14.78 (4.46–42.02)	16.03 (6.49–48.07)	10.03 (2.90–28.43)
CV%	62.25	74.53	72.29	66.97
Day 15†				
Geo. mean (range)	29.07 (7.29–53.48)	25.46 (10.54–65.40)	33.28 (5.03–208.80)	21.92 (7.76–61.31)
CV%	44.21	64.26	121.51	62.88
$t_{1/2}$ [h]‡				
Day 1				
Mean (SD)	0	0	2.10 (0.32)	0
Range			1.88–2.33	
Day 15				
Mean (SD)	1.80 (0.94)	2.76 (1.53)	7.70	1.91 (0.37)
Range	1.01–3.14	1.68–3.84	7.70–7.70	1.68–2.33
T_{max} [h]				
Day 1				
Median (range)	3.50 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (2.00–8.00)	6.00 (4.00–6.00)
Day 8				
Median (range)	4.00 (2.00–6.00)	5.00 (3.00–8.00)	5.00 (4.00–8.00)	4.00 (3.00–6.00)
Day 15				
Median (range)	4.00 (2.00–6.00)	4.00 (3.00–8.00)	5.00 (0.00–8.00)	4.00 (3.00–4.00)

*p = 0.64 for group effect; †p = 0.72 for group effect

‡On day 1, n = 2 in Child-Pugh group B and n = 0 in all other groups; on day 15, n = 4 in group A, 2 in group B, 1 in group C, and 3 in group D

AUC_{0-12}, area under the plasma concentration curve from time 0 up to 12 hours after dosing; AUC_{0-t}, area under the plasma concentration curve from time 0 to the last measurable concentration; C_{max}, maximum observed plasma concentration; CV, coefficient of variation; geo. Mean, geometric mean; n, number of subjects; SD, standard deviation; T_{max}, time to maximum observed plasma concentration; $t_{1/2}$, half-life

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Example 6

PK/PD Modeling Results

[00165] In the case of most drugs, the fraction of an orally administered dose which is removed and metabolized by the liver prior to reaching the systemic circulation (i.e. first pass effect) is not considered bioavailable, since it does not enter the systemic circulation and therefore is not able to reach its target organ or receptor. However, this is not the case for ammonia scavenging drugs described in this invention. Since hepatocytes and possibly enterocytes contain the enzymes necessary for conversion of PBA to PAA and conversion of PAA to PAGN and since glutamine is present in the splanchnic as well as the systemic circulation, it is likely that PBA can be converted to PAGN prior to reaching the systemic circulation (i.e. "pre-systemically") and that this PBA is fully effective with respect to ammonia scavenging (Figure 5); i.e. fully active. To verify this possibility, PK/PD modeling using NONMEM VI (Icon, Ellicot City, MD.) was carried out on plasma and urinary metabolite data (over 5000 data points) from the clinical studies described above involving healthy adults, subjects with cirrhosis and UCD subjects. The results of this PK/PD modeling have validated the model depicted in Figure 3. Moreover, the modeling has verified that HPN-100 exhibits slow release characteristics as compared with sodium PBA and provided an explanation for the poor correlation between blood levels of PBA/PAA and ammonia and the importance of urinary PAGN is dose adjustment. Key conclusions resulting from the PK/PD modeling were as follows

1. PBA is more slowly absorbed (~40% as fast) from the intestine after administration of HPN-100 versus sodium PBA (absorption rate constants and absorption half-lives for HPN-100 and sodium PBA are 0.544 h^{-1} vs. 1.34 h^{-1} and 1.27 h vs. 0.52 h, respectively).

2. The lower plasma levels of PBA following administration of HPN-100, as compared with sodium PBA, reflect results indicating a fractionally greater amount of PBA (31% vs. 1%) being converted pre-systemically (to PAA and PAGN) following administration of HPN-100 than Na PBA.

3. In a dataset containing healthy, cirrhotic, and UCD individuals, diagnosis was introduced as a covariate on the estimated bioavailability of HPN-100 revealing a 32% lower estimated bioavailability of PBA in healthy adults compared to adult UCD patients. Cirrhotic and UCD patients had similar PBA bioavailability following HPN-100 treatment.

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Example 7

ADME Study In Three Cynomolgous Monkeys

[00166] To assess the preclinical handling of ammonia scavenging drugs, 600 mg/kg of either radio labeled sodium PBA or radio labeled HPN-100 was administered as a single dose to 3 cynomolgous monkeys. These monkeys were chosen because, like humans (and unlike most other species), they metabolize PAA to PAGN and thus provide a useful model for testing prodrugs of PAA. This study corroborated clinical findings summarized in Examples 1-3, including the following: (a) dosing with oral sodium PBA or oral HPN-100 did not result in 100% conversion to urinary PAGN, (b) plasma PBA and PAA blood levels did not correlate consistently with ammonia scavenging activity as reflected by urinary PAGN output, and (c) HPN-100 exhibited slow release characteristics as compared with sodium PBA.

[00167] Radio labeled PBA and PAA entered the systemic circulation rather slowly following administration of radio labeled HPN-100 [Cmax for PBA was achieved 1.5 hours post-dosing (52.2 μg/mL) and Cmax for PAA was achieved 8 hours post dosing (114 μg/mL)], corroborating the findings observed in humans (including the PK/PD modeling), and essentially no HPN-100 appeared in systemic circulation or in excretions. About 90% of radioactive material derived from HPN-100 that was excreted in urine was PAGN, accounting for 39% of the administered HPN-100. By contrast, when oral sodium PBA was administered, PAGN accounted for only 23% of the radio labeled material, and unchanged PBA accounted for 48% of the administered dosage of oral sodium PBA. Thus oral sodium PBA was utilized less efficiently than HPN-100, and an unexpectedly high amount of PBA was excreted unchanged.

Example 8

Biological and Anatomical Considerations

[00168] Unlike most drugs which act on a target organ/cell/receptor (etc.) perfused by systemic blood, ammonia scavenging drugs of the types covered by this invention do not act on a target organ, rather they act through the combination of PAA with glutamine to form PAGN (Figure 5). Since glutamine is present in the splanchnic as well as the systemic circulation and since the liver is a metabolically active organ capable of catalyzing all steps involved in the conversion of HPN-100 or PBA to PAA and then to PAGN, the data accumulated to date, including the PK/PD modeling, as well as anatomical consideration lead us to the conclusion that the formation of

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PAGN from PBA/PAA occurs to a significant degree before PBA/PAA reach the systemic circulation (e.g. within the liver). This is especially true when HPN-100 is administered as a PBA prodrug. This explains the poor correlation between plasma levels and ammonia trapping effects and leads to the conclusion that the dosing and dose adjustment of these PBA prodrugs should be based on urinary excretion of PAGN and total urinary nitrogen. Figure 5 illustrates how this occurs.

[00169] For certain clinical trials, particularly for comparing HPN-100 to PBA, HPN-100 will be administered at a dose that is equivalent (equimolar) to an amount of sodium PBA that would be considered suitable for the particular patient; and the dosage can then be adjusted by the methods described herein. For example, the HPN-100 dose range will match the PBA molar equivalent of the approved sodium PBA (sodium phenylbutyrate) (NaPBA) dose range. HPN-100 will be administered three times a day (TID) with meals. Note that the conversion of the dose of NaPBA to the dose of HPN-100 involves correction for their different chemical forms (i.e. HPN-100 consists of glycerol in ester linkage with 3 molecules of PBA and contains no sodium) (NaPBA [g] x 0.95 = HPN-100 [g]) as well as correction for the specific gravity of HPN-100, which is 1.1 g/mL.

HPN-100 Dose Ranges Corresponding to Recommended Daily Doses of Sodium PBA

Sodium PBA	HPN-100 PBA Equivalent Dose (mg)	HPN-100 PBA Equivalent Dose (mL)
450-600 mg/kg/day (patients ≤ 20 kg)	428 – 570 mg/kg/day	0.39-0.52 mL/kg/day
9.9-13.0 g/m2/day (patients > 20 kg)	9.4 – 12.4 g/m2/day	8.6-11.2 mL/m2/day
Maximum Daily Dose: 20 g	Maximum Daily Dose: 19 g	17.4 mL

[1] 20 g of sodium PBA contains ~17.6 g of phenylbutyric acid; 19 g of HPN-100 contains ~17.6 g of phenylbutyric acid

Example 9

Determination of a Starting Dosage and Dose Adjustment of HPN-100

[00170] A patient having a nitrogen retention state (e.g. an inherited urea cycle disorder, cirrhosis complicated by hepatic encephalopathy or chronic renal failure) who is currently not being treated with an ammonia scavenging agent as described in this invention is determined clinically to be in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of HE in patients with cirrhosis, elevated blood

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ammonia levels in UCD patients, levels of urea and symptoms of uremia in patients with chronic renal failure).

[00171]. The starting dosage is based on clinical considerations, including the estimation of residual urea synthetic capacity (an infant with UCD presenting with hyperammonemia in the first few days of life would be presumed to have no significant urea synthesis capacity) and appropriate dietary protein intake (i.e., infants with UCD require increased dietary protein to support body growth, but long-term dietary protein restriction in patients with cirrhosis is usually ineffective or counterproductive, and the methodology outlined in this invention.

[00172] For example, an adult with limited residual urea synthetic capacity is treated with an initial dosage of HPN-100 of 19 g per day and placed on a protein-limited diet containing about 25 g of protein per day. The patient's daily urinary output of PAGN is monitored. The daily intake of HPN-100 amounts to 19 g of HPN-100, at a molecular weight of ~530, which is 0.0358 mol HPN-100. Each mole of HPN-100 can theoretically be converted into three moles of PAA and thus three moles of PAGN, so the 19 g daily dosage of HPN-100 could produce 0.108 mol of PAGN in vivo. If entirely converted into PAGN and all of the PAGN is excreted in the urine, the theoretical quantity of PAGN would be 28.4 g per day, which would be sufficient to mediate the waste nitrogen excretion resulting from ~41 grams of dietary protein, assuming that 16% of dietary protein is nitrogen and ~47% of dietary nitrogen is excreted as waste nitrogen (see Brusilow).

[00173] However, as demonstrated herein, HPN-100 is typically converted into urinary PAGN with an efficiency of about 40% to 70% (typically about 54% conversion was found in UCD patients), thus the physician would expect to observe about 17 g of urinary PAGN output per day from this dosage of HPN-100. This corresponds to ~25 grams of dietary protein – which is similar to the prescribed amount, but less than the theoretical amount (41 grams) this dosage of HPN-100 might have been expected to account for theoretically. Thus the adjustment for 40-70% efficiency significantly affects the overall treatment program, and knowing what efficiency to expect enables the treating physician to avoid putting the patient on a diet containing too much protein for the patient to manage on this dosage of HPN-100.

[00174] When monitoring the patient, if the doctor observes a higher output of urinary PAGN than expected, the dosage of HPN-100 is reduced proportionally; thus if 21 g of urinary PAGN per day is observed, the physician will reduce the dosage of HPN-100 to (17/21)*19g = 15 g. Similarly, if urinary PAGN output is below that expected amount, such as 12 g per day, the amount of HPN-100 would be increased: if 12 g is observed and 17 is expected, the physician could adjust

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the HPN-100 dosage to (17/12) * 19g = 27 g HPN-100 per day, if that dosage is within a range considered safe to administer to the patient. Either the dosage of HPN-100 or dietary protein intake could be adjusted to optimize the treatment plan for this subject. Alternatively, if the urinary PAGN output exceeds about 10 g per day, based on the correlation described above, the treating physician will recognize that this correlates with high likelihood of achieving normal ammonia levels, and may monitor ammonia and/or urinary PAGN for a time to assess whether the dosage being administered is generally sufficient for this subject.

[00175] Optionally, the urinary PAGN output may be determined as a ratio of urinary PAGN concentration to urinary creatinine concentration; creatinine levels are typically stable enough for a given individual to provide a normalization factor for urine volume so that rather than determining total daily urinary PAGN, the physician can estimate total daily urinary PAGN from testing a single urine sample.

[00176] The physician may also monitor the plasma ammonia levels and dietary protein intake in the patient to ascertain whether the patient's dietary protein intake and drug treatment combined are producing the appropriate therapeutic effect. Dietary protein intake or drug dosage or both could be adjusted to attain a normal or desired plasma ammonia level, e.g., a level below about 40 umol/L. However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA to adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00177] If the 19g dose of HPN-100 is determined to be inadequate (e.g. patient requires an increase in dietary protein which would result in excretion of waste nitrogen exceeding his or her urea synthesis capacity and PAGN excretion), HPN-100 dose would be increased sufficiently to cover the necessary dietary protein and the same methodology of dose adjustment based on urinary PAGN excretion would be applied to determine that dosage of HPN-100.

[00178] In a subject having little or no urea synthesis capacity where essentially all urinary nitrogen would be accounted for by PAGN, the ammonia scavenging effect may be monitored by determination of total urinary nitrogen (TUN), rather than directly measuring PAGN levels in the urine.

[00179] Optionally, the TUN can be used as a measure of urea synthesis capacity, by subtracting the amount of nitrogen present as PAGN.

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Example 10

Determination of a Dosage of HPN-100 for a Patient already on sodium PBA

[00180] A patient with a UCD already on sodium PBA who is to be transitioned to HPN-100 would undergo assessment of dietary protein and measurement of urinary PAGN excretion.

[00181] If the patient is judged to be adequately controlled on sodium PBA , then the starting dose of HPN-100 would be the amount necessary to deliver the same amount of PAA (e.g. 19 grams of HPN-100 would correspond to 20 grams of sodium PBA). Subsequent dose adjustment would be based on repeated measurement of urinary PAGN as well as assessment of dietary protein and ammonia. , However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA either to determine the initial dosage of HPN-100 or adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00182] If the patient is determined to be inadequately controlled on sodium PBA , then the starting dose of HPN-100 would be selected to deliver an amount of PAA higher than the dose of sodium PBA provided such HPN-100 dosage is otherwise appropriate. Subsequent dose adjustment would be based on repeated measurement of urinary PAGN as well as assessment of dietary protein and plasma ammonia. However, as demonstrated by the observations described herein, the physician would not use plasma levels of PAA or PBA either to determine the initial dosage of HPN-100 or adjust the dosage of HPN-100 or otherwise guide treatment, as those levels do not correlate well with the ammonia scavenging effect of the administered HPN-100.

[00183] Optionally, for example in a 'fragile' UCD patient with a history of repeated episodes of hyperammonemia, the conversion from sodium PBA to HPN-100 might occur in more than one step, whereby, at each step, the dose of sodium PBA would be reduced in an amount corresponding to the amount of PAA delivered by the incremental dose of HPN-100.

[00184] If the dose of HPN-100 is determined to be inadequate (e.g. patient requires an increase in dietary protein which would result in production of waste nitrogen exceeding his or her urea synthesis capacity and PAGN excretion), HPN-100 dose would be increased sufficiently to cover the necessary dietary protein and the same methodology of dose adjustment based on urinary PAGN excretion would be applied.

[00185] The examples set forth herein are illustrative only, and should not be viewed as limiting the invention.

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Claims

1. A method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100,

wherein monitoring the effect consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output;

and determining from the urinary PAGN output whether and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect.

2. The method of claim 1, wherein urinary PAGN output is determined as total urinary PAGN output for a 24 hour period following a first dosage of HPN-100 on a day, or total urinary PAGN output for a 12-24 hr period following the first dosage of HPN-100 on a day, or a as a ratio of the concentration of urinary PAGN to urinary creatinine in a sample of the patient's urine, which is used to estimate total urinary PAGN output for 24 hrs or for 12-24 hrs.

3. The method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy, a urea cycle disorder or chronic renal failure.

4. The method of any one of claims 1-3, wherein determining how to adjust the initial dosage comprises calculating an adjusted dosage based on conversion of about 54% of orally administered HPN-100 into urinary PAGN.

5. The method of claim 1, wherein determining from the urinary PAGN output whether to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect comprises comparing the patient's urinary PAGN output to a cut-off level of urinary PAGN output that correlates with achieving effective ammonia control for comparable patients.

6. A method to determine a dosage of HPN-100 for a patient having a nitrogen retention disorder, which comprises calculating the dosage of HPN-100 based on a

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conversion efficiency for HPN-100 conversion into PAGN of about 40% to about 70%.

7. The method of claim 6, wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake.

8. The method of claim 7, wherein the dosage of HPN-100 is adjusted to account for the patient's estimated residual urea synthesis capacity, if any.

9. A method to determine a dosage of a PAA prodrug for a patient having a nitrogen retention disorder, comprising:

a) estimating the patient's residual urea synthesis capacity, if any;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

d) determining an amount of the PAA prodrug needed to produce the target amount of urinary PAGN,

wherein the dosage of PAA prodrug is determined based on a conversion efficiency whereby about 40% to about 70% of the PAA prodrug is converted into urinary PAGN.

10. The method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof.

11. The method of claim 9, wherein the PAA prodrug is HPN-100.

12. A method to treat a patient having a nitrogen retention disorder with a suitable dosage of a PAA prodrug, comprising:

a) determining the patient's residual urea synthesis capacity;

b) determining the patient's dietary protein intake;

c) estimating from a) and b) the patient's target urinary PAGN output;

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d) determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 40% to about 70% conversion of the PAA prodrug into urinary PAGN; and

e) administering to the patient the suitable dosage of the PAA prodrug.

13. The method of claim 12, wherein the PAA prodrug is phenylbutyrate or a pharmaceutically acceptable salt thereof, or HPN-100.

14. The method of claim 12, wherein the PAA prodrug is HPN-100, the patient is a patient with clinically significant residual urea synthetic capacity, and the HPN-100 is administered in two or three doses per day.

15. A method to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, comprising:

determining a replacement amount of HPN-100 to replace at least a portion of the phenylacetate or phenylbutyrate;

substituting the replacement amount of the HPN-100 for the phenylacetate or phenylbutyrate; and

monitoring the amount of urinary PAGN excreted by the patient to assess the effectiveness of the replacement amount of the HPN-100.

16. The method of claim 15, wherein an increase in the amount of urinary PAGN caused by the transition indicates that the amount of phenylacetate, phenylbutyrate, and/or HPN-100 should be reduced, and optionally adjusting the patient's dosage of PAA, PBA, or HPN-100.

17. A method to transition a patient taking an initial daily dosage of phenylbutyrate from phenylbutyrate to HPN-100, comprising

a) determining a suitable amount of HPN-100 to replace at least a portion of the initial daily dosage of phenylbutyrate;

b) administering the suitable amount of HPN-100 to the subject along with an amount of phenylbutyrate corresponding to the initial daily dosage of

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phenylbutyrate minus an amount corresponding to the portion replaced by HPN-100;

c) determining the level of excreted urinary PAGN for the subject; and

d) repeating steps a-c until all of the phenylbutyrate is replaced by HPN-100.

18. A method to initiate treatment with phenylacetate, phenylbutyrate or a HPN-100 in a step-wise fashion, comprising:

a) estimating or measuring dietary nitrogen intake for the patient; and/or

b) estimating the patient's need for urinary waste nitrogen excretion based upon diet and urea synthetic capacity; then

c) administering a starting dose of the drug estimated to provide a fraction of the necessary waste nitrogen clearance as urinary PAGN taking into account an estimated 40% to 70% conversion of the administered drug into PAGN; and

d) increasing the dose of drug as needed, and repeating the steps above, to reach a maintenance dose of the drug that provides a normal blood level of ammonia.

19. A method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA without increasing the patient's exposure to PBA as judged by the AUC and Cmax for PBA when the patient receives the PBA prodrug, when compared to the AUC and Cmax observed when the patient receives an equimolar amount of PBA.

20. The method of claim 19, wherein the PBA prodrug is HPN-100.

21. The method of claim 20, wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20%.

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22. The method of claim 20, wherein the effectiveness of the HPN-100 treatment is assessed based at least partly based on urinary output of PAGN.

23. A method to determine a suitable dietary protein level for a patient having a nitrogen retention disorder, comprising:

a) estimating the patient's endogenous capacity for waste nitrogen excretion;

b) calculating from the patient's endogenous capacity for waste nitrogen excretion an amount of dietary protein the patient can process without the aid of a nitrogen scavenging drug; and

c) adding an amount of protein that the patient should be able to process with the assistance of a selected dosage of a nitrogen scavenging drug

to arrive at an amount of dietary protein the patient can ingest while being treated with the selected dosage of the nitrogen scavenging drug, taking into account the of protein required for health and body growth.

24. The method of claim 23, wherein the nitrogen scavenging drug is HPN-100.

25. The method of claim 24, wherein the selected dosage of HPN-100 is up to about 19 grams per day, and wherein the amount of dietary protein the patient should be able to process with the assistance of this amount of HPN-100 is about 1 g of protein per gram of HPN-100 per day.

26. A method to treat a patient with a PBA prodrug, comprising administering HPN-100 at a daily dose in excess of 19 g per day to a subject having HE or UCD.

27. The method of claim 26, wherein the daily dose of HPN-100 is between about 19g and about 57 g.

28. A method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100, wherein the AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered.

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29. The method of claim 28, wherein the subject's plasma ammonia levels are on average normal when treated with HPN-100.

30. A method to determine whether a subject having a nitrogen retention disorder has achieved adequate ammonia control or needs further testing or a modified treatment program, comprising measuring the subject's urinary PAGN output level, and comparing this output level to a cut-off value for urinary PAGN output determined for comparable subjects, and classifying the subject as one in need of further testing or a modified treatment program if the subject's urinary PAGN falls below the cut-off value.

31. The method of claim 30, wherein the subject is an adult UCD patient, and the cut-off value is about 10 g of urinary PAGN per day.

32. The method of claim 30, wherein the subject has chronic renal failure.

33. The method of claim 30, wherein the subject has a UCD or HE.

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Figure 1a
Nitrogen Retention States

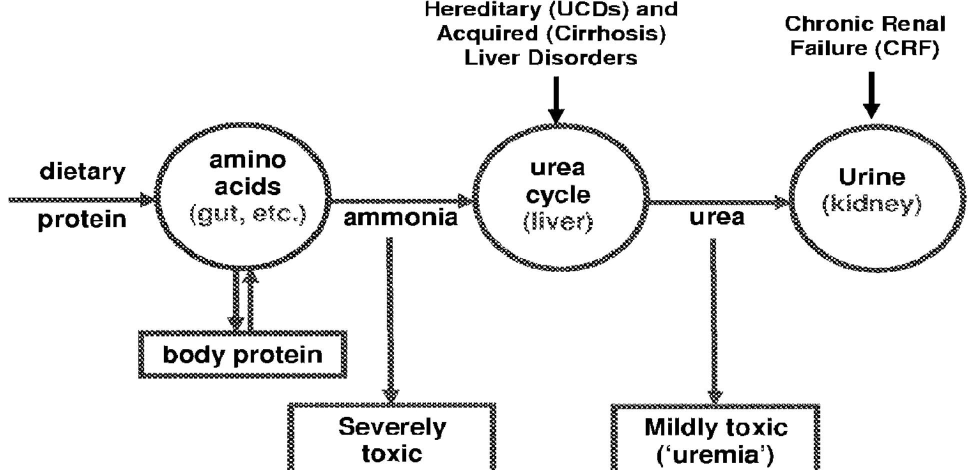

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Figure 1b

The Urea Cycle

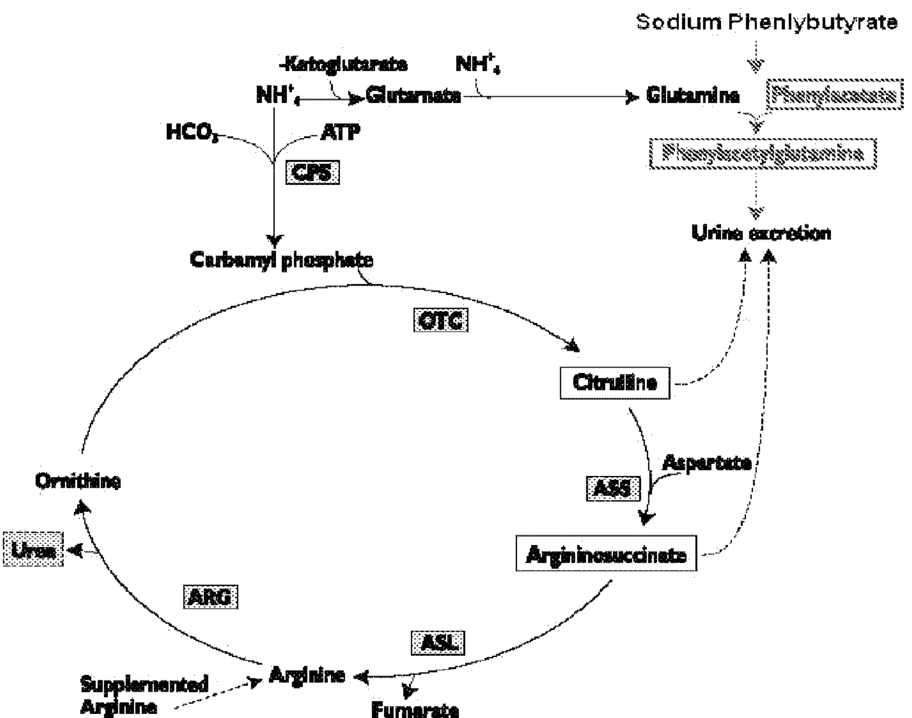

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Figure 2

A conventional clinical pharmacology model in which only drug reaching the central (systemic) circulation is assumed to be active.

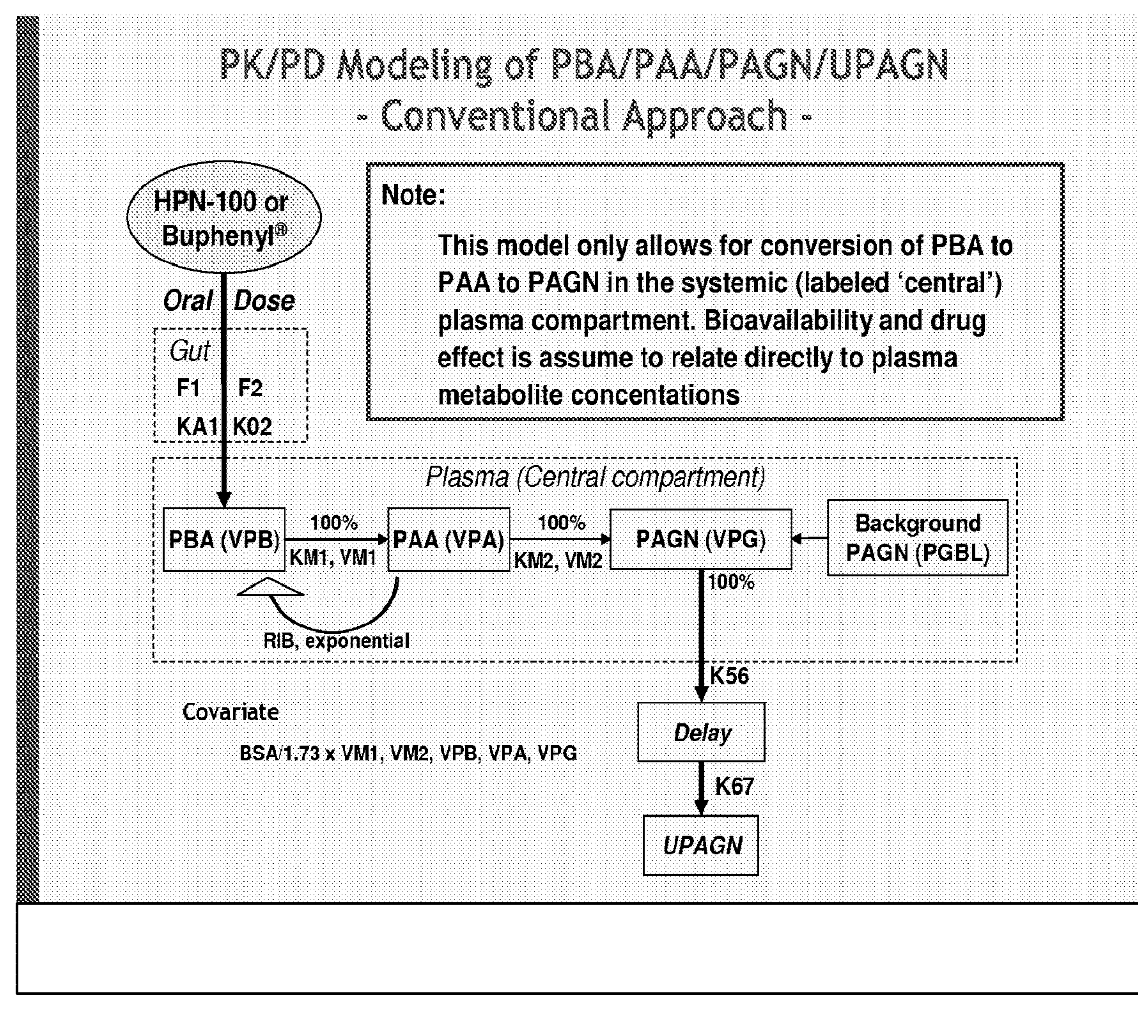

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Figure 3

A modified clinical pharmacology model as described in this application in which an ammonia scavenging agent converted into PAGN prior to reaching the systemic circulation is fully active with respect to excretion of waste nitrogen. As a corollary, concentrations of metabolites in the systemic circulation do not correlate consistently with drug effect.

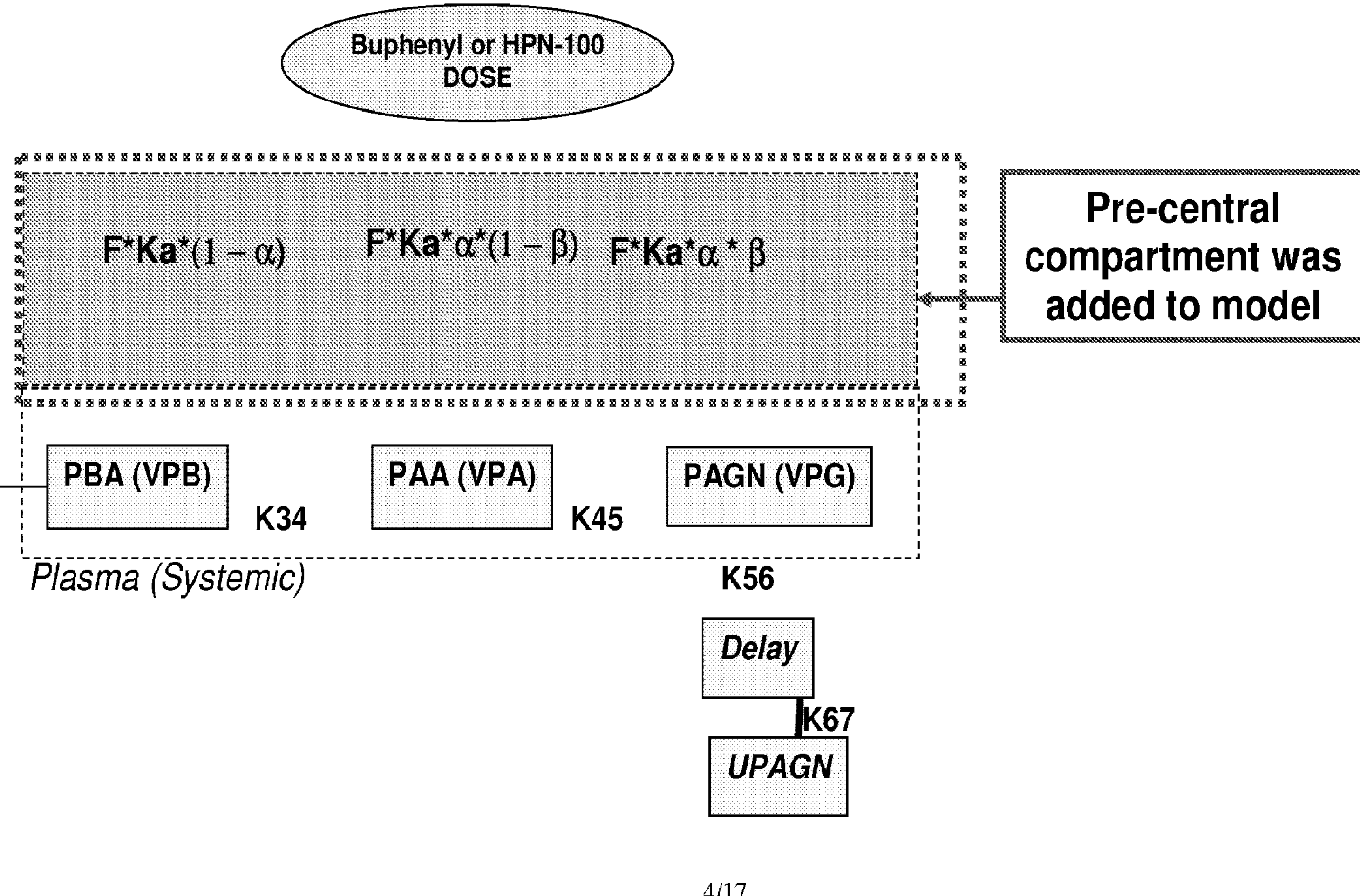

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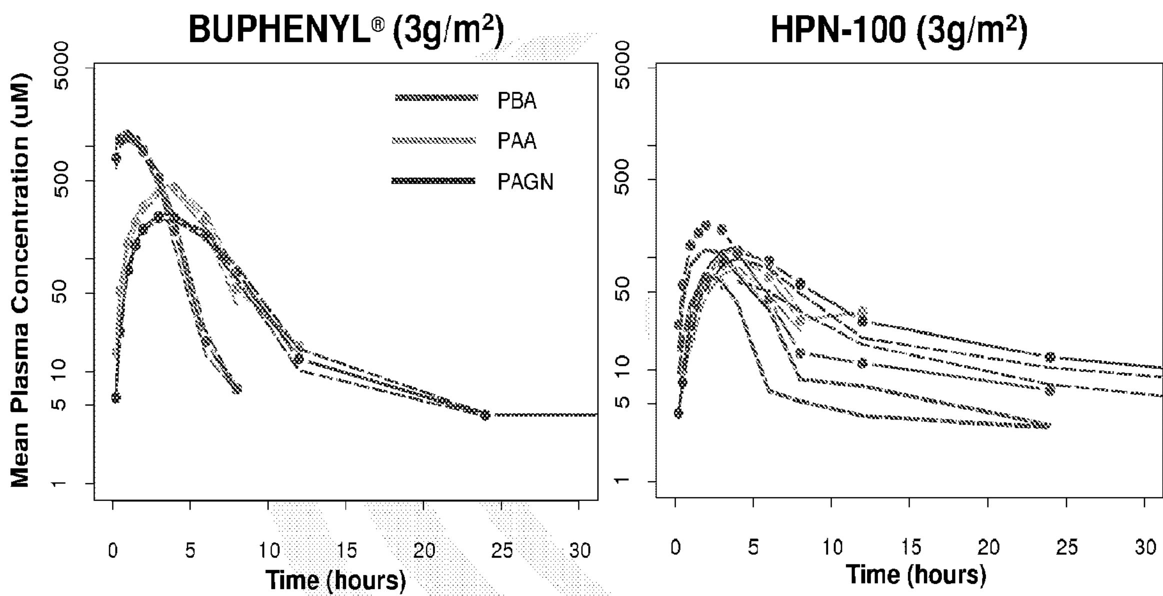
Figure 4



In each panel, the curves represent measured levels of PBA, PAA or PAGN in subjects receiving BUPHENYL® (sodium phenylbutyrate) (sodium PBA) at $3g/m^2$ dosage, or HPN-100 in an amount calculated to provide an equimolar amount of PBA to that provided by the sodium PBA dosage. Three curves for each material are for three subjects who received the specified dosages of sodium PBA or HPN-100. In the left panel, the upper curve represents PBA levels; the intermediate one represents PAA levels; and the lowest of the three sets of lines represents PAGN levels. In the right panel, the three lowest curves at the 10-15 hour time span are all for PBA; and the highest three curves at 15-25 hours represent PAGN levels. PAA levels were not determined after approximately12 hours, and fall generally close to the PAGN curves up to that time.

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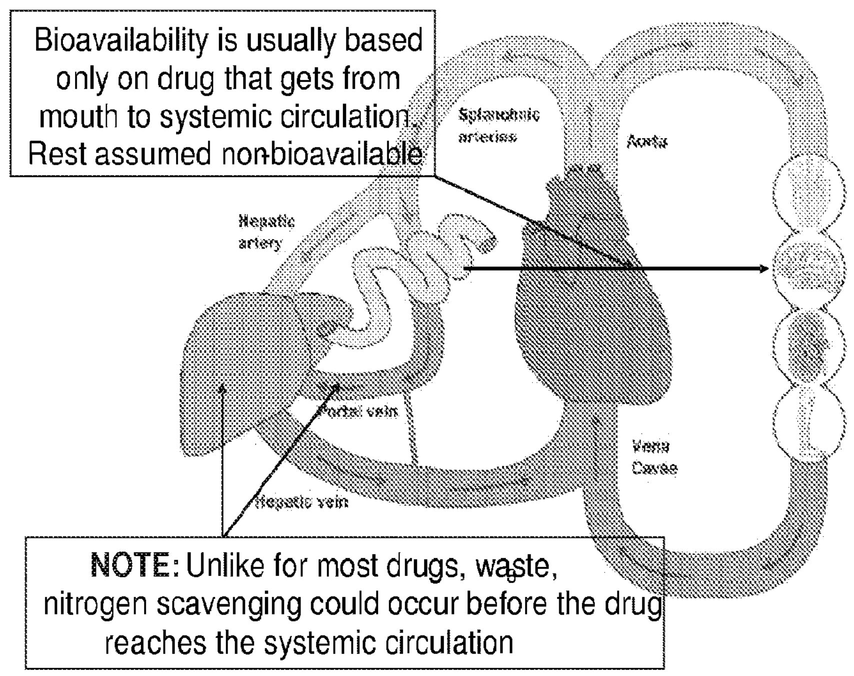
Figure 5



Schematic anatomic depiction of the systemic and presystemic (represented by the portal vein) compartments. Unlike the case for most drugs which need to pass through the liver to the systemic circulation to exert an effect, PAA converted to PAGN prior to reaching the systemic circulation (e.g. in the liver) is still effective in clearing waste nitrogen from the body.

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Figure 6

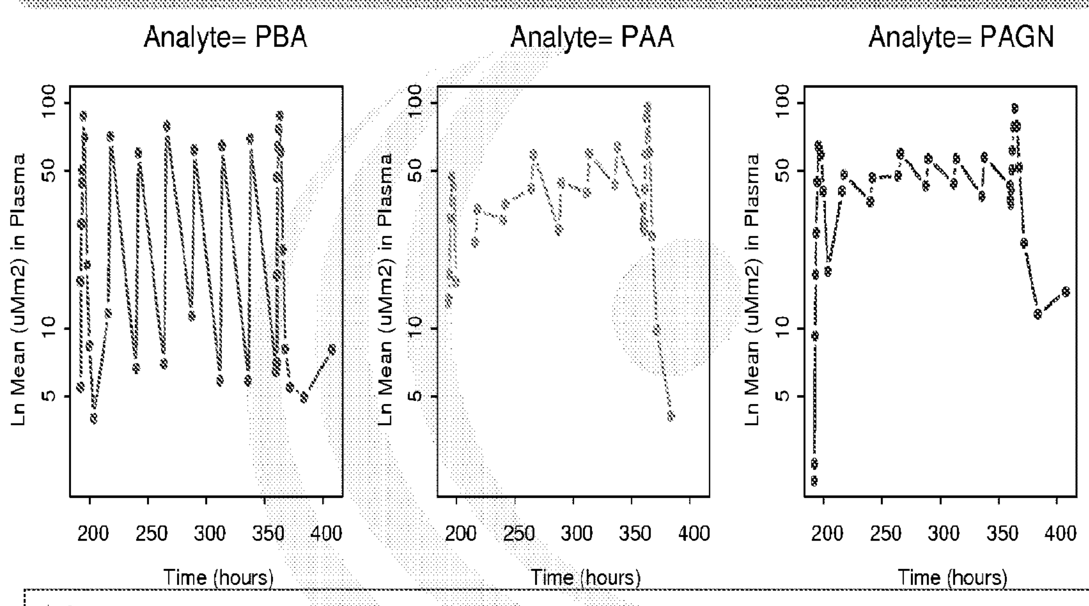

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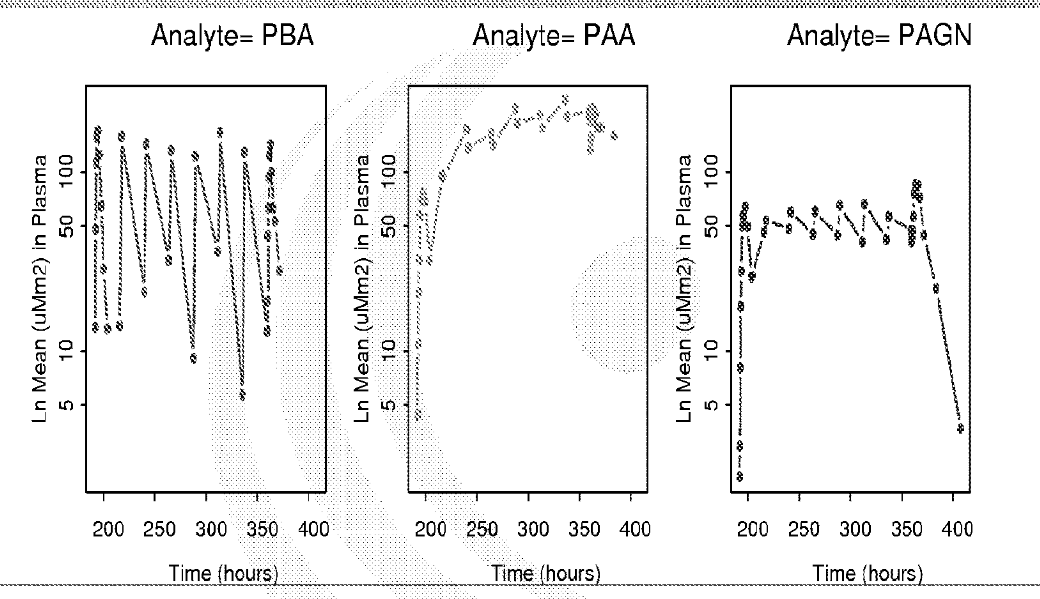
Figure 7

UP1204-002: Blood Metabolite Concentrations Vs. Time In Patients With Cirrhosis (Childs-Pugh C)



* Shows BID dosing from days 8-15. Plasma PBA levels returned to near predose level between doses on each day during multiple dosing in cirrhotics. PAA levels increase and require 4 days to reach steady-state with BID dosing

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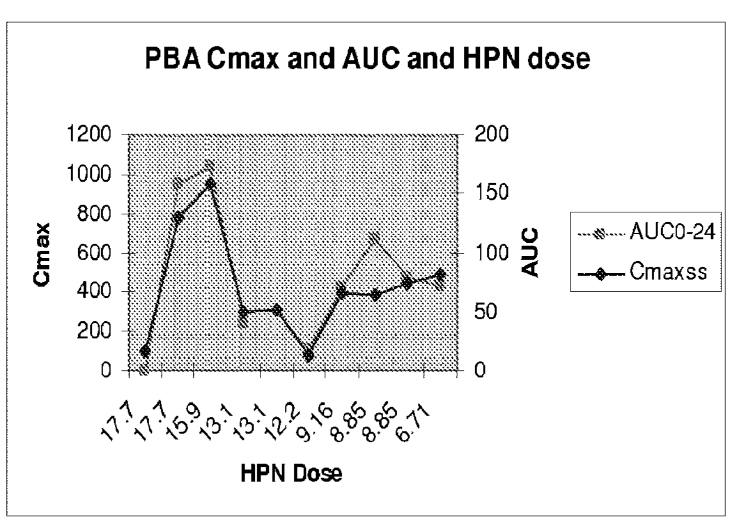
Figure 8a

Figure 8 depicts the lack of correlation between drug dose and plasma PBA (8a) and plasma PAA (8b), as compared with a significant correlation with urinary output of PAGN (8c).



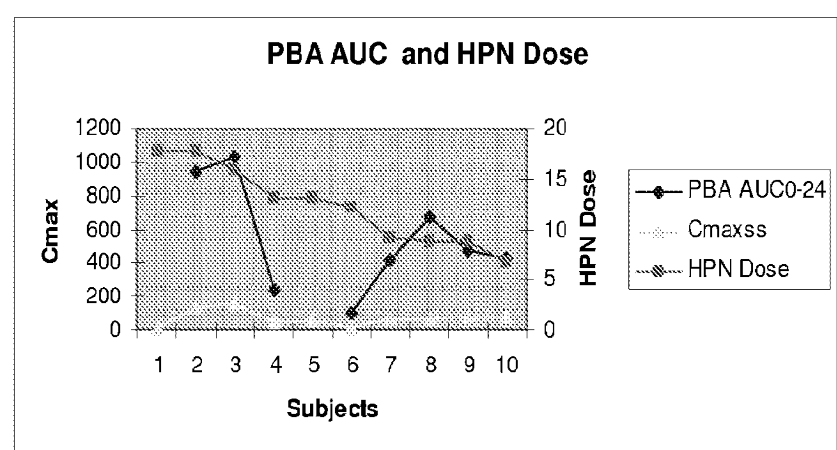


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Figure 8b

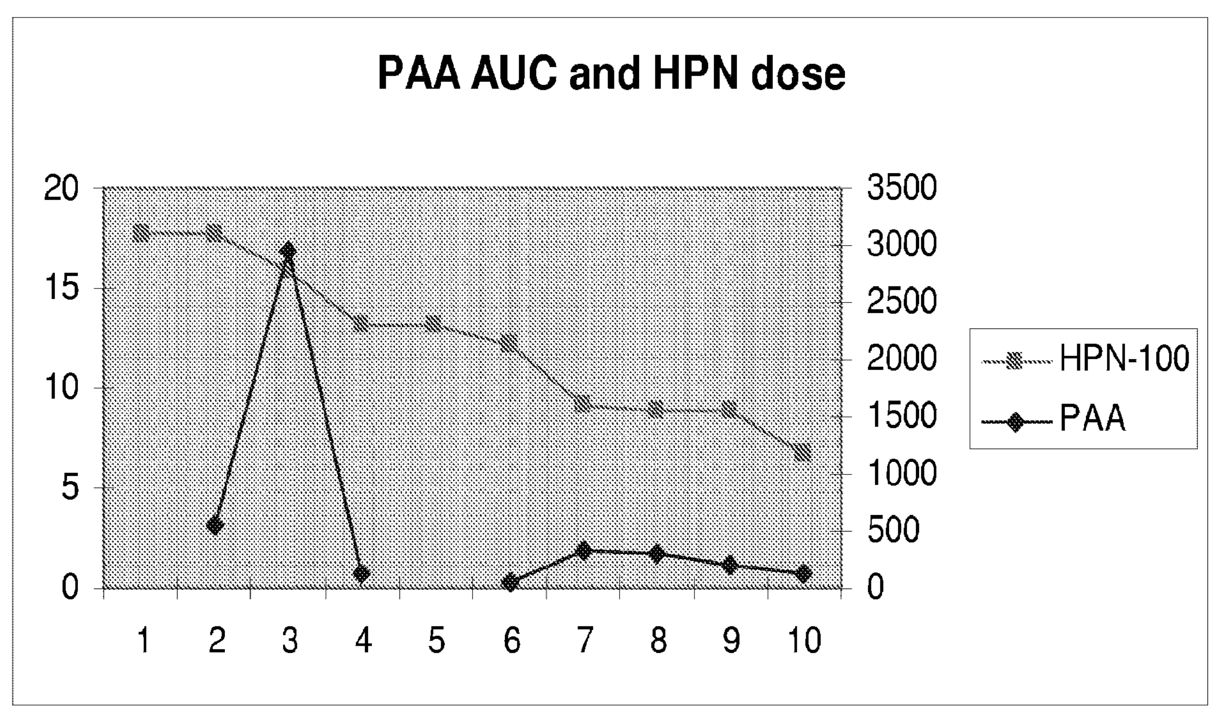

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Figure 8c

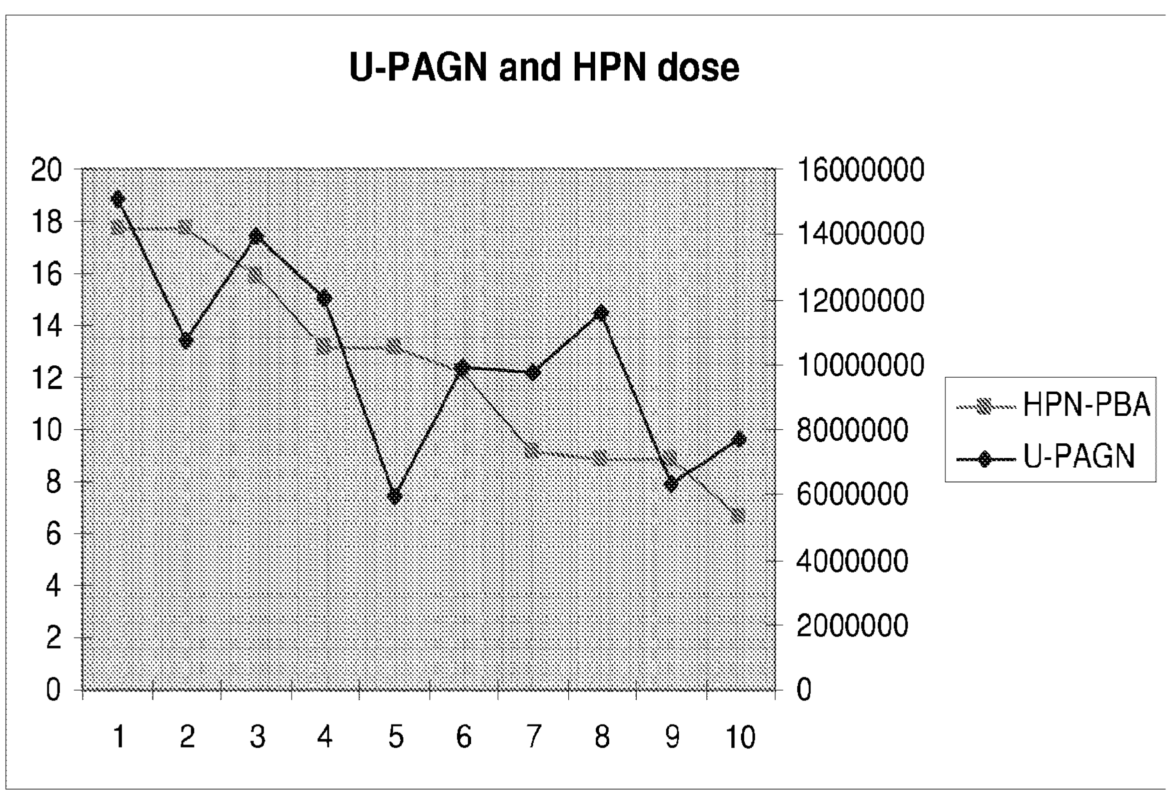

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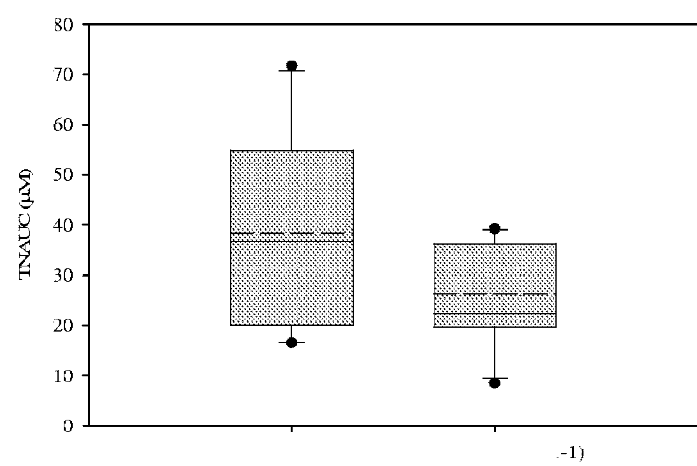
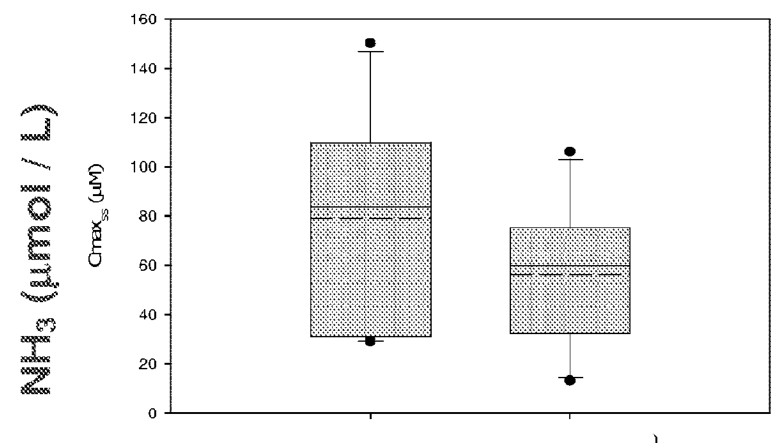
Figure 9





	BUPHENYL®	HPN-100
AUC	38.4 +/- 19.6	26.1 +/- 10.3
Cmax	79.1 +/- 40.1	56.3 +/- 27.9

——— Mean

- - - - - - Median

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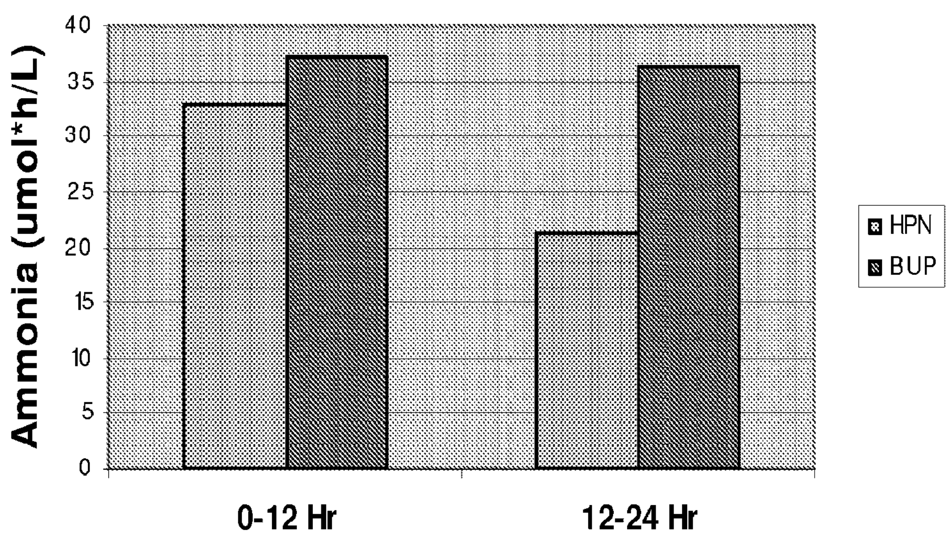
Figure 10



Plasma ammonia levels (time-normalized area under the curve [TN-AUC or AUC]) during the day and night in 10 UCD patients treated for seven days with either sodium PBA (BUP) or a PBA equimolar dose of HPN-10.

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Figure 11

Plasma ammonia levels (time-normalized area under the curve [TN-AUC) in 10 UCD patients treated for seven days with sodium PBA (BUP) followed by seven days with a PBA equimolar dose of HPN-100.

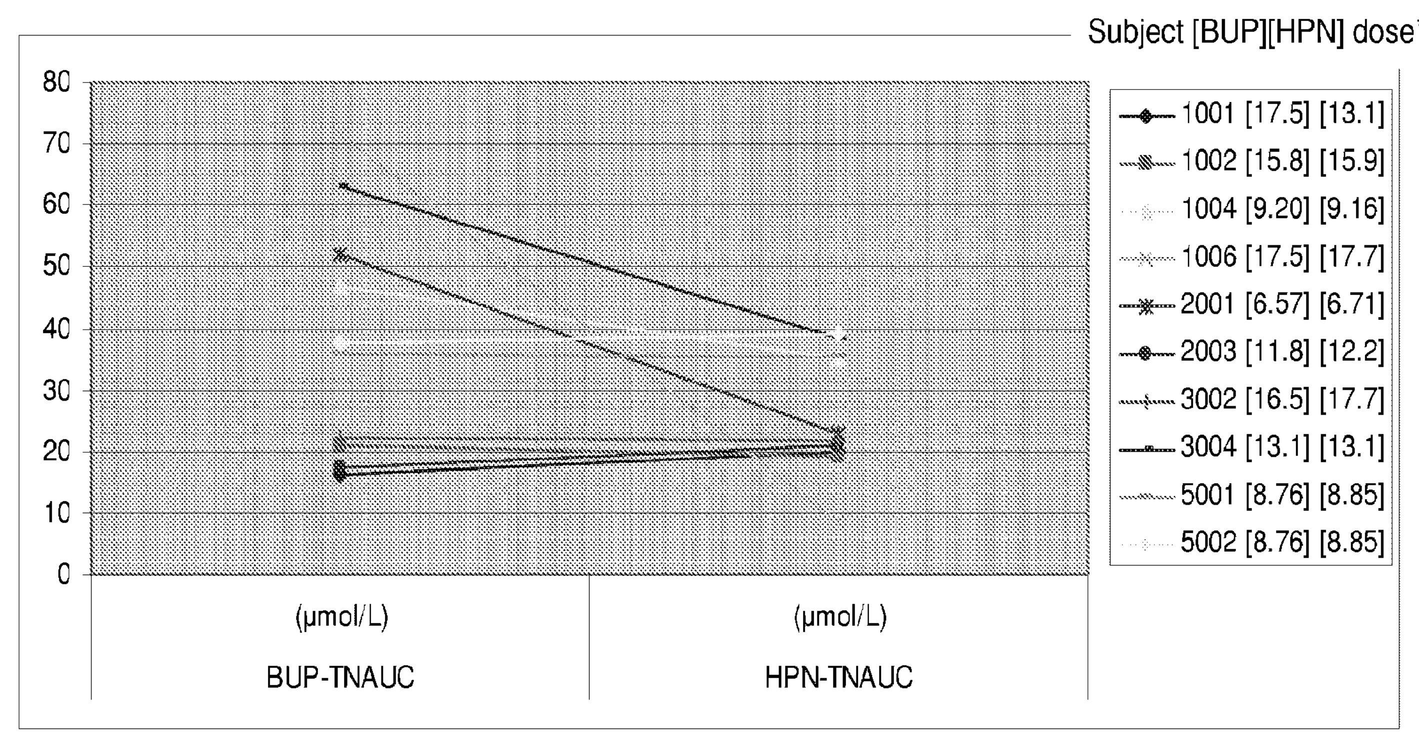

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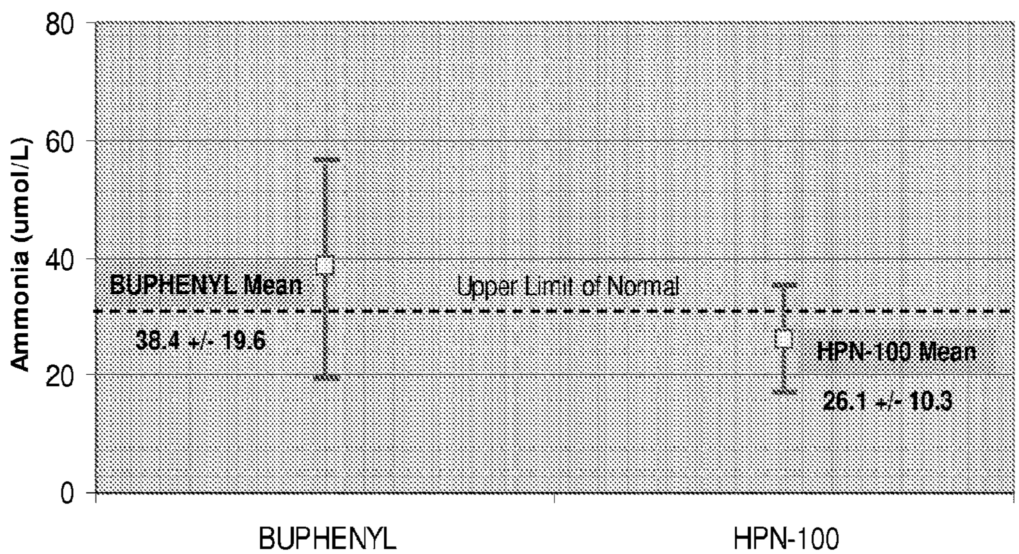
Figure 12



Mean plasma ammonia levels (time-normalized area under the curve [TN-AUC]) in 10 UCD patients treated for seven days with sodium PBA followed by seven days with a PBA equimolar dose of HPN-100.

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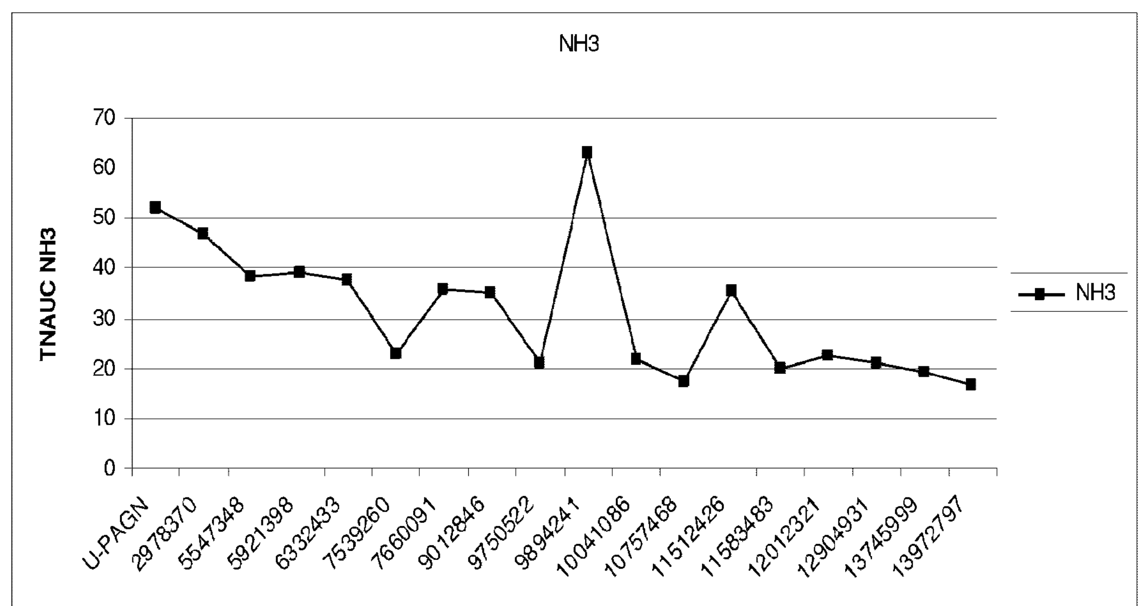
Figure 13



The X-axis is the average ammonia level assessed as time-normalized area under the curve (Y-axis) for subjects receiving HPN-100; the X-axis is urinary PAGN output. Blood ammonia correlated inversely ($r = -0.80$; $p < 0.001$) with uPAGN output. One subject was excluded from this post hoc analysis since TNAUC was calculable for only 6 hours during treatment with HPN-100.

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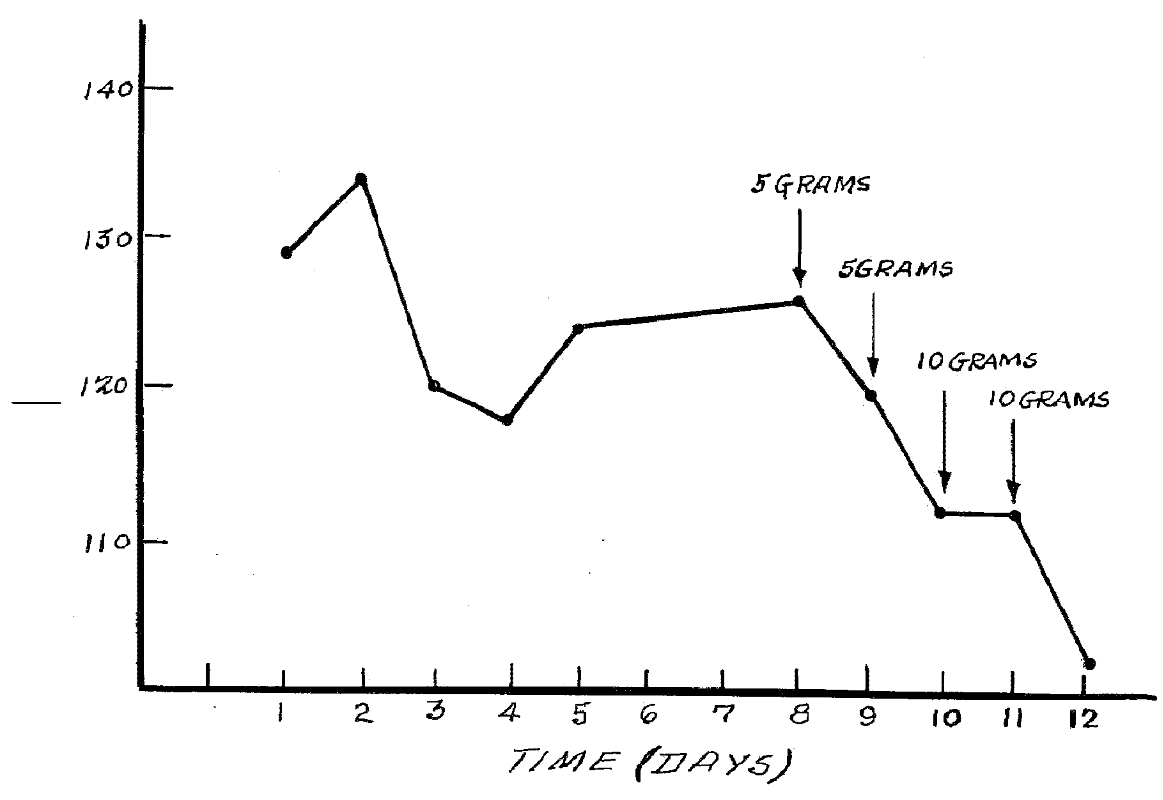
Figure 14

EFFECT OF SODIUM BENZOATE ADMINISTRATION ON SERUM UREA NITROGEN IN TREATING UREMIA

From US Patent # 4,284,647 (Brusilow; August 1981)



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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/055256

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01), pages 1117-1121, XP009127277 ISSN: 0031-3998 the whole document	30-33 1-29

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2009	30/12/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Moreno de Vega, C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/055256

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MACARTHUR ROBERT B ET AL: "Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers" MOLECULAR GENETICS AND METABOLISM, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 81, no. Suppl.1, 1 April 2004 (2004-04-01), pages S67-S73, XP009127291 ISSN: 1096-7192 the whole document	1-33
Y	TANNER L M ET AL: "Nutrient intake in lysinuric protein intolerance" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER ACADEMIC PUBLISHERS, DO, vol. 30, no. 5, 21 June 2007 (2007-06-21), pages 716-721, XP019548954 ISSN: 1573-2665 page 716 - page 717	1-33
X	LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01), page 91, XP009127344 ISSN: 0141-8955	1-5, 15-17, 19-22, 30-33
Y	the whole document	1-33

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(54) **Title**: POROUS POLYSACCHARIDE SCAFFOLD COMPRISING NANO-HYDROXYAPATITE AND USE FOR BONE FORMATION

(57) **Abstract**: The present invention relate to three dimensional porous polysaccharide matrices able to induce mineralisation of a tissue in osseous site, as well as in non- osseous site, in the absence of stem cells or growth factors.

Porous polysaccharide scaffold comprising nano-hydroxyapatite and use for bone formation

5 **FIELD OF THE INVENTION**

The present invention relates to a method for preparing a porous polysaccharide scaffold comprising hydroxyapatite, preferably nano-hydroxyapatite, that supports mineralization of tissues. The present invention further provides a porous polysaccharide scaffold obtainable by said method, and its use for bone 10 formation.

BACKGROUND OF THE INVENTION

The topic of bone-related disorders has gained considerable attention over the past years. The use of autologous and allograft bones has been popularly 15 implemented in clinics for overcoming bone related disorders, such as bone defect. However, the use of autologous bone is known to result in secondary trauma and allograft bone induces immune repulsion. In addition, autologous and allograft bones present serious limitations since their uses are dependent on the size and the localisation of the defect. For example, it was reported that grafts in large defects 20 were resorbed by the body before the completion of osteogenesis, which leaves a doubt about the success of this therapy (Hoexter DL. *Bone regeneration graft materials* J Oral Implantol. 2002;28(6); Delloye C, Cornu O, Druez V, Barbier O. *Bone allografts: What they can offer and what they cannot.* J Bone Joint Surg Br. 2007 May;89(5):574-9).

25 To remedy to those drawbacks, many works have focus their interest into replacing natural bone by synthetically prepared implants, capable of inducing mineralisation and of supporting new bone formation. Three dimensional scaffolds have thus been explored to repair tissues that do not self develop spontaneously. Thus, scaffold-based tissues engineering has become a promising strategy in 30 regenerative medicine, because cells alone lack the ability to form three dimensional tissues without the support of an artificial structure.

Prior art discloses porous scaffolds suitable for tissue engineering since their porous structure promotes cell colonization and tissue formation within the scaffold.

However, using said scaffolds for the treatment of bone related disorders still present various drawbacks related to the disease to be treated, as it depends on the type, size, and localisation of the damaged bone, as well as on the nature, age and sex of the subject to be treated.

Currently, many works are based on the use of bioactive and biocompatible material such as hydroxypatite. Indeed, hydroxyapatite, which is able to bond with the bone, is used as a filler to replace amputated bone or as a coating to promote bone ingrowth into prosthetic implants. However, the use of hydroxyapatite presents limitations since it is mainly effective on osseous sites.

There is currently no available technique providing bone formation which does not present any risk of rejection and which may be independent of the size and localisation of the bone to regenerate.

Consequently, there is a need for a biocompatible porous material, which can be used on any subject, independently of the type, size and localisation of the damaged bone, and is capable of promoting bone formation and providing osteoinductive properties.

SUMMARY OF THE INVENTION

The inventors have prepared porous three-dimensional polysaccharide scaffold able to provide an ideal environment for bone formation and facilitate the growth of vasculature into the material. Surprisingly and unexpectedly, the inventors have shown that polysaccharide scaffold comprising nanocristalline hydroxyapatite induce mineralisation of a tissue. Thus, by stimulating undifferentiated cells *in situ* into bone cell lineages, the invention overcomes the limitations of the prior art strategies of treatment of bone related disorders.

The inventors have thus found out very promising polysaccharide scaffolds for bone formation, in a non-osseous site, in the absence of growth factors or stem cells. The invention hence challenges the currently acknowledged techniques for treating bone related disorders and offers a wide range of possibilities disclosed hereafter.

The invention relates to a method for preparing a porous polysaccharide scaffold comprising the following step:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii),

wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably nano-hydroxyapatite.

The invention also relates to a method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide and one cross-linking agent,

b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b), wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite, preferably nano-hydroxyapatite,

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

The invention further relates to a porous polysaccharide scaffold obtainable by the method of the invention.

The invention further relates to a porous polysaccharide scaffold obtainable according to the method of the invention, for use in the treatment of bone related disorders.

DETAILED DESCRIPTION OF THE INVENTION

Definition

As used herein, the term **"polysaccharide"** refers to a molecule comprising two or more monosaccharide units.

As used herein, the term **"alkaline solution"** refers to a solution having a pH strictly superior to 7.

As used herein, the term **"aqueous solution"** refers to a solution in which the solvent is water.

5 As used herein, the term **"porogen agent"** refers to any solid agent which has the ability to form pores within a solid structure.

As used herein, the term **"cross-linking"** refers to the linking of one polysaccharide chain to another one with covalent bonds.

As used herein, the term **"cross-linking agent"** encompasses any agent able 10 to introduce cross-links between the chains of the polysaccharides of the invention.

As used herein, the term "**scaffold**" or **"matrix"** refers to a semi-solid system comprising a three-dimensional network of one or more species of polysaccharide chains. Depending on the properties of the polysaccharide (or mixtures of polysaccharides) used, as well as on the nature and density of the network, such 15 structures in equilibrium can comprise various amounts of water. In the following, the terms "scaffold" and "matrix" are interchangeable.

As used herein, the term '**hydroxyapatite**', or "**micro-hydroxyapatite**" or "**HA**" refers to a naturally occurring mineral form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$ to denote that the 20 crystal unit cell comprises two entities. The OH^- ion can be replaced by fluoride, chloride or carbonate, producing fluorapatite or chlorapatite. Preferably, for the purpose of the invention, the OH^- is not replaced. Hydoxyapatite is the major component of bone and teeth matrix and gives bones and teeth their rigidity. Typically, the size of the microparticles of hydroxyapatite is comprised between 1 to 25 20 μm, preferably 5 and 15 μm.

As used herein, the term "**nanocristalline hydroxyapatite**", or "**nano-hydroxyapatite**", or "**n-HA**", refers to hydroxyapatite crystal particles having a size comprised between 10 and 100 nm, preferably 20 and 80 nm, preferably 30 and 70 nm, preferably between 30 and 60 nm, and most preferably about 50 nm. Preferably, 30 the n-HA particles are needle-shaped. Preferably, the n-HA suitable for carrying out the present invention is a n-HA prepared by chemical precipitation at room temperature, for example by precipitation of a solution of phosphoric acid with a solution of calcium hydroxide.

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As used herein, the term “**porous composite polysaccharide scaffold**” refers to a porous scaffold comprising polysaccharides associated with n-HA according to the invention.

As used herein, the term "**biodegradable**" refers to materials that degrade in
5 vivo to non-toxic compounds, which can be excreted or further metabolized.

As used herein, the term “**sublimation**” refers to the physical phase transition from a solid state directly to a vapor state. More specifically, sublimation is a process in which a substance goes from a solid to a gas without going through a liquid phase. Sublimation of a solution may be obtained through the freeze-drying process.

10 As used herein, the term “**freeze-drying**” refers the drying of a deep-frozen material under high vacuum by freezing out the solvent (ie. water) and then evaporating it in the frozen state.

As used herein, the terms "**treating**", “**treatment**” and "**therapy**" refer to therapeutic treatment and prophylactic, or preventative manipulations, or
15 manipulations which stimulate bone cell differentiation or bone formation. Such expression also encompasses manipulations which postpone the development of bone disorder symptoms, and/or reduce the severity of bone disorders and/or such symptoms that will or are expected to develop from a bone disorder. The terms further include ameliorating existing bone disorder symptoms, preventing additional
20 symptoms, or preventing or promoting bone growth.

As used herein, the expression "**bone tissue**" refers to calcified tissues (e.g., calvariae, tibiae, femurs, vertebrae, teeth), bone trabeculae, the bone marrow cavity, the cortical bone, which covers the outer peripheries of the bone trabeculae and the bone marrow cavity, and the like. The expression “bone tissue” also encompasses
25 bone cells that are generally located within a matrix of mineralized collagen; blood vessels that provide nutrition for the bone cells; bone marrow aspirates: joint fluids: bone cells that are derived from bone tissues; and may include fatty bone marrow. Finally, bone tissue includes bone products such as whole bones, sections of whole bone, bone chips, bone powder, bone tissue biopsy, collagen preparations, or
30 mixtures thereof. For the purposes of the present invention, the term "bone tissue" is used to encompass all of the aforementioned bone tissues and products, whether human or animal, unless stated otherwise.

As used herein, the expression **"bone-related disorders"** includes disorders of bone formation and bone resorption. Preferably, the expression "bone related disorders" refers to diseases associated with insufficiency of bone formation or bone loss.

5 Non-limiting examples of bone related disorders are rickets, osteoporosis osteomalacia, osteopenia, bone cancer, arthritis, rickets, bone fracture, bone defects, osteolytic bone disease, osteomalacia, bone frailty, loss of bone mineral density achondroplasia, cleidocranial dysostosis, Paget's disease, osteogenesis imperfecta, osteopetrosis, sclerotic lesions, pseudoarthrosis, periodontal disease, anti-epileptic 10 drug induced bone loss, weightlessness induced bone loss, postmenopausal bone loss, osteoarthritis, infiltrative disorders of bone, metabolic bone diseases, organ transplant related bone loss, adolescent idiopathic scoliosis, glucocorticoid-induced bone loss, heparin-induced bone loss, bone marrow disorders, malnutrition, calcium deficiency, rheumatoid arthritis, hypogonadism, HIV associated bone loss, tumor-15 induced bone loss, cancer-related bone loss, hormone ablative bone loss, multiple myeloma drug- induced bone loss, facial bone loss associated with aging, cranial bone loss associated with aging, jaw bone loss associated with aging, skull bone loss associated with aging, and bone loss associated with space travel.

Preferably, the bone related disorders, as used herein, are bone fracture, large 20 bone defects, rickets, osteoporosis, osteogenesis imperfecta, osteomalacia, osteopenia, bone cancer, osteolytic bone disease, bone frailty and/or loss of bone mineral density.

Porous polysaccharide scaffolds and methods for preparing thereof

25 In a first object, the invention relates to a method for preparing a porous polysaccharide scaffold comprising the following step:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

30 ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii), wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably
5 nano-hydroxyapatite.

The concentration of the porogen agent affects both the total porosity and the size of the pores formed in the scaffolds, so that the porosity and the pore size can be under the control of the concentration of said porogen agent.

Non-limiting examples of porogen agents are sodium chloride, calcium
10 chloride, ammonium carbonate, ammonium bicarbonate, calcium carbonate, sodium carbonate, and sodium bicarbonate and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich (St. Louis, Michigan, US).

Preferably, in the context of the present invention, the porogen agent is
15 chosen from sodium chloride, calcium chloride or mixtures thereof.

Alternatively, the porogen agent may be an inorganic salt that can be dissolved once the cross-linked polysaccharide scaffold is immersed in water. An example of such a porogen agent includes saturated salt solution, which would be dissolved progressively.

20 Typically, the weight ratio of the polysaccharide to the porogen agent is in a range1:50 to 50:1, preferably from 1:30 to 30:1, preferably from 1:12 to 12:1.. In a preferred embodiment, said weight ratio of the polysaccharide to the porogen agent is about 12:14.

Typically, the aqueous solution of step iii) is water.

25 Alternatively, the aqueous solution of step iii) is a buffer solution. Non-limiting examples of buffer solution are PBS (Phosphate buffered saline), EDTA (ethylenediaminetetraacetic acid), TAPS (3-{ [tris(hydroxymethyl)methyl]amino} propanesulfonic acid), Bicine (N,N-bis(2-hydroxyethyl)glycine), Tris (tris(hydroxymethyl)methylamine), Tricine (N-tris(hydroxymethyl)methylglycine),
30 HEPES (4-2-hydroxyethyl-1-piperazineethanesulfonic acid), MOPS (3-(N-morpholino)propanesulfonic acid), PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid), Cacodylate (dimethylarsinic acid), SSC (saline sodium citrate), MES (2-(N-morpholino)ethanesulfonic acid) and mixtures thereof.

Alternatively, the aqueous solution of step iii) is an acidic solution. The acid may be selected from the group consisting of citric acid, hydrochloric acid, acetic acid, formic acid, tartaric acid, salicylic acid, benzoic acid, and glutamic acid.

Preferably, the aqueous solution of step iii) is a buffer solution. Most
5 preferably, the aqueous solution of step iii) is phosphate buffer saline (PBS).

Preferably, the solvent of step ii) is an inorganic solvent.

In one embodiment, the method of the invention may comprise a further step, consisting of freeze-drying the scaffold obtained at step iv). Freeze-drying may be performed with any apparatus known in the art. There are essentially three categories
10 of freeze dryers: rotary evaporators, manifold freeze dryers, and tray freeze dryers. Such apparatus are well known in the art and are commercially available such as a freeze-dryer Lyovac (GT2, STERIS Rotary vane pump, BOC EDWARDS). Basically, the vacuum of the chamber is from 0.1 mBar to about 6.5 mBar. The freeze-drying is performed for a sufficient time sufficient to remove at least 98.5 %
15 of the water, preferably at least 99% of the water, more preferably at least 99.5%.

In another embodiment, the method of the invention may comprise a further step consisting of hydrating the scaffold as prepared according to the invention. Said hydration may be performed by submerging the scaffold in an aqueous solution (e.g., de-ionized water, water filtered via reverse osmosis, a saline solution, or an aqueous
20 solution containing a suitable active ingredient) for an amount of time sufficient to produce a scaffold having the desired water content. Typically, when a scaffold comprising the maximum water content is desired, the scaffold is submerged in the aqueous solution for an amount of time sufficient to allow the scaffold to swell to its maximum size or volume. Typically, the scaffold is submerged in the aqueous
25 solution for at least about 1 hour, preferably at least about 2 hours, and more preferably about 4 hours to about 24 hours. It is understood that the amount of time necessary to hydrate the scaffold to the desired level will depend upon several factors, such as the composition of the used polysaccharides, the size (e.g., thickness) of the scaffold, and the temperature of the aqueous solution, as well as other factors.

30

Preferably, the hydrated scaffold comprises more than 80% of water, preferably 90% of water, most preferably 95 % of water.

In a second aspect, the invention relates to a method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, and one cross-linking agent,

5 b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b), wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite, preferably nano-hydroxyapatite,

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

10 It is an essential feature of the invention that step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a). Typically, temperature and time are the main factors to control the cross-linking of the aqueous solution. To avoid or to seriously limit the cross-linking of the polysaccharide, the aqueous solution may be prepared at a temperature under 37°C, more preferably 15 comprised between 4°C and 25°C. Moreover, the step b) may be performed as quickly as possible to avoid the cross-linking of said polysaccharide.

Once the aqueous solution is prepared, it is frozen. The freezing of the aqueous solution may be performed at different rates (e.g., °C/min). Typically, the freezing may be performed at rate from about 1°C/min to about 200°C/min, 20 preferably from about 1°C/min to about 20°C/min, and most preferably from about 5°C/min to about 10°C/min. The solution may be frozen in liquid nitrogen or in dried ice.

When the aqueous solution is frozen, sublimation may take place. In a preferred embodiment, the method for preparing porous polysaccharide scaffolds 25 according to the present invention includes a freeze-drying process. Therefore, according to the invention, the freeze-drying process has to take place before the cross-linking process occurs in the aqueous solution. Freeze-drying may be performed with any apparatus known in the art. There are essentially three categories of freeze dryers: rotary evaporators, manifold freeze dryers, and tray freeze dryers. 30 Such apparatus are well known in the art and are commercially available such as a freeze-dryer Lyovac (GT2, STERIS Rotary vane pump, BOC EDWARDS). Basically, the deep-frozen aqueous solution is placed in a chamber. Then the chamber temperature is increased to a level higher than the boiling point of the

liquefied vapour, whereby the vapour is vaporized and removed. Typically, the temperature of chamber may be from -70 °C to -1°C, preferably from -70°C to -40°C, further preferably about -50°C to -40°C. The heating of the chamber is accompanied with a vacuum flow to decrease the pressure of the chamber. Typically, 5 the vacuum of the chamber is from 0.1 mBar to about 6.5 mBar. Typically, the freeze-drying is performed for a sufficient time sufficient to remove at least 98.5 % of the water, preferably at least 99% of the water, more preferably at least 99.5%.

The freezing of the aqueous solution causes the formation of ice particles from the water. Without to be bound by any theory, under the temperature and 10 pressure condition described above, water included in the frozen solution is sublimed, and thus, thereby leaving interstices in the material in the spaces previously occupied by the ice particles, and accordingly porous polysaccharide scaffolds are produced. Surprisingly, the cross-linking process occurs during the freeze-drying process.

15 The material density and pore size of the resultant scaffold may be therefore varied by controlling the rate of freeze-drying of the frozen aqueous solution. The essential parameter in a freeze-drying process is the vacuum rate.

For the purpose of the present invention, any type of polysaccharide can be used. Synthetic or natural polysaccharide may be alternatively used in the context of 20 the invention. Non-limiting examples of suitable polysaccharide for implementing the present invention are dextran, agar, alginic acid, hyaluronic acid, inulin, pullulan, heparin, fucoidan, chitosan, scleroglucan, curdlan, starch, cellulose and mixtures thereof. Chemically modified polysaccharides bearing for instance acidic groups (carboxylate, sulphate, phosphate), amino groups (ethylene amine, 25 diethylaminoethylamine, propylamine), hydrophobic groups (alkyl, benzyl) can be included. Saccharide structures and oligosaccharides that may be used to produce the desired materials include but are not limited to ribose, glucose, mannose, galactose, fructose, sorbose, sorbitol, mannitol, iditol, dulcitol and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich 30 (St. Louis, Michigan, US).

Typically, the average molecular weight of the polysaccharides is from about 5,000 Daltons to about 2,000,000 Daltons, preferably from about 100,000 Daltons to

about 500,000 Daltons. Typically, the polysaccharide used to prepare the scaffold of the invention is a neutral polysaccharide such as dextran, agar, pullulan, inulin, scleroglucan, curdlan, starch, cellulose and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a positively charged
5 polysaccharide such as chitosan, DEAE-dextran, DEAE-pullulan, EA-pullulan and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a negatively charged polysaccharide such as alginic acid, hyaluronic acid, heparin, fucoidan and mixtures thereof. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a mixture of neutral and negatively charged
10 polysaccharides. Typically, the negatively charged polysaccharides represent 1 to 20%, preferably 5 to 10% of the mixture. Alternatively, the polysaccharide used to prepare the scaffold of the invention is a mixture of neutral and positively charged polysaccharides. Typically, the positively charged polysaccharides represent 1 to 20%, preferably 5 to 10% of the mixture.

15 Preferably, for the purpose of the invention, said polysaccharide is selected in the group consisting of dextran, pullulan, agar, alginic acid, starch, hyaluronic acid, inulin, heparin, fucoidan, chitosan and mixtures thereof. In one particular embodiment of the invention, said polysaccharide is a mixture of pullulan and dextran. Typically, the weight ratio of pullulan/dextran is in a range from 95:5 to
20 95:5 (w/w), preferably in a ration of 75:25 (w/w). In another embodiment of the invention, said polysaccharide is a mixture of pullulan, dextran and fucoidan. Typically, the weight ratio of pullulan/dextran/fucoidan is in a range from about 70:20:10 to about 50:20:30, preferably from about 70:20:10 to about 50:30:20, and most preferably in a ratio of about 73:22:5 (w/w). The presence of fucoidan in the
25 porous polysaccharide scaffold of the invention is highly advantageous since fucoidan promotes vascularisation.

Typically, the covalent cross-linking agent is selected from the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride ($POCl_3$), epichlorohydrin, formaldehydes, carbodiimides, glutaraldehydes, any other
30 compound that is suitable for crosslinking a polysaccharide and mixtures thereof. Many of these compounds are available commercially from companies such as Sigma-Aldrich (St. Louis, Michigan, US). Preferably, for the purpose of the present invention, said cross-linking agent is STMP. Typically, the concentration of the

covalent cross-linking agent in the aqueous solution (w/v) is from about 1% to about 6%, more preferably from about 2% to about 6%, most preferably from about 2% to about 3%. Typically, the weight ratio of the polysaccharide to the cross-linking agent is in a range from 20:1 to 1:1, preferably from 10:1 to 2:1.

5 In the context of the present invention, nano-hydroxyapatite may be a commercial nano-hydroxyapatite, such as those commercialised by Inframat Corporation or Fluidinova. Preferably, nanocristalline hydroxyapatite useful in the context of the present invention is obtained through chemical precipitation at room temperature of a solution of phosphoric acid, at a concentration comprised between 10 0.3 to 1M, preferably 0.6M, with a solution of calcium hydroxide, at a concentration comprised between 0.5 to 1.5M, preferably 1M. Typically, the concentration of hydroxyapatite in the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v). Typically, the concentration of nano-hydroxyapatite in 15 the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v).

In one embodiment, the alkaline aqueous solution of step a) or step i) comprising hydroxyapatite, preferably nano-hydroxyapatite, may be poured in a 20 mould before step b) or step ii), so that the porous polysaccharide scaffold obtained with the method of the invention can take a desired form. Any geometrical moulds may be used according to the invention. Different sizes may also be envisaged. The mould may be made of any material, but preferred material includes non sticky surfaces such as Teflon.

25 Alternatively, the scaffolds of the invention may be cut and shaped to take a desired size and form.

The methods of the invention can further include the step of sterilizing the scaffold using any suitable process. The scaffold can be sterilized at any suitable point, but preferably is sterilized before the scaffold is hydrated. A suitable 30 irradiative sterilization technique is for example an irradiation with Cesium 137, 35 Gray for 10 minutes. Suitable non-irradiative sterilization techniques include, but are not limited to, UV-exposure, gas plasma or ethylene oxide methods known in the art. For example, the scaffold can be sterilized using a sterilisation system which is

available from Abtox, Inc of Mundelein, Illinois under the trade mark PlazLyte, or in accordance with the gas plasma sterilization processes disclosed in US-5413760 and US-5603895.

The scaffold produced by the methods of the invention can be packaged in any suitable packaging material. Desirably, the packaging material maintains the sterility of the scaffold until the packaging material is breached.

In a further embodiment, the alkaline solution of step i) or a) further comprises a drug. The invention thus provides porous polysaccharide scaffold comprising a drug. Typically, said drug is a drug having an acknowledged therapeutic effect, such as hormones radioactive substance, fluorescent substance, chemotactic agent, antibiotic, steroidal or non-steroidal analgesic, immunosuppressant, or anti-cancer drug, drugs belonging to the pharmaceutical class of statins. Preferably, said drug belongs to the pharmaceutical class of statins. As used herein, "statins" refers to a pharmaceutical class of HMG-CoA reductase inhibitors. It has been recently shown that some of the drugs from this pharmaceutical class play a role in the process of bone formation. Preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin pitavastatin, rosuvastatin, pravastatin, fluvastatin and simvastatin. More preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin and simvastatin. Said statins are highly appropriate in the context of the present invention since they play a role in the bone formation.

In a further embodiment, the alkaline solution further comprises a bioactive substance. Typically, said bioactive substance is a substance known for playing an important role in various mechanisms such as modification of cellular pathways and modification of cellular or tissular responses. Said bioactive substance is chosen among growth factors, cytokines (lymphokines, interleukins, and chemokines), antioxidant molecules, angiogenic molecule, anti-angiogenic agents, immunomodulating agents, proinflammatory cytokines, antiinflammatory cytokines, plasma-derived bioactive substances, PRP (platelet rich plasma)-derived substances, soluble adhesion molecules.

In a third aspect, the invention relates to porous polysaccharide scaffolds obtainable by the methods of the invention. These porous polysaccharide scaffolds are indeed the only ones which have the remarkable properties provided by the

invention. When the method of preparing the porous polysaccharide scaffold according to the invention involves the use of a porogen agent, the concentration of the porogen agent affects the size of the pores formed in the scaffolds. Therefore, in this particular embodiment, the size of the pores can be under the control of the
5 concentration of said porogen agent. Typically, the average pore size of the scaffold is from about 1μm to about 500 μm, preferably from about 10 μm to about 200 μm. Typically, the density of the pores (or porosity) is from about 4% to about 75%, preferably from about 4% to about 50%. The person skilled in the art may provide desired properties to the porous polysaccharide scaffold according to the invention.
10 Typically, the person skilled in the art may add one or more compounds chosen in the group consisting of a biomolecule, a bioactive agent, a drug, an anti-inflammatory agent, an additive, an antimicrobial agent, a colorant, a surfactant and a differentiation agent. The techniques for incorporating said compounds in the porous polysaccharide scaffold of the invention completely falls within the ability of the
15 person skilled in the art. Typically, said compounds may be added directly the alkaline solution of step i) or a) of the method of the invention. In this particular embodiment, the compound would be within the structure of the porous polysaccharide scaffold of the invention. Alternatively, said compounds can be incorporated into the porous polysaccharide scaffold during a step consisting of
20 hydrating said scaffold with a solution of the compound.

In one embodiment, the porous polysaccharide scaffold of the invention further comprises one or more biomolecules. Non-limiting examples of biomolecules are drugs, hormones, radioactive substances, fluorescent substances, chemicals or agents, chemotactic agents, antibiotics, steroidal or non-steroidal analgesics,
25 immunosuppressants, anti-cancer drugs, short chain peptides, glycoprotein, lipoprotein, cell attachment mediators, biologically active ligands, integrin binding sequence, ligands, small molecules that affect the up-regulation of specific growth factors, tenascin-C, hyaluronic acid, chondroitin sulphate, fibronectin, decorin, thromboelastin, thrombin-derived peptides, and mixtures thereof. The presence of
30 said biomolecules in the porous polysaccharide scaffold of the invention may enhance treatment effects, enhance visualization, indicate proper orientation, resist infection, promote healing, may increase softness or any other desirable effects. In another embodiment, the porous polysaccharide scaffold of the invention further

comprises a bioactive substance. Typically, said bioactive substance is a substance known for playing an important role in various mechanisms such as modification of cellular pathways and modification of cellular or tissular responses. Said bioactive substance is chosen among growth factors, cytokines (lymphokines, interleukins, and
5 chemokines), antioxidant molecules, angiogenic molecule, anti-angiogenic agents, immunomodulating agents, proinflammatory cytokines, antiinflammatory cytokines, plasma-derived bioactive substances, PRP (platelet rich plasma)-derived substances, and soluble adhesion molecules.

In a further embodiment, the porous polysaccharide scaffold of the invention
10 further comprises one or more drug. Typically, said drug is a drug having an acknowledged therapeutic effect, such as hormones radioactive substance, fluorescent substance, chemotactic agent, antibiotic, steroidal or non-steroidal analgesic, immunosuppressant, or anti-cancer drug, drugs belonging to the pharmaceutical class of statins. Preferably, said drug belongs to the pharmaceutical
15 class of statins. Preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin pitavastatin, rosuvastatin, pravastatin, fluvastatin and simvastatin. More preferably, said statins is selected from the group consisting of lovastatin, atorvastatin, mevastatin and simvastatin. Said statins are highly appropriate in the context of the present invention since they play a role in the bone
20 formation

In another embodiment, the porous polysaccharide scaffold of the invention further comprises anti-inflammatory agents. Non-limiting examples of anti-inflammatory agents are indomethacin, salicylic acid acetate, ibuprofen, sulindac, piroxicam, and naproxen; thrombogenic agents, such as thrombin, fibrinogen,
25 homocysteine, and estramustine; and radio-opaque compounds, such as barium sulfate, gold particles and iron oxide nanoparticles (USPIOs) and mixtures thereof.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises additives. The amount of the additive used depends on the particular application of the porous polysaccharide scaffold of the invention and
30 may be readily determined by the person skilled in the art using routine experimentation.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises an antimicrobial agent. Suitable antimicrobial agents are

well known in the art. Non-limiting examples of suitable antimicrobial agents are alkyl parabens, such as methylparaben, ethylparaben, propylparaben, and butylparaben; cresol; chlorocresol; hydroquinone; sodium benzoate; potassium benzoate; triclosan and chlorhexidine and mixture thereof. Other examples of
5 antibacterial agents and of anti-infectious agents that may be used are, in a non-limiting manner, rifampicin, minocycline, chlorhexidine, silver ion agents and silver-based compositions and mixtures thereof.

In a further embodiment, the porous polysaccharide scaffold of the invention further comprises at least one colorant to enhance the visibility of the scaffold.
10 Suitable colorants include dyes, pigments, and natural coloring agents. Non-limiting examples of suitable colorants are alcian blue, fluorescein isothiocyanate (FITC) and FITC dextran and mixtures thereof.

In still another embodiment, the porous polysaccharide scaffold of the invention further comprises at least one surfactant. Surfactant, as used herein, refers
15 to a compound that lowers the surface tension of water. The surfactant may be an ionic surfactant, such as sodium lauryl sulfate, or a neutral surfactant, such as polyoxyethylene ethers, polyoxyethylene esters, and polyoxyethylene sorbitan and mixtures thereof.

In one embodiment, the porous polysaccharide scaffold of the invention
20 further comprises a differentiation agent. Preferably, such a differentiation agent is an agent involved in bone formation. Alternatively, such a differentiation agent is an agent involved in osteogenesis, angiogenesis or wound healing. Preferably, such a differentiation agent is a growth factor. Non-limiting examples of growth factor suitable for the purpose of the present invention are epidermal growth factor (EGF),
25 insulin-like growth factor (IGF-I, IGF-II), transforming growth factor beta (TGFβ), heparin binding growth factor (HBGF), stromal derived factor (SDF-1), vascular endothelial growth factors (VEGF), fibroblast growth factors (FGFs), platelet derived growth factors (PDGF), parathyroid hormone (PTH), parathyroid hormone related peptide (PTHrP), basic fibroblast growth factor (bFGF); TGFβ superfamily factors;
30 bone morphogenetic proteins (BMPs) preferably BMP2, BMP3, BMP4, BMP5, BMP7, somatropin, growth differentiation factor (GDF) and mixtures thereof.

Typically, the growth factor is present at a concentration comprised from 1 ng to 100 μg per porous polysaccharide scaffold of the invention.

In another embodiment, the porous polysaccharide scaffold of the invention further comprises cells, such as yeast cells, mammalian cells, insect cells, and plant cells.

Preferably, said cell is a mammalian cell. Non-limiting examples of mammalian cells suitable for the purpose of the invention are differentiated cells such as chondrocytes, fibrochondrocytes, osteocytes, osteoblasts, osteoclasts, synoviocytes, epithelial cells and hepatocytes or stem cells, embryonic stem cells, induced progenitor stem cells (iPS), mesenchymal stem cells from different sources, bone marrow, adipose tissue, peripheral blood progenitor cells, cord blood progenitor cells, genetically transformed cells and mixtures thereof. Most preferably, the mammalian cells comprised in the porous polysaccharide scaffold according to the invention are adipose derived stroma cells. Typically, the mammalian cells comprised in the porous polysaccharide scaffold are present at a cell density comprised between 200 cells/mm^3 to 35 000 cells/mm^3.

In a fourth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for bone generation.

As used herein, the expression “bone generation” encompasses “bone repair” and “bone development”.

In a fifth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for stimulating ectopic mineralized tissue formation. In the context of the present invention, the expression "ectopic" refers to a non osseous tissue. Therefore, the invention also relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use for inducing mineralized tissue in a non-osseous site.

Preferably, said stimulation of ectopic mineralization occurs in absence of stem cells and/or growth factors. Indeed, the inventors have shown that the porous polysaccharide scaffold according to the invention has the ability to induce mineralized tissue in a non-osseous site and in an osseous site (calvaria site or femoral condyle), even in the absence of stem cells and/or growth factors. Therefore, the invention provides a porous polysaccharide scaffold useful for stimulating mineralized tissue formation in osseous site, as well as in non-osseous site, in the presence as well as in the absence of stem cells and/or growth factors.

Use of the porous polysaccharide scaffold according to the invention

The inventors have shown that implanting porous polysaccharide scaffold according to the invention lead to the stimulation of a dense collagen network and blood vessel formation as well as the recruitment of osteoblast-like cells. Said implantation of scaffolds according to the invention in subcutaneous site leads to the formation of a dense mineralized tissue, and thus to bone formation.

The inventors have shown that the scaffold of the invention, when implanted, retains growth factor such as VEGF and BMP. The inventors also evidenced that the ability of retaining said growth factor was higher for the scaffold comprising n-HA, compared to a scaffold not comprising n-HA.

In a sixth aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use in the treatment of bone related disorders. The inventors have indeed shown the ability of the porous polysaccharide scaffold according to the invention to stimulate the production of an extracellular mineralized matrix, probably through differentiation of cells into bone cells. Thus, the inventors evidenced that the scaffold of the invention is useful for the treatment of bone related disorders.

In a seventh aspect, the invention relates to a porous polysaccharide scaffold obtainable according to the method of the invention for use as a polysaccharide scaffold.

Typically, the size and the shape of the porous polysaccharide scaffold can be adapted to the type and size of the bone to replace, and to the localization of said bone. Preferably, the shape of the scaffold is a sphere, a cylinder, a cube or a rectangular cuboid. Preferably, the size of said scaffold is comprised between 0.5 mm and 30 cm. Typically, the polysaccharide scaffold of the invention may be is implanted as follows: the lyophilized scaffold is placed within the defect and its size is adapted to the size of defect. For example, for the implantation in calvaria site in mouse, defects of 4 mm of diameter and 500 μm of depth were performed and the matrices were apposed onto the host tissue. In mice, bone defect performed in the femoral condyle is around $1mm^3$. In rat, the critical size defect performed in the

femoral condyle is 5 mm of diameter and 3 mm of depth. These bone defects are filled with the matrices. For segmental bone defect in large animal (sheep or goat), a resection of 2.5 cm is performed at metatarsus and cylinder of polysaccharide scaffold is placed within the defect. Analysis of the newly formed tissue within the defect is performed between 15 days to 12 months. The person skilled in the art is award of the routine suitable techniques for analyzing said newly formed tissue. Typically, said analysis may be performed using several invasive methods such as histomorphometry as gold standard technique. Alternatively, said analysis may be performed using non invasive imaging approaches such as Magnetic Resonance Imaging (MRI), X Ray micro Computed Tomography (micro-CT), Single Photon Emission Computarized Tomography (SPECT) or radiological analysis. The choice of the suitable technique is dependent on the type of bone in small and large animals, or humans.

FIGURES LEGENDS

Figure 1: Porous polysaccharide scaffold.

Macroscopic view of hybrid porous discs with n-HA before (Figure 1A) and after (Figure 1B) rehydration with phosphate buffer saline (PBS). The scale bar corresponds to 1 mm.

Figure 2: Electron Microscopy of a freeze-dried polysaccharide scaffold.

The morphology of freeze-dried scaffolds was analyzed by scanning electron microscopy (Figure 2A). After rehydration in PBS, porosity of hydrated scaffolds was observed with Environnemental Scanning Electron Microscopy (ESEM Philips XL 30) (Figure 2B).

Figure 3: Healing of critical size defects in nude mice by the polysaccharide-based matrices.

Micro-CT images of calvaria defects filled with polysaccharide matrices without n-HA (Figure 3A), or with the polysaccharide scaffold (Figure 3B), loaded (on left side) or not (on right side) with $5x10^5$ differentiated adipose derived stromal cells (ADSCs). Imaging on the same animal for each type of scaffold was performed after 15, 30, 60 and 84 days of implantation, and resulting images are respectively referred to as D15, D30, D60, D84. Quantitative analysis of the Tissue Mineral

Density (TMD) of implanted polysaccharide scaffold. Calvaria bone was used as a control (Figure 3C).

Figure 4: Ectopic mineralized tissue formation in subcutaneous site induced by the polysaccharide scaffold.

5 **(A)** Micro-CT images at Days 15, 30 and 60 of a mouse implanted with two discs of the polysaccharide scaffold (n-HA/scaffold) (left site) and one disc previously seeded with $5x10^5$ differentiated ADSCs (right site).

(B) Macroscopic view at D60.

(C) Quantitative analysis of the tissue mineral density (TMD).

10 **(D)** Histological examination of undecalcified (D1; magnification x10) (stained by Goldner's trichrome) and decalcified (D2; magnification x2) (D3; magnification x20) sections (Masson's staining) obtained at Day 60.

(E) Von Kossa staining performed on explanted materials at Day 30 and Day 60. Control was performed using the paraffin-embedded composite matrix before 15 implantation (magnification x2).

Figure 5 : Matrix+n-HA (MATRI+) induces mineralization in ectopic site of mice.

(A) Representative micro-CT images of the subcutaneous implantation of the Matrix alone on the left side (indicated by an arrowed doted line) and Matrix+n-HA 20 (MATRI+) on right side (indicated by an arrowed plain line), after 15 (D15), 30 (D30) and 60 days (D60) of implantation in Balb/c mice.

(B) Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from reconstructed three-dimensional micro-CT images with Microview Image analyser of the Matrix (white rectangle) and Matrix+n-HA (MATRI+) (black 25 rectangle). Data are presented as means ± standard deviation for n=8. The symbol ** indicates a statistically significant difference compared to the other groups <0.01.

Figure 6 : Matrix+n-HA induces formation of a collagen-based mineralized tissue: histological analyis of the newly formed tissue.

(A) Representative histological undecalcified sections of the Matrix and 30 Matrix+n-HA (MATRI+) samples implanted subcutaneously in mice, after 15 days (D15) and 60 days (D60) : Von Kossa staining.

(B) Representative histological decalcified sections of Matrix+n-HA (MATRI+) 60 days after implantation : Goldner staining, The images showed a high

dense collagen tissue around the implant that colonizes the scaffold, with osteoblast-like cells as indicated by the white arrows, and numerous vessels inside the collagen tissue indicated by the black arrows.

Figure 7 : XRD patterns of matrices before surgery (D0) and 15 days (D15) after subcutaneously implantation in mice.

(A) Matrix+n-HA (MATRI+) ; (B) Matrix without n-HA

Specific peaks of hydroxyapatite (HA) are only observed in the XRD patterns after 15 days of implantation of MATRI+. Peaks of Halite (H) due to sample processing, are observed in all spectra. The XRD patterns obtained at day 30 and day 60 are similar than those observed at D15 for both groups (data not shown).

Figure 8 : Matrix+n-HA (MATRI+) retained endogeneous osteoinductive and angiogenic factors.

Measurement by ELISA of BMP2 (A) and VEGF165 (B), retained in the tissue formed within the Matrix (white rectangle) and Matrix+n-HA (MATRI+) (black rectangle) when implanted subcutaneously at D15, D30 and D60. Results are expressed in pg of growth factors retained per μg of proteins quantified by BCA. Data are presented as means ± standard deviation for n=6 samples. The symbols * and ** indicate a statistically significant difference compared to the other groups with $p<0.05$ and <0.01, respectively.

Figure 9 : Matrix+n-HA (MATRI+) induces a high mineralization of tissue in a critical size bone defect performed in the femoral condyle of rats.

(A) Representative micro-CT images of the femoral condyle of rats, 15 days (D15), 30 days (D30) and 90 days (D90) after implantation without scaffold (empty), with Matrix or Matrix+n-HA (MATRI+).

(B) Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from reconstructed three-dimensional micro-CT images of the empty group (white rectangle), the Matrix group (grey rectangle) and Matrix+n-HA (MATRI+) (black rectangle). Data are presented as means ± standard deviation for n=4. The symbol ** indicates a statistically significant difference compared to the other groups with $p<0.01$.

Figure 10 : Matrix+nHA (MATRI+) induces a high mineralized bone tissue in a critical size bone defect performed in the femoral condyle of rats after 90 days of implantation; histological analysis of the newly formed tissue.

(A) Representative histological undecalcified sections of Empty, Matrix and Matrix+n-HA (MATRI+) samples implanted in the femoral condyle of rats, after 90 days of implantation: Von Kossa staining. The arrows indicated the position of the bone defect.

5 **(B)** Representative histological decalcified sections of of Empty, Matrix and Matrix+nHA samples 90 days after implantation : Goldner staining, A fibrous tissue was formed in the empty bone defect, while bone formation occurred in direct contact of the matrix and was enhanced within the MATRIX+ implant.

10

EXAMPLE

Example 1 : Implantation of the scaffold of the invention in calvaria site of athymic mice.

5 **Materials and Methods**

Nano-hydroxyapatite preparation

Nano-hydroxyapatite (n-HA) was prepared by wet chemical precipitation using a 0.6M solution of Phosphoric acid (H_3PO_4 Rectapur, Prolabo®, France) and a 1M solution of calcium hydroxide ($CaOH_2$ Alfa Aesar, Germany). 100 ml of H_3PO_4 10 solution were added dropwise in 100 ml of $CaOH_2$ solution during 30 minutes under vigorous stirring at room temperature. At the end of reaction, pH was adjusted to 9 using $0.4.10^{-3}$ mol of a 0.6 M sodium hydroxide solution, then stirring was continued during 12 hours.

Nano-hydroxyapatite (n-HA) has been characterized by transmission electron 15 microscopy (TEM), scanning electron microscopy and by FTIR analysis. TEM revealed n-HA needle-shaped crystals of 50 nm long. FTIR analysis showed specific bands of phosphate ions of at 559 cm^{-1}, 601 cm^{-1} and 1018 cm^{-1} and a non-specific carbonate band 1415 cm^{-1}.

20 ***Preparation of composite polysaccharide scaffolds (MATRI+)***

Macroporous composite scaffolds (MATRI+) were prepared using a blend of pullulan/dextran 75:25 (pullulan, MW 200,000, Hayashibara Inc, Dextran MW 500,000, Pharmacia), prepared by dissolving 9 g of pullulan and 3 g of dextran into 27 mL of distilled water containing 14g of NaCl and 13 mL of nano-hydroxyapatite 25 suspension (n-HA, 6.36% w/v). Chemical cross-linking was carried out using trisodium trimetaphosphate STMP (Sigma) under alkaline condition. Briefly, 1 mL of 10M sodium hydroxide was added to 10 g of the polysaccharide blend, followed by the addition of 1 mL of water containing 300 mg of STMP. After incubation at 50°C for 15 min, resulting scaffolds were cut into 6mm diameter discs, neutralized in 30 PBS 10X (pH 7.4) then washed extensively with a 0.025% NaCl solution. After a freeze-drying step, porous composite polysaccharide scaffolds were stored at room temperature until use. Fluorescent scaffolds were prepared by adding 1% of

Fluorescein IsoThioCyanate (FITC) dextran (Sigma, St. Louis MO, USA) to the mixture before cross-linking.

ADSC cultures and osteogenic differentiation

5 Adipose Derived Stromal Cells (ADSCs) were isolated from human adipose tissue after a digestion with 0.1% (w/v) collagenase type I and cultured as previously described by Gimble et al, 2007. The remaining Stromal Vascular Fraction (SVF) was cultured in a basal medium (DMEM F12 medium (Invitrogen) supplemented with 10% (v/v) Foetal Bovine Serum (FBS) or in an osteogenic medium for inducing 10 osteoblastic differentiation of ADSCs (IMDM medium (Invitrogen), supplemented with 10% (v/v) FBS (Lonza), 10^{-8} M dexamethasone (Sigma), 50 mg/ml ascorbic acid (Sigma) and 10 mM β-glycerophosphate (Sigma)).

Experimental models in nude mice

15 *Orthotopic new bone formation* was assessed on calvaria site of athymic mice. Twelve weeks-old nude mice were anesthetized with an isoflurane/N2O mixture and were subjected to surgery to make a 4 mm diameter full thickness on the left and right parietal bone using a trephine dental burr. Disk-shaped matrices without n-HA (Group 1) and composite polysaccharide scaffold MATRI+ containing 20 n-HA (Group 2) were implanted on top of the periosteum of the parietal bone. Group 3 corresponds to mice implanted with the composite polysaccharide scaffold associated with differentiated ADSCs one week before implantation.

To study ectopic bone formation, polysaccharide-based matrices (Group 1), composite polysaccharidescaffold without cells (Group 2), or matrices previously 25 seeded with differentiated ADSCs (Group 3), were implanted into dorsal, subcutaneous spaces of athymic mice (female, 12 weeks old). Four scaffolds were implanted by mice. Bone formation was followed by a non invasive high resolution X-ray tomography (micro-CT) analysis performed 15, 30 and 60 days after implantation and by histological examination at the end of the experiment (D60).

30

High resolution X-ray tomography (micro-CT) analysis

Mice were scanned in an *in vivo* Explore Locus SP X-Ray micro-computerized tomography (micro-CT) device (General Electric) at an isotropic

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resolution of 45 μm. Reconstruction of the parietal and subcutaneous region was performed following correction of rotation centre and calibration of mineral density. Bone analysis was performed using the "Advanced Bone Analysis"™ software (GE). Thresholding of grey values was performed using the histogram tool in order to
5 separate mineralized elements from background. The density of mineralized tissue (TMD) was determined in the region of interest (ROI).

Histological evaluation

At the end of the experimental periods, mice were euthanized and samples
10 were dissected out and fixed in 3.7% (v/v) paraformaldehyde in PBS 0.1M pH 7.4. One part of the samples were decalcified and embedded in paraffin. Permanent sections of 7 micron were stained with hematoxylin and eosin and Masson trichrome dye. The other part of the samples were embedded in methylmethacrylate as described by Schenk et al, 1984. Longitudinal sections (15 μm thick) were prepared
15 using a Leica microtome and tungsten carbide blades. Sections were stained with Goldner's trichrome, Von Kossa, and observed using a Nikon Eclipse 80i microscope. Pictures were generated using a DXM 1200 C (Nikon) CCD camera.

Results

20 3D porous matrices (Figure 1) were obtained according to the methods disclosed in the PCT patent applications WO2009/047346 and WO2009/047347, with n-HA included in the starting formulation. n-HA in suspension (6.36% (w/v)) allowed an homogeneous dispersion of the HA nanoparticles in the resulting 3D matrices. The n-HA matrices contained in the dry state, 2.8+/- 0.1% (w/w) of HA.
25 The use of n-HA in the dry form instead of a n-HA suspension, induced large aggregates inside the matrices. The 3D matrices in the presence of n-HA are porous (Figure 2) with pore sizes controlled by the patented process.

Discs of 4 mm in diameter of 3D porous matrices with or without n-HA (composite scaffold) and previously seeded or not with human adipose derived
30 mesenchymal stem cells (ADSCs) were then evaluated in two mice models.

Orthotopic new bone formation on calvariae site of athymic mice revealed that only the polysaccharide-based matrices associated with n-HA (composite scaffold) induced formation of a mineralized tissue in nude mice. The porous

matrices without n-HA do not induce any mineralization within 60 days. The orthotopic new bone formation was observed with composite matrices in absence of human mesenchymal stem cells, and even if the scaffold moved out of the bone defect (Figure 3B). The mineralization occured four weeks after implantation and
5 increased with time (Figure 3C). Histological examination (Goldner's trichrome staining) revealed a fibrous tissue formed when polysaccharide-based matrices without n-HA were implanted, whereas the composite polysaccharide scaffold provides an efficient scaffold for local production of collagen network within the matrices.

10 Since the n-HA matrix (composite scaffold) was found to induce mineralization outside the bone defect, the inventors next examined its potency to stimulate ectopic bone formation. They observed that implantation of matrices without n-HA did not form any mineralized tissue at day 60. In contrast, implantation of n-HA matrices (composite polysaccharide scaffold of the invention) in
15 subcutaneous site lead to the formation of a dense mineralized tissue (Figure 4A and 4B) four weeks after implantation and without ADSCs seeding. The mineralization increased with time. Quantification indicated that the TMD of the calcified tissue was about 420 mg/cm^3 and close to the density of the implanted composite matrix in orthotopic site (Figure 4C) 60 days after implantation. Histological analysis on
20 undecalcified (Figure 4D1) and decalcified (Figure 4D2) sections of the ectopically induced mineralized tissue revealed that n-HA matrices (composite polysaccharide scaffold MATRI+) stimulated a dense collagen network and blood vessel formation as well as the recruitment of osteoblast-like cells (Figure 4D3). To visualize the level of calcification in the newly formed tissue, sections of n-HA/scaffold were stained
25 according to Von Kossa technique at day 30 and day 60 (Figure 4E). Controls were performed on the paraffin-embedded composite polysaccharide. This staining showed a well-calcified tissue of n-HA/scaffold that increases with time of implantation. To the knowledge of the inventors, no material so far in the absence of stem cells or growth factors, was able to give this effect.

30 The inventors further investigated for comparison the role of n-HA alone on non-osseous site. For this purpose, they proceed to the implantation of n-HA alone in subcutaneous site. After 15 days and 30 days, they only observed a classical reaction to a foreign body. Indeed, the histological examination of undecalcified section

(Cyanine Solochrome staining) of non-osseous site implanted with n-HA alone did not show the presence of any mineralized tissue. Implantation of n-HA alone hence did not lead to the formation of a mineralized tissue.

The inventors have thus shown that the porous composite polysaccharide 5 scaffold of the invention provides unexpected results by stimulating mineralized tissue formation in osseous site, as well as in non-osseous site, in the absence of stem cells or growth factors.

Example 2 : Implantation of the scaffold according to the invention in a non 10 osseous site in mice and osseous site in rat.

Materials and Methods

Nanohydroxyapatite and scaffold according to the invention were prepare as described in Example 1. The inventors assessed the implantation of said scaffold in 15 animal. Both the procedure and the animal treatment complied with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research. The studies were carried out in accredited animal facilities at the University of Bordeaux Segalen, under authorization (N°: 3300048 of the Ministere de l'Agriculture, France) and were approved by the Animal Research Committee of 20 Bordeaux University.

Non-osseous implantation in mice: ectopic bone formation analysis

The two different formulations of scaffolds: disk-shaped matrices without n-HA (Group 1) and the composite scaffold containing n-HA (MATRI+) (Group2) 25 (cylinders of 4 mm diameter and 6 mm depth) were inserted into subcutaneous pockets created in the dorsum of the 12-week-old Balb/c mice weighing 25–30 g (Charles River Laboratories, France). Samples were retrieved after 15, 30 and 60 days of implantation and treated for micro-CT and histological analysis. Eight samples were used for histological observation and micro-CT in each group.

30

Osseous implantation in rats: orthotopic new bone formation analysis

Medial holes, 5 mm diameter and 6 mm depth were created in both left and right femoral condyles of Wistar rats weighing 150-200 g (Charles River

Laboratories, France) using trephine dental burr. Bone pieces were removed from the bone defect, the hole was rinsed with physiological solution (NaCl 0.9 % (w/v) before introducing the scaffold within the defect. The two different scaffold formulations (matrices without n-HA and composite scaffold containing n-HA) were implanted into each bone defect. A control experiment without scaffold was also conducted. Implants were retrieved 15, 30, 60 and 90 days after surgery and treated for micro-CT and histological analysis. Six samples were used for micro-CT and histological observation in each group.

Histological procedure

At the end of each implantation period, animals were euthanized by injecting an overdose of pentobarbital sodium (Nembutal®). Immediately afterwards, the implants and surrounding tissue were retrieved, fixed with 4% (w/v) paraformaldehyde in a 0.1 M phosphate buffer and scanned with micro-CT before histology. The samples were then prepared for histological analysis. One part was decalcified, dehydrated and embedded in paraffin. Thin sections (7 μm in thickness) were prepared and stained with hematoxylin and eosin and with Goldner's Trichrome for osteoid staining. The other part were dehydrated in a graded series of ethanol, and then embedded with methylmethacrylate, which was subsequently polymerized. Ten to 15 μm transverse sections were made using a modified diamond blade microtome (Leica Microsystems SP1600, Rijswijk, The Netherlands), with four sections obtained from each implant. Sections were stained with Goldner's trichrome, Von Kossa, and observed using a Nikon Eclipse 80i microscope. Pictures were generated using a DXM 1200 C (Nikon) CCD camera.

Micro-computed tomography (micro-CT)

Micro-CT was used to develop three-dimensional images of the implants and surrounding tissue; these models were used to quantify the bone formation at each implant site. An *ex vivo* General Electric (GE) micro-CT (Explore LP Locus, General Electric), with a source voltage of 80 kV, a current of 60 μA, and 15 μm resolution, was used to acquire X-ray radiographs. *In vivo* micro-CT (General Electric) was performed with a source voltage of 150 mV, a current of 450 μA, and 45 μm resolution. After scanning, cross-sectional slices were reconstructed and 3D analyses

were performed using Microview software. Each scan result was reconstructed using the same threshold values to distinguish bone and air. Bone Mineral Content (BMC) and Bone Mineral density (BMD) volume were measured for each group and statistically analyzed using the Student's *t*-test.

5

Protein extraction from subcutaneous implants and ELISA analysis of osteogenic and angiogenic growth factors retained within the implants.

Subcutaneous implants retrieved after 2, 15, 30 and 60 days of implantation were crushed on ice with an electric crusher in PBS containing a cocktail of protease
10 inhibitors (10 µg/ml Aprotinine (Sigma), 10 µg/ml Leupeptin (Sigma) and 1 mM (4-(2-Aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF) (Fluka). The lysates were then centrifuged at 16 000 rpm and 4°C for 20 min. The supernatant was collected and then frozen at -80°C for ELISA analysis. Quantification of the protein was performed using bicinchoninic acid (BCA) protein assay kit (Thermoscientific)
15 described by Smith PK *et al.* (1985). Absorbance was read at 550 nm. There were eight matrices without n-HA (Group 1) and composite scaffold MATRI+ containing n-HA samples (Group 2), respectively for each time of implantation. The amounts of VEGF165 and BMP2 retained within the two different formulations of implants were quantified with the mouse VEGF immunoassay kit (MMV00, Quantikine ®, R&D
20 sytems), and BMP-2 immunoassay kit (DBP200, Quantikine ®, R&D sytems), respectively.

X-ray diffraction analysis

Subcutaneous implants of matrices without n-HA and composite scaffold
25 MATRI+ containing n-HA were retrieved after 15, 30 and 60 days of implantation. In order to obtain a fine powder without any organic tissues, they were treated with bleach for 2 hours at room temperature and then centrifuged to keep only the pellet. Structural properties were explored by X-ray diffraction (XRD) using PANalytical X'pert MPD diffractometer (Bragg Brentano t-t geometry) equipped with a
30 secondary monochromator and uses a copper radiation (mean λ= 1,5418 A°), the working tension and intensity were 40 kV and 40 mA, respectively.

Samples were placed on a single-crystalline wafer sample holder made of silicium. Diffractograms were all measured with the same parameters: angular range

from 8 to 80° (2t), step: 0,02°, measure time: one hour; Following X-ray diffraction (XRD) analysis of the material, phase identification through JCPDS-ICDD data (Diffract-Plus Eva Software, Bruker©) was compatible with a carbonated hydroxyapatite [Ca10(PO4)3(CO3)0,01(OH)1,3], displaying hexagonal lattice
5 parameters (a = 9.3892 A° ; c = 6.9019 A°; α=β=90° and g=120°; space group:P63/m(176)).

Statistical analysis

All data were expressed as means ± standard deviation (SD) and were
10 analyzed using standard analysis of Student's t-test. Differences were considered significant when $p \leq 0.05$ (a) or $p \leq 0.01$ (b).

Results

Two different scaffolds, matrices without n-HA (Group 1) and the composite
15 scaffold MATRI+ containing n-HA (Group 2), were implanted in Balb/c mice for 15, 30 and 60 days. Micro-CT, quantification of mineralization (BMC and BMD analysis) and histological studies were performed for both groups. Implantation of matrices without n-HA did not form any mineralized tissue from day 15 to day 60, as showed by micro-CT (Figure 5A) and BMC and BMD quantification (Figure 5B). In
20 contrast, implantation in subcutaneous site of matrices containing n-HA (without any cells and growth factors) lead to the formation of a dense mineralized tissue (Figure 5A) as quantified by BMC (Bone Mineral Content) and BMD (Bone Mineral Density) measured at each time (Figure 1B). The mineralization process starts at day 15 from the periphery of the scaffold (Figure 1A) and lead to a high and dense
25 mineralized tissue after 60 days of implantation.

From histological data, the porous n-HA matrices exhibited favorable mineralized tissue responses at D15 and D60, as demonstrated by von Kossa staining of undecalcified sections of MATRI+ (Figure 6A), compared to matrix without n-HA. Von kossa staining is high after 60 days of implantation of MATRI+, compared
30 to the same scaffold at day 15. The n-HA matrices before implantation stained with von kossa revealed a slight staining, due to the presence of the nanohydroxyapatite within the scaffold (not shown). However, the staining is much lower than that observed after 30 and 60 days of implantation.

Moreover, Goldner staining performed 60 days after implantation on decalcified sections of MATRI+ (Figure 6B), revealed, a dense fibrous collagen tissue, mainly around the implant. Some collagen tissue penetrate within the scaffold, exhibiting some lining osteoblast-like cells indicated by white arrows, in contact with 5 the scaffold and numerous vessels marked by black arrows on the histological picture. No inflammatory event was detectable with both scaffolds, whatever the time of implantation.

The XRD patterns of powder of n-HA matrices before implantation (D0) or retrieved at day 15 (D15) revealed specific peaks of hydroxyapatite at D15 on the 10 spectrum (Figure 7A). Peaks of Halite (H), probably due to the treatment of the samples with bleach, were observed in all spectra. The XRD patterns obtained at day 30 and day 60 were similar than those observed at D15 for both groups (data not shown).

The inventors also explored whether the n-HA matrices compared to matrices 15 without n-HA could interact with endogeneous osteogenic and angiogenic growth factors. They have tested two major growth factors that play a fundamental role in angiogenesis and osteogenesis, the isoform VEGF165 and BMP2, an osteoinductive factor that could, by itself, induces mineralization and bone formation. Two days of implantation, corresponding to the inflammatory phase observed following material 20 implantation, both samples retained the two growth factors but to a different extent. Strikingly, the amount of BMP2 retained on MATRI+ is 1.41 pg /µg protein extracted from the samples, while the matrix without n-HA retained only 0.12 pg /µg protein. For VEGF165, the amount retained in MATRI+ and matrix without n-HA are 0.089 pg/µg protein and 0.055 pg/µg protein, respectively. With time of 25 implantation, and during the formation of the dense mineralized tissue, the concentration of BMP2 (Figure 8A) and VEGF165 (Figure 8B) decreased in both groups, compared to data obtained after 2 days, but remains significantly higher in the MATRI+ group after 30 and 60 days of implantation, compared to matrix without n-HA.

30 The scaffolds, matrices without n-HA (Group 1) and the composite scaffold MATRI+ containing n-HA (Group 2), were implanted in a critical size bone defect of 5 mm diameter and 6 mm depth in the femoral condyle of rats, for 15, 30 and 90 days. Micro-CT, quantification of mineralization (BMC and BMD analysis) and

histological analysis were performed for both groups. As showed by micro-CT, matrices with n-HA (MATRI+) (Figure 9A) formed within the bone defect, a highly dense mineralized tissue, compared to matrix without n-HA. Mineralization increases with time of implantation as shown by quantification analysis of the BMD and BMC 5 (Figure 9B) from day 15 to day 90 of implantation. BMC and BMD in the control group (empty) remain lower than in the other groups, whatever the time of implantation.

Histological data after 90 days of implantation confirmed, a high staining by von Kossa of the matrices with n-HA (MATRI+) compared with the matrix alone 10 without n-HA or the empty group (Figure 10A). Goldner staining evidenced a fibrous tissue in the empty bone defect, while bone formation was enhanced within the MATRI+ implant after 90 days of implantation and occurred in direct contact of the MATRI+ implant (Figure 10B).

CLAIMS

1. A method for preparing a porous polysaccharide scaffold comprising the following steps:

i) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, an amount of a cross-linking agent and an amount of a porogen agent,

ii) transforming the solution into a hydrogel by placing said solution at a temperature from about 4°C to about 80°C for a sufficient time to allow the cross-linking of said amount of polysaccharide,

iii) submerging said hydrogel into a solvent, preferably an aqueous solution, and

iv) washing the porous polysaccharide scaffold obtained at step iii),

wherein the alkaline aqueous solution of step i) further comprises hydroxyapatite, preferably nano-hydroxyapatite.

2. The method according to claim 1 wherein the porogen agent is selected in the group consisting of sodium chloride, calcium chloride, ammonium carbonate, ammonium bicarbonate, calcium carbonate, sodium carbonate, and sodium bicarbonate and mixtures thereof.

3. The method according to claim 1 or 2 wherein the weight ratio of the polysaccharide to the porogen agent is in a range from 1:50 to 50:1, preferably from 1:30 to 30:1, preferably from 1:12 to 12:1, preferably 12:14.

4. A method for preparing a porous polysaccharide scaffold comprising the following steps:

a) preparing an alkaline aqueous solution comprising an amount of at least one polysaccharide, and one cross-linking agent,

b) freezing the aqueous solution of step a),

c) sublimating the frozen solution of step b),

wherein the alkaline aqueous solution of step a) further comprises hydroxyapatite preferably nano-hydroxyapatite.

and wherein step b) is performed before the cross-linking of the polysaccharide occurs in the solution of step a).

5. The method of any one of claims 1 to 4, wherein said polysaccharide is selected from the group consisting of dextran, pullulan, agar, alginic acid, starch, hyaluronic acid, inulin, heparin, fucoidan, chitosan and mixtures thereof .

6. The method of any one of claims 1 to 5, wherein said polysaccharide is a mixture of pullulan/dextran in a ratio in a range from 95:5 to 5:95, preferably in a ratio of 75:25 (w/w).

7. The method of any one of claims 1 to 5, wherein said polysaccharide is a mixture of pullulan/dextran/fucoidan in a ratio in a range from about 70:20:10 to about 50:20:30, preferably from about 70:20:10 to about 50:30:20, and most preferably in a ratio of about 73:22:5 (w/w).

8. The method according to any one of claims 1 to 7, wherein said cross-linking agent is selected from the group consisting of trisodium trimetaphosphate (STMP), phosphorus oxychloride ($POCl_3$), epichlorohydrin, formaldehydes, carbodiimides, glutaraldehydes, and mixtures thereof.

9. The method according to any one of claims 1 to 8, wherein said nano-hydroxyapatite is obtained from a solution of phosphoric acid, at a concentration comprised between 0.3 to 1M, preferably 0.6M, with a solution of calcium hydroxide, at a concentration comprised between 0.5 to 1.5M, preferably 1M and preferentially through chemical precipitation at room temperature.

10. The method according to claim 9, wherein the concentration of nano-hydroxyapatite in the alkaline solution of polysaccharide (w/v) is comprised between 0.01 and 10% (w/v), preferably between 0.1 and 0.5% (w/v), more preferably between 0.1 and 0.3% (w/v).

11. A porous polysaccharide scaffold obtainable by the method according to any one of claims 1 to 10.

12. The porous polysaccharide scaffold of claim 11, wherein the size of the pores is
5 comprised from about 1 µm to about 500 µm, preferably from about 10 µm to about 200 µm, and the porosity is from about 4% to about 75%, preferably from about 4% to about 50%.

13. A porous polysaccharide scaffold obtainable according to method of claim 1 to
10 10, for use for bone generation, preferably bone repair and/or bone development.

14. A porous polysaccharide scaffold obtainable according to method of claim 1 to 10, for use for stimulating ectopic mineralized tissue formation.

15 15. A porous polysaccharide scaffold obtainable according to method of claim 1 to 10, for use in the treatment of bone related disorders.

FIGURES

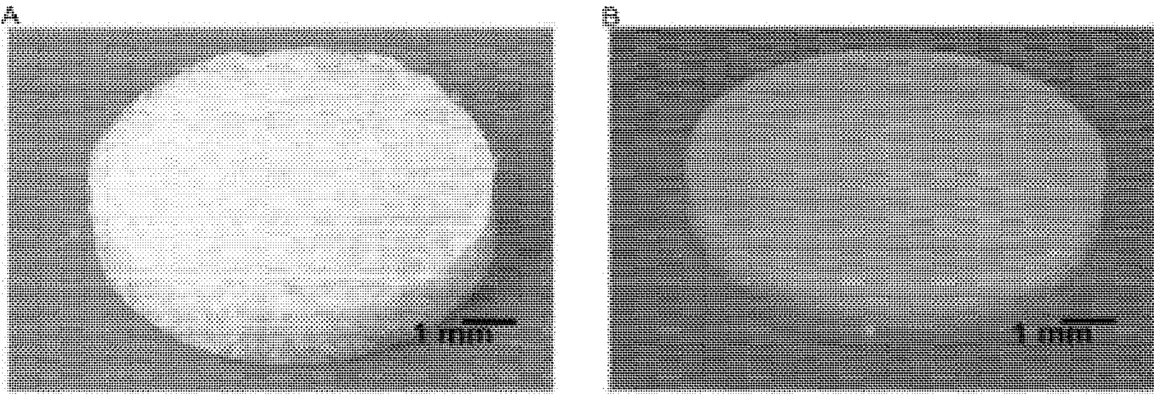


Figure 1

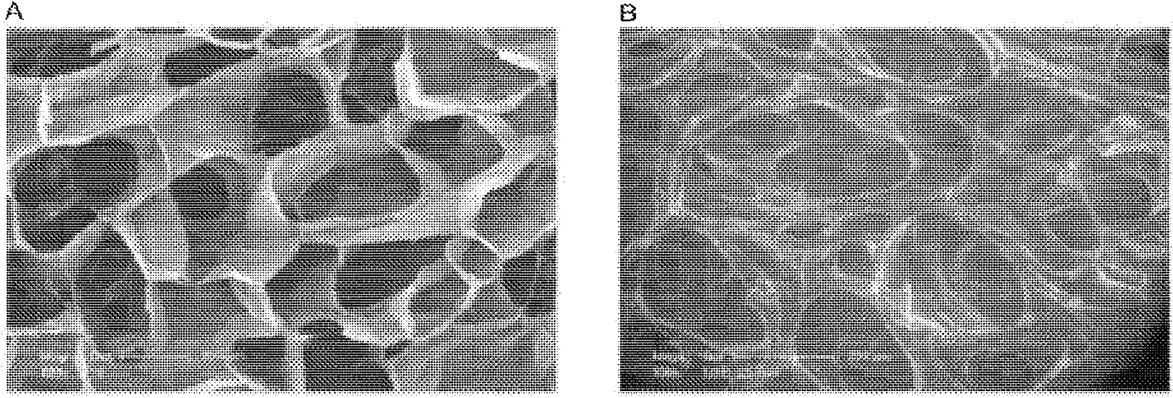


Figure 2

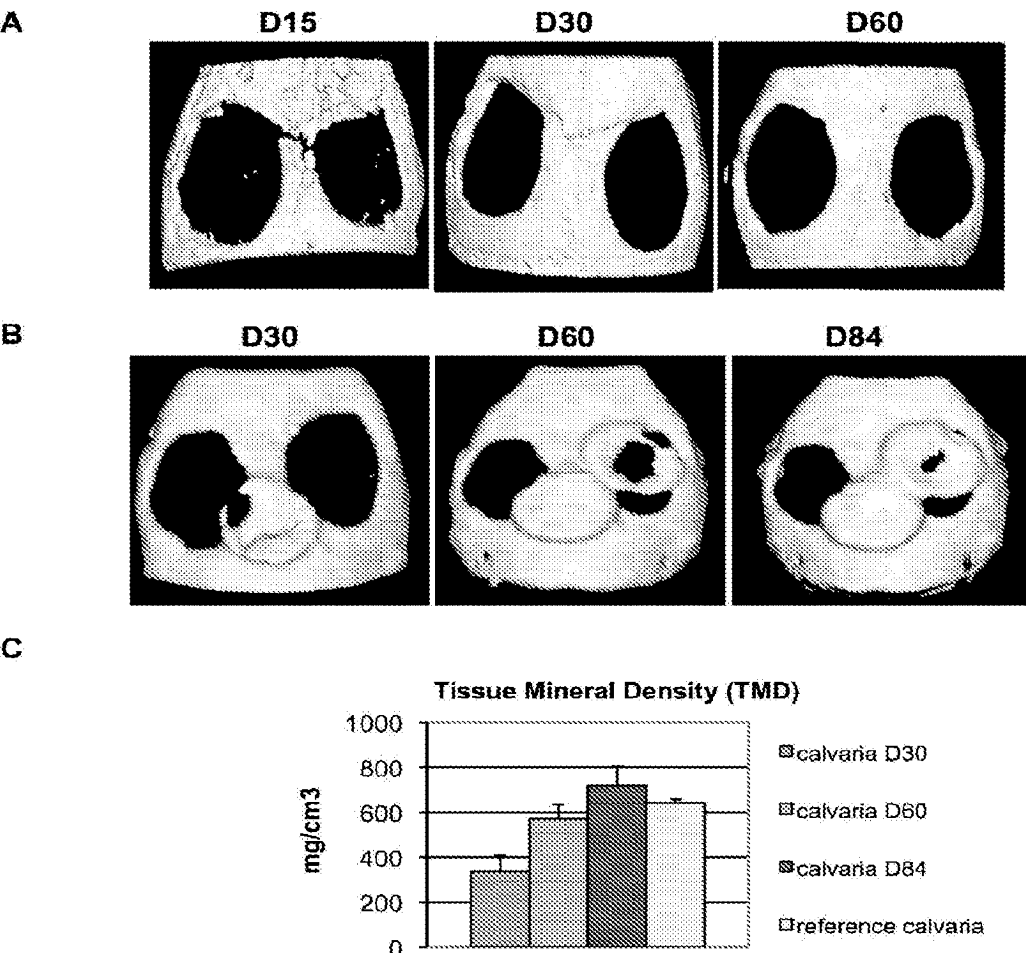
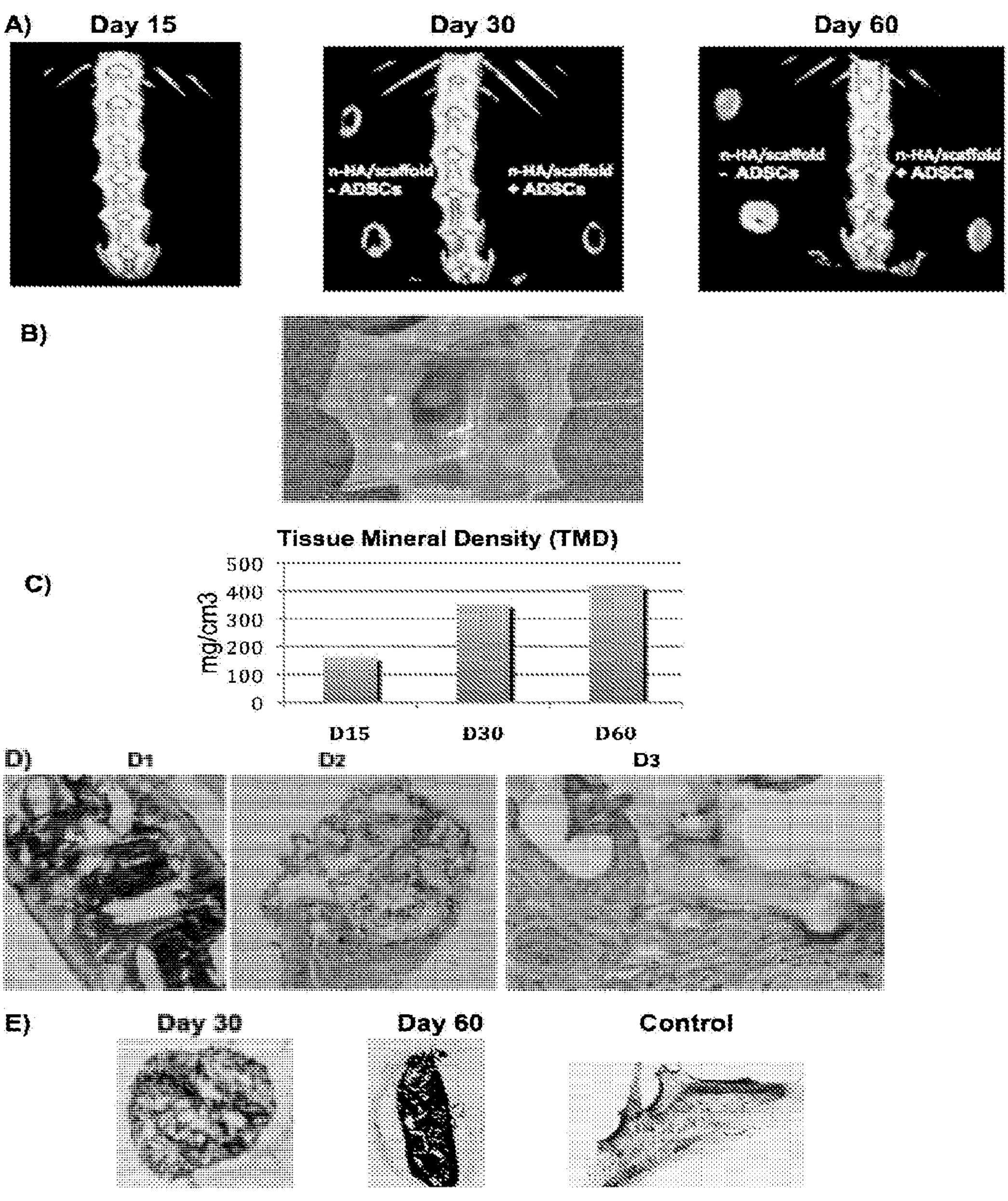


Figure 3



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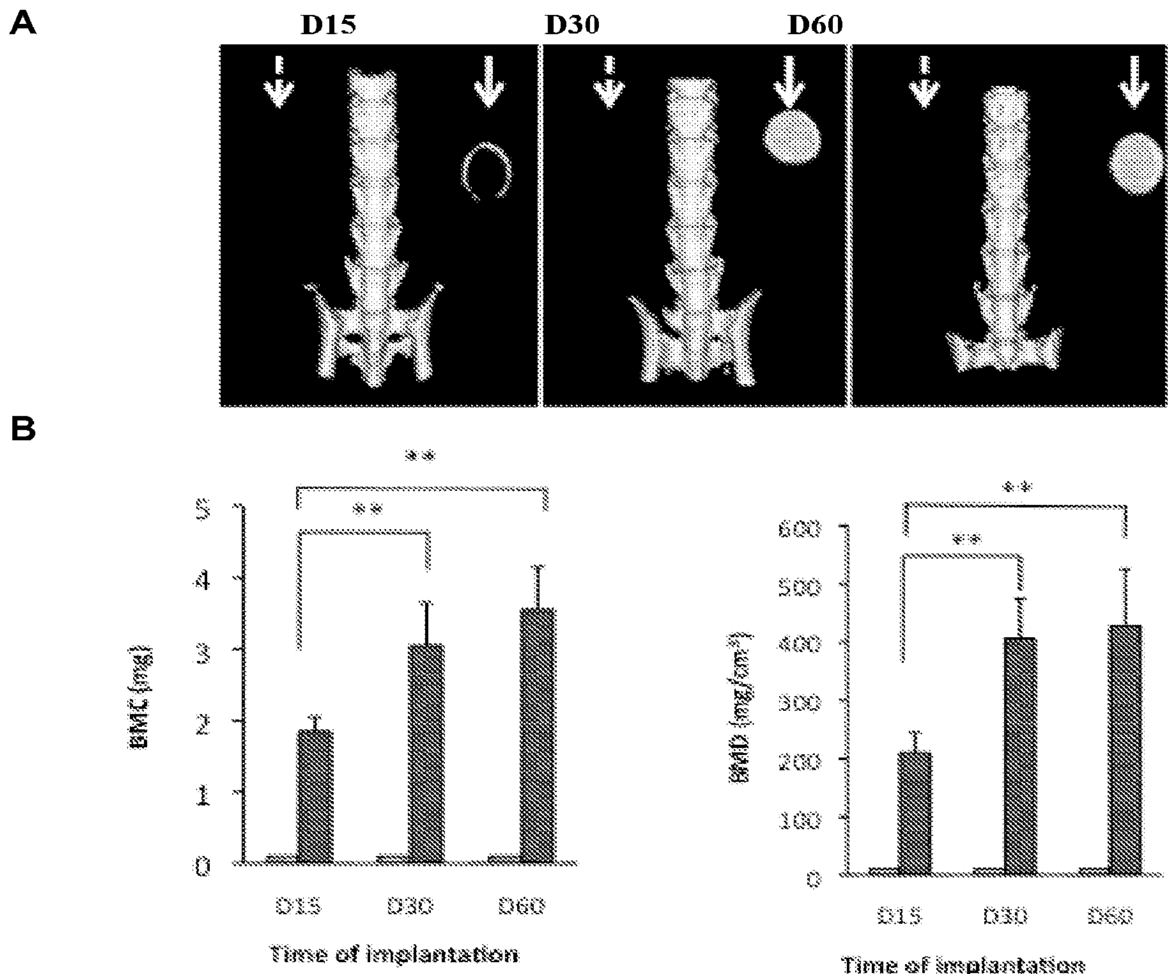
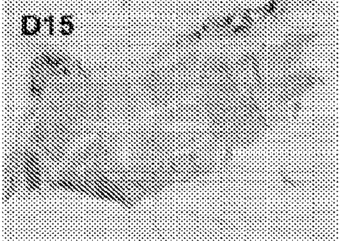
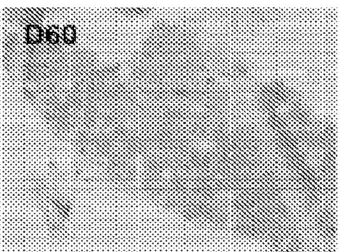


Figure 5

A

Matrix without n-HA
Von Kossa staining





Matrix + n-HA
(MATRI+)
Von Kossa staining

B

Matrix + n-HA
(MATRI+)
Goldner staining
At D60

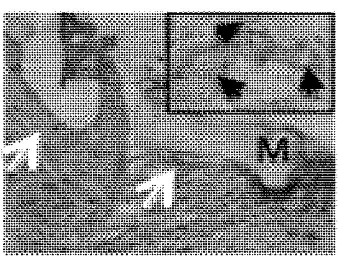


Figure 6

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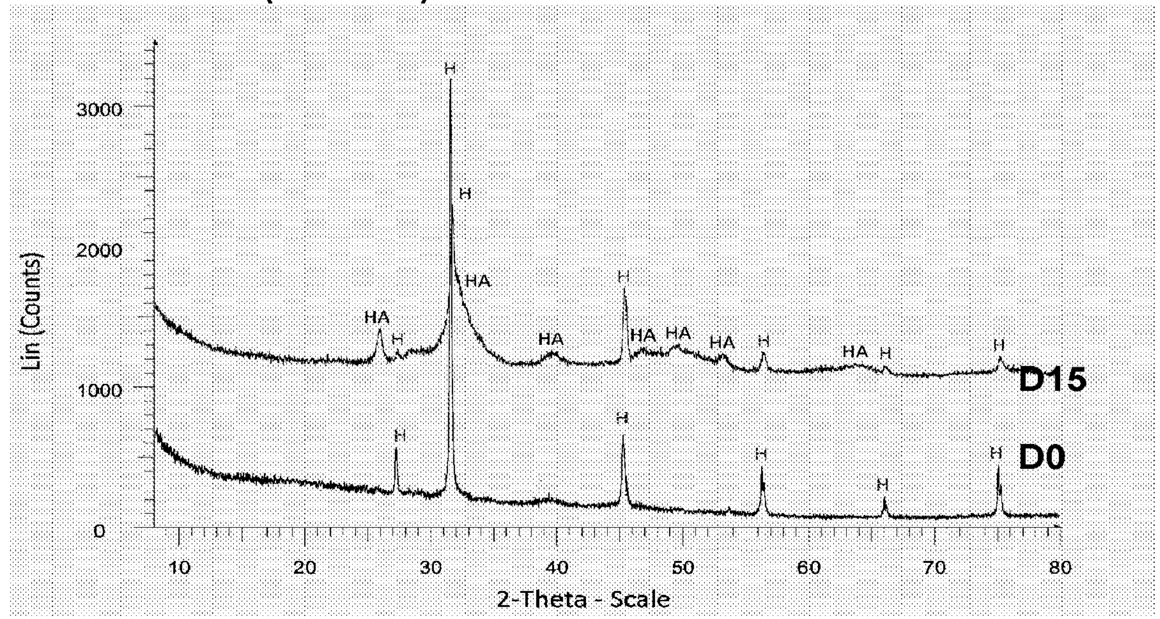
A : Matrix + n-HA (MATRI+)



B : Matrix without n-HA

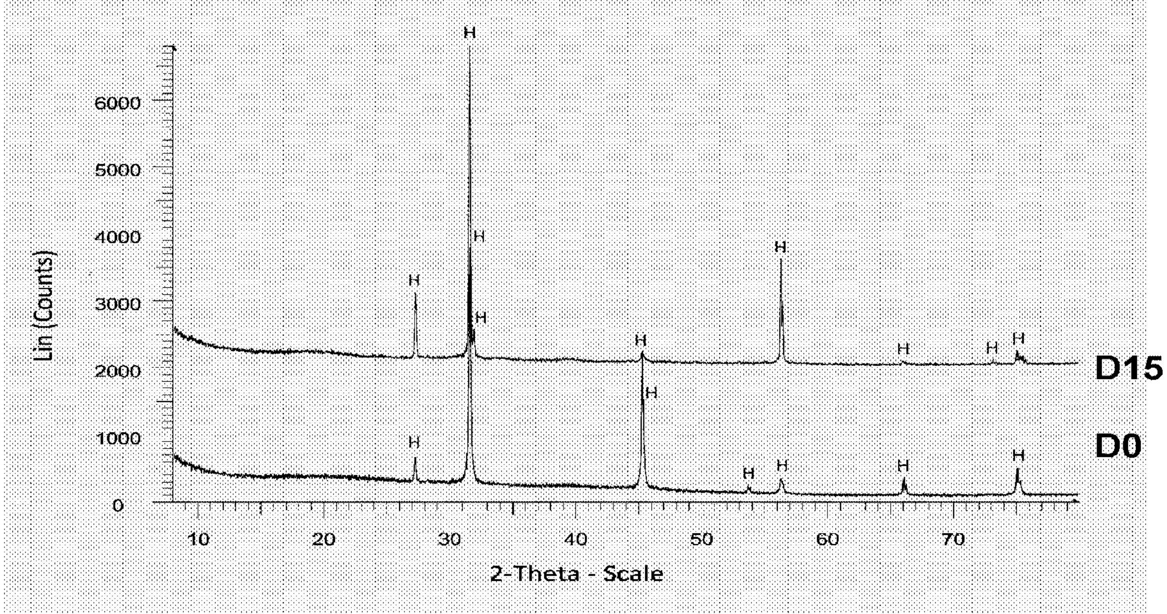
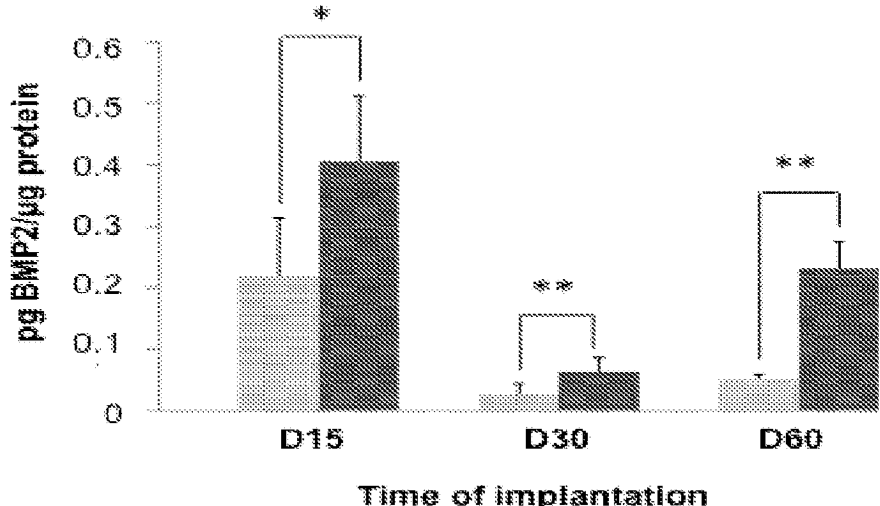


Figure 7



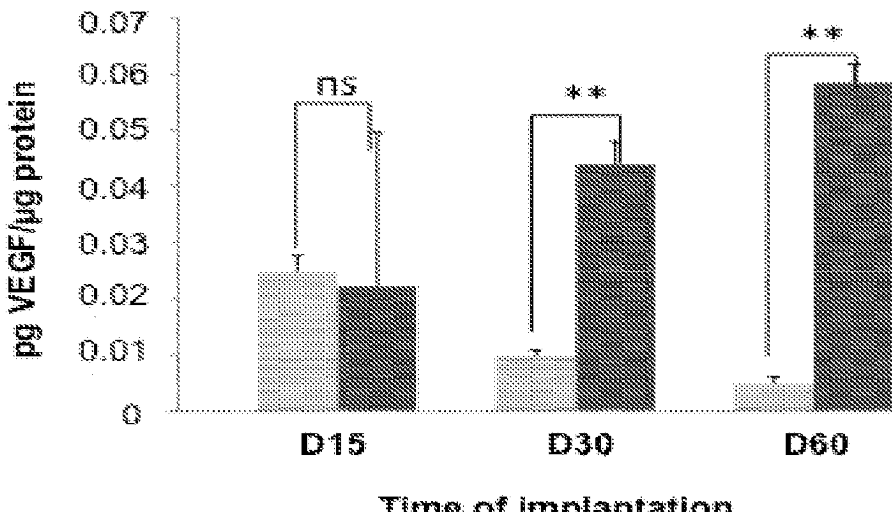
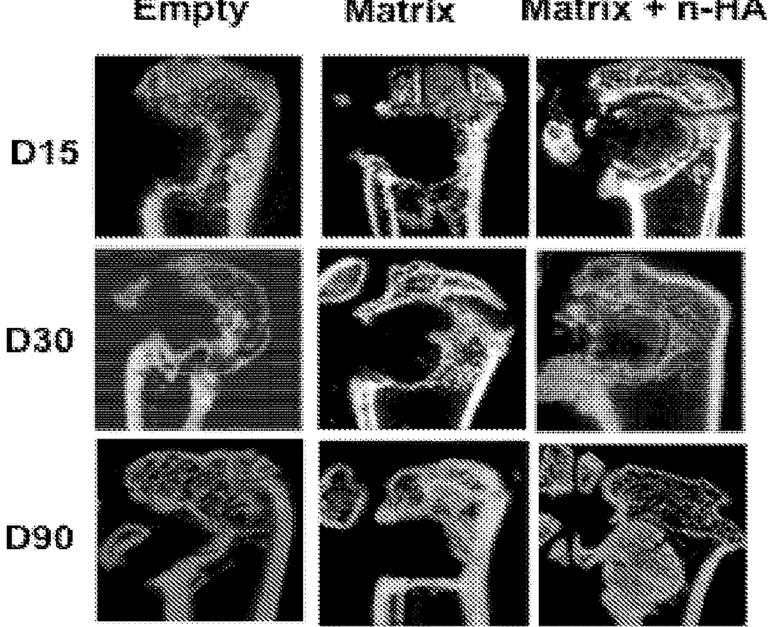


Figure 8



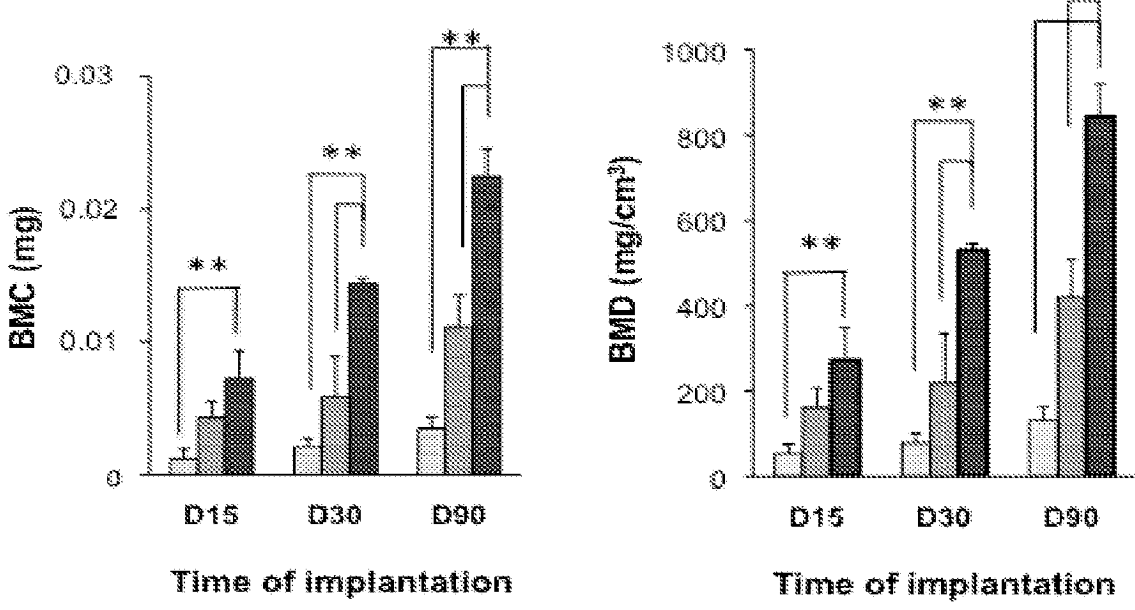


Figure 9

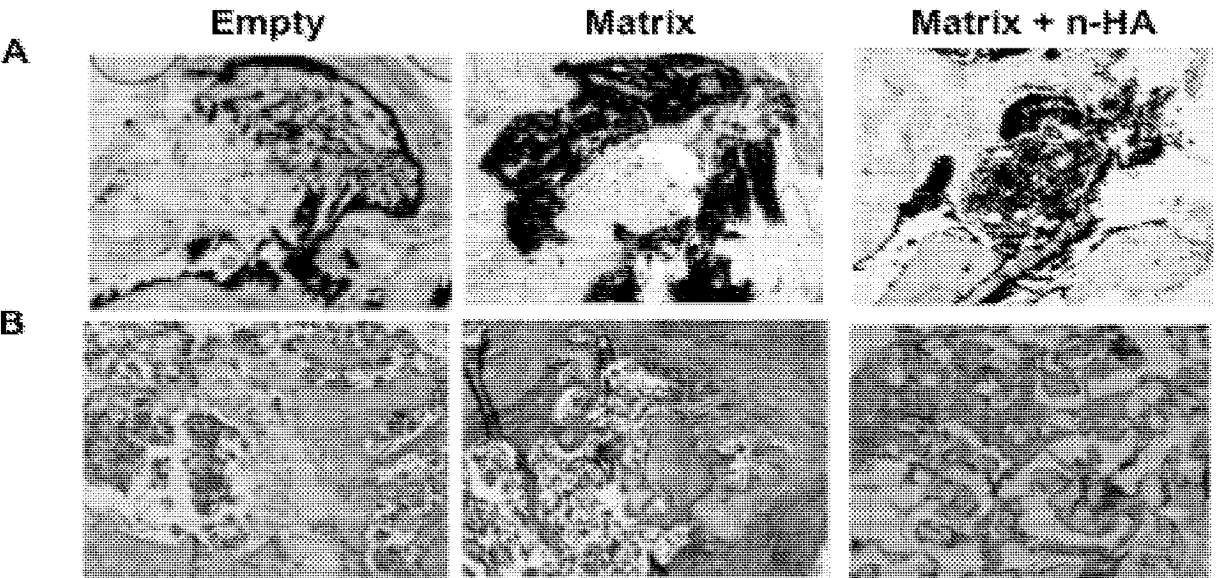


Figure 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2011/064924

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/20 A61L27/46 A61L27/56
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/153814 A1 (LIAO CHUN-JEN [TW] ET AL) 13 July 2006 (2006-07-13) paragraph [0028] - paragraph [0035] paragraphs [0038], [0042]; claims; examples	1,5, 11-15
X	WO 2009/047346 A1 (INST NAT SANTE RECH MED [FR]; UNIV PARIS 7 DENIS DIDEROT [FR]; LE VISA) 16 April 2009 (2009-04-16) page 3, lines 11-23 pages 5,6 page 14, lines 16-19; claims; examples	2,3,6-10
X	WO 2009/047347 A1 (INSERM INST NAT DE SANTE ET DE [FR]; UNIV PARIS 7 DENIS DIDEROT [FR];) 16 April 2009 (2009-04-16) page 3, line 24 - page 4, line 8; claims; examples	4

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2011	20/12/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Derrien, Anne-Cécile

2

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIUYUN, YUBAO, LI, JIANGUO: "preparation and properties of a novel bone repair composite : nano HAP/chitosan/CMC", J. MATER SCI: MAT MED, vol. 19, 1 January 2008 (2008-01-01), pages 981-987, XP002608050, DOI: 10.1007/s10856-007-3208-1 the whole document -----	1-15
A	KONG, GAO, CAO, GONG, ZHAO, ZHANG: "preparation and characterization of nano-hydroxyapatite/chitosan composite scaffolds", J. BIOMED MAT RES, vol. 75A, no. 2, 1 November 2005 (2005-11-01), pages 275-282, XP002608051, DOI: 10.1002/jbm.a.30414 the whole document -----	1-15

2

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Par v. Horizon, IPR of Patent No. 9,561,197
Page 497 of 1354

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2011/064924

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		EP 2200671 A1	30-06-2010
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		US 2010221301 A1	02-09-2010
		WO 2009047347 A1	16-04-2009

Form PCT/ISA/210 (patent family annex) (April 2005)

Electronic Acknowledgement Receipt

EFS ID:	20745483
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Lara J. Dueppen/Deborah Muench
Filer Authorized By:	Lara J. Dueppen
Attorney Docket Number:	079532-8004.US01
Receipt Date:	19-NOV-2014
Filing Date:	11-SEP-2012
Time Stamp:	17:05:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal Letter	2014-11-19_IDS_Transmittal_795328004US01.pdf	83884 f91ea2fa56d647908cdcde2f5880c2ba5a42a70a	no	3

Warnings:

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2	Information Disclosure Statement (IDS) Form (SB08)	2014-11-19_IDS_Form_PTO-1449_795328004US01.pdf	176488 588cd998cca83e49841a2b64dc169373e9520492	no	11
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3	Foreign Reference	WO2005053607A2.PDF	662823 53db1ac7d911f0a5d14213b3472a1cc735ab777f	no	12
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4	Foreign Reference	WO2006056794.PDF	2594219 edcdbb3473b6fd92756447fc3593721ae2666850	no	61
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6	Foreign Reference	WO2009087474A2.PDF	3777890 9cca4e5b3be5a80b0360dec55d494634306d1c9c	no	67
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7	Foreign Reference	WO2009134460.PDF	5092295 624e0a389b5341fcd3085836fd24cafd94549d75	no	77
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8	Foreign Reference	WO2010025303A1.PDF	5270084 84fcce502f190436bd38f075d396d715d61c6456	no	99
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9	Foreign Reference	WO2012028620.PDF	2882713 c0b0a285b0bd13be60dca656aa0e60019d11b934	no	48
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10	Non Patent Literature	Batshaw_M_1980_JPediat_97_893-900.PDF	647574 b787bf330e1e5e2f6ee675c784cb8f14527b1021	no	8

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11	Non Patent Literature	Batshaw_M_1982_NEnglJMed_306_1387-92.PDF	985789 0f04db4a221e31ebcb01ec7a5700b2fe0a4d3252	no	6
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12	Non Patent Literature	Batshaw_M1984_CurrProblPediatr_2-69.PDF	3738234 e50ebed93b7a26ba3383935975f4e7bc0168e9e4	no	35
Warnings:					
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13	Non Patent Literature	Batshaw_M_1981_Pediatrics_68_290-297.PDF	1417261 7b232b6720fe9fcece6df359d2b6c08cf0e1717d	no	10
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14	Non Patent Literature	Berry_G_2001_JPediatr_138_S56-S61.PDF	757473 0ad683e7728a63f0d24ec20999732664c71831e8	no	6
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15	Non Patent Literature	Brahe_C_2005_EurJHumGenet_13_256-259.PDF	115135 8b0fab1febdd66cad229d931f42430338d393cb9	no	4
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16	Non Patent Literature	Brunetti-Pierri_2011_HumMolGenet_20_631-640.PDF	262898 2b9223a07189ae82aedb9e49accea256a84692fc	no	10
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17	Non Patent Literature	Brusilow_SW_1979_Lancet_2_452-454.PDF	664185 7366e8e3a38ada9b9be48ab8f707a9bb341f93cf	no	4
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Warnings:					
Information:					
20	Non Patent Literature	Brusilow_SW_1991_ChurchillLivingstone_Ch5_79-94.PDF	925647 8f8919028954b9213afafbf2264a74d432db0c95	no	18
Warnings:					
Information:					
21	Non Patent Literature	Brusilow_SW_1993_JMetabolism_42_1336-1339.PDF	394026 1572d5d73bd837d8520dca6a790bd5a20ce7cc69	no	4
Warnings:					
Information:					
22	Non Patent Literature	Brusilow_SW_1995_ProgLivDis_12_293-309.PDF	766755 29e49a8537ed78b61512d5661b0a912f6c5be39d	no	17
Warnings:					
Information:					
23	Non Patent Literature	Brusilow_SW_1995_MetMolBasesInherDis_1187-1232.PDF	2259764 29892c95c3df02f1ed1b78a61edb4019b2a40f14	no	48
Warnings:					
Information:					
24	Non Patent Literature	Brusilow_SW_1996_AdvPediatr_43_127-770.PDF	1894248 a542b21db24ce5b41b663ba44aff8c9b293f4f1e	no	23
Warnings:					
Information:					
25	Non Patent Literature	Calloway_D_1971_JNutrition_101_775-786.PDF	1972162 aaf2419ad3d9c012fbf426dac7c4d9a2e9cb27f9	no	12
Warnings:					
Information:					
26	Non Patent Literature	Calloway_D_1971_JNutrition_101_205-216.PDF	1644481 dea403dcc6757379313f5b32aeb53837e61626c9	no	12
Warnings:					
Information:					
27	Non Patent Literature	Camacho_L_2007_InvestNewDrugs_25_131-138.PDF	299331 30e854ca3e83b36e1910e960e30f49f5b907ed93	no	8
Warnings:					
Information:					
28	Non Patent Literature	Chang_J_PNAS_2001_98_9808-9813.PDF	730431 ce6cd92a92687c6b9cbf74675a3533ffffd41569	no	6

Warnings:					
Information:					
29	Non Patent Literature	Chung_YL_ClinCancerRes_2000_6_1452-1458.PDF	750618 2ff26b5a3a6db9969211cd8c2f43dd2e14910501	no	8
Warnings:					
Information:					
30	Non Patent Literature	Clinical_Trial_SNCT00551200.PDF	204266 4ea1586f47c9d9868f32f18593da17c8c253fe07	no	4
Warnings:					
Information:					
31	Non Patent Literature	Comte_B_2002_JMassSpectrometry_37_581-590.PDF	683712 4aacf9f4ff1743204bd225424f98f5dccf76f5c5	no	10
Warnings:					
Information:					
32	Non Patent Literature	Cudkowicz_M_2009_AmyotrophicLateralSclerosis_10_99-1106.PDF	123014 c38180d9a9bd4706d6ca6dce4c617a9021961a49	no	8
Warnings:					
Information:					
33	Non Patent Literature	Deferrari_G_1981_KidneyInternational_20_505-510.PDF	598880 4d33336f30f5d517b402d70c702ecb27b4a0a814	no	6
Warnings:					
Information:					
34	Non Patent Literature	Diaz_GA_2011_MolGenetMetab_102_276.PDF	168683 21d1de3dcae3b6a51cf84586bc0e7b79ab521bdc	no	1
Warnings:					
Information:					
35	Non Patent Literature	Enns_GM_2007_NEngJMed_356_2282-2292.PDF	222044 8c38e91725e2fb538cc51886287f207545f9b762	no	11
Warnings:					
Information:					
36	Non Patent Literature	FDA_Label_BUPHENYL_6pages.PDF	534147 71a0a732deddb3f9a225ebb6aa26c9b50be7754e	no	6
Warnings:					
Information:					
37	Non Patent Literature	Ghabril_M_2012_DigestiveDisWeek.PDF	74328 16047e1c078dfa0ab08829e4d9691340480687e3	no	1

Warnings:					
Information:					
38	Non Patent Literature	Gropman_A_2008_MolGenetMetab_95_21-30.PDF	1349320 ad58901fe3bb63afb3bf59d284f59ecbf5e3232d	no	10
Warnings:					
Information:					
39	Non Patent Literature	Gropman_A_2010_MolGenetMetab_100_S20-S30.PDF	1566360 f2ef8b75ca076e6672115c6db4239569c794dc86	no	11
Warnings:					
Information:					
40	Non Patent Literature	Hines_P_2008_PediatrBloodCancer_50_357-359.PDF	106950 0f5c83da84f176268df5f58ec38f5743c6538b47	no	3
Warnings:					
Information:					
41	Non Patent Literature	Hogarth_P_2007_MovDisorders_22_1962-1964.PDF	57861 7c1d94ea74bd8ac5dad1ac1de21d3be6fc801908	no	3
Warnings:					
Information:					
42	Non Patent Literature	Huang_H_2012_Hepatology_56_248-258.PDF	1455550 ffe291036302ede5513c387082ac265daa701689	no	11
Warnings:					
Information:					
43	Non Patent Literature	HyperionPressRes_10232007.PDF	87009 4ebd52e262a117abe39c5b2bc90109b59740be2d	no	1
Warnings:					
Information:					
44	Non Patent Literature	HyperionPressRes_03302009.PDF	187356 7a2889a74ab5eeef376a18d1587554a549d4163c	no	3
Warnings:					
Information:					
45	Non Patent Literature	HyperionPressRes_06022009.PDF	206909 cb6a7cc26ae4bac59a6fb3536cc50d08a80fa5c9	no	3
Warnings:					
Information:					
46	Non Patent Literature	James_MO_1972_ProcRSocLond_182_25-25.PDF	1188944 45fa8296c4a5068c81f082772d0ab950c3a43ddc	no	11

Warnings:					
Information:					
47	Non Patent Literature	John_BA_ACMG_2009_ADME_Abstract.PDF	54507 af12217c09f108ef736e0193b2986d523c8c8c60	no	1
Warnings:					
Information:					
48	Non Patent Literature	Kasumov_T_2004_DrugMetabDisp_32_10-19.PDF	1167314 7ee7cefb36b54ad2745e955d678bca216b20bba0	no	10
Warnings:					
Information:					
49	Non Patent Literature	Lee_B_2008_JInheritMetabolDis_31_91_362-P.PDF	121649 da88cf35faaec62aa50b083cfedaed9190182495	no	1
Warnings:					
Information:					
50	Non Patent Literature	Lee_B_2009_ICIEM_Poster.PDF	265527 53d5a8909673dc73e4efa8bde3d01d92e5acf455	no	1
Warnings:					
Information:					
51	Non Patent Literature	Lee_B_2009_ACMG_UCD_phase_II_abstract_FINAL.PDF	44330 c57838f3af084511732ead17e5512c037b1668a5	no	1
Warnings:					
Information:					
52	Non Patent Literature	Lee_B_2009_ACMG_17pgs.PDF	991128 b0eb092c5a1742655a7f682a361290903c4c4add	no	17
Warnings:					
Information:					
53	Non Patent Literature	Lee_B_2010_MolGenetMetab_100_221-228.PDF	750661 a68aca842a29e4c2ab318dcdb0df43024560acd6	no	9
Warnings:					
Information:					
54	Non Patent Literature	Liang_K-Y_1986_Biometrika_73_13-22.PDF	588858 fca9e1c6564162ecc877106021b95c5f8e065d70	no	10
Warnings:					
Information:					
55	Non Patent Literature	Lichter-Konecki_2011_MolGenetMetab_103_323-329.PDF	705287 95395548b41e64b01837ff15f8bfb5a15e0d7bee	no	7

Warnings:					
Information:					
56	Non Patent Literature	MacArthur_R_2004_MolGenetMetab_81_S67-S73.PDF	561963 746a835e6027bdc8736af5b80d226dbb8662c7e6	no	7
Warnings:					
Information:					
57	Non Patent Literature	Maestri_N_1992_JPediatr_121_259-261.PDF	126144 ea0ee41d1f3a1418566543be32e48848e8344550	no	3
Warnings:					
Information:					
58	Non Patent Literature	McGuire_B_2008_LiverInternational_28_743.PDF	61061 8f4d9eb4c227893220fae79d10a453589334eaef	no	1
Warnings:					
Information:					
59	Non Patent Literature	McGuire_B_2009_DDW_Poster.PDF	262060 26f9ff8c526e42dedfeeaee37dc1eeada5728b65	no	1
Warnings:					
Information:					
60	Non Patent Literature	McGuire_B_2010_Hepatology_51_2077-2085.PDF	815101 eeff9a09c15197cca51da025eefc8c91c5d33f25	no	9
Warnings:					
Information:					
		Total Files Size (in bytes):	58503613		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: BRUCE SCHARSCHMIDT ET AL.	CONF. NO: 1957
APPLICATION NO.: 13/610,580	ART UNIT: 1765
FILED: SEPTEMBER 11, 2012	
FOR: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS	

Information Disclosure Statement Within Three Months of Application Filing or Before First Action – 37 C.F.R. § 1.97(b)

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. Timing of Submission

This information disclosure is being filed within three months of the filing date of this application or date of entry into the national stage of an international application or before the mailing date of a first Office action on the merits, whichever occurs last [37 C.F.R. § 1.97(b)]. The references listed on the enclosed Form PTO-1449 (modified) may be material to the examination of this application; the Examiner is requested to make them of record in the application.

2. Cited Information

☒ Copies of the following references are enclosed:

- ☒ All cited references
- ☐ References marked by asterisks
- ☐ The following:

☐ Copies of the following references can be found in parent U.S. Application No. <>:

- ☐ All cited references
- ☐ All references
- ☐ The following:

☒ This application was filed after 30 June 2003 and no copies of U.S. patents nor published applications are enclosed (See Notice of Deputy Commissioner Kunin on 11 July 2003).

☐ The following references are not in English. For each such reference, the undersigned has enclosed (i) a translation of the reference; (ii) a copy of a communication from a foreign patent office or International Searching Authority citing the reference, (iii) a copy of a reference which appears to be an English-language counterpart, or (iv) an English-language abstract for the reference prepared by a third party. Applicant has not verified that the translation, English-language counterpart or third-party abstract is an accurate representation of the teachings of the non-English reference, though, and reserves the right to demonstrate otherwise.

- ☐ All cited references
- ☐ References marked by ampersands
- ☐ The following:

3. <u>Effect of Information Disclosure Statement (37 C.F.R. § 1.97(h))</u>

This Information Disclosure Statement is not to be construed as a representation that: (i) a search has been made; (ii) additional information material to the examination of this application does not exist; (iii) the information, protocols, results and the like reported by third parties are accurate or enabling; or (iv) the cited information is, or is considered to be, material to patentability. In addition, applicant does not admit that any enclosed item of information constitutes prior art to the subject invention and specifically reserves the right to demonstrate that any such reference is not prior art.

4. <u>Fee Payment</u>

No fees are believed due because this Information Disclosure Statement is being filed before the mailing date of the first Office Action.

☐ Applicant further submits that no fee is due in light of the following certification under 37 C.F.R. § 1.97(e) (check only one):

- ☐ In accordance with 37 C.F.R. § 1.97(e)(1), the undersigned hereby states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart

foreign application not more than three months prior to the filing of this statement; or

☐ In accordance with 37 C.F.R. § 1.97(e)(2), the undersigned hereby states that no item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart foreign application, or, to the knowledge of the person signing the certification after making reasonable inquiry, was known to any individual designated in 37 C.F.R. § 1.56(c), more than three months prior to the filing of this statement.

However, should the Commissioner determine that fees are due in order for this Information Disclosure Statement to be considered, the Commissioner is hereby authorized to charge such fees to Deposit Account No. 50-2586.

5. Patent Term Adjustment (37 C.F.R. § 1.704(d))

☐ The undersigned states that each item of information submitted herewith was cited in a communication from a foreign patent office in a counterpart application and that this communication was not received by any individual designated in 37 C.F.R. § 1.56(c) more than thirty days prior to the filing of this statement. 37 C.F.R. § 1.704(d).

Respectfully submitted,
Perkins Coie LLP

Date: November 19, 2014

/Patrick D. Morris/
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PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

MORRISON & FOERSTER LLP LEW
Attn. Smith, Michael G.
12531 High Bluff Drive, Suite 100
San Diego CA 92130-2040
ETATS-UNIS D'AMERIQUE

DOCKETED: RESP TO ISR
REMINDER: 1/30/10
FINAL DUEDATE: 2/28/10

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (*day/month/year*) 30/12/2009
Applicant's or agent's file reference 643982000141	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2009/055256	International filing date (*day/month/year*) 27/08/2009
Applicant Hyperion Therapeutics	DOCKETED: RESP TO WO / CH. II DEMAND. REMINDER: 3/29/10 FINAL DUEDATE: 6/29/10

1. [x] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the International Search Report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Fascimile No.: (41-22) 338.82.70

   For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Monika Langerova

Form PCT/ISA/220 (July 2009) *(See notes on accompanying sheet)*

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annex B).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, International Phase, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet or sheets containing a complete set of claims in replacement of all the claims previously filed must be submitted.

Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively in Arabic numerals (Section 205(a)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the *PCT Applicant's Guide*.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report and the written opinion of the International Searching Authority, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims,description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only (see *PCT Applicant's Guide*, Annex B).

The attention of the applicant is drawn to the fact that amendments to the claims under Article 19 are not allowed where the International Searching Authority has declared, under Article 17(2), that no international search report would be established (see *PCT Applicant's Guide*, International Phase, paragraph 296).

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet or sheets containing a complete set of claims in replacement of all the claims previously filed must be submitted.

Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively in Arabic numerals (Section 205(a)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

. ATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 643982000141	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2009/055256	International filing date *(day/month/year)* 27/08/2009	(Earliest) Priority Date *(day/month/year)* 29/08/2008
Applicant Hyperion Therapeutics		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language,** the international search was carried out on the basis of:
      [X] the international application in the language in which it was filed
      [ ] a translation of the international application into ____________, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
   b. [ ] This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. [ ] With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. [ ] **Certain claims were found unsearchable** (See Box No. II)
3. [ ] **Unity of invention is lacking** (see Box No III)
4. With regard to the **title,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established by this Authority to read as follows:
5. With regard to the **abstract,**
   [X] the text is approved as submitted by the applicant
   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority
6. With regard to the **drawings,**
   a. the figure of the **drawings** to be published with the abstract is Figure No. 1
      [ ] as suggested by the applicant
      [X] as selected by this Authority, because the applicant failed to suggest a figure
      [ ] as selected by this Authority, because this figure better characterizes the invention
   b. [ ] none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/055256

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01), pages 1117-1121, XP009127277 ISSN: 0031-3998	30-33
Y	the whole document	1-29

-/--

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 December 2009	30/12/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Fax: (+31–70) 340–3016	Moreno de Vega, C

1

Form PCT/ISA/210 (second sheet) (April 2005)

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 514 of 1354

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/055256

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MACARTHUR ROBERT B ET AL: "Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers" MOLECULAR GENETICS AND METABOLISM, ACADEMIC PRESS, SAN DIEGO, CA, US, vol. 81, no. Suppl.1, 1 April 2004 (2004-04-01), pages S67-S73, XP009127291 ISSN: 1096-7192 the whole document	1-33
Y	TANNER L M ET AL: "Nutrient intake in lysinuric protein intolerance" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER ACADEMIC PUBLISHERS, DO, vol. 30, no. 5, 21 June 2007 (2007-06-21), pages 716-721, XP019548954 ISSN: 1573-2665 page 716 - page 717	1-33
X	LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01), page 91, XP009127344 ISSN: 0141-8955	1-5, 15-17, 19-22, 30-33
Y	the whole document	1-33

1

PATENT COOPERATION TREATY

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JAN 05 2010
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SAN DIEGO DOCKETING

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2009/055256	27.08.2009	29.08.2008

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
Hyperion Therapeutics

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Moreno de Vega, C Telephone No. +49 89 2399-7486

Form PCT/ISA/237 (Cover Sheet) (April 2005)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in electronic form.

   ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-14, 18, 23-29
	No:	Claims	1-5, 15-17, 19-22, 30-33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-33
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01) , pages 1117-1121, XP009127277 ISSN: 0031-3998

D4 LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01) , page 91, XP009127344 ISSN: 0141-8955

1 Claims 12-18 and 26-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT.

The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Document D1 discloses the study of the metabolic changes caused by benzoate and phenylacetate and their pharmacokinetics in the treatment of an urea cycle disorder, the lysinuric protein intolerance, and that 54% of the single phenylacetate dose was excreted in urine as phenylacetylglutamine in 24 hours after the load. This document appears to be novelty destroying for claims 30-33.

3 Document D2 discloses the use of HPN-100, a PBA (phenylbutyrate) prodrug, in the treatment of urea cycle disorders and the assessment of its metabolites and the urinary excretion of nitrogen by phenylacetylglutamine measurement. This document appears to be novelty destroying for claims 1-5, 15-17, 19-22 and 30-33.

Thus, claims 1-5, 15-17, 19-22 and 30-33 do not meet the requirements of Article 33(2) PCT.

4 Considering D2 as the most relevant prior art, the technical problem to be solved by claims 6-14, 18 and 23-29 is the provision of a method for determining an effective dosis of HPN-100 for a patient in need of treatment for a nitrogen retention disorder. The solution proposed by said claims is based on a treatment with a known compound which is prodrug of compounds known from D1 to have an excretion rate as disclosed in the claims, and therefore cannot be considered to be inventive.

Thus, claims 1-33 do not meet the requirements of Article 33(3) PCT.

Possible steps after receipt of the international search report (ISR) and written opinion of the International Searching Authority (WO-ISA)

General information	For all international applications filed on or after 01/01/2004 the competent ISA will establish an ISR. It is accompanied by the WO-ISA. Unlike the former written opinion of the IPEA (Rule 66.2 PCT), the WO-ISA is not meant to be responded to, but to be taken into consideration for further procedural steps. This document explains about the possibilities.
Amending claims under Art. 19 PCT	Within 2 months after the date of mailing of the ISR and the WO-ISA the applicant may file amended claims under Art. 19 PCT directly with the International Bureau of WIPO. The PCT reform of 2004 did not change this procedure. For further information please see Rule 46 PCT as well as form PCT/ISA/220 and the corresponding Notes to form PCT/ISA/220.
Filing a demand for international preliminary examination	In principle, the WO-ISA will be considered as the written opinion of the IPEA. This should, in many cases, make it unnecessary to file a demand for international preliminary examination. If the applicant nevertheless wishes to file a demand this must be done before expiry of 3 months after the date of mailing of the ISR/WO-ISA or 22 months after priority date, whichever expires later (Rule 54bis PCT). Amendments under Art. 34 PCT can be filed with the IPEA as before, normally at the same time as filing the demand (Rule 66.1 (b) PCT). If a demand for international preliminary examination is filed and no comments/amendments have been received the WO-ISA will be transformed by the IPEA into an IPRP (International Preliminary Report on Patentability) which would merely reflect the content of the WO-ISA. The demand can still be withdrawn (Art. 37 PCT).
Filing informal comments	After receipt of the ISR/WO-ISA the applicant may file informal comments on the WO-ISA directly with the International Bureau of WIPO. These will be communicated to the designated Offices together with the IPRP (International Preliminary Report on Patentability) at 30 months from the priority date. Please also refer to the next box.
End of the international phase	At the end of the international phase the International Bureau of WIPO will transform the WO-ISA or, if a demand was filed, the written opinion of the IPEA into the IPRP, which will then be transmitted together with possible informal comments to the designated Offices. The IPRP replaces the former IPER (international preliminary examination report).
Relevant PCT Rules and more information	Rule 43 PCT, Rule 43bis PCT, Rule 44 PCT, Rule 44bis PCT, PCT Newsletter 12/2003, OJ 11/2003, OJ 12/2003

BNSDOCID: <XS_____200704010CK_I_>

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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)

(PCT Rule 44bis.1(c))

To:

REVIEWED
By Tom Herrera at 1:53 pm, Apr 11, 2014

079532-8003.WO00
PDM/CDK

MORRIS, Patrick D.
Perkins Coie LLP
P.O. Box 1208
Seattle, Washington 98111-1208
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)* 10 April 2014 (10.04.2014)	
Applicant's or agent's file reference 795328003WO	**IMPORTANT NOTICE**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2012/028620	09 March 2012 (09.03.2012)	30 September 2011 (30.09.2011)

Applicant

HYPERION THERAPEUTICS, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt03.pct@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 795328003WO	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2012/028620	International filing date *(day/month/year)* 09 March 2012 (09.03.2012)	Priority date *(day/month/year)* 30 September 2011 (30.09.2011)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant HYPERION THERAPEUTICS, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 April 2014 (01.04.2014)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Philippe Bécamel e-mail: pt03.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	20 JUN 2012

Applicant's or agent's file reference	FOR FURTHER ACTION
795328003WO	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2012/028620	09 March 2012	30 September 2011

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2

Applicant SCHARSCHMIDT, BRUCE

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	04 June 2012	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2012/028620

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [x] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
   - [ ] on paper
   - [ ] in electronic form

   b. (time)
   - [ ] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8	YES
	Claims	1-7, 9-12	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-7 and 9-12 lack novelty under PCT Article 33(2) as being anticipated by Scharschmidt et al. (hereafter Scharschmidt).

Regarding claim 1, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]);
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26. 1 umol/L), Para. [0201]).

Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0064]) comprising:

a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]);

Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]).

Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]).

Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPEA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]).

Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]).

Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]).

Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects is between 26 to 35 umol/L, Para. [0094]).

Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising:
c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and
e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Claim 8 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al.(hereafter Scharschmidt) in view of Ennis et al. (hereafter Ennis).

Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). It would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with lethal urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16).

Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Form PCT/ISA/237 (Supplemental Box) (July 2011)

079532-8003.W000
PDM / CDK

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247
USA

RECEIVED
PATENT DOCKETING
SEP 09 2013
PERKINS COIE LLP

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)
(PCT Rule 71.1)

Date of mailing (*day/month/year*)	04 SEP 2013

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
79532.8003.WO00	

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US12/28620	09 March 2012 (09.03.2012)	30 September 2011 (30.09.2011)

Applicant
HYPERION THERAPEUTICS, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary report on patentability and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary report on patentability. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the *PCT Applicant's Guide.*

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed invention is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the IPEA/ US	Authorized officer
Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	SAVITHA RAO Telephone No.

Form PCT/IPEA/416 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 79532.8003.WO00	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US12/28620	International filing date *(day/month/year)* 09 March 2012 (09.03.2012)	Priority date *(day/month/year)* 30 September 2011 (30.09.2011)
International Patent Classification (IPC) or national classification and IPC IPC: **A61B 5/11**(2006.01);**A61K 31/192**(2006.01);**A61K 49/00**(2006.01),**A61P 13/00** USPC: 424/9.2,514/568,600/322		
Applicant HYPERION THERAPEUTICS, INC.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:

   a. ☑ *(sent to the applicant and to the International Bureau)* a total of 11 sheets, as follows:

   ☑ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).

   ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4*bis*, 70.2(e), 70.16 and 91.2).

   ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No.I and the Supplemental Box (see Rule 70.16(b)).

   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
   _____ containing a sequence listing, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*bis* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
07 December 2013 (07.12.2013)	22 August 2013 (22.08.2013)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (571) 273-3201	Authorized officer SAVITHA RAO Telephone No.

Form PCT/IPEA/409 (cover sheet) (July 2011)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/US12/28620

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:
   - [x] the international application in the language in which it was filed.
   - [ ] a translation of the international application into English which is the language of a translation furnished for the purposes of:
     - [ ] international search (Rules 12.3(a) and 23.1(b)).
     - [ ] publication of the international application (Rule 12.4(a)).
     - [ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a) and (b)).

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
   - [ ] the international application as originally filed/furnished
   - [x] the description:
     pages 1-30 as originally filed/furnished
     pages* NONE received by this Authority on ______
     pages* NONE received by this Authority on ______
   - [x] the claims:
     pages NONE as originally filed/furnished
     pages* NONE as amended (together with any statement) under Article 19
     pages* 31-32 received by this Authority on 07 DECEMBER 2012 (07.12.2012)
     pages* NONE received by this Authority on ______
   - [x] the drawings:
     pages 1-3 as originally filed/furnished
     pages* NONE received by this Authority on ______
     pages* NONE received by this Authority on ______
   - [ ] a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:
   - [ ] the description, pages ______
   - [ ] the claims, Nos. ______
   - [ ] the drawings, sheets/figs ______
   - [ ] the sequence listing (*specify*): ______

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and(*c-bis*)):
   - [ ] the description, pages ______
   - [ ] the claims, Nos. ______
   - [ ] the drawings, sheets/figs ______
   - [ ] the sequence listing (*specify*): ______

5. [ ] This report has been established:
   - [ ] taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - [ ] without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.4*bis*) and 70.2(e)).

6. [ ] Supplementary international search report(s) from Authority(ies) ______ has/have been received and taken into account in establishing this report (Rule 45*bis*.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Form PCT/IPEA/409 (Box No. I) (July 2011)

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1-12	YES
	Claims NONE	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-12	NO
Industrial Applicability (IA)	Claims 1-12	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)
Please See Continuation Sheet

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

V. 2. Citations and Explanations:
Claim 1-12 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al. in view of Ennis et al.

Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising: a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]). Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0064]) comprising: a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon

Supplemental Box

ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]. Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising: a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximately 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]). Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]); Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]). Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]). Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl td-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPEA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]). Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]). Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]). Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects are between 26 to 35 umol/L, Para. [0094]). Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising: c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).
As such the teachings of Scharschmidt et al. clearly provides a person of ordinary skill in the art explicit suggestions and motivation to develop the instantly claimed methods. Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). it would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with leth'al urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16). Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

------------------- NEW CITATIONS -------------------

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 535 of 1354

CMP / MYD / FRGN TD

PCT/US2009/030362

PATENT COOPERATION TREATY

ADVANCE E-MAIL

PCT

From the INTERNATIONAL BUREAU

(R)

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)

(PCT Rule 44bis.1(c))

To:

SMITH, Michael, G.
Morrison & Foerster LLP
Suite 100
12531 High Bluff Drive
San Diego, CA 92130-2040
ETATS-UNIS D'AMERIQUE

PA

RECEIVED
MAR 22 2011
MORRISON & FOERSTER
SAN DIEGO DOCKETING

Date of mailing *(day/month/year)* 10 March 2011 (10.03.2011)		
Applicant's or agent's file reference 643982000140	IMPORTANT NOTICE	
International application No. PCT/US2009/030362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)	Priority date *(day/month/year)* 29 August 2008 (29.08.2008)
Applicant UCYCLYD PHARMA, INC. et al		

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

RECEIVED
MAR 29 2011
Morrison & Foerster LLP
Palo Alto

EM

PREVIOUSLY DOCKETED KH

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Simin Baharlou
Facsimile No. +41 22 338 82 70	e-mail: pt09.pct@wipo.int

Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 643982000140	FOR FURTHER ACTION	See item 4 below
International application No. PCT/US2009/030362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)	Priority date *(day/month/year)* 29 August 2008 (29.08.2008)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant UCYCLYD PHARMA, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 March 2011 (01.03.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Simin Baharlou
Facsimile No. +41 22 338 82 70	e-mail: pt09.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) 02 MAR 2009

Applicant's or agent's file reference		FOR FURTHER ACTION
643982000140		See paragraph 2 below
International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US 09/30362	07 January 2009 (07.01.2009)	29 April 2008 (29.04.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570

Applicant HYPERION THERAPEUTICS

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 February 2009 (24.02.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:
   a. type of material
      - [ ] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [ ] on paper
      - [ ] in electronic form

   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [ ] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-29	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-29	NO
Industrial applicability (IA)	Claims	1-29	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0229948 A1 to Summar, et al. (hereinafter "Summar") in view of US 4,284,647 A to Brusilow, et al. (hereinafter "Brusilow-647").

Regarding claim 1, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary PAGN output taught in Brusilow-647, in order to determine the effective dosage of HPN-100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Regarding claim 2, Brusilow-647 further teaches the method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine (Fig. 3; col 4, ln 35-46).

Regarding claim 3, Summar further teaches the method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy (para [0029]).

Regarding claim 4, Summar further teaches the method of claim 1, wherein administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Summar does not explicitly teach achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; col 4, ln 64-68).

Regarding claim 5, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary phenylacetyl glutamine output and total urinary nitrogen (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary phenylacetyl glutamine output taught in Brusilow-647, in order to determine the effective dosage of HPN -100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Claims 6-8, 19-22 and 28 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of US 5,968,979 A to Brusilow (hereinafter "Brusilow-979").

Regarding claim 6, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach HPN-100 conversion to PAGN. However, Brusilow-979 teaches HPN-100 conversion to PAGN (col 4, ln 1-26, "n = 2"; col 5, ln 3-15; col 5, ln 29-35). It would have been obvious to one of ordinary skill in the art to calculate the dosage of HPN-100 based on a utilization efficiency for HPN-100 conversion into PAGN of about 60% to about 75%, in order to achieve effective plasma concentrations of phenylacetate for acetylation of glutamine, by routine experimentation, as Brusilow-979 teaches the intermediate formation of phenylacetate that produces PAGN by acetylation of glutamine (col 3, ln 3-7).

==============================Continued in Supplemental Box==

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 7, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 6. Neither Summar nor Brusilow teaches a method wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100, in order to effectively deplete accumulated nitrogen via acetylation of glutamine, as taught in Brusilow-979 (col 3, ln 3-7), as the plasma level of glutamine would be likely to depend on the protein intake of the patient, as taught in Brusilow-979 (col 1, ln 41-45).

Regarding claim 8, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 7. Neither Summar nor Brusilow-979 teaches a method wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity. However, it would have been obvious to one of ordinary skill in the art to reduce the dosage to account for the patient's residual urea synthesis capacity, by routine experimentation, as urea synthesis would be likely to lesson the plasma nitrogen accumulation, as taught in Brusilow-979 (col 1, ln 27-34).

Regarding claim 19, Brusilow-979 teaches a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA (col 2, ln 25-34; col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach determining the AUC and Cmax for PBA when the patient receives the PBA prodrug. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), by comparing the AUC and Cmax for the prodrug with those when the patient receives an equimolar amount of PBA, by routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Regarding claim 20, Brusilow-979 further teaches the method of claim 19, wherein the PBA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claims 21 and 22, Brusilow-979 (col 2, ln 25-34; col 3, ln 42-59) and Summar (para [0035]) teach the method of claim 20. Neither Brusilow nor Summar teaches a method wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20% or by at least 30%. However, it would have been obvious to one of ordinary skill in the art to expect AUC for PBA exposure to be lower by 20-30% for PBA prodrug than with PBA, in order to treat UCD with minimum exposure to PBA, as taught in Brusilow-979 (col 2, ln 15-24), as the triglyceride of PBA would be likely to produce a stable drug level by gradual beta-oxidation of the prodrug, as taught in Brusilow-979 (col 2, ln 25-34).

Regarding claim 28, Brusilow-979 teaches a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach the AUC or Cmax of PBA. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug so that AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), through routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Claims 12-18 and 23-27 lack an inventive step under PCT Article 33(3) as being obvious over Brusilow-647 in view of Brusilow-979.

Regarding claim 12, Brusilow-979 teaches a method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug comprising administering to the patient the suitable dosage of the PAA prodrug (col 4, ln 1-26; col 3, ln 56-59). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output in a patient (col 2, ln 26-32; Fig 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output based on 60-75% convertion of the pro-drug, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, by the method taught in Brusilow-647, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN for a patient, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 13, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 14, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100, administered in fewer doses per day (col 3, ln 42-55; col 4, ln 1-26). Brusilow-979 does not teach administering two or three doses of HPN-100 per day. However, it would have been obvious to one of ordinary skill in the art to administer two or three doses of HPN-100 to the patient with clinically significant residual urea synthetic capacity, in order to reduce plasma ammonium to normal levels, as the urea synthetic capacity would be likely to aid in the depletion of nitrogen, as taught in Brusilow-979 (col 1, ln 27-34), thus reducing the number of doses per day of HPN-100 required to be administered to the patient.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 15, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 by the method taught in Brusilow-647, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 16, Brusilow-979 teaches the method of claim 15 (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach determining the urinary PAGN. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to reduce the amount of HPN-100 based on the increase in the amount of urinary PAGN caused by the transition, in order to effectively treat nitrogen-retention disorders, by routine experimentation, as a correlation between urinary PAGN output and HPN-100 is taught in Brusilow-979 (col 5, ln 3-15; ln 29-35).

Regarding claim 17, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to gradually transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100 in small amounts, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 in depleting waste nitrogen as PAGN, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 18, Brusilow-979 teaches a method of treatment with HPN-100 (col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to initiate treatment with HPN-100 in a step-wise fashion and increase the amount of HPN-100 gradually, by monitoring the urinary PAGN based on 60-75% convertion by the method taught in Brusilow-647, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the maintenance dose of HPN-100 effective for the treatment of nitrogen-retention disorders, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and HPN-100 administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 23, Brusilow-647 teaches a method to determine the nitrogen elimination capacity of a patient having a nitrogen retention disorder, being treated with a nitrogen scavenging drug (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine"). Brusilow-647 does not teach a method to determine a suitable dietary protein level for a patient. However, it would have been obvious to one of ordinary skill in the art to use the method taught in Brucilow-647 to determine the patient's endogenous nitrogen elimination capacity with and without the nitrogen scavenging drug, in order to determine the amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, through routine experimentation, since the dietary protein intake would be likely to influence the nitrogen elimination capacity of the patient, as taught in Brucilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 24, Brusilow-979 further teaches the method of claim 23, wherein the nitrogen scavenging drug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 25, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 24, wherein Brusilow-979 teaches the selected dosage of HPN-100 (col 4, ln 54-58). Neither Brusilow-647 nor Brusilow-979 teaches a dosage of HPN-100 of up to about 19 grams per day. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen, as PAGN as taught in Brusilow-979 (col 5, ln 3-15), by routine experimentation, as the patient's inherent ability to process nitrogen and the dietary protein intake would be likely to influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Regarding claim 26, Brusilow-979 teaches a method to treat a patient with a PBA prodrug, comprising administering HPN-100 to a subject having HE or UCD (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26; col 4, ln 54-58). Brusilow does not teach a daily dose in excess of 19 g per day of the prodrug. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, since the patient's inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

==================================Continued in Next Supplemental Box==================================

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 27, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 26. Neither Brusilow-647 nor Brusilow-979 teaches a daily dose of HPN-100 is between about 199 and about 57 g. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN, as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, as the patients inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Bruclilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Claims 9-11 and 29 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of Brusilow-647 and further in view of Brusilow-979.

Regarding claim 9, Summar teaches a method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder (para [0022],"glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not explicitly teach determining the patient's residual urea synthesis capacity or dietary intake or estimating the urinary PAGN output. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output for a patient based on 60-75% convertion of the pro-drug, by the method taught in Brusilow-647, by taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15; col 5, ln 29-35).

Regarding claim 10, Summar further teaches the method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof (para [0022]).

Regarding claim 11, Summar further teaches the method of claim 9, wherein the PAA prodrug is HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)").

Regarding claim 29, Brusilow-979 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26) and Summar (para [0035]) teach the method of claim 28, wherein Summar further teaches that administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Neither Brusilow-979 nor Summar explicitly teaches achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; ln 64-68).

Claims 1-29 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING TRANSMITTAL OF COPY OF INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY (CHAPTER I OF THE PATENT COOPERATION TREATY)

(PCT Rule 44bis.1(c))

To:

SMITH, Michael, G.
Morrison & Foerster LLP
12531 High Bluff Drive, Suite 100
San Diego, CA 92130-2040
ETATS-UNIS D'AMERIQUE

Date of mailing *(day/month/year)*
10 March 2011 (10.03.2011)

Applicant's or agent's file reference
643982000141

IMPORTANT NOTICE

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2009/055256	27 August 2009 (27.08.2009)	29 August 2008 (29.08.2008)

Applicant
UCYCLYD PHARMA, INC. et al

The International Bureau transmits herewith a copy of the international preliminary report on patentability (Chapter I of the Patent Cooperation Treaty)

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Form PCT/IB/326 (January 2004)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference 643982000141	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2009/055256	International filing date *(day/month/year)* 27 August 2009 (27.08.2009)	Priority date *(day/month/year)* 29 August 2008 (29.08.2008)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant UCYCLYD PHARMA, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 01 March 2011 (01.03.2011)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Masashi Honda e-mail: pt08.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

	Date of mailing (*day/month/year*) see form PCT/ISA/210 (second sheet)
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2009/055256	27.08.2009	29.08.2008

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
Hyperion Therapeutics

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Moreno de Vega, C Telephone No. +49 89 2399-7486

Form PCT/ISA/237 (Cover Sheet) (April 2005)

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in electronic form.

   ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-14, 18, 23-29
	No:	Claims	1-5, 15-17, 19-22, 30-33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-33
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01) , pages 1117-1121, XP009127277 ISSN: 0031-3998

D4 LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01) , page 91, XP009127344 ISSN: 0141-8955

1 Claims 12-18 and 26-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT.

The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Document D1 discloses the study of the metabolic changes caused by benzoate and phenylacetate and their pharmacokinetics in the treatment of an urea cycle disorder, the lysinuric protein intolerance, and that 54% of the single phenylacetate dose was excreted in urine as phenylacetylglutamine in 24 hours after the load. This document appears to be novelty destroying for claims 30-33.

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 549 of 1354

3 Document D2 discloses the use of HPN-100, a PBA (phenylbutyrate) prodrug, in the treatment of urea cycle disorders and the assessment of its metabolites and the urinary excretion of nitrogen by phenylacetylglutamine measurement. This document appears to be novelty destroying for claims 1-5, 15-17, 19-22 and 30-33.

Thus, claims 1-5, 15-17, 19-22 and 30-33 do not meet the requirements of Article 33(2) PCT.

4 Considering D2 as the most relevant prior art, the technical problem to be solved by claims 6-14, 18 and 23-29 is the provision of a method for determining an effective dosis of HPN-100 for a patient in need of treatment for a nitrogen retention disorder. The solution proposed by said claims is based on a treatment with a known compound which is prodrug of compounds known from D1 to have an excretion rate as disclosed in the claims, and therefore cannot be considered to be inventive.

Thus, claims 1-33 do not meet the requirements of Article 33(3) PCT.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

	Date of mailing (*day/month/year*) see form PCT/ISA/210 (second sheet)
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2009/055256	27.08.2009	29.08.2008

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
Hyperion Therapeutics

1. This opinion contains indications relating to the following items:

   - ☒ Box No. I Basis of the opinion
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

   If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

   If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

   For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Moreno de Vega, C Telephone No. +49 89 2399-7486

Form PCT/ISA/237 (Cover Sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2009/055256

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in electronic form.

   ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	6-14, 18, 23-29
	No:	Claims	1-5, 15-17, 19-22, 30-33
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-33
Industrial applicability (IA)	Yes:	Claims	1-33
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 SIMELL O ET AL: "Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate in lysinuric protein intolerance" PEDIATRIC RESEARCH, WILLIAMS AND WILKINS, BALTIMORE, MD, US, vol. 20, no. 11, 1 January 1986 (1986-01-01) , pages 1117-1121, XP009127277 ISSN: 0031-3998

D4 LEE B ET AL: "Preliminary data on adult patients with urea cycle disorders (UCD) in an open-label, switch-over, dose-escalation study comparing a new ammonia scavenger, glyceryl tri(4-phenylbutyrate) (HPN-100), to buphenyl (sodium phenylbutyrate (PBA))" JOURNAL OF INHERITED METABOLIC DISEASE, KLUWER, DORDRECHT, NL, vol. 31, no. suppl. 1, 1 August 2008 (2008-08-01) , page 91, XP009127344 ISSN: 0141-8955

1 Claims 12-18 and 26-29 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv) / 67.1(iv) PCT.

The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for use in a first or further medical treatment.

2 Document D1 discloses the study of the metabolic changes caused by benzoate and phenylacetate and their pharmacokinetics in the treatment of an urea cycle disorder, the lysinuric protein intolerance, and that 54% of the single phenylacetate dose was excreted in urine as phenylacetylglutamine in 24 hours after the load. This document appears to be novelty destroying for claims 30-33.

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 554 of 1354

3 Document D2 discloses the use of HPN-100, a PBA (phenylbutyrate) prodrug, in the treatment of urea cycle disorders and the assessment of its metabolites and the urinary excretion of nitrogen by phenylacetylglutamine measurement. This document appears to be novelty destroying for claims 1-5, 15-17, 19-22 and 30-33.

Thus, claims 1-5, 15-17, 19-22 and 30-33 do not meet the requirements of Article 33(2) PCT.

4 Considering D2 as the most relevant prior art, the technical problem to be solved by claims 6-14, 18 and 23-29 is the provision of a method for determining an effective dosis of HPN-100 for a patient in need of treatment for a nitrogen retention disorder. The solution proposed by said claims is based on a treatment with a known compound which is prodrug of compounds known from D1 to have an excretion rate as disclosed in the claims, and therefore cannot be considered to be inventive.

Thus, claims 1-33 do not meet the requirements of Article 33(3) PCT.

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing *(day/month/year)*	02 MAR 2009
Applicant's or agent's file reference 643982000140	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 09/30362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)
Applicant HYPERION THERAPEUTICS	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
   1211 Geneva 20, Switzerland, Facsimile No.: +41 22 740 14 35

   For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MICHAEL G. SMITH
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE, SUITE 100
SAN DIEGO, CA 92130-2040

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	02 MAR 2009

Applicant's or agent's file reference	FOR FURTHER ACTION
643982000140	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US 09/30362	07 January 2009 (07.01.2009)	29 April 2008 (29.04.2008)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570

Applicant HYPERION THERAPEUTICS

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 February 2009 (24.02.2009)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 09/30362

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:
   - a. type of material
     - [ ] a sequence listing
     - [ ] table(s) related to the sequence listing
   - b. format of material
     - [ ] on paper
     - [ ] in electronic form
   - c. time of filing/furnishing
     - [ ] contained in the international application as filed
     - [ ] filed together with the international application in electronic form
     - [ ] furnished subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-29	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-29	NO
Industrial applicability (IA)	Claims	1-29	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-5 lack an inventive step under PCT Article 33(3) as being obvious over US 2004/0229948 A1 to Summar, et al. (hereinafter "Summar") in view of US 4,284,647 A to Brusilow, et al. (hereinafter "Brusilow-647").

Regarding claim 1, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary PAGN output taught in Brusilow-647, in order to determine the effective dosage of HPN-100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Regarding claim 2, Brusilow-647 further teaches the method of claim 1, wherein urinary PAGN output is determined as a ratio of the concentration of urinary PAGN to urinary creatinine (Fig. 3; col 4, ln 35-46).

Regarding claim 3, Summar further teaches the method of claim 1, wherein the nitrogen retention disorder is chronic hepatic encephalopathy (para [0029]).

Regarding claim 4, Summar further teaches the method of claim 1, wherein administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Summar does not explicitly teach achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; col 4, ln 64-68).

Regarding claim 5, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder, which comprises monitoring the effect of an initial dosage of HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach monitoring the patient's urinary phenylacetyl glutamine (PAGN) output. However, Brusilow-647 teaches a method of determining the patient's urinary phenylacetyl glutamine output and total urinary nitrogen (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to use the method of determining the urinary phenylacetyl glutamine output taught in Brusilow-647, in order to determine the effective dosage of HPN -100 for a patient and/or how to adjust the initial dosage of HPN-100 to produce a desired ammonia scavenging effect, as a correlation of phenylacetyl glutamine to phenylacetate administration is disclosed in Brusilow-647 (col 2, ln 26-32), a correlation similar to which would be likely between the administration of HPN-100 and urinary phenylacetyl glutamine output, phenyl acetate being a metabolite of HPN-100 (Summar, para [0005]).

Claims 6-8, 19-22 and 28 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of US 5,968,979 A to Brusilow (hereinafter "Brusilow-979").

Regarding claim 6, Summar teaches a method to determine an effective dosage of HPN-100 for a patient in need of treatment for a nitrogen retention disorder (para [0022], "glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not teach HPN-100 conversion to PAGN. However, Brusilow-979 teaches HPN-100 conversion to PAGN (col 4, ln 1-26, "n = 2"; col 5, ln 3-15; col 5, ln 29-35). It would have been obvious to one of ordinary skill in the art to calculate the dosage of HPN-100 based on a utilization efficiency for HPN-100 conversion into PAGN of about 60% to about 75%, in order to achieve effective plasma concentrations of phenylacetate for acetylation of glutamine, by routine experimentation, as Brusilow-979 teaches the intermediate formation of phenylacetate that produces PAGN by acetylation of glutamine (col 3, ln 3-7).

==========================Continued in Supplemental Box==

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.

PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 7, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 6. Neither Summar nor Brusilow teaches a method wherein the dosage of HPN-100 is calculated from the patient's dietary protein intake. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100, in order to effectively deplete accumulated nitrogen via acetylation of glutamine, as taught in Brusilow-979 (col 3, ln 3-7), as the plasma level of glutamine would be likely to depend on the protein intake of the patient, as taught in Brusilow-979 (col 1, ln 41-45).

Regarding claim 8, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, ln 1-26; col 5, ln 29-35) teach the method of claim 7. Neither Summar nor Brusilow-979 teaches a method wherein the dosage of HPN-100 is reduced to account for the patient's residual urea synthesis capacity. However, it would have been obvious to one of ordinary skill in the art to reduce the dosage to account for the patient's residual urea synthesis capacity, by routine experimentation, as urea synthesis would be likely to lesson the plasma nitrogen accumulation, as taught in Brusilow-979 (col 1, ln 27-34).

Regarding claim 19, Brusilow-979 teaches a method to treat a UCD patient with a PBA prodrug, wherein the prodrug produces equivalent or better ammonia level control compared to PBA (col 2, ln 25-34; col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach determining the AUC and Cmax for PBA when the patient receives the PBA prodrug. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), by comparing the AUC and Cmax for the prodrug with those when the patient receives an equimolar amount of PBA, by routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Regarding claim 20, Brusilow-979 further teaches the method of claim 19, wherein the PBA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claims 21 and 22, Brusilow-979 (col 2, ln 25-34; col 3, ln 42-59) and Summar (para [0035]) teach the method of claim 20. Neither Brusilow nor Summar teaches a method wherein the AUC for PBA exposure is lower with the prodrug than with PBA by at least about 20% or by at least 30%. However, it would have been obvious to one of ordinary skill in the art to expect AUC for PBA exposure to be lower by 20-30% for PBA prodrug than with PBA, in order to treat UCD with minimum exposure to PBA, as taught in Brusilow-979 (col 2, ln 15-24), as the triglyceride of PBA would be likely to produce a stable drug level by gradual beta-oxidation of the prodrug, as taught in Brusilow-979 (col 2, ln 25-34).

Regarding claim 28, Brusilow-979 teaches a method to treat a patient having a nitrogen retention disorder with the PBA prodrug HPN-100 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26). Brusilow-979 does not teach the AUC or Cmax of PBA. However, Summar teaches determining the blood levels of phenyl butyrate in a patient (para [0035]). It would have been obvious to one of ordinary skill in the art to determine the effective dosage of the PBA prodrug so that AUC for PBA is less than about 600 and the Cmax for PBA is less than about 100 when the PBA prodrug is administered, in order to treat UCD without the excessive sodium intake associated with administration of phenyl butyrate, as taught in Brusilow-979 (col 2, ln 15-24), through routine experimentation, as the pharmacokinetic parameters would be a measure of the plasma-level of PBA in the patient, measurement of which for determining dosage has been disclosed in Summar (para [0035], "sodium phenyl butyrate and its metabolites").

Claims 12-18 and 23-27 lack an inventive step under PCT Article 33(3) as being obvious over Brusilow-647 in view of Brusilow-979.

Regarding claim 12, Brusilow-979 teaches a method to treat a patient having an ammonia retention disorder with a suitable dosage of a PAA prodrug comprising administering to the patient the suitable dosage of the PAA prodrug (col 4, ln 1-26; col 3, ln 56-59). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output in a patient (col 2, ln 26-32; Fig 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output based on 60-75% convertion of the pro-drug, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, by the method taught in Brusilow-647, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN for a patient, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 13, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 14, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100, administered in fewer doses per day (col 3, ln 42-55; col 4, ln 1-26). Brusilow-979 does not teach administering two or three doses of HPN-100 per day. However, it would have been obvious to one of ordinary skill in the art to administer two or three doses of HPN-100 to the patient with clinically significant residual urea synthetic capacity, in order to reduce plasma ammonium to normal levels, as the urea synthetic capacity would be likely to aid in the depletion of nitrogen, as taught in Brusilow-979 (col 1, ln 27-34), thus reducing the number of doses per day of HPN-100 required to be administered to the patient.

==============================Continued in Next Supplemental Box==

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 15, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 by the method taught in Brusilow-647, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 16, Brusilow-979 teaches the method of claim 15 (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach determining the urinary PAGN. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to reduce the amount of HPN-100 based on the increase in the amount of urinary PAGN caused by the transition, in order to effectively treat nitrogen-retention disorders, by routine experimentation, as a correlation between urinary PAGN output and HPN-100 is taught in Brusilow-979 (col 5, ln 3-15; ln 29-35).

Regarding claim 17, Brusilow-979 teaches a method of treatment to a patient comprising substituting HPN-100 for phenylacetate or phenylbutyrate (col 2, ln 25-34; col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to gradually transition a patient receiving treatment with an initial amount of phenylacetate or phenylbutyrate to a final amount of HPN-100 in small amounts, by monitoring the amount of urinary PAGN excreted by the patient, in order to assess the effectiveness of the replacement amount of the HPN-100 in depleting waste nitrogen as PAGN, by routine experimentation, as the urinary PAGN output would be a measure of the effectiveness of the waste nitrogen depletion by the drug administered, as taught in Brusilow-647 (col 2, ln 26-32).

Regarding claim 18, Brusilow-979 teaches a method of treatment with HPN-100 (col 3, ln 42-55). Brusilow-979 does not teach a method of determining the urinary PAGN output of the patient. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to initiate treatment with HPN-100 in a step-wise fashion and increase the amount of HPN-100 gradually, by monitoring the urinary PAGN based on 60-75% convertion by the method taught in Brusilow-647, taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the maintenance dose of HPN-100 effective for the treatment of nitrogen-retention disorders, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and HPN-100 administration is disclosed in Brusilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 23, Brusilow-647 teaches a method to determine the nitrogen elimination capacity of a patient having a nitrogen retention disorder, being treated with a nitrogen scavenging drug (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine"). Brusilow-647 does not teach a method to determine a suitable dietary protein level for a patient. However, it would have been obvious to one of ordinary skill in the art to use the method taught in Brucilow-647 to determine the patient's endogenous nitrogen elimination capacity with and without the nitrogen scavenging drug, in order to determine the amount of dietary protein the patient can have while being treated with the selected dosage of the nitrogen scavenging drug, through routine experimentation, since the dietary protein intake would be likely to influence the nitrogen elimination capacity of the patient, as taught in Brucilow-979 (col 1, ln 27-34; ln 41-45; col 5, ln 3-15; ln 29-35).

Regarding claim 24, Brusilow-979 further teaches the method of claim 23, wherein the nitrogen scavenging drug is HPN-100 (col 4, ln 1-26, "n = 2").

Regarding claim 25, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 24, wherein Brusilow-979 teaches the selected dosage of HPN-100 (col 4, ln 54-58). Neither Brusilow-647 nor Brusilow-979 teaches a dosage of HPN-100 of up to about 19 grams per day. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen, as PAGN as taught in Brusilow-979 (col 5, ln 3-15), by routine experimentation, as the patient's inherent ability to process nitrogen and the dietary protein intake would be likely to influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Regarding claim 26, Brusilow-979 teaches a method to treat a patient with a PBA prodrug, comprising administering HPN-100 to a subject having HE or UCD (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26; col 4, ln 54-58). Brusilow does not teach a daily dose in excess of 19 g per day of the prodrug. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, since the patient's inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

===================================Continued in Next Supplemental Box=====================================

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US 09/30362

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 27, Brusilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46) and Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15) teach the method of claim 26. Neither Brusilow-647 nor Brusilow-979 teaches a daily dose of HPN-100 is between about 199 and about 57 g. However, it would have been obvious to one of ordinary skill in the art to determine the dosage of HPN-100 based on the dietary protein the patient intake of the patient, in order to provide effective elimination of waste nitrogen as PAGN, as taught in Brusilow-979 (col 5, ln 3-15), through routine experimentation, as the patients inherent ability to process nitrogen and the dietary protein intake would likely influence the nitrogen elimination capability, measured by the method taught in Brucilow-647 (col 2, ln 26-32; Fig. 3; col 4, ln 35-46, "urinary phenylacetyl glutamine").

Claims 9-11 and 29 lack an inventive step under PCT Article 33(3) as being obvious over Summar in view of Brusilow-647 and further in view of Brusilow-979.

Regarding claim 9, Summar teaches a method to determine a dosage of a PAA prodrug for a patient having an ammonia retention disorder (para [0022],"glyceryl-tri(4-phenyl butyrate)"; para [0029], "hepatic encephalopathy"; para [0035]). Summar does not explicitly teach determining the patient's residual urea synthesis capacity or dietary intake or estimating the urinary PAGN output. However, Brusilow-647 teaches a method of determining the urinary PAGN output (col 2, ln 26-32; Fig. 3; col 4, ln 35-46). It would have been obvious to one of ordinary skill in the art to estimate the target urinary PAGN output for a patient based on 60-75% convertion of the pro-drug, by the method taught in Brusilow-647, by taking into account the residual urea synthesis capacity and dietary protein intake of the patient, in order to determine the amount of the PAA prodrug needed to produce the target amount of urinary PAGN, as a correlation of urinary PAGN output to the residual urea synthesis capacity and dietary protein intake of the patient and to PAA prodrug administration is disclosed in Brusilow-979 (col 1, ln 27-34; col 1, ln 41-45; col 5, ln 3-15; col 5, ln 29-35).

Regarding claim 10, Summar further teaches the method of claim 9, wherein the PAA prodrug is phenylbutyric acid (PBA) or a pharmaceutically acceptable salt thereof (para [0022]).

Regarding claim 11, Summar further teaches the method of claim 9, wherein the PAA prodrug is HPN-100 (para [0022], "glyceryl-tri(4-phenyl butyrate)").

Regarding claim 29, Brusilow-979 (col 3, ln 42-59, "triglycerides of phenyl alkanoic acid"; col 4, ln 1-26) and Summar (para [0035]) teach the method of claim 28, wherein Summar further teaches that administering the effective dosage of HPN-100 to the patient produces a change in plasma ammonia level in the patient (para [0035]). Neither Brusilow-979 nor Summar explicitly teaches achieving normal plasma ammonia levels. However, it would have been obvious to one of ordinary skill in the art to produce normal plasma ammonia levels by administration of HPN-100, as a reduction in plasma ammonium levels following administration of a metabolite of HPN-100, namely phenyl acetic acid, is taught in Brusilow-647 (col 4, ln 46-50; ln 64-68).

Claims 1-29 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 643982000140	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 09/30362	International filing date *(day/month/year)* 07 January 2009 (07.01.2009)	(Earliest) Priority Date *(day/month/year)* 29 April 2008 (29.04.2008)
Applicant HYPERION THERAPEUTICS		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into ________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. 4
      ☒ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/30362

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 37/10; A61K 31/19 (2009.01)
USPC - 514/570
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
IPC(8): A01N 37/10; A61K 31/19 (2009.01)
USPC: 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
US WEST(PGPB,USPT,EPAB,JPAB), Google Scholar, Dialog PRO (Engineering)
ammonia scavenging, accumulation, retention, hepatic encephalopathy, urea cycle disorder, phenylacetyl glutamine, PAGN, HPN-100, phenyl butyrate, glyceryl tri-(4-phenyl butyrate)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2004/0229948 A1 (SUMMAR, et al.) 18 November 2004 (18.11.2004), para [0022], [0029], [0035]	1-11, 19-22, 28, 29
Y	US 4,284,647 A (BRUSILOW, et al.) 18 August 1981 (18.08.1981) col 2, ln 26-32; Fig. 3; col 4, ln 35-46.	1-5, 9-18, 23-27, 29
Y	US 5,968,979 A (BRUSILOW) 19 October 1999 (19.10.1999), col 1, ln 27-34; col 1, ln 41-45; col 2, ln 25-34; col 3, ln 3-7; col 3, ln 42-59; col 4, ln 1-26; col 4, ln 54-58; col 5, ln 3-15; ln 29-35	6-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 2009 (24.02.2009)	02 MAR 2009

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

79532-8003.WO00
PDM/CDK

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JUN 25 2012

PERKINS COIE LLP

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

DOCKETED TO CPI
[x] Deadline
[ ] Follow up
[ ] Previously
[ ] Abandoned
[ ] Transferred
[ ] Dedocketed

1/30/13
7/30/13

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (*day/month/year*) 20 JUN 2012
Applicant's or agent's file reference 795328003WO	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US2012/028620	International filing date (*day/month/year*) 09 March 2012
Applicant SCHARSCHMIDT, BRUCE	

1. [X] The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. [ ] **With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:**

   [ ] the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   [ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within 19 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300
Facsimile No. 571-273-3201	Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328003WO	**FOR FURTHER ACTION** see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US2012/028620	International filing date *(day/month/year)* 09 March 2012	(Earliest) Priority Date *(day/month/year)* 30 September 2011
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ **With regard to any nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (see Box No. II).
3. ☐ **Unity of invention is lacking** (see Box No. III).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. 2
      ☒ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/028620

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61B 5/00; A61K 31/192; A61K 49/00; A61P 13/00 (2012.01)
USPC - 424/9.2; 514/568; 600/322, 341

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patent, Google, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2010/0008859 A1 (SCHARSCHMIDT) 14 January 2010 (14.01.2010) entire document	1-7, 9-12 --- 8
Y	ENNS et al., Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders, N Engl J Med 356; 22, 31 May 2007. entire document.	8
A	US 6,219,567 B1 (EGGERS et al) 17 April 2001 (17.04.2001) entire document	1-12
A	LEE et al., Phase 2 Comparison of A Novel Ammonia Scavenging Agent with Sodium Phenylbutyrate in Patients with Urea Cycle Disorders: Safety, Pharmacokinetics, and Ammonia Control. Mol. Genet Metab. 100(3) July 2010 entire document	1-12
A	LICHTER-KONECKI et al., Ammonia Control with Urea Cycle Disorders (UCDs); Phase 2 comparison of sodium phenylbutyrate and glycerol phynylbutyrate. Mol. Genet. Metab. 103 5 May 2011. entire document	1-12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 June 2012	20 JUN 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	20 JUN 2012
Applicant's or agent's file reference: 795328003WO	FOR FURTHER ACTION: See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2012/028620	09 March 2012	30 September 2011

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00 (2012.01)
USPC - 424/9.2

Applicant SCHARSCHMIDT, BRUCE

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	04 June 2012	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2012/028620

Box No. I — Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into __________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
   - [ ] on paper
   - [ ] in electronic form

   b. (time)
   - [ ] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY	International application No. PCT/US2012/028620

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	8	YES
	Claims	1-7, 9-12	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-7 and 9-12 lack novelty under PCT Article 33(2) as being anticipated by Scharschmidt et al. (hereafter Scharschmidt).

Regarding claim 1, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to increase a dosage of a nitrogen scavenging drug in a subject (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) currently receiving the nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]);
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level ((comparing fasting with) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) to determine whether to increase the dosage of a nitrogen scavenging drug (determining and adjusting the dose of an ammonia scavenging drug, Para. [0041]), wherein the dosage needs to be increased if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (if the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]; ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26. 1 umol/L), Para. [0201]).

Regarding claim 2, Scharschmidt discloses the method (method, Para. [0039]) for determining whether to administer a nitrogen scavenging drug (adjusting the schedule and dose of orally administered nitrogen scavenging drugs, Para. [0020]) to a subject having a nitrogen retention disorder (retention states including urea cycle disorders and liver disease, Para. [0064]) comprising:

a) measuring a fasting blood ammonia level for the subject (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and

b) comparing the fasting blood ammonia level to the upper limit of normal for blood ((comparing) normal upper limit for venous (blood) ammonia, Para. [0201], plasma upper limit of normal, Para. [0094]) ammonia levels to determine whether to administer a nitrogen scavenging drug to the subject (determining the dose of an ammonia scavenging drug to be administered, Para. [0041]), wherein a nitrogen scavenging drug needs to be administered to the subject if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (adjusting the initial dosage of the new drug based upon ammonia control, Para. [0099]; (ammonia value after HPN-100 treatment (26.1 umol/L) was within the normal range and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 570 of 1354

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 3, Scharschmidt discloses the method (method, Para. [0039]) of treating a subject with a nitrogen retention disorder (dosing schedule and dose adjustments necessary for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy, Para. [0064]) who has previously been administered a nitrogen scavenging drug (method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage (already receiving a drug), Para. [0044]) comprising:

a) measuring a fasting blood ammonia level (PK/PD modeling (a measurement) of ammonia in fasted and fed (subjects), Para. [0212]) for the subject (subjects, Para. [0213]); and
b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia level and administering an increased dosage of the nitrogen scavenging drug (if the ammonia control is inadequate, the dosage of the nitrogen scavenging drug can be increased, Para. [0083]) if the fasting blood ammonia level is greater than half the upper limit of normal for blood ammonia level (ammonia value after HPN-100 (26.1 umol/L) was within the normal range of 26 to 35 umol/L and above the upper limit of normal (ULN) after sodium PB (upper limit of normal is approximatley 26 to 35 umol/L; half the upper limit of normal is about 13 to 17.5 umol/L which is greater than 26.1 umol/L), Para. [0201]).

Regarding claim 4, Scharschmidt discloses the method of claim 1. Scharschmidt discloses further comprising: c) administering an increased dosage of the nitrogen scavenging drug if the need exists (treatment with an ammonia scavenging agent as described in this invention is determined clinically if the subject is in need of such treatment. This clinical determination would be based upon a variety of factors (e.g. signs and symptoms of hepatic encephalopathy in patients with cirrhosis, elevated blood ammonia levels), Para. [0221]);

Regarding claim 5, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen retention disorder is selected from the group consisting of a urea cycle disorders and hepatic encephalopathy (urea cycle disorder, Para. [0221], hepatic encephalopathy, Para. [0041]).

Regarding claim 6, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the nitrogen scavenging drug is a PAA prodrug (prodrugs of PAA, Para. [0217]).

Regarding claim 7, Scharschmidt discloses the method of claim 6. Scharschmidt discloses wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (HPN-100, Para. [0020]).

Regarding claim 9, Scharschmidt discloses the method of claim 3 or 4. Scharschmidt discloses wherein administering an increased dosage of the nitrogen scavenging drug produces a normal average daily ammonia level in the subject (administering the effective dosage of HPN-100 (effective dose may require increasing or decreasing the drug) to the patient preferably produces a normal plasma ammonia level in the patient, Para. [0142]); nitrogen scavenging drug may need to be increased, Para. [0083]).

Regarding claim 10, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses further comprising the step of determining an upper limit of normal for blood ammonia level for the subject prior to step (b) (monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dose of HPN-100 to the patient produces a normal plasma ammonia level. Plasma ammonia in the patient can be a level of about 35 or about 40 umol/L (determining the upper limit of normal for the subject via urinary excretion of PAGN prior to step b), Para. [0142]); the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 umol/L, Para. [0201]).

Regarding claim 11, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt discloses wherein the upper limit of normal blood ammonia level is 35 umol/L (upper limit of normal for subjects is between 26 to 35 umol/L, Para. [0094]).

Regarding claim 12, Scharschmidt discloses the method of claim 6. Scharschmidt discloses further comprising:
c) measuring urinary PAGN excretion (measuring PAGN excretion, Para. [0096]); and
e) determining an effective dosage of the PAA (effective dose, Para. [0140]), prodrug based on a mean conversion of PAA prodrug to urinary PAGN of 60-75% (determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60% to about 75% conversion of the PAA prodrug into urinary PAGN, Para. [0148]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY	International application No. PCT/US2012/028620

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Claim 8 lacks an inventive step under PCT Article 33(3) as being obvious over Scharschmidt et al.(hereafter Scharschmidt) in view of Ennis et al. (hereafter Ennis).

Regarding claim 8, Scharschmidt discloses the method of any of claims 1-3. Scharschmidt fails to explicitly disclose wherein the nitrogen scavenging drug is sodium benzoate. Ennis is in the field of treating urea cycle disorders with phenylacetate and benzoate and teaches the use of sodium benzoate to treat patients with ammonia disorders (sodium benzoate therapy in patients, Pg. 1, Lns.1-16). It would have been obvious to one of ordinary skill in the art at the time of the invention to use the therapeutic drug sodium benzoate as taught by Ennis with the method of Scharschmidt. The motivation would have been to lower plasma ammonium levels and improve the survival of patients with lethal urea-cycle enzyme defects (Ennis, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects, Pg. 1, Lns. 1-16).

Claims 1-12 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

...

Form PCT/ISA/237 (Supplemental Box) (July 2011)

Docketed:
Amend Claims: 05/28/14
File Response: 09/21/14

REVIEWED
By Renee George at 3:07 pm, Apr 02, 2014

079532-8005.W000
PDM / CDK

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

RECEIVED
PATENT DOCKETING
APR 02 2014
PERKINS COIE LLP

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

	Date of mailing (*day month year*) 28 MAR 2014
Applicant's or agent's file reference 795328005WO0	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 13/71333	International filing date (*day month year*) 21 November 2013 (21.11.2013)
Applicant SCHARSCHMIDT, BRUCE	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

   For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until **30 months** from the priority date (in some Offices even later); otherwise, the applicant must, within **20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Lee W. Young
Facsimile No. 571-273-3201	Telephone No. PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795326005WO0	FOR FURTHER ACTION	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US 13/71333	International filing date *(day/month/year)* 21 November 2013 (21.11.2013)	(Earliest) Priority Date *(day/month/year)* 21 November 2012 (21.11.2012)
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   ☐ the text is approved as submitted by the applicant.
   ☒ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. 1
      ☒ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

Box No. IV Text of the abstract (Continuation of item 5 of the first sheet)

The present disclosure provides methods for treating hepatic encephalopathy (HE) and for optimizing and adjusting nitrogen scavenging drug dosage for subjects with HE, comprising administering a nitrogen scavenging drug at a dosage sufficient to maintain a fasting blood ammonia level at or below a specified threshold level which is 1.5 times the upper limit of normal for blood ammonia. The nitrogen scavenging drug administered in the method is a phenylacetic acid prodrug selected from HPN-100, PBA, NaPBA, sodium benzoate, or any combination thereof (i.e., any combination of two or more of HPN-100, PBA, NaPBA).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/71333

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/9.1; 514/570

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase (AU BE BR CA CH CN DE DK EP ES FI FR GB IN JP KR SE TH TW US WO), PubWest, FreePatentsOnline, Google Web
search terms: hepatic encephalopathy blood plasma ammonia NH3 nitrogen phenylacetyl glutamine PAGN scavenging PAA phenylacetic acid prodrug sodium benzoate glyceryl tri phenylbutyrate (HPN-00) phenylbutyric acid PBA NaPBA measure

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	US 2010/0008859 A1 (Scharschmidt) 14 January 2010 (14.01.2010), para [0020], [0039], [0041], [0044], [0064], [0094], [0099], [0142], [0148], [0201], [0212], [0217]	9-12 1-8
Y	Stauch et al., "Oral L-ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study" Journal of Hepatology, May 1998, Vol 28, Issue 5, Pages 856-864, [retrieved from internet: <URL: http://www.sciencedirect.com] pg 860, col 1, para 2- col 2, para 3; pg 862, col 1, para 3; pg 863, col 1, para 1	1-8
Y	Enns et al., "Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders", N Engl J Med., 31 May 2007 (31.05.2007), Vol. 356, pages 2282-2292, [retrieved from the internet: <URL: http://www.nejm.org] abstract, para 1-4	5-6
A	US 2012/0220661 A1 (Lee) 30 August 2012 (30.08.2012), para [0034], [0090], [0094]	1-12
A	Lee et al., "Phase 2 comparison of a novel ammonia scavenging agent with sodium phenylbutyrate in patients with urea cycle disorders: Safety, pharmacokinetics and ammonia control", Molecular Genetics and Metabolism, July 2010, Vol 100, Issue 3, pages 221-228, [retrieved from the Internet: <URL: http://www.sciencedirect.com] pg 1-4	1-12
X, P	US 2013/0210914 A1 (Scharschmidt et al.) 15 August 2013 (15.08.2013), para [0010]-[0012], [0019], [0023]-[0031], [0036], [0042], [0057]	1-12

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
28 February 2014 (28.02.2014)

Date of mailing of the international search report
28 MAR 2014

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*) 28 MAR 2014

Applicant's or agent's file reference	FOR FURTHER ACTION
795328005WO0	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US 13/71333	21 November 2013 (21.11.2013)	21 November 2012 (21.11.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00, A61K 31/19 (2014.01)
USPC - 424/9.2; 514/568

Applicant SCHARSCHMIDT, BRUCE

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	28 February 2014 (28.02.2014)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
   - [ ] on paper
   - [ ] in electronic form

   b. (time)
   - [ ] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-8	YES
	Claims	9-12	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-12	NO
Industrial applicability (IA)	Claims	1-12	YES
	Claims	none	NO

2. Citations and explanations:

Claims 9-12 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0008859 A1 (Scharschmidt).

Regarding claim 9, Scharschmidt teaches a method (para [0039]) of treating hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) in a subject (para [0142], patient) in need thereof
comprising:
(a) determining a target urinary phenylacetyl glutamine (PAGN) output (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen);
(b) calculating an effective initial dosage of a PAA prodrug (para [0142], a method to determine an effective dosage of HPN-100 for a patient...monitoring the effect of an initial dosage of HPN-100) to achieve the target PAGN output based on a mean conversion of PAA prodrug to urinary PAGN of 52% to 63% (para [0148], determining an amount of the PAA prodrug needed to mobilize the target amount of urinary PAGN based on about 60 percent to about 75 percent conversion of the PAA prodrug into urinary PAGN) and
(c) administering the effective initial dosage of PAA prodrug to the subject (para [0142], administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level in the patient).

Regarding claim 10, Scharschmidt teaches a method of claim 9, wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (para [0020], administered nitrogen scavenging drugs, including sodium phenylbutyrate (NaPBA) and glyceryl tri-[4-phenylbutyrate] (HPN-100)).

Regarding claim 11, Scharschmidt teaches a method of claim 9, further comprising a step of determining the upper limit of normal for blood ammonia for the subject (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level...can be a level of about 35 or about 40 micro mol/L; para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Regarding claim 12, Scharschmidt teaches a method of claim 9, wherein the upper limit of normal blood ammonia is 35 Lmol/L (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Claims 1-4, 7, and 8 lack an inventive step under PCT Article 33(3) as being obvious over Scharschmidt, in view of the article titled "Oral L-ornithine-L-aspartate therapy of chronic hepatic encephalopathy: results of a placebo-controlled double-blind study" to Stauch et al. (hereinafter 'Stauch').

Regarding claim 1, Scharschmidt teaches a method (para [0039]) of treating hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) in a subject comprising:
(a) measuring a fasting blood ammonia level (para [0212], PK/PD modeling (a measurement) of ammonia in fasted and fed);
(b) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L...; para [0094], plasma levels of ammonia are acceptable when they are at or below a level considered normal...the upper limit of normal for the subjects was between 26 and 35 micro mol/L); and
(c) administering a nitrogen scavenging drug to the subject (para [0041], determining and adjusting the dose of an ammonia scavenging drug to be administered to a patient with liver disease, including hepatic encephalopathy).
Scharschmidt does not specifically teach administering a nitrogen scavenging drug to the subject if the fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia.

Stauch teaches treatment of hepatic encephalopathy (HE) with OA (L-ornithine-L-aspartate) and further teaches OA decreases hyperammonemia (blood ammonia level) and improves HE (pg 862, col 1, para 3, confirms the beneficial effects already proven for OA-infusions in terms of the reduction in hyperammonemia and improvement in overt HE; pg 860, col 2, para 2, OA-treated patients with hepatic encephalopathy revealed a reduction in the fasting venous blood ammonia level (day 0: 87.5 +/- 28.8 micro mol/L; day 14: 52.2 +/- 27.8 micro mol/L).

-- Please see Supplemental Box for continuation of claim 1 --

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 13/71333

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V. No 2: Citations and Explanations:
(Continued from Claim 1)

Stauch further teaches measuring the fasting blood ammonia level prior to and after administering OA to a subject (patient), and the pretreatment fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia (pg 860, col 1, para 2, pretreatment fasting blood ammonia concentrations (median) were similar in the placebo (84 micro mol/L) and OA groups (77 micro mol/L); pg 863, col 1, para 1, upper limit of normal (50 micro mol/L)).
To a person of ordinary skill in the art it would have been obvious to measure the fasting blood ammonia level prior to administering a drug to a subject (patient), and to check if it is greater than 1.5 times the upper limit of normal for blood ammonia as taught by Stauch when administering a nitrogen scavenging drug to a subject as taught by Scharschmidt. This is because both Scharschmidt (para [0201], patients with higher ammonia levels greater decreases in ammonia values following administration of HPN-100) and Stauch (pg 5, para 3) are directed toward the treatment of hepatic encephalopathy through lowering blood ammonia level.

Regarding claim 2, Scharschmidt in view of Stauch teach a method of claim 1 as discussed above. Scharschmidt further teaches wherein the subject has previously been administered a first dosage of a nitrogen scavenging drug (para [0044], method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate).

Regarding claim 3, Scharschmidt in view of Stauch teach a method of claim 2 as discussed above. Scharschmidt further teaches wherein the dosage of nitrogen scavenging drug administered in step (c) is greater than the first dosage (para [0083], plasma or blood level of ammonia is optionally also determined...If the ammonia control is inadequate, the dosage of the nitrogen scavenging drug may need to be increased).

Regarding claim 4, Scharschmidt teaches a method (para [0039]) of optimizing the dosage of a nitrogen scavenging drug (para [0020], adjusting the schedule and dose of orally administered nitrogen scavenging drugs) for the treatment of hepatic encephalopathy (HE) (para [0064], for treatment of nitrogen retention states including urea cycle disorders and liver disease complicated by hepatic encephalopathy) comprising:
(a) administering a first dosage of a nitrogen scavenging drug (para [0044], method involves administering an initial dosage of the prodrug that is selected based on the patient's current dosage of phenylacetate or phenylbutyrate);
(b) measuring a fasting blood ammonia level (para [0212], PK/PD modeling (a measurement) of ammonia in fasted and fed);
(c) comparing the fasting blood ammonia level to the upper limit of normal for blood ammonia (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L...; para [0094], plasma levels of ammonia are acceptable when they are at or below a level considered normal...the upper limit of normal for the subjects was between 26 and 35 micro mol/L) to determine whether to increase the dosage of a nitrogen scavenging drug (para [0041], determining and adjusting the dose of an ammonia scavenging drug to be administered), wherein the dosage needs to be increased (para [0099], adjusting the initial dosage of the new drug as needed to provide an adjusted dosage based upon ammonia control); and
(d) administering a second dosage of the nitrogen scavenging drug based on the determination in (c) (para [0113], this method comprises adjusting the amount of the prodrug and administering an adjusted amount of the prodrug).

Scharschmidt does not specifically teach dosage needs to be increased if the fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia.

Stauch teaches treatment of hepatic encephalopathy (HE) with OA (L-ornithine-L-aspartate) and further teaches OA decreases hyperammonemia (blood ammonia level) and improves HE (pg 862, col 1, para 3, confirms the beneficial effects already proven for OA-infusions in terms of the reduction in hyperammonemia and improvement in overt HE; pg 860, col 2, para 2, OA-treated patients with hepatic encephalopathy revealed a reduction in the fasting venous blood ammonia level (day 0: 87.5 +/- 28.8 micro mol/L; day 14: 52.2 +/- 27.8 micro mol/L).

Stauch further teaches measuring fasting blood ammonia level prior to and after administering OA to a subject (patient), and the pretreatment fasting blood ammonia level is greater than 1.5 times the upper limit of normal for blood ammonia (pg 860, col 1, para 2, pretreatment fasting blood ammonia concentrations (median) were similar in the placebo (84 micro mol/L) and OA groups (77 micro mol/L); pg 863, col 1, para 1, upper limit of normal (50 micro mol/L)).
To a person of ordinary skill in the art it would have been obvious to measure the fasting blood ammonia level prior to administering a drug to a subject (patient), and to check if it is greater than 1.5 times the upper limit of normal for blood ammonia as taught by Stauch when administering a nitrogen scavenging drug to a subject as taught by Scharschmidt. This is because both Scharschmidt (para [0201], patients with higher ammonia levels greater decreases in ammonia values following administration of HPN-100) and Stauch (pg 5, para 3) are directed toward the treatment of hepatic encephalopathy through lowering blood ammonia level.

Regarding claim 7, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above. Scharschmidt further teaches comprising a step of determining the upper limit of normal for blood ammonia for the subject (para [0142], monitoring the effect of the initial dosage of HPN-100 consists essentially of determining the patient's urinary phenylacetyl glutamine (PAGN) output and/or total urinary nitrogen. Administering the effective dosage of HPN-100 to the patient preferably produces a normal plasma ammonia level...can be a level of about 35 or about 40 micro mol/L; para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

Regarding claim 8, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above. Scharschmidt further teaches wherein the upper limit of normal blood ammonia is 35 .mol/L (para [0201], the normal upper limit for venous (blood) ammonia varied among the study sites from 26 to 35 micro mol/L).

-- Please see Next Supplemental Box --

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Previous Supplemental Box:

Claims 5-6 lack an inventive step under PCT Article 33(3) as being obvious over Scharschmidt, in view of Stauch, further in view of the article titled "Survival after Treatment with Phenylacetate and Benzoate for Urea-Cycle Disorders" to Enns et al. (hereinafter 'Enns') .

Regarding claim 5, Scharschmidt in view of Stauch teach a method of claim 1 or 4 as discussed above.
Scharschmidt further teaches wherein the nitrogen scavenging drug is a PAA prodrug (para [0217], the preclinical handling of ammonia scavenging drugs, 600 mg/kg of either radio labeled sodium PBA or radio labeled HPN-100 was administered as a single dose to 3 cynomolgous monkeys. These monkeys were chosen because, like humans (and unlike most other species), they metabolize PAA to PAGN and thus provide a useful model for testing prodrugs of PAA).

Scharschmidt does not specifically teaches the nitrogen scavenging drug is sodium benzoate.

Enns teaches treating urea cycle disorder (relates to hepatic encephalopathy) with phenylacetate and benzoate and further teaches the use of sodium benzoate to treat patients with ammonia disorders (abstract, para 1-4, sodium benzoate therapy in patients). To a person of ordinary skill in the art it would have been obvious to use the therapeutic drug sodium benzoate as taught by Enns with the method of Scharschmidt in view of Stauch. The motivation would be to lower plasma (blood) ammonia levels and improve the survival of patients with lethal urea-cycle enzyme defects (Enns) (abstract, para 1-4, lower plasma ammonium levels and improve survival in small cohorts of patients with historically lethal urea-cycle enzyme defects).

Regarding claim 6, Scharschmidt in view of Stauch, further in view of Enns teach a method of claim 5, wherein Scharschmidt further teaches the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA (para [0020], administered nitrogen scavenging drugs, including sodium phenylbutyrate (NaPBA) and glyceryl tri-[4-phenylbutyrate] (HPN-100)).

Claims 1-12 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

795328004WO00
PDM/CDK

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

RECEIVED
PATENT DOCKETING
NOV 21 2012
PERKINS COIE LLP

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Date of mailing (day/month/year): 20 NOV 2012

Applicant's or agent's file reference: 795328004WO0

DOCKETED TO CPI
8/24/13
9/20/14
☐ Follow up ☐ Previously ☐ Abandoned ☐ Transferred ☐ Unchecked

FOR FURTHER ACTION: See paragraphs 1 and 4 below

International application No.: PCT/US 12/54673

International filing date (day/month/year): 11 September 2012 (11.09.2012)

Applicant: SCHARSCHMIDT, BRUCE

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 82 70

For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 – 9.011.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to any protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. Following the expiration of 30 months from the priority date, these comments will also be made available to the public.

Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90*bis*.1 and 90*bis*.3).

Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

For details about the applicable time limits, Office by Office, see www.wipo.int/pct/en/texts/time_limits.html and the *PCT Applicant's Guide*, National Chapters.

Name and mailing address of the ISA/	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450	Lee W. Young PCT Helpdesk: 571-272-4300
Facsimile No. 571-273-3201	Telephone No. PCT OSP: 571-272-7774

Form PCT/ISA/220 (July 2010)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328004WO0	**FOR FURTHER ACTION** see Form PCT/ISA/220 as well as, where applicable, item 5 below.	
International application No. PCT/US 12/54673	International filing date *(day/month/year)* 11 September 2012 (11.09.2012)	(Earliest) Priority Date *(day/month/year)* 20 April 2012 (20.04.2012)
Applicant SCHARSCHMIDT, BRUCE		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into ________________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (see Box No. II).
3. ☐ **Unity of invention is lacking** (see Box No. III).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. ________
      ☐ as suggested by the applicant.
      ☐ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/54673

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/216; A61K 31/185
USPC - 514/533; 514/576; 514/532, 514/553; 554/220, 554/227
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/216; A61K 31/185 (2012.01)
USPC: 514/533; 514/576

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/532, 514/553; 554/220, 554/227 (search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST, PatBase, Google Scholar: Plasma, PAA, PAGN, nitrogen retention, phenylacetic acid, phenylacetylglutamine, levels, NaPBA, NPH-100, nitrogen retention disorders, target range, dose

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2012/0022157 A1 (SCHARSCHMIDT) 26 January 2012 (26.01.2012); para [0021], [0089], [0097], [0106], [0116], [0118], [0160], [0173], [0174], [0181], [0297]	1-13
Y	MCGUIRE et al., Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis, HEPATOLOGY, June 2010, Vol. 51, pages 2077-2085; abstract; page 2079, col 2, para 3, page 2081, col 1, para 2;	1-13

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 October 2012 (24.10.2012)	20 NOV 2012

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: PATRICK MORRIS
PERKINS COIE LLP
P.O. BOX 1208
SEATTLE, WA 98111-1208

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	20 NOV 2012

Applicant's or agent's file reference	FOR FURTHER ACTION
795328004WO0	See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 12/54673	11 September 2012 (11.09.2012)	20 April 2012 (20.04.2012)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 31/216; A61K 31/185 (2012.01)
USPC - 514/533; 514/576; 514/532, 514/553; 554/220, 554/227

Applicant SCHARSCHMIDT, BRUCE

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	24 October 2012 (24.10.2012)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 12/54673

Box No. I — Basis of this opinion

1. With regard to the language, this opinion has been established on the basis of:
   - [X] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
      - [ ] on paper
      - [ ] in electronic form

   b. (time)
      - [ ] in the international application as filed
      - [ ] together with the international application in electronic form
      - [ ] subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 586 of 1354

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY	International application No. PCT/US 12/54673

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-13	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-13	NO
Industrial applicability (IA)	Claims	1-13	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-13 lack an inventive step under PCT Article 33(3) as being obvious over US 2012/0022157 A1 (Scharschmidt) in view of the article entitled, 'Pharmacology and Safety of Glycerol Phenylbutyrate in Healthy Adults and Adults with Cirrhosis' by McGuire et al. (hereinafter 'McGuire').

Regarding claim 1, Scharschmidt teaches a method of treating a nitrogen retention disorder in a subject (para [0173]) comprising:
(a) administering a first dosage of a PAA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]).
Scharschmidt however, fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (page 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 2, Scharschmidt teaches a method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug (para [0106], [0173]) comprising:
(a) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (b) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(d) administering a second dosage of the P AA prodrug based on the determination in (c) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

*****Continued in Supplemental Box*****

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V.2. Citations and Explanations:

Regarding claim 3, Scharschmidt teaches a method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject (para [0116], [0173]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the P AA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 4, Scharschmidt teaches a method of treating a condition for which PAA prodrug administration is expected to be beneficial in a subject (para [0116]) who has previously been administered a first dosage of a P AA prodrug (para [0106]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 5, Scharschmidt teaches a method of adjusting the dosage of a PAA prodrug (para [0021]) comprising:
(a) administering a first dosage of a P AA prodrug (para [0173]) and
(b) measuring PAGN levels (para [0174]), but fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (c) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(e) administering a second dosage of the P AA prodrug based on the determination in (d) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

*****Continued in Next Supplemental Box*****

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Prior Supplemental Box:

Regarding claim 6, Scharschmidt teaches a method of optimizing the therapeutic efficacy of a PAA prodrug in a subject (para [0297], [0173]) who has previously been administered a first dosage of a PAA prodrug (para [0106]) comprising:
(a) measuring PAGN levels (para [0174]), however fails to teach wherein the PAGN levels are measured in plasma or wherein plasma PAA levels are measured as well or (b) calculating a plasma PAA:PAGN ratio.
Scharschmidt further teaches
(c) determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]), but fails to teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.
Scharschmidt goes on to teach
(d) administering a second dosage of the P AA prodrug based on the determination in (c) (para [0106], [0174]).
McGuire teaches measuring metabolites in blood and urine after administration of a PAA prodrug (abstract) and further teaches wherein these metabolites include plasma PAA and PAGN (pg 2079, col 2, para 3). McGuire further teaches comparing these measured concentrations in a ratio (pg 2081, col 1, para 2).
In light of the teachings of McGuire that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1) and that metabolites important in the monitoring of PAA prodrugs include PAA in addition to PAGN, it would have been obvious to one of ordinary skill in the art to modify the method taught by Scharschmidt by incorporating comparing of PAA to PAGN in plasma of a subject, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 7, the combination of Scharschmidt and McGuire makes obvious the method of claim 1, and Scharschmidt further teaches wherein the nitrogen retention disorder is selected from the group consisting of UCD (para [0097]).

Regarding claim 8, the combination of Scharschmidt and McGuire makes obvious the method of claim 3, and Scharschmidt further teaches wherein the disorder is a metabolic disorder (para [0046]).

Regarding claims 9-10, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 1, but fails to teach wherein the target range is 1 to 2.5 or wherein the target range is 1 to 2. It would have been obvious to an artisan of ordinary skill to determine the optimal target range for the plasma PAA:PAGN ratio for the subject being treated, based on routine experimentation, in order to more accurately assess the patient's response to PAA prodrugs and to suitably adjust the dosage of said prodrug based on the measured plasma PAA:PAGN ratio.

Regarding claim 11, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 1, and Scharschmidt further teaches wherein measurement PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state (para [0160]), but fails to teach wherein the PAA levels are measured. It would have been obvious to one of ordinary skill in the art to further measure the PAA at the same time as the PAGN in order to maintain comparable results.

Regarding claim 12, the combination of Scharschmidt (para [0106], [0173], [0174]) and McGuire (pg 2079, col 2, para 3; pg 2081, col 1, para 2) makes obvious the method of claim 11, and Scharschmidt further teaches wherein measurement of PAGN levels is carried out 48 hours to 1 week after the first dosage of PAA prodrug is administered (para [0160], 3 days), but fails to teach wherein the PAA levels are measured. It would have been obvious to one of ordinary skill in the art to further measure the PAA at the same time as the PAGN in order to maintain comparable results.

Regarding claim 13, the combination of Scharschmidt and McGuire makes obvious the method of claim 1, and Scharschmidt further teaches wherein the PAA prodrug is HPN-100 (para [0118]).

Claims 1-13 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 589 of 1354

Electronic Acknowledgement Receipt

EFS ID:	20747936
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Lara J. Dueppen/Deborah Muench
Filer Authorized By:	Lara J. Dueppen
Attorney Docket Number:	079532-8004.US01
Receipt Date:	19-NOV-2014
Filing Date:	11-SEP-2012
Time Stamp:	20:33:28
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Mercuri_E_2004_Neuromuscul Disord_14_130-135.PDF	214339 0bc7927cef71f03b15712509842fb202317242bc	no	6

Warnings:

Information:

2	Non Patent Literature	Mokhtarani_M_2012_MolGenetMetab_Abstract_105_342-343.PDF	61319 9cfbc082c1d3e87683b45d47d0eecd6bc505fcd6	no	2
Warnings:					
Information:					
3	Non Patent Literature	Monteleone_J_2012_MolGenetMetab_105_343.PDF	242644 4c69fa985e537a906beb0d1dc79b0ffa2404851e	no	2
Warnings:					
Information:					
4	Non Patent Literature	Ong_J_2003_AmJMed_114_188-193.PDF	113922 1cf4dd4f85d5bc2a8902ccabdc1e7031539bc3f8	no	6
Warnings:					
Information:					
5	Non Patent Literature	Piscitelli_S_1995_JClinPharmacol_35_368-373.PDF	1044496 928e2be0335d3787e1eb7aa7b2fca5bf1ae12e15	no	6
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6	Non Patent Literature	Propst_A_1995_DigestiveDisSci_40_1805-1815.PDF	963865 953917f33369accc759bc6363967b51d0aa47a9c	no	11
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Information:					
7	Non Patent Literature	Riley_T_2001_AmFamPhysician_64_1735-1740.PDF	70659 0f9b16e38519e6fda32244602d038bb2384c2705	no	6
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8	Non Patent Literature	Rudman_D_1973_JClinInvest52_2241-2249.PDF	758124 f4a2a8576f6f5907951cc6189285b142fff60b92	no	9
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Information:					
9	Non Patent Literature	Ryu_H_2005_JNeurochem_93_1087-1098.PDF	819106 f46c5411bbf08d35b97164d59b415d0f9b75f2de	no	12
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Information:					
10	Non Patent Literature	Simell_O_1986_PedRes_20_1117-1121.PDF	557829 b0d328ee2f9ba774739c7ebfcf252554ed1161a2	no	5
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Information:					

11	Non Patent Literature	Singh_Supp_J_Pediatrics_138_S1-S5.PDF	467433 4bdacafe03e85bfd9631616ffc2bf3e76b9898e4	no	5
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Information:					
12	Non Patent Literature	Stauch_S_1998_JHepatology_28_856-864.PDF	296302 bcff3f692e8a5aafbded52152a4b2060a766e604	no	9
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Information:					
13	Non Patent Literature	Summar_M_2007_JInheritMetabDis_30_82_327-O.PDF	65765 7d561a42b3513c0aff67e4463a3e415df5af4fa9	no	1
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14	Non Patent Literature	Summar_M_2008_Acta_Paediatrica_97_1420-1425.PDF	635881 35d13b5f4bbbc90b3763059d10e7680aa78d32b9	no	6
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15	Non Patent Literature	SwedishOrphans.PDF	466059 ec43b4bca9a695012811761b5cdde20fb80f24c1	no	1
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Information:					
16	Non Patent Literature	Tanner_L_2007_JInheritedMetabDis_30_716-721.PDF	633161 85f481690faad443082dda685381831aa8d3f44f	no	6
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17	Non Patent Literature	Thibault_A_1994_CancerRes_54_1690-1964.PDF	1141364 413e4f7133cd39ca6c14b8e5a870fee52242b239	no	5
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18	Non Patent Literature	Thibault_A_1995_Cancer_75_2932-2938.PDF	1185711 62189db432e0059d97bbd0a4d90500338b912ebd	no	7
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19	Non Patent Literature	Waterlow_J_1963_AmJClinNutrition_12_235-240.PDF	1142177 116160303c47be7a71f360d2773d2fc0b2e1bddd	no	6
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20	Non Patent Literature	Xie_G_2012_Gastroenterology_142_918-927.PDF	6518066 3257c9a1c2831745b17a551bed9a2f6a2512081a	no	16
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21	Other Reference-Patent/App/Search documents	2009-10-09_Combined_Search_Exam_Report_GB0915545-8.PDF	350321 3ab5a7af97ac6810699e47be4348b4fa1828ba48	no	5
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22	Other Reference-Patent/App/Search documents	2010-09-09_Combined_Search_Exam_Report_GB1013468-2.PDF	645030 5459bd9ddf4ce11a2650fcfda3c5f611d81da000	no	6
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23	Other Reference-Patent/App/Search documents	2011-11-02_eESR-EP09739263.PDF	497036 470bc7c9f91e5a73ccffcfe2cdd0ef670989d6cd	no	6
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24	Other Reference-Patent/App/Search documents	2009-12-30_ISR-EPandWO.PDF	1249139 4eb380a535bfb8943ff342a7c27b292b139a12ad	no	13
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25	Other Reference-Patent/App/Search documents	2010-02-05_GB_Examination_Report-GB0915545-8.PDF	171095 b535c06df87edabc7861918247f08c2a484b68e2	no	2
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26	Other Reference-Patent/App/Search documents	2010-05-11_GB_Examination_Report-GB0915545-8.PDF	112112 fcaf295119d337b48fabaaa22934e5f995f8a458	no	1
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27	Other Reference-Patent/App/Search documents	2010-10-27_GB_Examination_Report-GB0915545-8.PDF	145446 0ff1e9b5785acfd7d7f42cae6eab7f46f680a408	no	2
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28	Other Reference-Patent/App/Search documents	2011-10-28_GB_Examination_Report-GB1013468-2.PDF	257798 c985e27cc2abd0ad7ebf08418b52277508a49f87	no	2
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29	Other Reference-Patent/App/Search documents	2014-04-10_IPRP_Ch_I-PCTUS2012028620.PDF	1648474 99fc5ffdcf3dc82c792003669d6ead0e30b7deaa	no	7
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30	Other Reference-Patent/App/Search documents	2013-09-04_IPRP_Ch_II-PCTUS2012028620.PDF	458238 24b2b74ecc36a476dd2cfe125acf3a958ae18fca	no	6
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31	Other Reference-Patent/App/Search documents	2011-03-10_IPRPandWO_Ch_I_PCTUS2009030362.PDF	982809 53a00976818ea104f01be34eb34b3bf6c05a1640	no	8
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32	Other Reference-Patent/App/Search documents	2011-03-10_IPRPandWO_Ch_I_PCTUS2009055256.PDF	247203 97d02ac3bdb8635429fc3c7f9a22601813c6a500	no	12
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33	Other Reference-Patent/App/Search documents	2009-03-02_ISRandWO.PDF	568842 4464dfcb205cfed0e99babd63236056be8926404	no	9
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Information:					
34	Non Patent Literature	Ambrose_A_1933_JBiolChem_101_669.PDF	676103 9b884d7dbc620e63b71d5af59990f4799f36c2ed	no	8
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35	Non Patent Literature	Brusilow_SW_1991_PediatrRes_29_147-150.pdf	740584 ad33d7be2aa4d955609c5d32a1b543d8e795742f	no	4
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36	Non Patent Literature	Brusilow_SW_1994_IND_Protocol.pdf	653110 65a48f72e749b3878402c5015a2cd33df529f763	no	14
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37	Non Patent Literature	Diaz_SIMD_ph_III_final_abstract.pdf	84490 6bda481ae62d34fa0faed8de3e80e888df6e9ef3	no	2
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38	Non Patent Literature	FDA_Buphenyl_9pgs.pdf	135331 6392bcfefcdf0ec928f2d53b1cf74736f2f99183	no	9
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39	Non Patent Literature	Gargosky_S_2005-08-02_SSIEM_Poster.pdf	354373 53d6759183ed6ffc301ef972d5ba6f264c95fa8c	no	6
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40	Non Patent Literature	Gropman_A_2008_MolGenetMetab_94_52-60.pdf	2009379 6a905a0c46a36606d3fab34ad6bd30705fafd994	no	18
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41	Non Patent Literature	John_B_2009ACMG_ADMEposter.pdf	1029585 7d017266b21fa05b753189d86c2d05391f761073	no	2
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Information:					
42	Non Patent Literature	Lee_B_2009_ICIEM_Abstract_uPAGN_biomarker_FINAL.pdf	112033 c681c28ec1302272cf999650e8031199390f37ef	no	1
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43	Non Patent Literature	Gargosky_S_2005-10-14_ASHG_Poster_AMMONU.pdf	326324 e5ad1a2c8a96f2ea061ec393d3864c4e0d069eb9	no	1
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44	Non Patent Literature	Gargosky_S_2006_ACMGPoster_AMMONUL_UCY05015E.pdf	315656 bcee5c0db92b55703caf614dfe355ac5def49da8	no	1
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45	Non Patent Literature	Lewis_HB_1914_JBiolChem_18_225-231.pdf	914918 5cf9cb28f049124b71300774a5a690360bcd400c	no	7
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46	Non Patent Literature	Mansour_A_1997_Surgery_122_730-736.pdf	1431221 f714e5c14580d08220be85bf44453e03543054d9	no	7
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47	Non Patent Literature	McGuire_B_2008_IntlSymposium_Italy_April.pdf	135415 56420bafad374516e6c021151a5290adfb3be625	no	2
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48	Non Patent Literature	McGuire_B_2009_DDW_Abstract.pdf	51360 555db4272f211746903efba7e7380d19de3df8a7	no	2
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Information:					
49	Non Patent Literature	McQuade_P_1984_ProgNeuro-PsychopharmacolBiolPsychiat_8_607-614.pdf	1382043 8bdc36233509b9afb1d15377499e29082fd8ea61	no	8
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50	Non Patent Literature	Moldave_K_1957_JBiolChem_229_463-476.pdf	2053969 968a689dd7a55fe35c5463db9c6ef61e597dd4af	no	15
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51	Non Patent Literature	Perrine_S_2008_PediatrAnn_37-339-346.pdf	1247020 b5ec8c0239677236665acd8e1afb6989e7be57ec	no	10
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52	Other Reference-Patent/App/Search documents	2012-06-20_ISRandWO.pdf	6733198 0b9bb6f10def7c9daa55ae6d97dc6e935153e0dd	no	8
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53	Other Reference-Patent/App/Search documents	2014-03-28_ISRandWO.pdf	17460908 17b517e60a6d0eb90abccee544ebd21970139cd1	no	9
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54	Non Patent Literature	Shiple_G_1922_JAmChemSoc_44_618-624.pdf	7240266 40e6bae25189afbc952cd61146d414d91adef5f0	no	7
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55	Non Patent Literature	Tuchman_M_2008_MolGenetMetab_94_397-402.pdf	2086642 8bba4d92c6e04f7a46230d1b733a1a5e4463117a	no	6
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56	Non Patent Literature	Zeitlin_P_2002_MolTher_6_119-126.pdf	1284571 5479cb1a2e85f8fcc9b8eaf0e9117bf157dad589	no	8
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57	Other Reference-Patent/App/Search documents	2012-11-20_ISRandWO.pdf	10693349 55b8bd529db22fd272ab401381cc766c8b4e2954	no	8
Warnings:					
Information:					
		Total Files Size (in bytes):	83883613		

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/610,580	09/11/2012	Bruce Scharschmidt	079532-8004.US01	1957

34055 7590 02/27/2015
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

EXAMINER
TOWNSLEY, SARA ELIZABETH

ART UNIT	PAPER NUMBER
1629	

NOTIFICATION DATE	DELIVERY MODE
02/27/2015	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

patentprocurement@perkinscoie.com

PTOL-90A (Rev. 04/07)

Office Action Summary	Application No. 13/610,580	Applicant(s) SCHARSCHMIDT ET AL.	
	Examiner SARA E. TOWNSLEY	Art Unit 1629	AIA (First Inventor to File) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11/4/2014*.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ______.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ______; the restriction requirement and election have been incorporated into this action.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5)☒ Claim(s) *1,2,5-7 and 9-13* is/are pending in the application.

5a) Of the above claim(s) ______ is/are withdrawn from consideration.

6)☐ Claim(s) ______ is/are allowed.

7)☒ Claim(s) *1,2,5-7 and 9-13* is/are rejected.

8)☐ Claim(s) ______ is/are objected to.

9)☐ Claim(s) ______ are subject to restriction and/or election requirement.

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☐ The drawing(s) filed on ______ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a)☐ All b)☐ Some** c)☐ None of the:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. ______.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date *11/19/2014*.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. ______ .

4) ☐ Other: ______.

NON-FINAL REJECTION

The present application is being examined under the pre-AIA first to invent provisions.

This application, filed Sep. 11, 2012, claims benefit of priority to provisional application 61/636,256, filed Apr. 20, 2012.

Claims 1, 2, 5-7, and 9-13, as amended, are pending.

Priority

Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c) is acknowledged.

Election/Restrictions

Applicant's election without traverse of the compound species HPN-100 (glycerol phenylbutyrate, CAS Registry No. 611168-24-2), and urea cycle disorder as the species of medical condition treated, in the reply filed on Nov. 4, 2014 is acknowledged.

Information Disclosure Statement

The information disclosure statement (IDS) submitted on Nov. 19, 2014 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement has been considered by the examiner.

Claim Objections

1. Claims 1, 2, 5-7, and 9-13 are objected to because of the following informalities: the first recitation of “PAA” should spell out in full the term for which it is an abbreviation, phenylacetic acid. Similarly, the first recitation of “PAGN” should spell out in full the term for which it is an abbreviation, phenylacetyl glutamine.

Appropriate correction is required.

Claim Rejections - 35 USC § 103

2. The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

4. Claims 1, 2, 5-7, and 9-13 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Scharschmidt (US Pub. 2012/0022157) in view of McGuire et al.

(*Hepatology* 51, 2077-2085 (2010)) (cited as references A9 and C66, respectively, on the IDS dated Nov. 19, 2014).

Independent claim 1 recites a method of treating a nitrogen retention disorder in a subject; and **independent claim 5** recites a method of adjusting the dosage of a PAA prodrug, each comprising the steps of

(a) administering a first dosage of a PAA prodrug,

(b) measuring plasma PAA and PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range,

where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased, and

a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the PAA prodrug based on the determination in (d).

Scharschmidt discloses a method of treating a nitrogen retention disorder in a subject (para [0173]) comprising:

(a) administering a first dosage of a PAA prodrug (para [0173]) and

(b) measuring urinary PAGN levels (para [0174]).

However, Scharschmidt does not disclose measuring PAA or PAGN levels in plasma, or (c) calculating a plasma PAA:PAGN ratio.

Scharschmidt further teaches the step of determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]).

However, Scharschmidt does not teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.

Scharschmidt also discloses the step of (e) administering a second dosage of the PAA prodrug based on the determination in (d) (para [0106], [0174]).

McGuire discloses measuring metabolites in blood and urine after administration of a PAA prodrug (abstract), wherein the metabolites include plasma PAA and PAGN (page 2079, col 2, para 3), and comparing these values as a ratio (pg 2081, col 1, para 2). McGuire further teaches that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1), and that metabolites important in the monitoring of PAA prodrugs include both PAA and PAGN.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Scharschmidt by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, and evaluate any need to adjust the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN.

Independent claim 2 recites a method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug; and **independent claim 6** recites a method of optimizing the therapeutic efficacy of a PAA prodrug in a subject who has previously been administered a first dosage of a PAA prodrug, each comprising the steps of

(a) measuring plasma PAA and PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first PAA prodrug dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the PAA prodrug based on the determination in (c).

Scharschmidt teaches a method of treating a nitrogen retention disorder in a subject who has previously been administered a first dosage of a PAA prodrug (para [0106], [0173]) comprising measuring PAGN levels (para [0174]). Scharschmidt also teaches a method of optimizing the therapeutic efficacy of a PAA prodrug in a subject (para [0297],[0173]) who has previously been administered a first dosage of a PAA prodrug (para [0106]) comprising measuring PAGN levels (para [0174]).

However, Scharschmidt does not disclose measuring PAA or PAGN levels in plasma, or (c) calculating a plasma PAA:PAGN ratio.

Scharschmidt further teaches the step of determining whether the PAA prodrug dosage needs to be adjusted based on whether the measured levels of PAGN falls within a target range (para [0174], [0106]).

However, Scharschmidt does not teach wherein the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased.

Scharschmidt also discloses the step of (d) administering a second dosage of the PAA prodrug based on the determination in (c) (para [0106], [0174]).

McGuire discloses measuring metabolites in blood and urine after administration of a PAA prodrug (abstract), wherein the metabolites include plasma PAA and PAGN (page 2079, col 2, para 3), and comparing these values as a ratio (pg 2081, col 1, para 2). McGuire further teaches that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1), and that metabolites important in the monitoring of PAA prodrugs include both PAA and PAGN.

Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Scharschmidt by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, and evaluate any need to adjust (optimize) the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN.

Scharschmidt discloses that in some embodiments, the nitrogen retention disorder is the elected condition, a UCD (urea cycle disorder), as recited by claim 7; and that the PAA prodrug can be the elected compound, HPN-100 (para. [0097]), as recited by claim 13.

While Scharschmidt does not disclose that the PAA:PAGN ratio falls within a target range of 1 to 2.5, as recited by claim 9, or within a target range of 1 to 2, as recited by claim 10, it would have been *prima facie* obvious to an ordinarily skilled clinician to determine the optimal target range for the plasma PAA:PAGN ratio for the subject being treated, by routine experimentation.

Scharschmidt further teaches measurement PAGN levels is carried out after the first dosage of PAA prodrug has had sufficient time to reach steady state (para [0160]), but does not disclose measurement of PAA levels, as recited by claim 11. However, it would have been *prima facie* obvious to an ordinarily skilled clinician to further measure PAA as well as PAGN in order to maintain comparable results, by routine experimentation.

Scharschmidt further teaches measurement of PAGN levels 48 hours to 1 week after the first dosage of PAA prodrug is administered (para (0160), 3 days), but does not disclose measurement of PAA levels, as recited by claim 12. However, it would have been *prima facie* obvious to an ordinarily skilled clinician to further measure PAA as well as PAGN in order to maintain comparable results, by routine experimentation.

The rationale to combine and modify Scharschmidt and McGuire is premised on the findings that (1) the prior art includes each element claimed, with the only difference

between the claimed invention and the prior art being the lack of actual combination of the elements in a single prior art reference; (2) one of ordinary skill in the art could have combined the elements as claimed by known methods, and that in combination, each element merely performs the same function as it does separately; and (3) one of ordinary skill in the art would have recognized that the results of the combination were predictable.

As recognized by MPEP §2143, combining prior art elements according to known methods to yield predictable results would motivate the skilled artisan to modify the references with a reasonable expectation of success. The rationale to support a conclusion of *prima facie* obviousness is that all the claimed elements were known in the prior art, and a skilled artisan could have combined the elements as claimed by known methods with no change in their respective functions, and the combination yielded nothing more than predictable results to one of ordinary skill in the art. See *KSR Int'l Co. v. Teleflex Inc.* (550 U.S. 398, 409).

Double Patenting

5. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit http://www.uspto.gov/forms/. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

6. Claims 1, 2, 5-7, and 9-13 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 6, 8, 11, and 12 of **U.S. Patent No. 8,642,012** in view of McGuire et al. (*Hepatology* 51, 2077-2085 (2010), cited above).

Reference claims 1, 3, and 6 are drawn to methods of treating a patient having a urea cycle disorder comprising

(a) determining a target urinary phenylacetyl glutamine (PAGN) output

(b) calculating an effective initial dosage of a phenylacetic acid (PAA) prodrug, e.g., HPN-100, wherein the effective dosage of PAA prodrug is calculated based on a mean conversion of PAA prodrug to urinary PAGN of about 60%; and

(c) administering the effective initial dosage of PAA prodrug to the patient;

wherein administration of the effective initial dosage of PAA prodrug produces a normal plasma ammonia level in the patient.

Reference claims 8, 11, and 12 are drawn to methods of administering a phenylacetic acid (PAA) prodrug, e.g., HPN-100, to a patient having a urea cycle disorder comprising

(a) administering a first dosage of the PAA prodrug;

(b) determining urinary phenylacetyl glutamine (PAGN) excretion following administration of the first dosage of the PAA prodrug;

(c) determining an effective dosage of the PAA prodrug based on the urinary PAGN excretion, wherein the effective dosage is based on a mean conversion of PAA prodrug to urinary PAGN of about 60%; and

(d) administering the effective dosage to the patient,

wherein administration of the effective dosage of PAA prodrug produces a normal plasma ammonia level in the patient.

McGuire discloses measuring metabolites in blood and urine after administration of a PAA prodrug (abstract), wherein the metabolites include plasma PAA and PAGN (page 2079, col 2, para 3), and comparing these values as a ratio (pg 2081, col 1, para 2). McGuire further teaches that urinary testing is not as complete and thorough as plasma testing (pg 2081, col 2, para 1), and that metabolites important in the monitoring of PAA prodrugs include both PAA and PAGN.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the methods of the reference claims by measuring plasma levels of PAA and PAGN, instead of urinary PAA and PAGN, and comparing them as a ratio, in order to more accurately assess the patient's metabolism of PAA prodrugs, and evaluate any need to adjust (optimize) the dosage, with a reasonable expectation of success, because McGuire teaches that urinary testing is not as complete and thorough as testing for plasma levels of PAA and PAGN. In addition, it

would have been *prima facie* obvious to an ordinarily skilled clinician to further measure PAA as well as PAGN, and to determine the optimal target range for the plasma PAA:PAGN ratio by routine experimentation.

Conclusion

Claims 1, 2, 5-7, and 9-13 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SARA E. TOWNSLEY whose telephone number is 571-270-7672. The examiner can normally be reached on Mon-Fri from 9:00 am to 5:00 pm (EST). If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeff S. Lundgren, can be reached at 571-272-5541. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://portal.uspto.gov/ external/portal. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SARA E. TOWNSLEY/
Examiner, Art Unit 1629

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BIB DATA SHEET

CONFIRMATION NO. 1957

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13/610,580	09/11/2012 RULE	514	1629	079532-8004.US01

APPLICANTS

INVENTORS
Bruce Scharschmidt, San Francisco, CA;
Masoud Mokhtarani, Walnut Creek, CA;

** **CONTINUING DATA** *************************
This appln claims benefit of 61/636,256 04/20/2012

** **FOREIGN APPLICATIONS** *************************

** **IF REQUIRED, FOREIGN FILING LICENSE GRANTED** ** ** SMALL ENTITY **
09/24/2012

Foreign Priority claimed ☐ Yes ☑ No 35 USC 119(a-d) conditions met ☐ Yes ☑ No Verified and Acknowledged /SARA ELIZABETH TOWNSLEY/ Examiner's Signature	☐ Met after Allowance Initials	STATE OR COUNTRY	SHEETS DRAWINGS	TOTAL CLAIMS	INDEPENDENT CLAIMS
		CA	7	10	4

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TITLE

METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

FILING FEE RECEIVED	FEES: Authority has been given in Paper	
1139	No.____________ to charge/credit DEPOSIT ACCOUNT No.____________ for following:	☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other ________________ ☐ Credit

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Form PTO-1449 (Modified)
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Sheet	1	of	11

COMPLETE IF KNOWN	
Application Number	13/610,580
Confirmation Number	1957
Filing Date	September 11, 2012
First Named Inventor	SCHARSCHMIDT, Bruce
Group Art Unit	1765
Examiner Name	
Attorney Docket No.	79532.8004.US01

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	A12	2013/0210914		SCHARSCHMIDT	08/15/2013	

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	B3	WO	2007/005633		Navinta LLC	01/11/2007		
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	B7	WO	2012/028620		INSERM	03/08/2012		

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EXAMINER /Sara E. Townsley/	DATE CONSIDERED 12/01/2014

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT Form PTO-1449 (Modified) (Use several sheets if necessary)				**COMPLETE IF KNOWN**	
				Application Number	13/610,580
				Confirmation Number	1957
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				First Named Inventor	SCHARSCHMIDT, Bruce
				Group Art Unit	1765
				Examiner Name	
Sheet	2	of	11	Attorney Docket No.	79532.8004.US01

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EXAMINER /Sara E. Townsley/	DATE CONSIDERED 12/01/2014

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	C95	EUROPEAN PATENT OFFICE, Extended European Search Report for EP09739263 completed November 2, 2011.	
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	C104	International Preliminary Report on Patentability for PCT/US2009/055256, completed on August 27, 2009, mailed on March 10, 2011.	
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Index of Claims	Application/Control No. 13610580	Applicant(s)/Patent Under Reexamination SCHARSCHMIDT ET AL.
	Examiner SARA E TOWNSLEY	Art Unit 1629

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☐ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47

CLAIM		DATE								
Final	Original	10/05/2014	02/20/2015							
	1	÷	✓							
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CPC- SEARCHED

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CPC COMBINATION SETS - SEARCHED

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US CLASSIFICATION SEARCHED

Class	Subclass	Date	Examiner

SEARCH NOTES

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61/636,256 considered	2/20/2015	set
Inventor name/assignee search (PALM, EAST)	2/20/2015	set
EAST keyword search (USPAT, PGPub, USOCR, EPO, JPO, Derwent)	2/20/2015	set

INTERFERENCE SEARCH

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EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L5	7	"8404215".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2015/02/23 10:20
L6	674	scharschmidt.in. or mokhtarani.in. or hyperion.as.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2015/02/23 10:22
L7	539	urea cycle disorder	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2015/02/23 10:22
L8	14	L6 and L7	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2015/02/23 10:22
L9	20	L6 and plasma.clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2015/02/23 10:22
S1	2	"20130281530".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 12:37
S2	2	"5968979".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 12:38
S3	2	WO "2009134460"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:05
S4	2	"8642012".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:06
S5	2	"20100008859".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:07
S6	666	scharschmidt.in. or mokhtarani.in. or hyperion.as.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:10
S7	72	HPN-100 or HPN100 or HPN "100"	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:10
S8	14	S6 and S7	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:10
S9	517	urea cycle disorder	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:11
S10	13	S7 and S9	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:12
S11	29	brusilow.in.	US-PGPUB; USPAT; USOCR; EPO; JPO;	ADJ	ON	2014/11/26 13:13

			DERWENT			
S12	3	PAA WITH PAGN WITH ratio	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:13
S13	3	"4284647".pn.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:34
S14	77	HPN-100 or HPN100 or HPN "100" or glycerol phenylbutyrate	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	ADJ	ON	2014/11/26 13:39

EAST Search History (Interference)

<This search history is empty>

2/23/2015 10:25:32 AM
C:\Users\stownsley\Documents\EAST\Workspaces\13610580.wsp

PTO/AIA/80 (07-12)
Approved for use through 11/30/2014. OMB 0651-0035
U.S. Patent and Trademark Office; U.S DEPARTMENT OF COMMERCE
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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: **101325**

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number: **101325**

OR

☐

Firm or Individual Name			
Address			
City		State	Zip
Country			
Telephone		Email	

Assignee Name and Address: Horizon Therapeutics, Inc.
533 Bryant, Suite #6
Palo Alto, CA 94301

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be Filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature	[signature]	Date	5/1/15
Name	Brian K. Beeler	Telephone	847-502-5250
Title	Senior VP, Legal		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: HORIZON THERAPEUTICS, INC.

Application No./Patent No.: As set forth on the attached Schedule A Filed/Issue Date: As set forth on the attached Schedule A

Titled: ______

HORIZON THERAPEUTICS, INC. , a Delaware Corporation

(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☑ The assignee of the entire right, title, and interest.

2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):

☐ The extent (by percentage) of its ownership interest is ______%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.

☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (*e.g.*, bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel See Schedule A, Frame See Schedule A, or for which a copy thereof is attached.

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: ______ To: ______

The document was recorded in the United States Patent and Trademark Office at

Reel ______, Frame ______, or for which a copy thereof is attached.

2. From: ______ To: ______

The document was recorded in the United States Patent and Trademark Office at

Reel ______, Frame ______, or for which a copy thereof is attached.

This collection of information is required by37 CFR3.73(b). The information is required toobtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentialityis governed by35 U.S.C. 122and 37 CFR1.11 and1.14. Thiscollection is estimated to take 12 minutes to complete, including gathering, preparing, and submittingthe completed application form to the USPTO.Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent tothe Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS.**SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: ______________________ To: ______________________

The document was recorded in the United States Patent and Trademark Office at

Reel ____________, Frame ____________, or for which a copy thereof is attached.

4. From: ______________________ To: ______________________

The document was recorded in the United States Patent and Trademark Office at

Reel ____________, Frame ____________, or for which a copy thereof is attached.

5. From: ______________________ To: ______________________

The document was recorded in the United States Patent and Trademark Office at

Reel ____________, Frame ____________, or for which a copy thereof is attached.

6. From: ______________________ To: ______________________

The document was recorded in the United States Patent and Trademark Office at

Reel ____________, Frame ____________, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Dennis A. Bennett/	May 15, 2015
Signature	Date
Dennis A. Bennett	Attorney of Record, Reg No. 34547
Printed or Typed Name	Title or Registration Number

Schedule A

Docket No.	Application No.	Application Date	Reel/Frame No.	Recordation Date
079532-8001.US01	12/350,111	2009-01-07	022305 / 0387 025031 / 0014 028014 / 0894 035638 / 0305	02/24/2009 09/22/2010 04/09/2012 05/14/2015
079532-8003.US02	13/417,137	2012-03-09	028014 / 0894 035638 / 0305	04/09/2012 05/14/2015
079532-8003.US03	13/775,000	2013-02-22	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8004.US01	13/610,580	2012-09-11	029337 / 0054 035638 / 0305	11/21/2012 05/14/2015
079532-8005.US02	14/086,870	2013-11-21	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US00	61/890,827	2013-10-14	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US01	62/044,168	2014-08-29	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015
079532-8007.US02	14/514,334	2014-10-14	035361 / 0777 035638 / 0305	04/08/2015 05/14/2015

Electronic Acknowledgement Receipt

EFS ID:	22364015
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	34055
Filer:	Dennis A. Bennett/Ronnie Almira
Filer Authorized By:	Dennis A. Bennett
Attorney Docket Number:	079532-8004.US01
Receipt Date:	15-MAY-2015
Filing Date:	11-SEP-2012
Time Stamp:	17:06:22
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	HorizonTherapeutics-POA_Assignee.pdf	96506 cb08b2aa2de030cfca8e0ff6ce3b34f18d522e9f	no	1

Warnings:

Information:

2	Assignee showing of ownership per 37 CFR 3.73	HOR_373-Statment_Schedule_A.pdf	157428 6c05c96d65f079637c44f6e854cbea479726c476	no	3

Warnings:

Information:

Total Files Size (in bytes):	253934

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/610,580	09/11/2012	Bruce Scharschmidt	079532-8004.US01

CONFIRMATION NO. 1957

POA ACCEPTANCE LETTER

OC000000075260399

101325
GLOBAL PATENT GROUP - HOR
1005 NORTH WARSON ROAD
SUITE 404
SAINT LOUIS, MO 63132

Date Mailed: 05/20/2015

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/15/2015.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ytdemisse/

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/610,580	09/11/2012	Bruce Scharschmidt	079532-8004.US01

CONFIRMATION NO. 1957

POWER OF ATTORNEY NOTICE

OC000000075259839

34055
PERKINS COIE LLP - LOS General
POST OFFICE BOX 1247
SEATTLE, WA 98111-1247

Date Mailed: 05/20/2015

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/15/2015.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at **(571) 272-4000** or **(571) 272-4200** or **1-888-786-0101**.

/ytdemisse/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Scharschmidt et al. | Confirmation No. 1957
Application No.: 13/610,580 | Examiner: Sara Elizabeth Townsley
Filing Date: September 11, 2012 | Group Art Unit: 1629

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT

Sir:

This Amendment is responsive to the Non-Final Official Action mailed February 27, 2015 regarding the above-referenced patent application. Please amend the above-identified application as shown and reconsider the rejections of the claims for at least the reasons presented in the following remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page **2** of this paper.

Remarks follow the Amendments to the Claims.

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Currently Amended) A method of treating urea cycle disorders ~~a nitrogen retention disorder~~ in a subject comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~,

(b) measuring plasma phenylacetic acid (PAA) [[PAA]] and phenylacetyl glutamine (PAGN) [[PAGN]] levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ based on the determination in (d).

2. (Currently Amended) A method of treating urea cycle disorders ~~a nitrogen retention disorder~~ in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~ comprising:

(a) measuring plasma phenylacetic acid (PAA) [[PAA]] and phenylacetyl glutamine (PAGN) [[PAGN]] levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the first ~~PAA prodrug~~ dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(d) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~

based on the determination in (c).

3. (Cancelled)

4. (Cancelled)

5. (Currently Amended) A method of adjusting the dosage of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~ comprising:

(a) administering a first dosage of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~,

(b) measuring plasma phenylacetic acid (PAA) [[PAA]] and phenylacetyl glutamine (PAGN) [[PAGN]] PAGN levels,

(c) calculating a plasma PAA:PAGN ratio,

(d) determining whether the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ dosage needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ based on the determination in (d).

6. (Currently Amended) A method of optimizing the therapeutic efficacy of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~ in a subject who has previously been administered a first dosage of glyceryl tri-[4-phenylbutyrate] ~~a PAA prodrug~~ comprising:

(a) measuring plasma phenylacetic acid (PAA) [[PAA]] and phenylacetyl glutamine (PAGN) [[PAGN]] PAGN levels,

(b) calculating a plasma PAA:PAGN ratio,

(c) determining whether the ~~PAA prodrug~~ dosage of glyceryl tri-[4-phenylbutyrate] needs to be adjusted based on whether the PAA:PAGN ratio falls within a target range, where a PAA:PAGN ratio below the target range indicates that the dosage potentially needs to be increased and a PAA:PAGN ratio above the target range indicates that the dosage needs to be decreased, and

(e) administering a second dosage of the glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ as necessary based on the determination in (c).

7. (Cancelled)

8. (Cancelled)

9. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein the target range is 1 to 2.5.

10. (Previously Presented) The method of any of claims 1, 2, 5, or 6, wherein the target range is 1 to 2.

11. (Currently Amended) The method of any of claims 1, 2, 5, or 6, wherein measurement of PAA and PAGN levels is carried out after the first dosage of glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ has had sufficient time to reach steady state.

12. (Currently Amended) The method of claim 11, wherein measurement of PAA and PAGN levels is carried out 48 hours to 1 week after the first dosage of glyceryl tri-[4-phenylbutyrate] ~~PAA prodrug~~ is administered.

13. (Cancelled)

REMARKS

Claims 7 and 13 have been cancelled with prejudice or disclaimer. Claims 1, 2, 5, 6, 11, and 12 have been amended. No new matter has been added by these amendments. Upon entry of this amendment, claims 1, 2, 5, 6, and 9-12 are pending.

Claim Objections

The Office has objected to the claims because of certain informalities. Specifically, the Office requests that the first recitations of "PAA" and "PAGN" should spell out in full the respective terms for which they stand. Applicant has amended the claims accordingly. Applicant requests that the objections be withdrawn.

Rejections under 35 U.S.C. § 103(a) (pre-AIA)

Claims 1, 2, 5-7, and 9-13 have been rejected under 35 U.S.C. § 103(a), as allegedly obvious over US Pub 2012/0022157 ("Scharschmidt") in view of McGuire et al. Hepatology 51, 2077-2085 (2010) ("McGuire"). The Office alleges that Scharschmidt discloses at [0173] a method of treating a nitrogen disorder in a subject, comprising (a) administering a PAA prodrug ([0173]), and (b) measuring urinary PAGN levels ([0174]). The Office acknowledges that Scharschmidt does not teach measuring the PAA and PAGN levels in plasma, or calculating the PAA/PAGN ratio. The Office also acknowledges that Scharschmidt does not teach using the PAA/PGN ratio in comparison to a target range to determine whether the PAA prodrug dosage needs to be decreased or increased. The Office alleges that the teachings in McGuire regarding measuring metabolites, including PAA and PAGN, of PAA prodrugs in plasma, and comparing these values as a ratio, together with the teachings of Scharschmidt, would lead the person of ordinary skill in the art at the time the present invention was made to measure plasma levels of PAA and PAGN in a patient taking a PAA prodrug, and use the PAA/PAGN ratio to adjust the dosage of the PAA prodrug. Applicant respectfully disagrees.

McGuire describes a statistical approach to assess bioequivalency of 2 different drugs (glycerol phenylbutyrate [GPB] as compared with sodium phenylbutyrate [NaPBA]). The ratio referred to by McGuire is a ratio of the geometric means of the systemic exposure to the same individual metabolites (PBA, PAA or PAGN) during dosing with GPB as compared NaPBA that is calculated as follows:

$$\text{Ratio} = \frac{\text{(PBA blood levels on GPB)}}{\text{(PBA blood levels on NaPBA)}}$$

wherein the systemic exposure is calculated based on PBA levels taken at multiple time points from multiple patients during dosing with each of the two different drugs (multiple samples from multiple patients on two different drugs). McGuire simply utilizes the conventional methodology for assessing bioequivalence of one drug to another, which involves comparing the ratio of the systemic exposure to the same metabolite, in this case PBA, during dosing with GPB as compared with NaPBA wherein the comparison of the two is expressed as a ratio. The same approach would be used for the other metabolites, including PAA and PAGN. Calculating the ratio of geometric means is a well-established statistical approach that is accepted by the field and regulatory authorities for assessing bioequivalence of 2 different drugs.

Importantly, McGuire does not teach the novel and unexpected finding that the ratio of two *different* metabolites; i.e,. PAA and PAGN, taken at the *same* time from the *same* patient receiving either glyceryl tri-[4-phenylbutyrate] (GPB) is of utility in assessing the effectiveness of PAA to PAGN conversion and, therefore, useful in identifying patients who are likely to experience high levels of PAA, a potentially toxic metabolite, and in whom dose reduction may be needed. The present invention teaches use of the following formula:

$$\text{Ratio} = \frac{\text{(PAA blood level on GPB)}}{\text{(PAGN blood level on GPB)}}$$

wherein the ratio represents the plasma level of PAA divided by the plasma level of PAGN and where both blood samples are taken from the same patient at exactly the same time (one sample from one patient on one drug).

Applicants have discovered that measuring the PAA/PAGN ratio provides an unexpectedly accurate measure of PAA prodrug metabolism in subjects with nitrogen retention disorders and/or hepatic impairment. This is important because high levels of PAA in circulation cause reversible toxicity (see specification at paragraph [0010]), and conversion of PAA to PAGN is a saturable process that varies considerably among individuals (specification at paragraph [0028]. Because PAA, PAGN, and ammonia levels do not provide information on whether a subject is effectively converting a PAA prodrug to PAGN, before the present invention was made there was lacking a

method to evaluate conversion of a PAA prodrug to PAGN on an individual basis, to provide improved methods of adjusting PAA prodrug dosage.

McGuire teaches the comparison of the same metabolite in patients taking different drugs, for the purpose of assessing bioequivalence of two different drugs. Nothing in McGuire teaches or suggests measuring two different metabolites from glyceryl tri-[4-phenylbutyrate] in the same patient, and using the ratio of the two metabolites from the same patient to adjust the dosage of the glyceryl tri-[4-phenylbutyrate]. Because the element of measuring plasma levels of PAA and PAGN in a single patient following treatment with glyceryl tri-[4-phenylbutyrate], and calculating the PAA/PGN ratio and comparing to a target range, is not taught or suggested by McGuire, the combination of references cited by the Office fails to teach all elements of the claimed invention. For at least these reasons, Applicant respectfully requests that the rejection be withdrawn.

Double Patenting

The claims have been rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1, 3, 6, 8, 11, and 12 of U.S. Patent No. 8,642,012 in view of McGuire. Solely to expedite prosecution and without in any way conceding to the rejection, Applicant submits a terminal disclaimer herewith. Applicant requests that the rejection be withdrawn.

The Examiner is invited to contact the undersigned by telephone or email if it is felt that an interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees due and any other fees under 37 C.F.R. § 1.16 or § 1.17 during the pendency of this application to our Deposit Account No. 50-4297.

Respectfully submitted,

/Lauren L. STEVENS/

Lauren Stevens, Reg. No. 36,691
Attorney for Applicants
Phone: 650-387-3813
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Scharschmidt et al. | Confirmation No. 1957

Application No.: 13/610,580 | Examiner: Sara Elizabeth Townsley

Filing Date: September 11, 2012 | Group Art Unit: 1629

For: METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS

NOTICE OF RELATED LITIGATION

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Applicant hereby notifies the U.S. Patent and Trademark Office that the subject matter of the present application is involved in litigation in the United States.

Specifically, Par Pharmaceutical, Inc. ("Par") sent a PIV notice letter to Hyperion Therapeutics, Inc. ("Hyperion") on March 12, 2014 providing notice that Par had filed an Abbreviated New Drug Application ("ANDA") with respect to RAVICTI® (Glycerol Phenylbutyrate) Oral Liquid, with a certification under 21 U.S.C. § 355(j)(2)(A)(vii)(IV) ("Paragraph IV") alleging that U.S. Patent Nos. 8,404,215 and 8,642,012 are invalid, unenforceable and/or will not be infringed by the commercial manufacture, use or sale of the Watson drug product.

Under 21 U.S.C. § 355(j)(5)(B)(iii), Hyperion had forty-five days from receipt of the ANDA notice letter to file suit against Watson for patent infringement. Accordingly, on April 23, 2014, Hyperion brought suit on those patents against Par in the United States District Court for the Eastern District of Texas, Marshall Division. The Complaint alleged that Par infringes U.S. Patent Nos. 8,404,215 and 8,642,012. Subsequently, in May of 2015, Horizon Pharma plc ("Horizon") acquired Hyperion Therapeutics, Inc. through a merger. The subject application is a divisional of U.S. Patent No. 8,404,215. The Complaint is provided with an SB-08 filed concurrently herewith.

Respectfully submitted,

By /Lauren L. STEVENS/

Lauren L. Stevens
Attorney for Applicant
Registration No. 36,691
(650) 387-3813

Par Pharmaceutical, Inc. Ex. 1009
Par v. Horizon, IPR of Patent No. 9,561,197
Page 643 of 1354

Electronic Patent Application Fee Transmittal

Application Number:	13610580
Filing Date:	11-Sep-2012
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Filer:	Lauren Stevens/Valerie Lechner
Attorney Docket Number:	HOR0027-201-US

Filed as Large Entity

Filing Fees for **Utility under 35 USC 111(a)**

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 3 months with $0 paid	1253	1	1400	1400
Miscellaneous:				
		Total in USD ($)		**1400**

Electronic Acknowledgement Receipt

EFS ID:	23024484
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Lauren Stevens/Valerie Lechner
Filer Authorized By:	Lauren Stevens
Attorney Docket Number:	HOR0027-201-US
Receipt Date:	29-JUL-2015
Filing Date:	11-SEP-2012
Time Stamp:	11:13:48
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	$1400
RAM confirmation Number	11738
Deposit Account	504297
Authorized User	LECHNER, VALERIE

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		HOR0027_Response.pdf	101545 f728a833ba1f3e47e563364b6e945595082950e7	yes	7

Multipart Description/PDF files in .zip description

Document Description	Start	End
Amendment/Req. Reconsideration-After Non-Final Reject	1	1
Claims	2	4
Applicant Arguments/Remarks Made in an Amendment	5	7

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
2	Notice of concurrent proceedings / decisions	HOR0027_NoticeRelated_Litigation.pdf	91031 38f3b4126982094a78dfda1cfd313e60405e16c3	no	2

Warnings:

Information:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
3	Fee Worksheet (SB06)	fee-info.pdf	30897 5e25a84a085cecfdce1f88660704668e77e28b94	no	2

Warnings:

Information:

Total Files Size (in bytes):	223473

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT COOPERATION TREATY PCT/US2014/058489 16.01.2015

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 795328008WO0	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US14/58489	International filing date *(day/month/year)* 30 September 2014 (30.09.2014)	(Earliest) Priority Date *(day/month/year)* 30 September 2013 (30.09.2013)
Applicant HYPERION THERAPEUTICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into ____________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

DIAGNOSING, GRADING, MONITORING, AND TREATING HEPATIC ENCEPHALOPATHY

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

a. the figure of the **drawings** to be published with the abstract is Figure No. ____________

☐ as suggested by the applicant.

☐ as selected by this Authority, because the applicant failed to suggest a figure.

☐ as selected by this Authority, because this figure better characterizes the invention.

b. ☒ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

International application No.
PCT/US14/58489

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61B 5/00; A61K 31/192 (2014.01)
CPC - A61B 5/00; A61K 31/192
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61B 5/00; A61K 31/192 (2014.01)
CPC: A61B 5/00; A61K 31/192; USPC: 424/9.2; 514/570

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, GB-G, FR-G, FR-A, CN-G, CN-G, KR-G, KR-A, AU-A, AU-G, IN-A, RU-A, RU-G, RU-U); Google; Google Scholar; Google Patent; ProQuest; PubMed/Medline; Search terms used: "hepatic encephalopathy", "hepatic coma", *orientat*, letharg*, asterixis, somnolence, coma, questionnaire

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2010/0016207 A1 (WURTMAN, RJ et al.) January 21, 2010; paragraphs [0059], [0064]-[0065], [0069], [0071]; claim 35	1-2 -- 3-5
X -- Y	MUNOZ, SJ. "Hepatic Encephalopathy". The Medical Clinics of North America. 2008; Vol. 92, pages 797-799, 803	6 -- 7-10, 18-20
X -- Y	CORDOBA, J. "New assessment of hepatic encephalopathy". Journal of Hepatology 2011 vol. 54; pages 1030, 1032, 1038	11 -- 12-20
Y	US 8404215 B1 (SCHARSCHMIDT, B et al.) March 26, 2013; column 1, lines 44-46; column 3, lines 16-19; column 20, lines 10-30	3-5, 7-10
Y	US 2005/0273359 A1 (YOUNG, DE) December 8, 2005; figures 1-2, 5; paragraphs [0021], [0030], [0037], [0038], [0040], [0044]-[0045]	12-20
Y	US 8094521 B2 (LEVY, G) January 10, 2012; abstract; column 3, lines 1-12, 17-21, 26-28, 56-58	17

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
23 December 2014 (23.12.2014)

Date of mailing of the international search report
16 JAN 2015

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:
Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

PATENT COOPERATION TREATY PCT/US2014/058489 16.01.2015

From the
INTERNATIONAL SEARCHING AUTHORITY

To: Courney Prochnow
Perkins Coie LLP
P.O. Box 1208
Seattle, Washington 98111-1208
United States of America

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	16 JAN 2015

Applicant's or agent's file reference	FOR FURTHER ACTION
795328008WO0	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US14/58489	30 September 2014 (30.09.2014)	30 September 2013 (30.09.2013)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61B 5/00; A61K 31/192 (2014.01)
CPC - A61B 5/00; A61K 31/192

Applicant HYPERION THERAPEUTICS, INC.

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to th IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	23 December 2014 (23.12.2014)	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/58489

Box No. I **Basis of this opinion**

1. With regard to the **language**, this opinion has been established on the basis of:
   ☒ the international application in the language in which it was filed.
   ☐ a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
   a. (means)
      ☐ on paper
      ☐ in electronic form
   b. (time)
      ☐ in the international application as filed
      ☐ together with the international application in electronic form
      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US14/58489

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	3-20	YES
	Claims	1-2	NO
Inventive step (IS)	Claims	NONE	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1 and 2 lack novelty under PCT Article 33(2) as being anticipated by US 2010/0016207 A1 to Wurtman et al. (hereinafter 'Wurtman').

As per claim 1, Wurtman discloses a method of determining whether a subject is experiencing an overt HE episode (a diagnosis of grade 4 hepatic encephalopathy determined by a comatose state, with or without response to painful stimuli; paragraphs [0064]-[0065], [0069]) comprising: (a) determining whether the subject has been disoriented as to time, place, or person for at least one hour; (b) determining whether the subject has been lethargic for at least one hour and is exhibiting asterixis; and/or (c) determining that the subject is incapable of being assessed due to disorientation as to time, place, and person, somnolence, or coma (the subject is in a coma, without response to painful stimuli (incapable of being assessed); paragraph [0069]), wherein the subject is classified as experiencing an overt HE episode if they meet any one of the criteria set forth in (a), (b), or (c) (the subject is classified with grade 4 hepatic encephalopathy, as presented by a coma, without response to painful stimuli; paragraphs [0065], [0069]).

As per claim 2, Wurtman discloses the method of claim 1, wherein a therapeutic intervention is administered to the subject if the subject meets any one of the criteria set forth in (a), (b), or (c) (administering to a subject with grade 4 hepatic encephalopathy, large neutral amino acids (LNAA; leucine / isoleucine / valine / tyrosine / phenylalanine); paragraphs [0059], [0071]; claim 35).

Claim 6 lacks an inventive step under PCT Article 33(3) as being obvious over the article titled "Hepatic Encephalopathy" (MUNOZ).

As per claim 6, Munoz discloses a method of treating an HE episode in a subject in need thereof (Pharmacologic therapy; pages 803-804), comprising: (a) determining whether a subject is experiencing at least a grade 2 HE episode (Clinical presentation, Table 2; pages 797-799) by (i) determining whether the subject has been disoriented as to time, place, or person for and/or (ii) determining whether the subject has been lethargic (Table 2, Stage II, consciousness; page 797) and is exhibiting asterixis (Table 2, Stage II, Neuromuscular abnormalities; page 797), wherein the subject is classified as experiencing at least a grade 2 HE episode if they meet the criteria set forth in either (i) or (ii) (Table 2, Stage II; page 797); (b) determining whether a subject is experiencing at least a grade 3 HE episode (Clinical presentation, Table 2; pages 797-799) by (iii) determining whether the subject has been disoriented as to time, place, and person and/or (iv) determining whether the subject has been somnolent (Table 2, Stage III, consciousness; page 797), wherein the subject is classified as experiencing at least a grade 3 HE episode if they meet the criteria set forth in either (iii) or (iv) (Table 2, Stage III; page 797); and (c) determining whether a subject is experiencing at least a grade 3 HE episode by (v) determining whether the subject is comatose (Table 2, Stage IV; page 797), wherein the subject is classified as experiencing a grade 4 HE episode if they meet the criteria set forth in (v) (Table 2, Stage IV; page 797); and (d) administering a therapeutic intervention if the subject is classified as experiencing a grade 2, 3, or 4 HE episode under steps (a), (b), or (c) (lactulose is administered to patients with stage I to IV hepatic encephalopathy; page 803). Munoz does not disclose; determining whether the subject has been disoriented as to time, place, and person, or lethargic, or somnolent, for at least one hour. It would have been obvious to a person of ordinary skill in the art, at the time of the invention, and it would have required only routine experimentation, to have modified the method of Munoz to include periods of observations for the signs of HE, i.e. whether the subject has been disoriented as to time, place, and person, or lethargic, or somnolent of at least one hour (other signs can be intermittent, wax and wane, or inconsistently occur with a stage of HE; page 799), for the advantage of strengthening the diagnosis for each grade of an HE episode, based upon an extended period of observation, and thereby mitigating the grading of HE episodes based on any transitory observations.

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US14/58489

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Box V: Citations and Explanations-***-

Claim 11 lacks an inventive step under PCT Article 33(3) as being obvious over the article 'New assessment of hepatic encephalopathy' (CORDOBA).

As per claim 11, Cordoba discloses a tool for screening a subject in need thereof for HE symptoms (monitoring and assessment of episodic HE; page 1030, 1st column) comprising:(a) a first set of steps comprising one or more steps selected from: (i) determining whether the subject has unusual difficulty speaking, (ii) determining whether the subject is exhibiting unusual behavior (abnormal behavior; supplementary table 4; section 1; page 1041), and (iii) determining whether the subject is more forgetful or confused than usual (difficulty focusing attention; thinking disorganized or incoherent; supplementary table 4; sections 2, 3a); (b) a second set of steps comprising one or more steps selected from: (iv) determining whether the subject can stay awake when being spoken to (lethargy, stupor; supplementary table 4, section 3b), (v) determining whether the subject is disoriented as to person, (vi) determining whether the subject is disoriented as to place, and (vii) determining whether the subject is disoriented as to time, wherein the second set of steps is performed only if one or more criteria from the first set of steps are met (cognitive function assessment in HE can be performed with a categorical or a continuous approach; cognitive assessment: (i) exclude episodic HE, (ii) clinical scales to assess severity of episodic HE; figure 1; page 1032; page 1038, 2nd column). Cordoba does not disclose wherein a user is instructed to contact a physician if one or more criteria from the second set of steps are met. Nevertheless, at the time of the invention it was well known in the art, to contact a physician in the event of onset of neurological impairment; and it would have been obvious to one of ordinary skill in the art to contact a physician if the first steps and the second steps were observed in a patient to provide necessary medical intervention.

Claims 3-5 lack an inventive step under PCT Article 33(3) as being obvious over Wurtman in view of US 8,404,215 B1 to Scharschmidt, et al. (hereinafter 'Scharschmidt').

As per claim 3, Wurtman discloses the method of claim 2. Wurtman does not disclose wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal. Scharschmidt discloses wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal (a dosage of 24 pills of Buphyenyl per day, amino acid supplements, and restricted dietary protein intake resulted in a patient's fasting blood ammonia level between 1 and 1.5 times the upper limit of normal (ULN); column 1, lines 14-16; column 20, lines 10-21). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Wurtman to include the therapeutic intervention of Scharschmidt, for the advantage of treating overt hepatic encephalopathy with an approach that includes treating and monitoring ammonia levels within a patient.

As per claim 4, Wurtman discloses the method of claim 2. Wurtman does not disclose wherein the therapeutic intervention is a nitrogen scavenging drug. Scharschmidt discloses wherein the therapeutic intervention is a nitrogen scavenging drug (the intervention includes the nitrogen scavenging drug sodium benzoate (Buphyenyl); column 3, lines 16-19; column 20, lines 10-12). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Wurtman to include the nitrogen scavenging drug of Scharschmidt, for the advantage of treating hyperammonemia, which is commonly associated with hepatic encephalopathy (Scharschmidt; column 1, lines 44-46).

As per claim 5, Wurtman and Scharschmidt, in combination, disclose the method of claim 4. Wurtman does not disclose wherein the nitrogen scavenging drug is selected from the group consisting of a PAA pro drug and sodium benzoate. Scharschmidt discloses wherein the nitrogen scavenging drug is selected from the group consisting of a PAA pro drug and sodium benzoate (nitrogen scavenging drug comprising HPN-100 (a PAA pro drug) and sodium benzoate (Buphyenyl), or any combination thereof; column 3, lines 16-19; column 20, lines 10-12). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Wurtman to include the specific nitrogen scavenging drugs of Scharschmidt, for the advantage of treating hyperammonemia, with commonly used medications (Scharschmidt; column 1, lines 44-46).

Claims 7-10 lack an inventive step under PCT Article 33(3) as being obvious over Munoz in view of Scharschmidt.

As per claim 7, Munoz discloses the method of claim 6. Munoz does not disclose wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal. Scharschmidt discloses wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal (a dosage of 24 pills of Buphyenyl per day, amino acid supplements, and restricted dietary protein intake resulted in a patient's fasting blood ammonia level between 1 and 1.5 times the upper limit of normal (ULN); column 20, lines 10-21). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Munoz to include the therapeutic intervention of Scharschmidt, for the advantage of treating overt hepatic encephalopathy with an approach that includes treating and monitoring fasting ammonia levels within a patient.

As per claim 8, Munoz and Scharschmidt, in combination, disclose the method of claim 7. Munoz does not disclose wherein the therapeutic intervention is a nitrogen scavenging drug. Scharschmidt discloses wherein the therapeutic intervention is a nitrogen scavenging drug (the intervention includes the nitrogen scavenging drug sodium benzoate (Buphyenyl); column 3, lines 16-19; column 20, lines 10-12). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Munoz to include the nitrogen scavenging drug of Scharschmidt, for the advantage of treating hyperammonemia, which is commonly associated with hepatic encephalopathy (Scharschmidt; column 1, lines 44-46).

-***-Continued Within the Next Supplemental Box-***-

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US14/58489

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

-***-Continued from Previous Supplemental Box-***-

As per claim 9, Munoz and Scharschmidt, in combination, disclose the method of claim 8. Munoz does not disclose wherein the nitrogen scavenging drug is selected from the group consisting of a PAA pro drug and sodium benzoate. Scharschmidt discloses wherein the nitrogen scavenging drug is selected from the group consisting of a PAA pro drug and sodium benzoate (nitrogen scavenging drug comprising HPN-100 (a PAA pro drug) and sodium benzoate (Buphyenyl), or any combination thereof; column 3, lines 16-19; column 20, lines 10-12). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Munoz to include the specific nitrogen scavenging drugs of Scharschmidt, for the advantage of treating hyperammonemia, with commonly used medications (Scharschmidt; column 1, lines 44-46).

As per claim 10, Munoz and Scharschmidt, in combination, disclose the method of claim 9. Munoz does not disclose wherein the PAA prodrug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] (HPN-100), phenylbutyric acid (PBA), sodium PBA (NaPBA), and a combination of two or more of HPN-100, PBA, and NaPBA. Scharschmidt discloses wherein the PAA prodrug is glyceryl tri-[4-phenylbutyrate] (HPN-100) (column 20, lines 26-28). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method of Munoz to include the specific PAA prodrug of Scharschmidt, for the advantage of treating hyperammonemia, when a patient is non-compliant with the nitrogen scavenging drug, Buphyenyl (Scharschmidt; column 20, lines 10-30).

Claims 12-16 lack an inventive step under PCT Article 33(3) as being obvious over Cordoba in view of US 2005/0273359 A1 (YOUNG).

As per claim 12, Cordoba discloses the tool of claim 11. Cordoba does not disclose wherein the user is a caregiver of the subject in need thereof. Young discloses wherein the user is a caregiver of the subject in need thereof (health care provider 14 is a private physician of the patient 12; figure 1; paragraph [0030]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the tool of Cordoba to include the caregiver of the subject in need thereof of Young, for the advantage of having a medical professional in charge of the patient undergoing the evaluation.

As per claim 13, Cordoba and Young, in combination, disclose the tool of claim 12, and Cordoba further discloses the first set of steps (supplementary table 4; section 1) and, if necessary, second set of steps (figure 1; page 1032; page 1038, 2nd column). Cordoba does not disclose wherein the set of steps are performed on a daily basis to monitor the subject. It would have been obvious to a person of ordinary skill in the art, at the time of the invention, and it would have required only routine experimentation, to have modified the tool of Cordoba to include wherein the set of steps are performed on a daily basis to monitor the subject, since doing so could be readily achieved through routine experimentation and testing, for the advantage of daily monitoring the HE patient, and thereby allowing the caregiver to record any progression or stabilization of the disorder for the patient over time.

As per claim 14, Cordoba and Young, in combination, disclose the tool of claim 13, and Cordoba further discloses wherein the steps of the tool are provided in a questionnaire format (supplementary tables 3-4).

As per claim 15, Cordoba and Young, in combination, disclose the tool of claim 14. Cordoba does not disclose wherein the tool is provided in an electronic format with a branching logic algorithm. Young discloses wherein a tool is provided in an electronic format with a branching logic algorithm (questions are presented on a computer 42, with a branching chain logic (algorithm); paragraphs [0044]-[0045]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the tool of Cordoba to include the electronic format with a branching logic algorithm of Young, for the advantage of having a universally accessible questionnaire with a sequential logic, such that the patient may proceed to any next section, pending a reply to the previous section.

As per claim 16, Cordoba and Young, in combination, disclose the tool of claim 15. Cordoba does not disclose wherein the tool is provided on a web-enabled device. Young discloses wherein a tool is provided on a web-enabled device (computer system 30 is provided to host and access an Internet-based website, for access by computer 42; figures 2, 5; paragraphs [0021], [0037], [0038], [0040], [0044]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the tool of Cordoba to include the web-enabled device of Young, for the advantage of allowing the patient to complete the questionnaire in a non-physical format, such that the questionnaire is universally available to the patient upon logging into a website, via a web-enabled computer.

Claim 17 lacks an inventive step under PCT Article 33(3) as being obvious over Cordoba and Young in view of US 8,094,521 B2 (LEVY).

As per claim 17, Cordoba and Young, in combination, disclose the tool of claim 16. Cordoba does not disclose wherein daily reminders are electronically sent to the caregiver at the same time each day to remind the caregiver to use the tool. Levy discloses wherein daily reminders are electronically sent to the caregiver at the same time each day to remind the caregiver to use a tool (alert device assists caregivers in ensuring time-sensitive tasks are performed within a specific time frame, including daily reminders, and subsequent use thereof; abstract; column 3, lines 1-12, 17-21, 26-28, 56-58). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the tool of Cordoba to include the alerting device of Levy, for the advantage of have a means of a caregiver receiving daily reminders on a portable electronic device.

-***-Continued Within the Next Supplemental Box-***-

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

-***-Continued from Previous Supplemental Box-***-

Claims 18-20 lack an inventive step under PCT Article 33(3) as being obvious over Cordoba and Young in view of Munoz.

As per claim 18, Cordoba and Young, in combination, disclose the tool of claim 12. Cordoba does not disclose wherein the subject in need thereof is administered a therapeutic intervention according to the physician's recommendation if one or more criteria are met from the second set of steps. Munoz discloses wherein the subject in need thereof is administered a therapeutic intervention according to the physician's recommendation if one or more criteria are met from the second set of steps (lactulose is administered to a patient who develops stage III or IV encephalopathy at higher dosages of 30 mL per administration; section titled 'Lactulose', page 803). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, and it would have required only routine experimentation to have modified the tool of Cordoba to include the therapeutic intervention of Munoz, and to further include the intervention being based according to the physician's recommendation, since such recommendations are known in the art to be based upon a physician's recommendation.

As per claim 19, Cordoba, Young and Munoz, in combination, disclose the tool of claim 18. Cordoba does not disclose wherein if the subject was previously administered a dosage of lactulose, the dosage of lactulose is increased and the increased dosage of lactulose is administered to the subject. Munoz discloses wherein if the subject was previously administered a dosage of lactulose (lactulose is administered to a patient with stage I or II encephalopathy at dosages of 30 mL two to four times a day; section titled 'Lactulose', page 803), the dosage of lactulose is increased and the increased dosage of lactulose is administered to the subject (lactulose is administered to a patient who develops stage III or IV encephalopathy at higher dosages of 300 mL per administration; section titled 'Lactulose', page 803). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, and it would have required only routine experimentation to have modified the tool of Cordoba to include the increased dosages of Munoz, such that the same patient receives higher dosages of Lactulose, if that patient progresses to higher levels of hepatic encephalopathy, since doing so could be readily achieved through routine experimentation and testing, for the advantage of having a more effective treatment for higher stages of hepatic encephalopathy.

As per claim 20, Cordoba, Young and Munoz, in combination, disclose the tool of claim 18. Cordoba does not disclose wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal. Munoz discloses wherein the therapeutic intervention is administered at a dosage sufficient to maintain the subject's ammonia level (lactulose can decrease ammonia production and increase ammonia excretion; 'Pharmacologic therapy' and 'Lactulose', page 803). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, and it would have required only routine experimentation to have modified the tool of Cordoba to include the decreased ammonia levels of Munoz, such that the therapeutic intervention is administered at a dosage sufficient to maintain the subject's fasting blood ammonia level at or below a specified threshold of 1.5 times the upper limit of normal, since doing so could be readily achieved through routine experimentation and testing, for the advantage of keeping the levels of ammonia below toxic levels, and thereby mitigating the progression of hepatic encephalopathy.

Claims 1-20 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 079532-8007.WO00	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US2014/060543	International filing date *(day/month/year)* 14 October 2014	(Earliest) Priority Date *(day/month/year)* 14 October 2013
Applicant HYPERION THERAPEUTICS, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 2 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**
   a. With regard to the **language**, the international search was carried out on the basis of:
      ☒ the international application in the language in which it was filed.
      ☐ a translation of the international application into ______ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).
   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).
   c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.
2. ☐ **Certain claims were found unsearchable** (see Box No. II).
3. ☐ **Unity of invention is lacking** (see Box No. III).
4. With regard to the **title**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established by this Authority to read as follows:
5. With regard to the **abstract**,
   ☒ the text is approved as submitted by the applicant.
   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. With regard to the **drawings**,
   a. the figure of the **drawings** to be published with the abstract is Figure No. 1
      ☐ as suggested by the applicant.
      ☒ as selected by this Authority, because the applicant failed to suggest a figure.
      ☐ as selected by this Authority, because this figure better characterizes the invention.
   b. ☐ none of the figures is to be published with the abstract.

Form PCT/ISA/210 (first sheet) (July 2009)

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 49/00 (2014.01)
CPC - A61K 31/216 (2014.10)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61K 31/192, 49/00; A61P 13/00 (2014.01)
CPC - A61K 31/192, 31/216, 31/221 (2014.10) (keyword delimited)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/9.2; 514/432, 433, 533, 568, 570 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit, PubMed, Google Patents, Google Scholar
Search terms used: urea cycle disorder effective dosage phenylacetic acid (PAA) prodrug subject body surface area urinary glyceryl tri phenylbutyrate nitrogen scavenging Hyperion therapeutics

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	WO 2013/048558 A2 (HYPERION THERAPEUTIC INC) 04 April 2013 (04.04.2013) entire document	1, 5, 6, 14 ---- 2-4, 7-13, 15-20
Y	US 2012/0022157 A1 (SCHARSCHMIDT) 26 January 2012 (26.01.2012) entire document	2-4, 8-11, 15-20
Y	Monteleone et al. Population Pharmacokinetic Modeling and Dosing Simulations of Nitrogen-Scavenging Compounds: Disposition of Glycerol Phenylbutyrate and Sodium Phenylbutyrate in Adult and Pediatric Patients with Urea Cycle Disorders. J Clin Pharmacol. 53 (7): 699–710, July 2013. [retrieved on 15 December 2014]. Retrieved from the Internet. <URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3923458/pdf/nihms536149.pdf> entire document	7-13, 19, 20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 December 2014	23 JAN 2015

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: COURTNEY PROCHNOW
PERKINS COIE LLP
P.O. BOX 1247
SEATTLE, WA 98111-1247

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	23 JAN 2015

Applicant's or agent's file reference	FOR FURTHER ACTION
079532-8007.WO00	See paragraph 2 below

International application No.	International filing date (*day/month/year*)	Priority date (*day/month/year*)
PCT/US2014/060543	14 October 2014	14 October 2013

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - A61K 49/00 (2014.01)
CPC - A61K 31/216 (2014.10)

Applicant HYPERION THERAPEUTICS, INC.

1. This opinion contains indications relating to the following items:

- [X] Box No. I Basis of the opinion
- [ ] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [X] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1 *bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to tb IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Fom PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	15 December 2014	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2011)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/060543

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [x] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ______________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)
   - [ ] on paper
   - [ ] in electronic form

   b. (time)
   - [ ] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

Form PCT/ISA/237 (Box No. I) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/060543

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2-4, 7-13, 15-20	YES
	Claims	1, 5, 6, 14	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-20	NO
Industrial applicability (IA)	Claims	1-20	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1, 5, 6, and 14 lack novelty under PCT Article 33(2) as being anticipated by Hyperion Therapeutics Inc (hereafter Hyperion Therapeutics).

Regarding claim 1, Hyperion Therapeutics discloses a method of determining an effective dosage of a phenylacetic acid (PAA) prodrug for treating a urea cycle disorder (UCD) in a subject in need thereof (methods for determining whether to increase a dosage of a nitrogen scavenging drug in a subject with a nitrogen retention disorder is a UCD; wherein a determination is made to administer an increased dosage of nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA pro-drug, Pg. 3, [0010]) comprising: calculating a body surface area (BSA) for the subject; and comparing the BSA to a predetermined threshold value (Initial dosage is determined based on body surface area (BSA) or as otherwise instructed according to HPN-100 drug labeling. Patient A's body surface area is 1.4 m2, Pg. 24; [0080]), wherein the effective dosage is a first dosage if the BSA is at or above the predetermined threshold value or a second dosage if the BSA is below the predetermined threshold value, and wherein the second dosage is higher than the first dosage as a function of BSA (administering a first dosage of a nitrogen scavenging drug, measuring a fasting blood ammonia level for the subject, and comparing the fasting blood ammonia level to a ULN for blood ammonia. If the fasting blood ammonia level has a value that is greater than half the ULN, a second dosage of a nitrogen scavenging drug that is greater than the first dosage is administered to the subject, Pg. 11; [0038]).

Regarding claim 5, Hyperion therapeutics discloses the method of claim 1, wherein the PAA pro drug is selected from the group consisting of glyceryl tri-[4-phenylbutyrate] and phenylbutyrate (treatment of nitrogen retention disorders is glyceryl tri-[4-phenylbutyrate] (HPN-100), Pg. 2; [0005]) or a pharmaceutically acceptable salt thereof including sodium phenylbutyrate (sodium phenylbutyrate (NaPBA, Pg. 2; [0005]).

Regarding claim 6, Hyperion therapeutics discloses a method of evaluating compliance with a phenylacetic acid (PAA) pro-drug treatment regimen in a subject with a urea cycle disorder (UCD) being treated with a PAA pro-drug (the methods disclosed herein for adjusting the dosage of a nitrogen scavenging drug comprise an additional step of measuring urinary PAGN and calculating an effective dosage based on a mean conversion of PAA prodrug to urinary PAGN of 60-75%, Pg. 18; [0057]) comprising: classifying the subject into a dosage group based on the dosage of a PAA pro-drug the subject is currently receiving; determining a urinary phenylacetylglutamine (PAGN) level for the subject (Patient B is an 11-year UCD patient receiving 24 pills of BUPHENYL ®per day, amino acid supplements, and restricted dietary protein intake. Patient B does not consume BUPHENYL ® supplements, or food for approximately 6 hours prior to a fasting morning blood draw, Pg. 25; [0082]); and comparing the urinary PAGN level to a predetermined threshold urinary PAGN level (Based on the correlation of fasting ammonia level to average ammonia level, it is determined that Patient B 's fasting blood ammonia level falling between 1 and 1.5 times the Upper limit of normal (ULN) represents a 55% chance of having an average ammonia during the day that is greater than the normal range, and as high as a 65% chance that her ammonia will go above 52 micro mol/L or 1.5 times ULN during the day, Pg. 25; [0082]), wherein a urinary PAGN level below the predetermined threshold urinary PAGN level indicates that the subject is non-compliant with the PAA pro-drug treatment regimen (Based on discussion with the patient and her mother, the physician suspects that Patient B is noncompliant with her medication, and decides to change her to HPN-100, Pg. 25; [0083]).

Regarding claim 14, Hyperion therapeutics discloses a method of treating a urea cycle disorder (UCD) in a subject in need thereof comprising: classifying the subject into a body surface area (BSA) group based on the subject's BSA level (Initial dosage is determined based on body surface area, Pg. 24; [0080]. Population PK model building was performed on 65 UCD patients who data points from 53 adult and 11 pediatric UCD patients (ages 6-17) who participated in 3 switch over studies of NaPBA and GPB. The median GPB dose, expressed as grams of PBA per m2, was 8.85 and 7.01 for pediatric and adult subjects, Pg. 29; [0098]); and determining a urinary phenylacetylglutamine (PAGN) level for the subject; comparing the urinary PAGN level to a predetermined threshold urinary PAGN level; administering a dosage of a PAA pro-drug to the subject if the urinary PAGN level for the subject is below the predetermined threshold urinary PAGN level (The final model that best fit the data was characterized by (a) partial conversion of PBA to PAGN prior to reaching the systemic circulation, (b) saturable conversion of PAA to PAGN (Km about 161 micro g/ml), and (c) about 60% slower PBA absorption when delivered as GPB vs. NaPBA. Body surface area (BSA) was a significant covariate such that metabolite clearance was proportionally related to BSA. Fractional presystemic metabolism of PBA was higher for adults than for pediatric patients receiving GPB (43% vs. 14%), whereas the reverse was true for NaPBA (23% vs. 43%). Predicted median PAA exposure based on simulated GPB dosing at the PBA equivalent of 13g/m2 of NaPBA was about 13%-22% lower in adults than NaPBA (Cmax = 82 vs. 106 micro g/mL; AUC0_24 = 649 vs. 829 micro g.h/m) and about 13% higher in pediatric subjects ages 6-17 than NaPBA (Cmax = 154 vs. 138 micro g/mL; AUC 0_24 = 1286 vs. 1154 microg.h/ml), Pg. 29; [0099]).

Form PCT/ISA/237 (Box No. V) (July 2011)

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2014/060543

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Claims 2-4 and 15-18 lack an inventive step under PCT Article 33(3) as being obvious over hereafter Hyperion Therapeutics in view of Scharschmidt.

Regarding claim 2, Hyperion Therapeutics discloses the method of claim 1, wherein the predetermined threshold value is 1.4 m2 (Patient A's body surface area is 1.4 m2, and therefore the initial dosage is determined to be 9 mL per day or 3 mL TID (three-time-a-day), which is approximately 60% of the maximum allowed dosage per HPN-100 label, Pg. 24; [0080]), but fails to explicitly discose wherein the predetermined threshold value is 1.3 m2. Further, Scharschmidtteaches wherein the predetermined threshold value is 1.3 m2 (The threshold level for this analysis, 30 micro mol/L, was the average upper limit for normal ammonia levels among the study sites, Pg. 11; [0098]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the predetermined threshold value is 1.3 m2, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 3, Hyperion therapeutics discloses the method of claim 1, wherein the first dosage (the initial dosage is determined to be 9 mL per day or 3 mL TID, Pg. 24; [0080]; Predicted median PAA exposure based on simulated GPB dosing at the PBA equivalent of 13g/m2 of NaPBA), but fails to explicitly disclsoes wherein the first dosage is about 7.18 g.m2/day. Further, Scharschmidt in the field of nitrogen scavenging drug (Abstract) teaches wherein the first dosage is about 7.18 g/m2/day (9.9-13.0 g/m2/day, Pg. 4; Table 1; Single Dose- 3 g/m2/day, Pg. 5; Table 2; PBA is pro-drug for PAA; Pg. 5; [0041]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the first dosage is about 7.18 g/m2/day, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 4, Hyperion therapeutics discloses the method of claim 1, wherein the second dosage (Patient A's dosage of HPN-100 is increased by approximately one-third to 12 mL total or 4 mL TID, Pg. 24; [0080]; his maximal daily ammonia is not expected to exceed approximately 52 micro mol/L, i.e., approximately 1.5 times the ULN, Pg. 24; [0081]), but fails to explicitly disclose wherein the second dosage is about 8.35 g/m2/day Further, Scharschmidt in the field of nitrogen scavenging drug (Abstract) teaches wherein the second dosage is about 8.35 g/m2/day (For a subject weighing more than 20 kg, a dosage range for HPN-1 00 would be between 8.6 and 11.2 mL/m2; Pg. 14; [0114]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the second dosage is about 8.35 g/m2/day, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 15, Hyperion therapeutics discloses the method of claim 14, but fails to explicitly disclose wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary PAGN level for the subject's BSA group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary PAGN level for the subject's BSA group (HPN-100 that was excreted in urine was PAGN, accounting for 39% of the administered HPN-100. By contrast, when oral sodium PBA was administered, PAGN accounted for only 23% of the radio labeled material, and unchanged PBA accounted for 48% of the administered dosage of oral sodium PBA, Pg. 26-27; [0290]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary PAGN level for the subject's dosage group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 16, Hyperion therapeutics in view of Scharschmidt discloses the method of claim 15. Hyperion therapeutics discloses wherein the subject's BSA group is less than or equal to 1.3 m2 or greater than 1.3 m2 (Initial dosage is determined based on body surface area or as otherwise instructed according to HPN-100 drug labeling. Patient A's body surface area is 1.4 m2, Pg. 24; [0080]).

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:

Regarding claim 17, Hyperion therapeutics in view of Scharschmidt discloses the method of claim 16. Hyperion therapeutics fails to explicitly disclose wherein the 25th percentile urinary PAGN level is about 8390 micro g/mL for the less than or equal to 1.3 m2 BSA group; and the 25th percentile urinary PAGN level is about 5259 micro g/mL for the greater than 1. 3 m2 BSA group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the 25th percentile urinary PAGN level is about 8390 micro g/mL for the less than or equal to 1.3m2 BSA group; and the 25th percentile urinary PAGN level is about 5259 micro g/mL for the greater than l. 3m2 BSA group (9.9-13.0 g/m2/day of Sodium PBA; 9.4-12.4 g/m2/day of HPN-100 PBA, Pg. 27; Table ; HPN -100 is typically converted into urinary PAGN with an efficiency of about 40% to 70% (typically about 54% conversion was found in UCD patients), thus the physician would expect to observe about 17 g of urinary PAGN output per day from this dosage of HPN-100. Pg. 27; [0296], Pg. 14; [0114]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics teaches wherein the 25th percentile urinary PAGN level is about 8390 micro g/mL for the less than or equal to 1.3 m2 BSA group; and the 25th percentile urinary PAGN level is about 5259 microg/mL for the greater than 1. 3 m2 BSA group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 18, Hyperion therapeutics in view of Scharschmidt discloses the method of claim 16. Hyperion therapeutics fails to explicitly disclose wherein the 25th percentile urinary PAGN level is about 8000 micro g/mL for the less than or equal to 1.3 m2 BSA group; and the 25th percentile urinary PAGN level is about 5000 micro g/mL for the greater than l. 3m2 BSA group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the 25th percentile urinary PAGN level is about 8000 micro g/mL for the less than or equal to 1.3 m2 BSA group; and the 25th percentile urinary PAGN level is about 5000 micro g/mL for the greater than 1. 3 m2 BSA group (9.9-13.0 g/m2/day of Sodium PBA; 9.4-12.4 g/m2/day of HPN-100 PBA, Pg. 27; Table . HPN-100 is typically converted into urinary PAGN with an efficiency of about 40% to 70% (typically about 54% conversion was found in UCD patients), thus the physician would expect to observe about 17 g of urinary PAGN output per day from this dosage of HPN-100. Pg. 27; [0296], Pg. 14; [0114]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics teaches wherein the 25th percentile urinary PAGN level is about 8000 micro g/mL for the less than or equal to 1.3 m2 BSA group; and the 25th percentile urinary PAGN level is about 5000 micro g/mL for the greater than 1. 3 m2 BSA group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Claims 7, 12, and 13 lack an inventive step under PCT Article 33(3) as being obvious over Hyperion Therapeutics in view of Monteleone et al.

Regarding claim 7, Hyperion therapeutics discloses the method of claim 6, but fails to explicitly disclose wherein the subject is less than 6 years of age. However, Monteleone et al. in the field of impaired urea synthesis and hyperammonemia (Abstract) teach wherein the subject is less than 6 years of age (Patients collectively spanned ages 2 months to 72 years, Abstract and Pg. 5; 6th Para.). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the subject is less than 6 years of age, as taught by Monteleone et al. The motivation for doing so would be to provide a novel approach for particular attention to phenylacetic acid (PAA), which has been associated with adverse events in non-UCD populations (Monteleone et al., Abstract).

Regarding claim 12, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 7. Hyperion therapeutics discloses wherein a dosage of a PAA pro-drug is administered to the subject if the urinary PAGN level for the subject is below the predetermined threshold urinary PAGN level (In certain embodiments wherein a determination is made to administer an increased dosage of nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA pro-drug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA pro-drug based on a mean conversion of PAA pro-drug to urinary PAGN of 60-75%, Pg. 3; [0010]. These findings based on PopPK modeling and dosing simulations suggest that while most patients treated with PAA pro-drugs including NaPBA or HPN-100 will have PAA levels below those reportedly associated with toxicity, Pg. 29; [00100]).

Regarding claim 13, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 12. Hyperion therapeutics teaches wherein the dosage of the PAA pro drug is an effective dosage (a determination is made to administer an increased dosage of nitrogen scavenging drug and wherein the nitrogen scavenging drug is a PAA pro-drug, the methods include an additional step of measuring urinary PAGN excretion and determining an effective dosage of the PAA pro-drug, Pg. 3; [0010]).

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No. PCT/US2014/060543

Supplemental Box

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Claims 8-11 lack an inventive step under PCT Article 33(3) as being obvious over Hyperion Therapeutics in view of Monteleone et al. and Scharschmidt.

Regarding claim 8, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 7. Hyperion therapeutics fails to explicitly disclose wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary PAGN level for the subject's dosage group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary PAGN level for the subject's dosage group (HPN-100 that was excreted in urine was PAGN, accounting for 39% of the administered HPN-100. By contrast, when oral sodium PBA was administered, PAGN accounted for only 23% of the radio labeled material, and unchanged PBA accounted for 48% of the administered dosage of oral sodium PBA, Pg. 26-27; [0290]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the predetermined threshold urinary PAGN level is a 25th percentile urinary P AGN level for the subject's dosage group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 9, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 8. Hyperion therapeutics fails to explicitly disclose wherein the dosage group is selected from the group consisting of less than 6 mL/m2, 6 to 10 mL/m2, and greater than 10 mL/m2. However, Scharschmidt in the field of nitrogen scavenging drug (Abstract) teaches wherein the dosage group is selected from the group consisting of less than 6 mL/m2, 6 to 10 mL/m2 , and greater than 10 mL/m2 (a daily dosage in a range of 9.9-13.0 g/m2 set according to the subject's size for subjects over 20 kg in weight; and a dosage within a range of 450-600 mg/kg for subjects weighing less than or equal to 20 kg is indicated; For a subject weighing more than 20 kg, a dosage range for HPN-100 would be between 8.6 and 11.2 mL/m2. For a subject weighing less than 20 kg, a dosage range of about 390 to 520 microL/kg per day of HPN-100 would be appropriate, based on the use of an equimolar amount compared to the recommended doses of HPN-100, Pg. 14; [0114]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the dosage group is selected from the group consisting of less than 6 mL/m2, 6 to 10 mL/m2 , and greater than 10 mL/m2, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 10, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 9. Hyperion therapeutics fails to explicitly disclose wherein the 25th percentile urinary PAGN level is about 1256 micro g/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3053 micro g/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 6990 micro g/mL for the greater than 10 mL/m2 dosage group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the 25th percentile urinary PAGN level is about 1256 micro g/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3053 micro g/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 6990 micro g/mL for the greater than 10 mL/m2 dosage group (1 gram of PAA mediates the excretion of about 0.18 grams of waste nitrogen if completely converted to PAGN; 54% of the PAA delivered as the PBA pro-drug released from HPN-100 is converted to PAGN; 47% of dietary protein is excreted as waste nitrogen, and 16% of dietary protein consists of nitrogen, Pg. 15; [0124]). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics teaches wherein the 25th percentile urinary PAGN level is about 1256 micro g/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3053 micro g/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 6990 micro g/mL for the greater than 10 mL/m2 dosage group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Regarding claim 11, Hyperion therapeutics in view of Monteleone et al. discloses the method of claim 9. Hyperion therapeutics fails to explicitly disclose wherein the 25th percentile urinary PAGN level is about 1000 microg/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3000 microg/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 7000 microg/mL for the greater than 10 mL/m2 dosage group. However, Scharschmidt in the field of in the field of nitrogen scavenging drug (Abstract) teaches wherein the 25th percentile urinary PAGN level is about 1000 microg/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3000 microg/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 7000 microg/mL for the greater than 10 mL/m2 dosage group(Dose 1: 3 mL BID Corresponds to about 0.47 x the dose administered in Example 2, for a 70 kg adult and about 0.35 x the amount of PBA (about 6.1 g) delivered in the maximum approved dose of sodium PBA of 20 g expected to mediate excretion of waste nitrogen associated with about 8 g of dietary protein; Dose 2: 9 mL BID Corresponds to about 1.42x the dose administered in Example 2, for a 70 kg adult and about 1.1 x the amount of PBA (about 18.2 g) delivered in the maximum approved dose of sodium PBA of 20 g expected to mediate excretion of waste nitrogen associated with about 25 g of dietary protein; Dose 3; 15 mL BID Corresponds to about 2.36 x the dose administered in Example 2, for a 70 kg adult and about 1.73 x the amount of PBA (about 30.3 g) delivered in the maximum approved dose of sodium PBA of 20 g expected to mediate excretion of waste nitrogen associated with about 40 g of dietary protein, Pg. 15; Table 5). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics teaches wherein the 25th percentile urinary PAGN level is about 1000 micro g/mL for the less than 6 mL/m2 dosage group; the 25th percentile urinary PAGN level is about 3000 micro g/mL for the 6 to 10 mL/m2 dosage group; and the 25th percentile urinary PAGN level is about 7000 micro g/mL for the greater than 10 mL/m2 dosage group, as taught by Scharschmidt. The motivation for doing so would be to provide provides a novel approach for a determining and adjusting the schedule and dose of orally administered nitrogen scavenging drugs (Scharschmidt, Pg. 2; [0021]).

Supplemental Box

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Claims 19 and 20 lack an inventive step under PCT Article 33(3) as being obvious over Hyperion Therapeutics in view of Scharschmidt and Monteleone.

Regarding claim 19, Hyperion therapeutics in view of Scharschmidt discloses the method of claim 16. Hyperion therapeutics fails to explicitly disclose wherein the subject is 2 years of age or older and the 25th percentile urinary PAGN level is about 7412 micro g/mL for the less than or equal to 1.3 m2 BSA group. However, Monteleone et al. teach wherein the subject is 2 years of age or older (enrolled patients ages 2 months to 72 years, Abstract) and the 25th percentile urinary PAGN level is about 7412 micro g/mL for the less than or equal to 1.3 m2 BSA group (Body size (expressed as BSA/1.73) was significant on parameters of clearance, volume, and presystemic conversion (alpha and beta) resulting in small BSA individuals having smaller PK values for these parameters compared to individuals with larger BSA values, Pg. 7; second Para. The saturable, BSA-dependent conversion of PAA to PAGN is manifested as the generally higher PAA exposure observed in smaller (lower BSA) patients during maximal dosing. while median PAA levels are well below 500 micro g/mL, even at the maximal dose and in the youngest patients, the upper 95% confidence intervals suggest the theoretical possibility that some pediatric patients would be exposed to PAA values exceeding 500 micro g/mL, Pg. 9; 4th Para.). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the subject is 2 years of age or older and the 25th percentile urinary PAGN level is about 7412 micro g/mL for the less than or equal to 1.3 m2 BSA group, as taught by Monteleone et al. The motivation for doing so would be to provide a novel approach for dose simulations were performed with particular attention to phenylacetic acid (PAA), which has been associated with adverse events in non-UCD populations (Monteleone et al., Abstract).

Regarding claim 20, Hyperion therapeutics in view of Scharschmidt discloses the method of claim 16. Hyperion therapeutics fails to explicitly disclose wherein the subject is 2 years of age or older and the 25th percentile urinary PAGN level is about 7000 micro g/mL for the less than or equal to 1. 3 m2 BSA group. However, Monteleone et al. teach wherein the subject is 2 years of age or older (enrolled patients ages 2 months to 72 years, Abstract) and the 25th percentile urinary PAGN level is about 7000 micro g/mL for the less than or equal to l. 3m2 BSA group (Body size (expressed as BSA/1.73) was significant on parameters of clearance, volume, and presystemic conversion (alpha and beta) resulting in small BSA individuals having smaller PK values for these parameters compared to individuals with larger BSA values, Pg. 7; second Para. The saturable, BSA-dependent conversion of PAA to PAGN is manifested as the generally higher PAA exposure observed in smaller (lower BSA) patients during maximal dosing while median PAA levels are well below 500 micro g/mL, even at the maximal dose and in the youngest patients, the upper 95% confidence intervals suggest the theoretical possibility that some pediatric patients would be exposed to PAA values exceeding 500 micro g/mL, Pg. 9; 4th Para.). It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify Hyperion Therapeutics wherein the subject is 2 years of age or older and the 25th percentile urinary PAGN level is about 7000 micro g/mL for the less than or equal to 1. 3 m2 BSA group, as taught by Monteleone et al. The motivation for doing so would be to provide a novel approach for dose simulations were performed with particular attention to phenylacetic acid (PAA), which has been associated with adverse events in non-UCD populations (Monteleone et al., Abstract).

Claims 1-20 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

Electronic Acknowledgement Receipt

EFS ID:	23050946
Application Number:	13610580
International Application Number:	
Confirmation Number:	1957
Title of Invention:	METHODS OF THERAPEUTIC MONITORING OF PHENYLACETIC ACID PRODRUGS
First Named Inventor/Applicant Name:	Bruce Scharschmidt
Customer Number:	101325
Filer:	Lauren Stevens
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Attorney Docket Number:	HOR0027-201-US
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Time Stamp:	11:18:10
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Non Patent Literature	Maestri_EnglJMed_1996.pdf	85124 99d3e298ffce0cd990a4ddf9d112a14a95b8f0a3	no	5

Warnings:

Information:

2	Non Patent Literature	Majeed_2001.pdf	7550179 d6094c729956932eba37a92098817f3397fe19aa	no	12
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6	Non Patent Literature	MOKHTARANI_MolGenetMetab_2013.pdf	1552206 bfbc6f227533169108211e5d133c1968fd27e7ba	no	21
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47	Non Patent Literature	Lee_SSIEM_2008_UCDphII-1st_patients_poster.pdf	126740 295a77e73c21976a3ab0ccfc8029454c007c3dd6	no	1
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48	Non Patent Literature	Lee_SSIEM_2008_UCDphII-1st_patients_abstract.pdf	33415 00e992b949032cc8ba05c16bf2ea044f88cdabc5	no	1
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		Total Files Size (in bytes):	143660201		

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New International Application Filed with the USPTO as a Receiving Office
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Substitute for form 1449/PTO				Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	13/610,580
				Filing Date	September 11, 2012
Date Submitted: March 12, 2012				**First Named Inventor**	Bruce Scharschmidt
				Art Unit	1629
(use as many sheets as necessary)				**Examiner Name**	Sara Elizabeth Townsley
Sheet	1	of	10	**Attorney Docket Number**	HOR0027-201-US

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number: Number-Kind Code[2] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	P1	4,457,942	07-03-1984	Brusilow, S.W.	
	P2	5,654,333	08-05-1997	The United States Of America As Represented By The Department Of Health And Human Services	
	P3	8,094,521	01-10-2012	Nightengale Products LLC	
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Examiner Initials*	Cite No.[1]	Foreign Patent Document: Country Code[3-] Number[4-]Kind Code[5] (*if known*)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Documents	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	F1	WO1994/22494	10-13-1994	The DuPont Merck Pharmaceutical Company		
	F2	WO2013/048558	04-04-2013	Hyperion Therapeutics, Inc.		
	F3	WO2013/158145	10-24-2013	Hyperion Therapeutics, Inc.		

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Substitute for form 1449/PTO				*Complete if Known*	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT				**Application Number**	13/610,580
				Filing Date	September 11, 2012
Date Submitted: March 12, 2012				**First Named Inventor**	Bruce Scharschmidt
				Art Unit	1629
(use as many sheets as necessary)				**Examiner Name**	Sara Elizabeth Townsley
Sheet	2	of	10	**Attorney Docket Number**	HOR0027-201-US

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
	D1	AMODIO, P., et al., "Detection of Minimal Hepatic Encephalopathy: Normalization and Optimization of the Psychometric Hepatic Encephalopathy Score. A Neuropsychological and Quantified EEG Study," J. Hepatol. 49:346-353 (2008).	
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				Art Unit	1629
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Sheet	3	of	10	**Attorney Docket Number**	HOR0027-201-US

NON PATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.) date, page(s), volume-issue number(s), publisher, city and/or country where published.	T[6]
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	D26	European Medicines Agency, European Public Assessment Report: Summary for the Public for Ammonaps (2009).	
	D27	European Medicines Agency, Scientific Discussion for Ammonaps (2005).	
	D28	European Medicines Agency, Scientific Discussion for Carbaglu (2004).	
	D29	FDA Label for Carbaglu, seven pages. (Mar. 2010).	
	D30	Feillet, F. and Leonard, J.V., Alternative Pathway Therapy for Urea Cycle Disorders, 21 J. Inher. Metab. Dis. 101-111 (1998).	
	D31	Feoli-Fonseca, M. L., Sodium Benzoate Therapy in Children with Inborn Errors of Urea Synthesis: Effect on Carnitine Metabolism and Ammonia Nitrogen Removal, 57 Biochemical and Molecular Medicine 31 (1996).	
	D32	FERENCI, P., et al., "Hepatic Encephalopathy-Definition, Nomenclature, Diagnosis, and Quantification: Final Report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998," Hepatology 35:716-721 (2002).	
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