

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 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 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 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 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 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 laparotomy was performed, the bile duct was exposed, triply ligated with 4.0 silk suture, and severed between the second and third ligature. The 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 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 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 heparinised 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 according to manufacturers instructions. Amino acids were quantified by HPLC with  
5 fluorescence detection.

## Results

In the cirrhotic bile duct ligated rat model there is a substantial increase in the  
10 arterial plasma ammonia level ( $205 \pm 11$   $\mu\text{moles/L}$ , mean  $\pm$  SEM) compared with healthy controls ( $25.6 \pm 2$   $\mu\text{moles/L}$ ,  $p < 0.001$  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,  $0.22\text{g/Kg}$ ,  $n=6$ ), phenylbutyrate (PB,  $0.3\text{g/Kg}$ ,  $n=7$ ) and ornithine phenylbutyrate (OP,  $0.22\text{g/Kg} + 0.3\text{g/Kg}$ ,  $n=7$ ). \* 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, 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 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 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 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.

20  
25

**Results**

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

30

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

5 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.

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

**Methods**

15 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.

- 20
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.

25 ALF was induced by portal vein anastomosis 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

30 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 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 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 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 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 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 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.

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 effect of ammonia on neutrophil function. Thus, use of ornithine alone is more  
5 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 be 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 hyperammonemic 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 hyperammonemia 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

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.

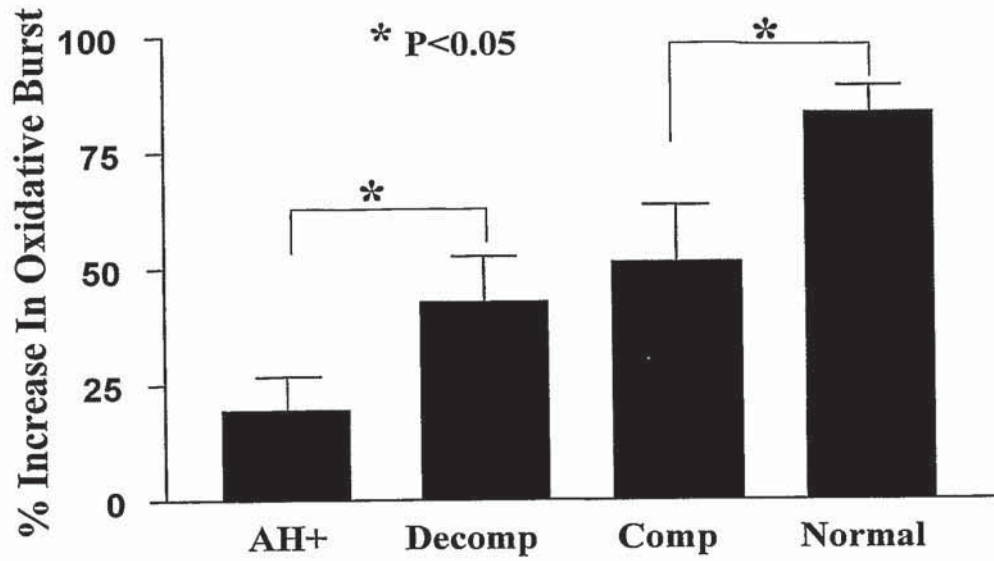
CLAIMS

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  
5 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.  
10
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.
- 15 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.  
20
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  
25 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.  
30
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.

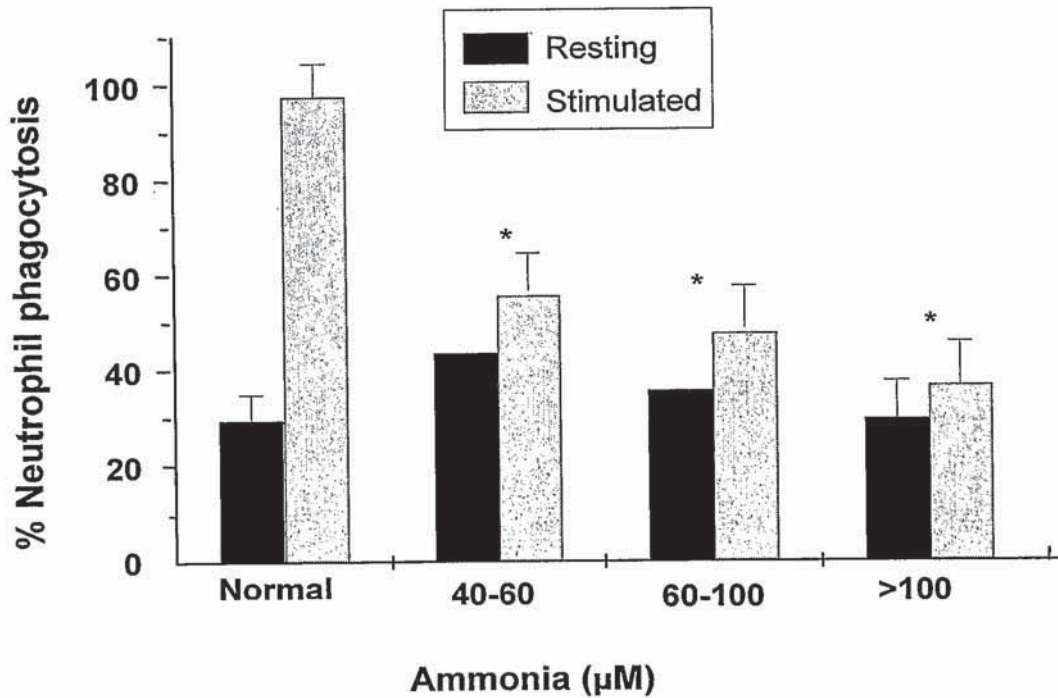
10. Use according to any one of the preceding claims wherein said ornithine is present as a free monomeric amino acid or physiologically acceptable salt.
11. Use according to any one of the preceding claims wherein the at least one of  
5 phenylacetate and phenylbutyrate is present as sodium phenylacetate 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.

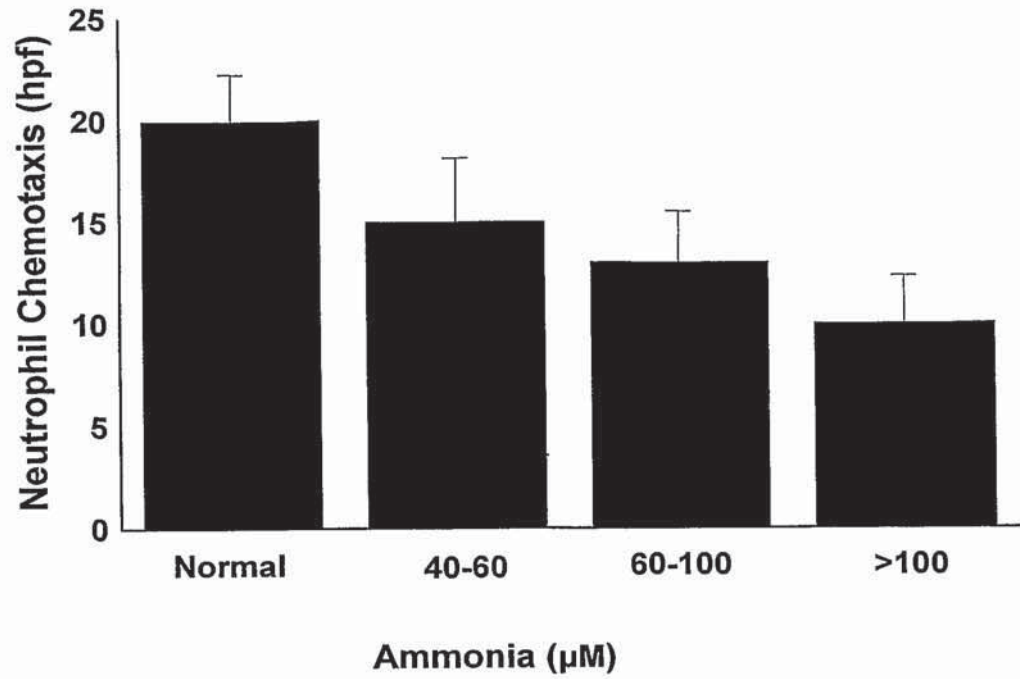
**Figure 1**



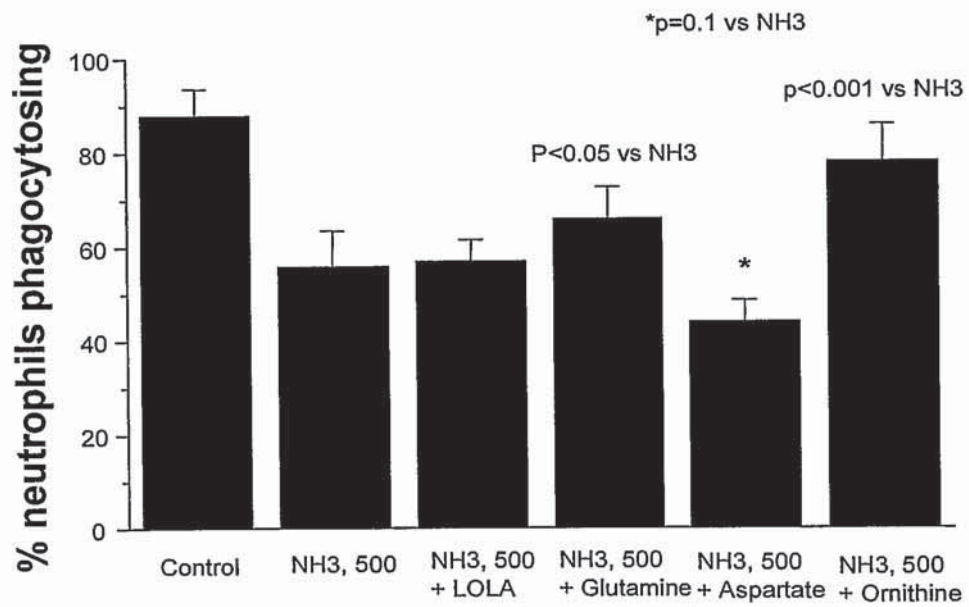
**Figure 2**



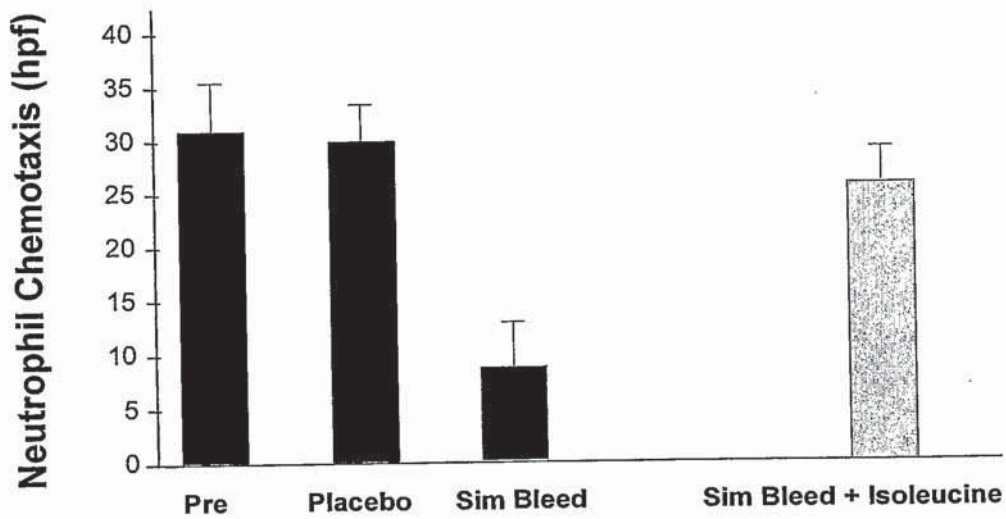
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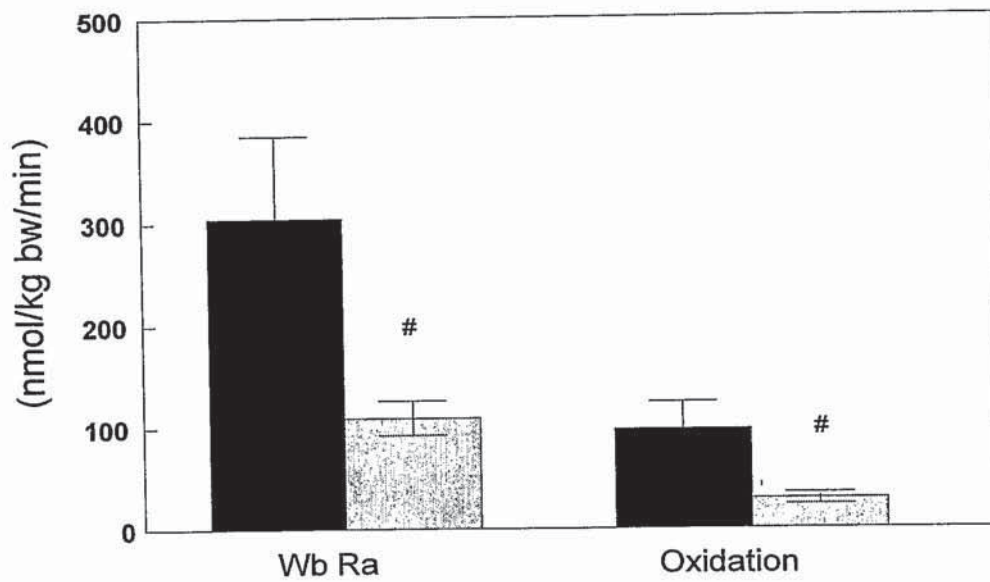
**Figure 4**



**Figure 5**

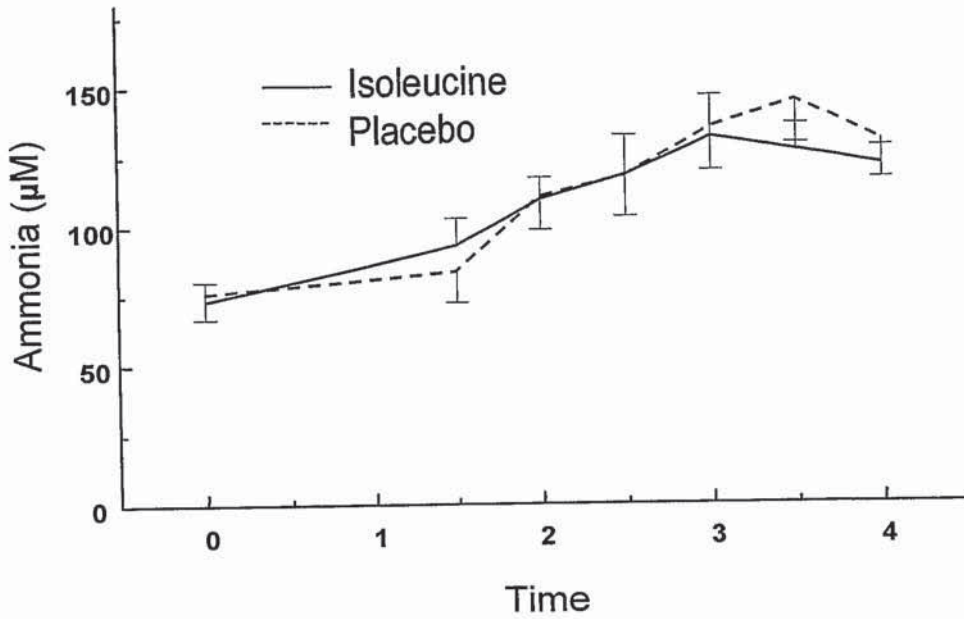


**Figure 6**

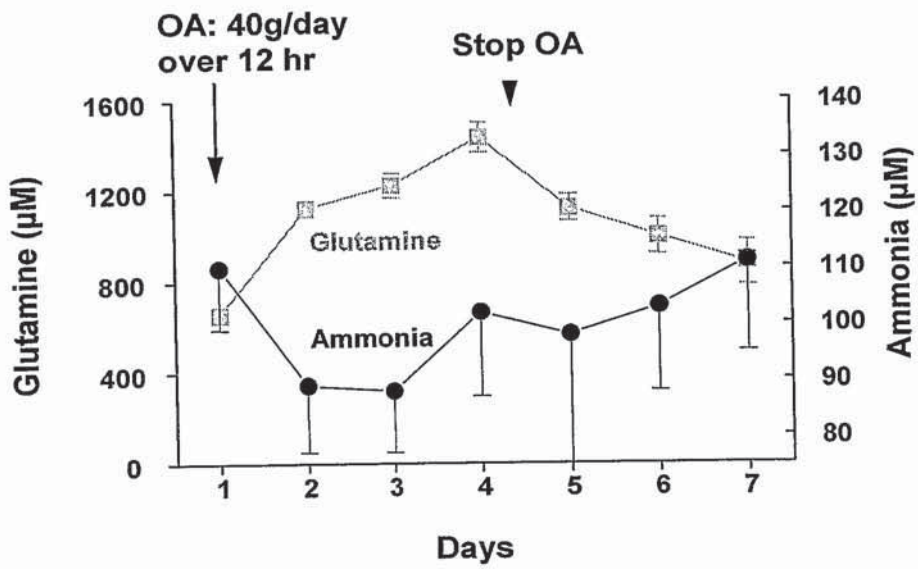




**Figure 7**

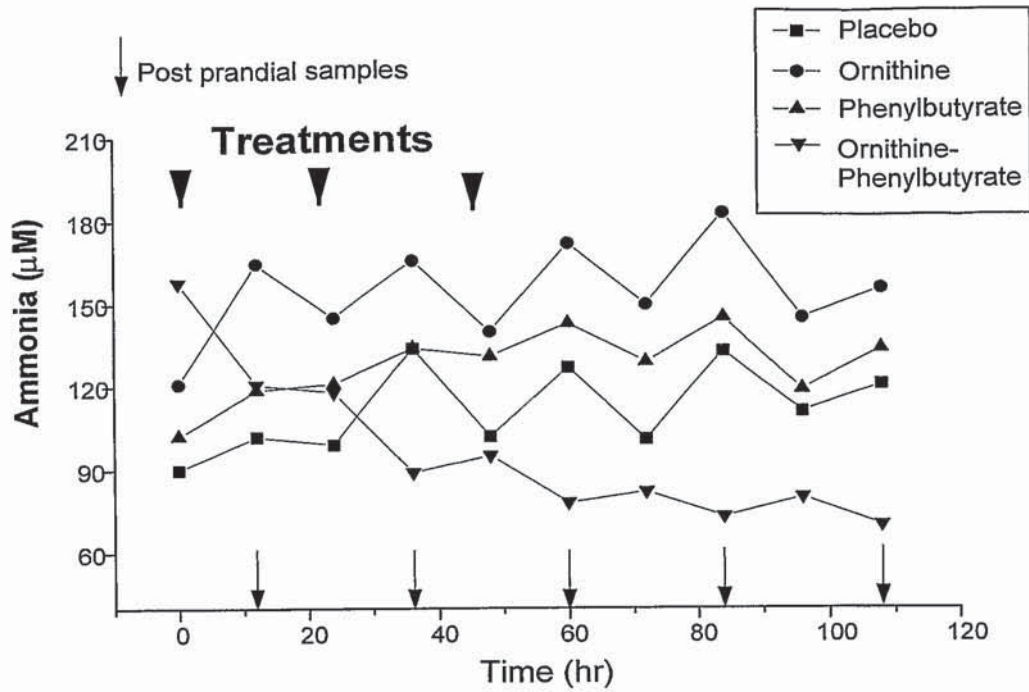


**Figure 8**

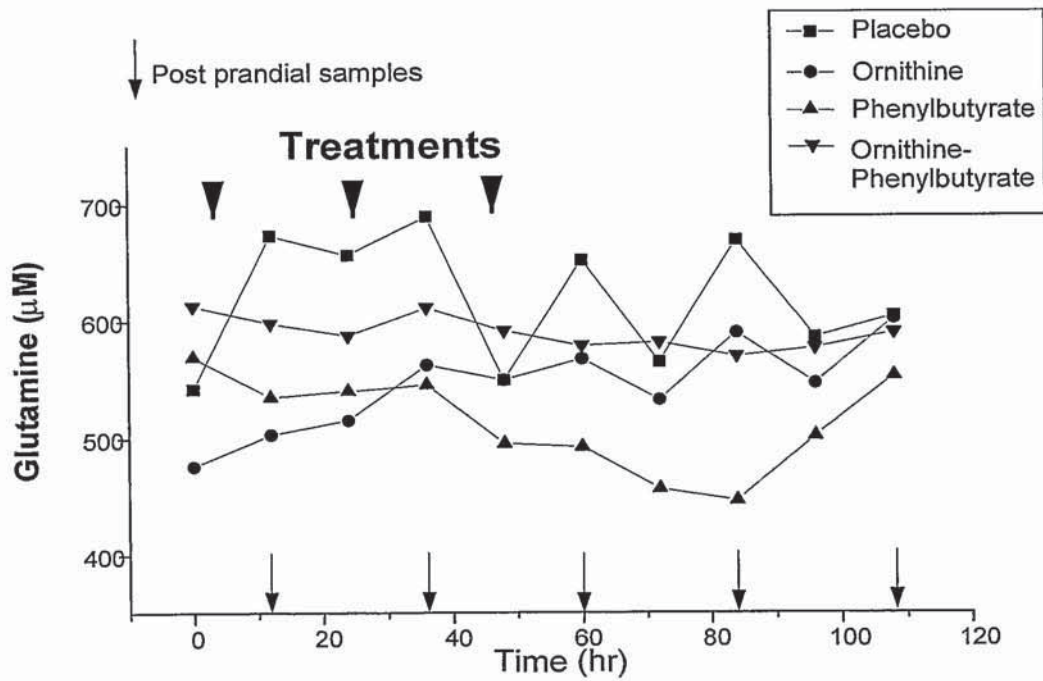




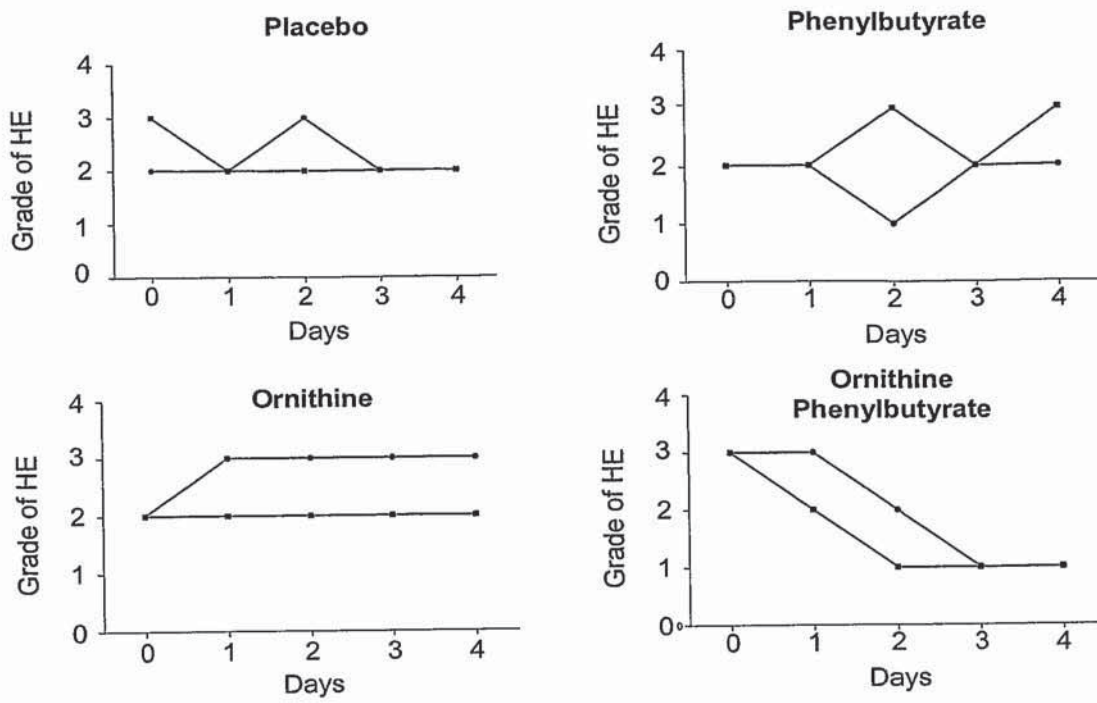
**Figure 11**



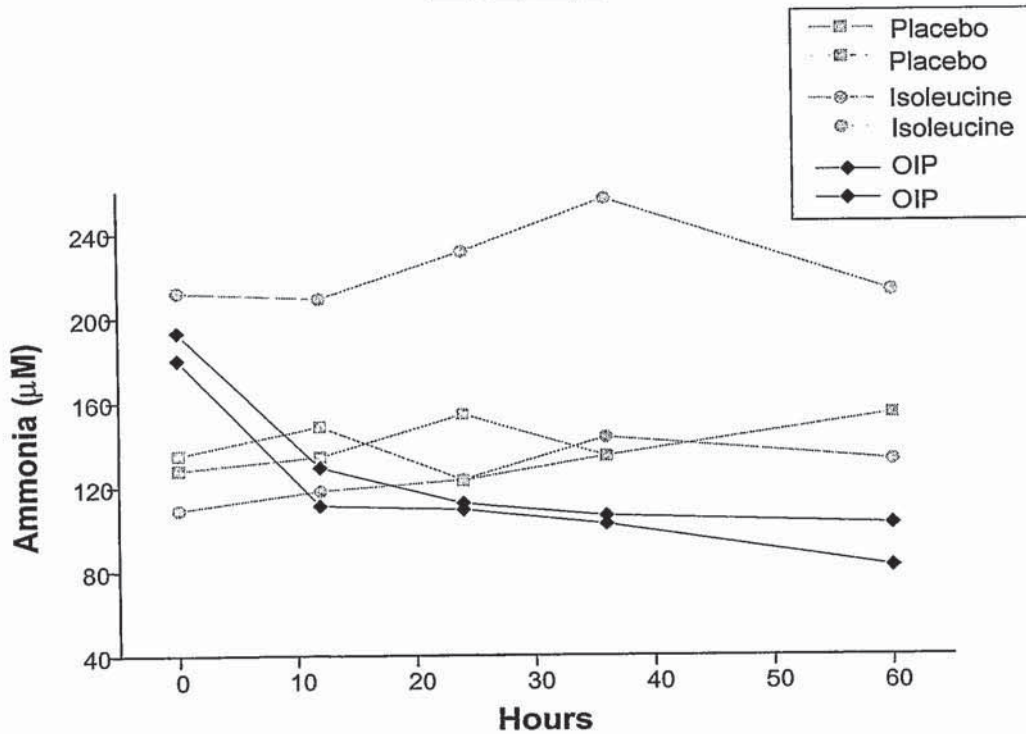
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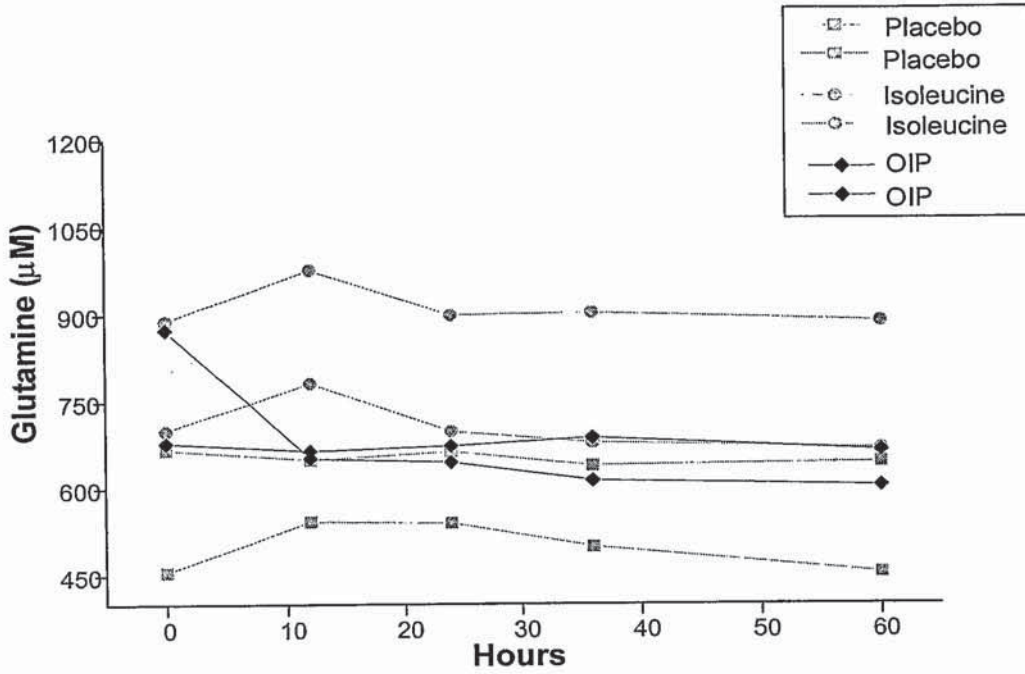
**Figure 13**



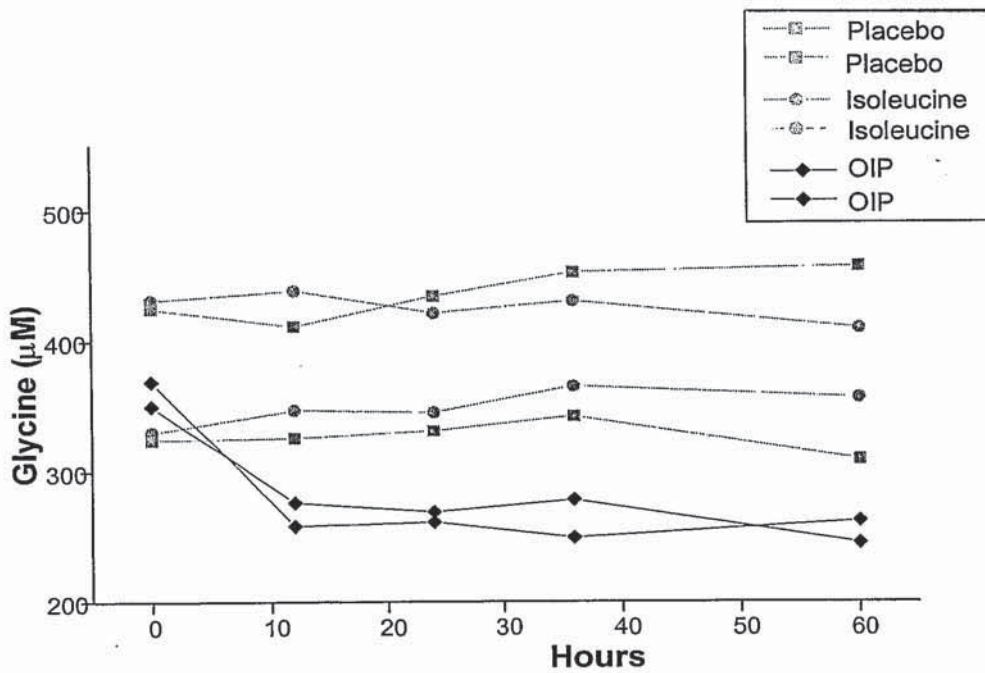
**Figure 14**



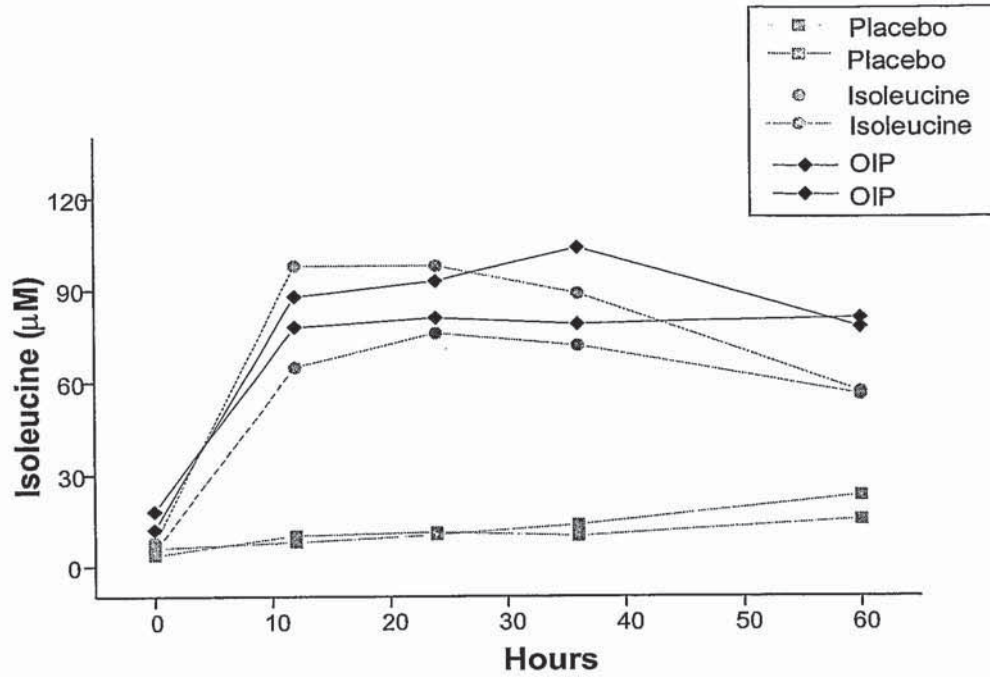
**Figure 15**



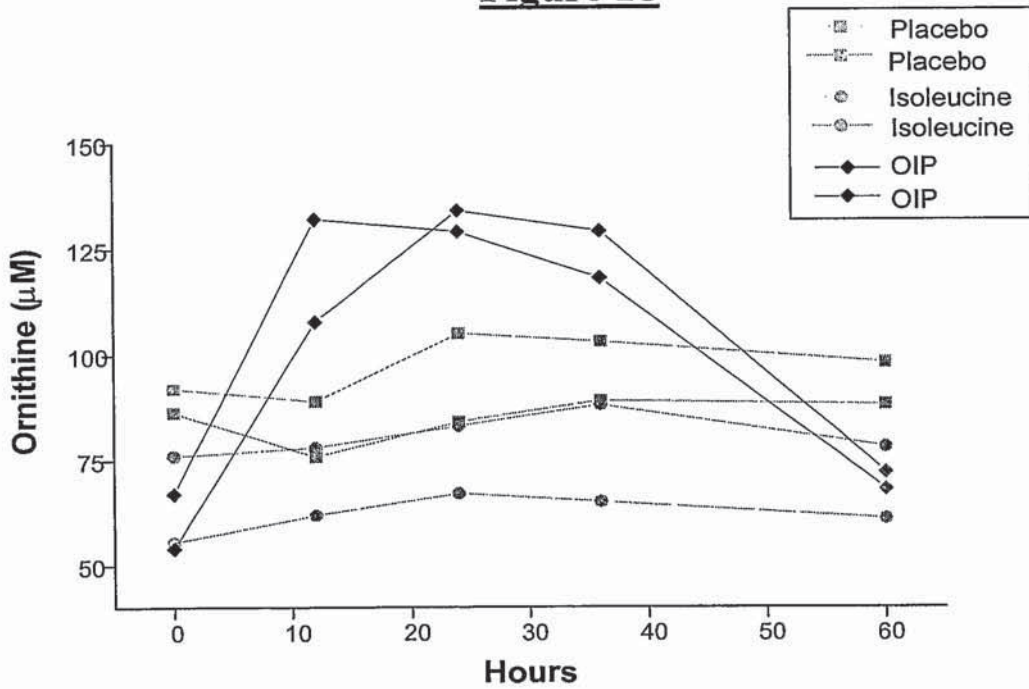
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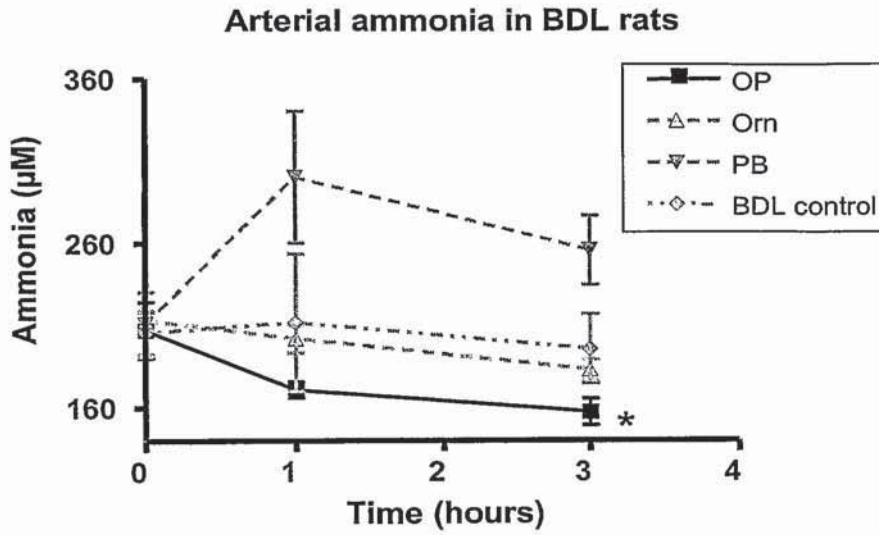
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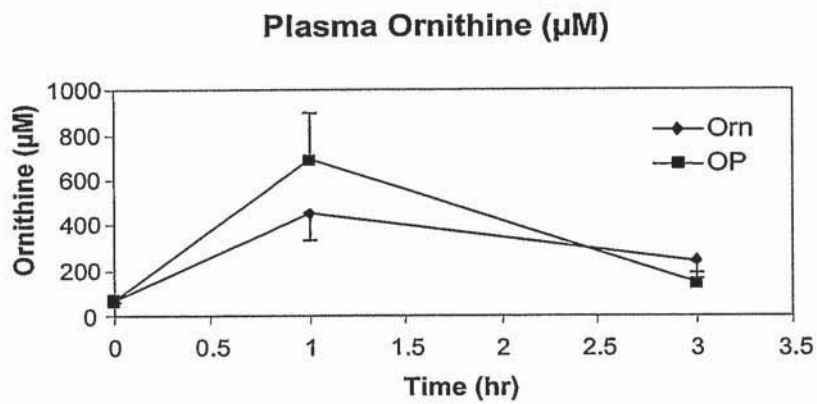
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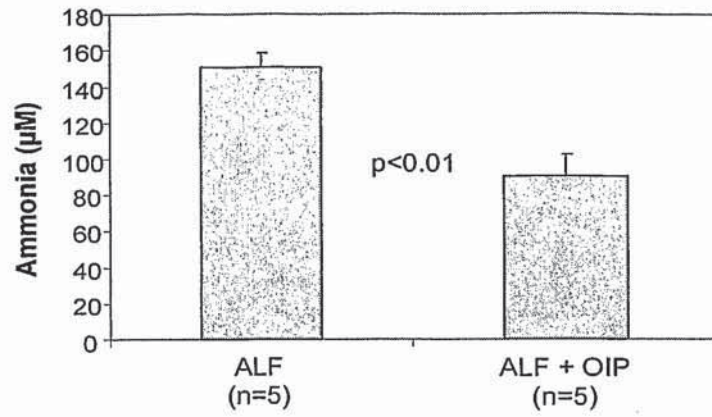
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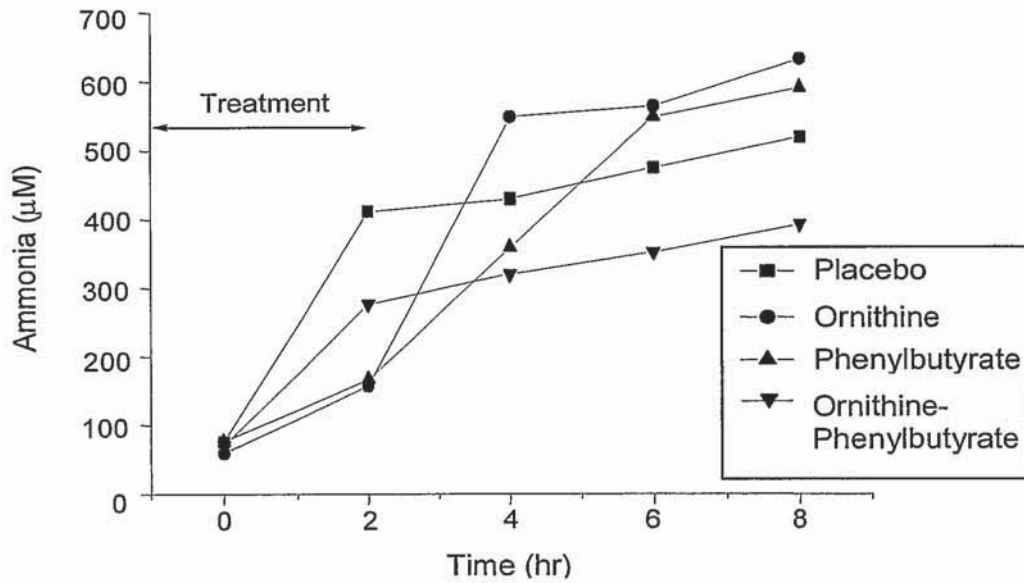
**Figure 20**



**Figure 21**

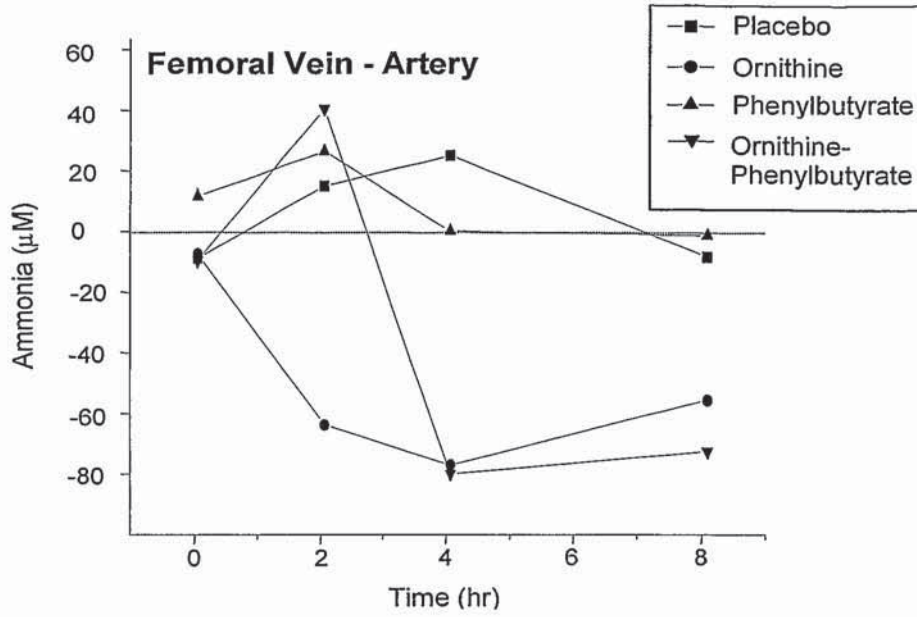


**Figure 22**

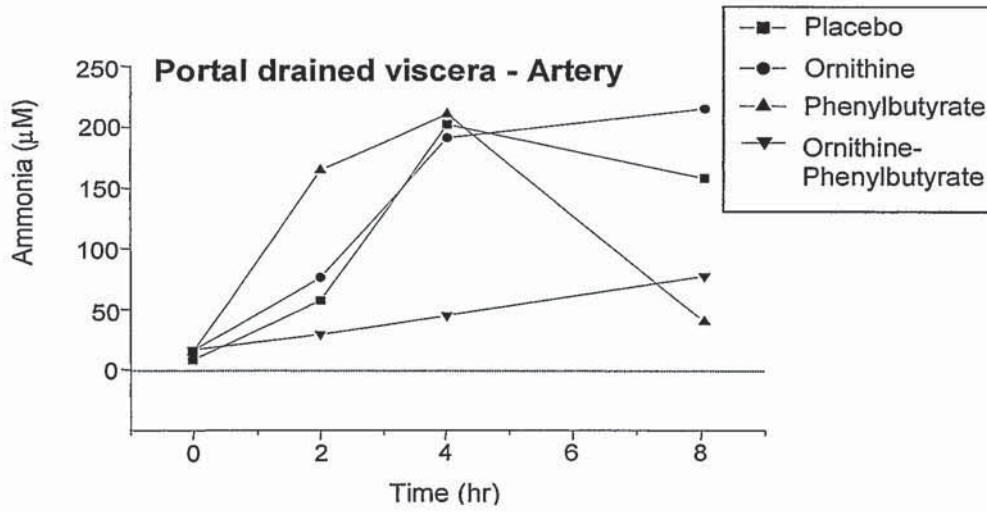




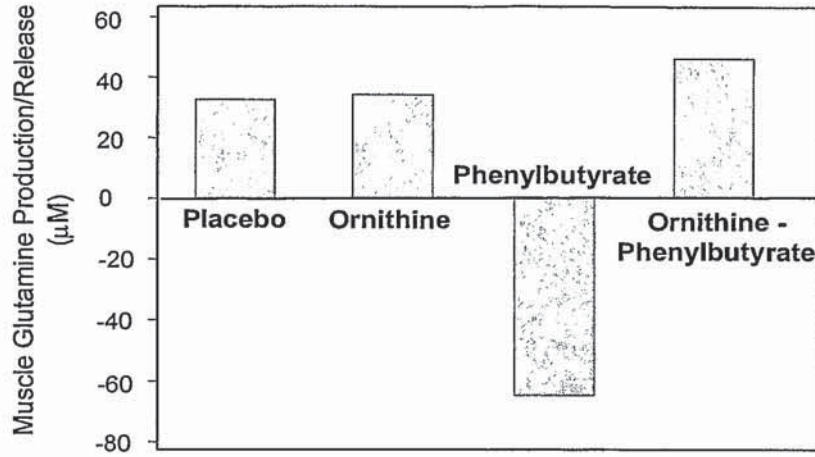
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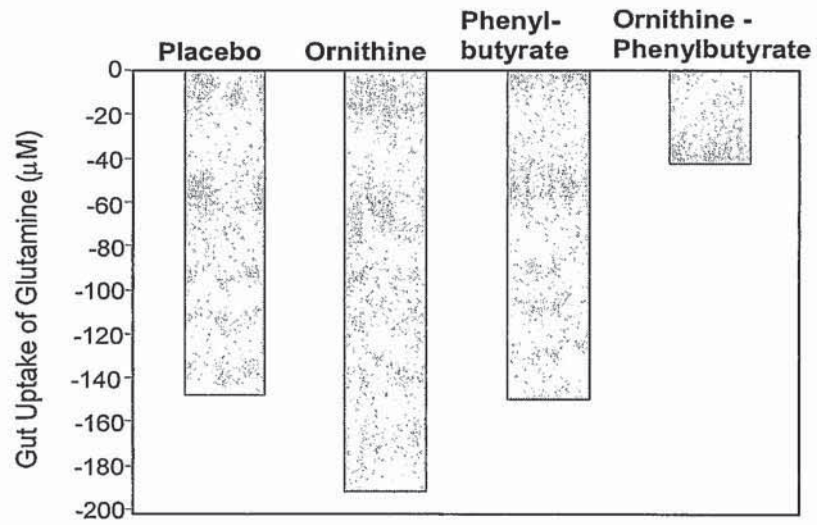
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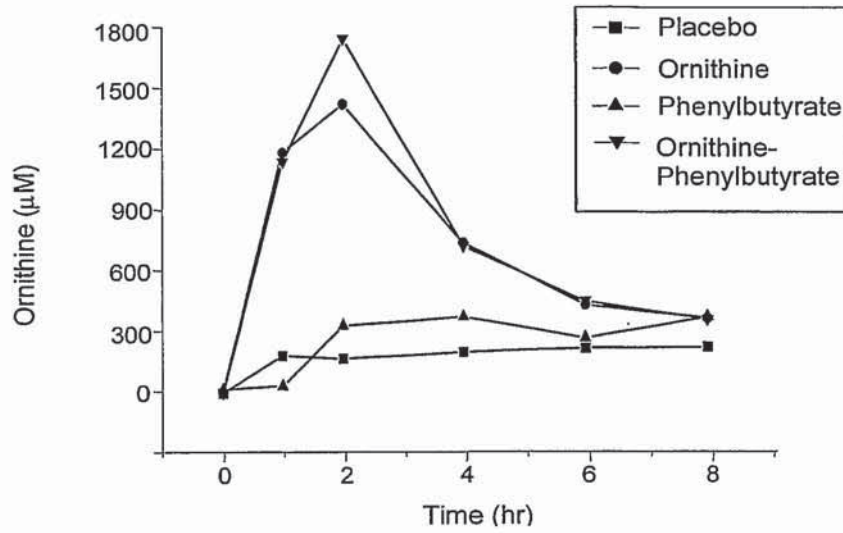
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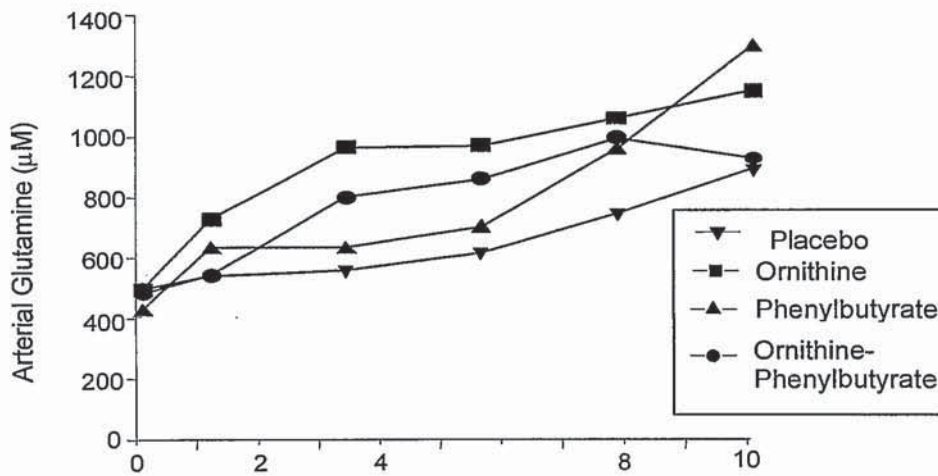
**Figure 26**



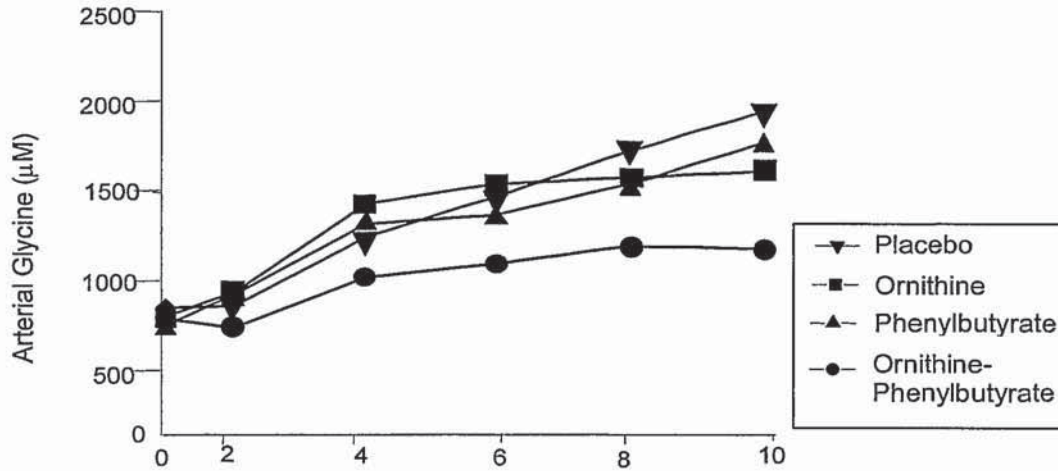
**Figure 27**



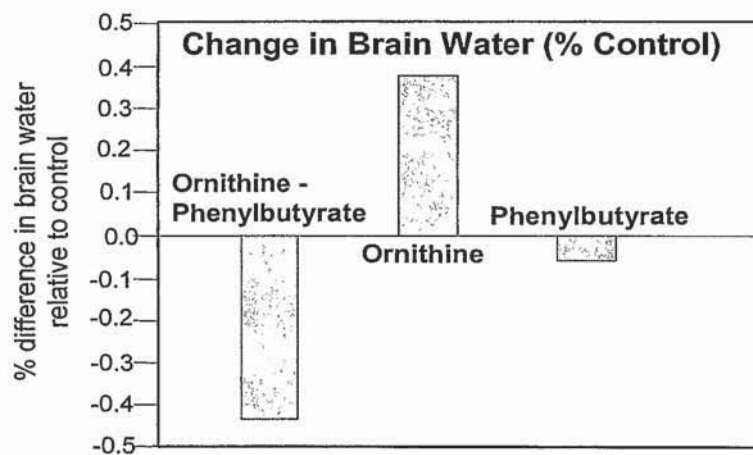
**Figure 28**



**Figure 29**



**Figure 30**



# INTERNATIONAL SEARCH REPORT

International application No  
PC 1 / 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
	----- -/--	

Further documents are listed in the continuation of Box C.       See patent family annex.

\* Special categories of cited documents :

<p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*E* earlier document but published on or after the international filing date</p> <p>*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)</p> <p>*O* document referring to an oral disclosure, use, exhibition or other means</p> <p>*P* document published prior to the international filing date but later than the priority date claimed</p>	<p>*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</p> <p>*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</p> <p>*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.</p> <p>*&amp;* document member of the same patent family</p>
--	--

Date of the actual completion of the international search  <p style="text-align: center;">27 January 2006</p>	Date of mailing of the International search report  <p style="text-align: center;">08/02/2006</p>
---	---

Name and mailing address of the ISA/ 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	Authorized officer  <p style="text-align: center;">Skjöldebrand, C</p>
---	--

# INTERNATIONAL SEARCH REPORT

Internal application No  
PC 1 / 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

International 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.  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 report covers 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

Information on patent family members

International application No  
PC 1 / GB2005/004539

Patent document cited in search report	A1	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
			CA 2468133	A1 05-06-2003
			CN 1596109	A 16-03-2005
			EP 1450781	A1 01-09-2004
			HU 0402240	A2 28-02-2005
			JP 2005518361	T 23-06-2005
			PL 353656	A1 02-06-2003
			SI 21542	A 28-02-2005
			WO 03045372	A1 05-06-2003
			US 2003105104	A1 05-06-2003
			US 4228099	A
AU 4515479	A 20-09-1979			
BE 874900	A1 16-07-1979			
CA 1115729	A1 05-01-1982			
DE 2910221	A1 20-09-1979			
DK 110879	A 18-09-1979			
ES 478710	A1 16-01-1980			
FI 790901	A 18-09-1979			
FR 2419723	A1 12-10-1979			
GB 2017094	A 03-10-1979			
IE 48336	B1 12-12-1984			
IT 1202904	B 15-02-1989			
JP 1204098	C 25-04-1984			
JP 54163518	A 26-12-1979			
JP 58038421	B 23-08-1983			
LU 81057	A1 07-09-1979			
NL 7902136	A 19-09-1979			
NO 790903	A 18-09-1979			
SE 446724	B 06-10-1986			
SE 7902401	A 16-10-1979			
CN 1383815	A	11-12-2002	NONE	
US 2004229948	A1	18-11-2004	AU 2003218361	A1 27-10-2003
			WO 03086074	A1 23-10-2003
			US 2003195255	A1 16-10-2003



Substitute for form 1449/PTO			<b>Complete if Known</b>	
<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  <i>(Use as many sheets as necessary)</i>			Application Number	12/350,111
			Filing Date	January 7, 2009
			First Named Inventor	Bruce SCHARSCHMIDT
			Art Unit	1614
			Examiner Name	Not Yet Assigned
			Attorney Docket Number	643982000100
Sheet	1	of	1	

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code <sup>2</sup> (if known)			
	1.	US-5,968,979	10/1999	Brusilow	
	2.	US-2004/0229948	11/2004	Summar et al.	
	3.	US-2006/0135612	06/2006	Ferrante	
	4.	US-2008/0119554	05/2008	Jalan et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. <sup>1</sup>	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T <sup>6</sup>
		Country Code <sup>3</sup> -Number <sup>4</sup> -Kind Code <sup>5</sup> (if known)				
	5.	WO-2006/056794	06/2006			

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

\*EXAMINER: Initial if information considered, whether or not citation 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 applicant. <sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> See Kinds Codes of USPTO Patent Documents at [www.uspto.gov](http://www.uspto.gov) or MPEP 901.04. <sup>3</sup> Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). <sup>4</sup> For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. <sup>5</sup> Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. <sup>6</sup> Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. <sup>1</sup>	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 <sup>2</sup>
	6.	BERRY et al., J Pediatrics (2001) 138:S56-S61	
	7.	BRUSILOW, Pediatric Research (1991) 29:147-150	
	8.	BRUSILOW, Progress in Liver Diseases (1995) 12:293-309	
	9.	BRUSILOW and FINKELSTEIN, J Metabolism (1993) 42:1336-1339	
	10.	CHANG et al., PNAS USA (2001) 98(17):9808-9813	
	11.	FDA Label for BUPHENYL, 6 pages	
	12.	KASUMOV et al., Drug Metabolism and Disposition (2004) 32(1):10-19	
	13.	RUDMAN et al., J Clin Invest (1973) 52:2241-2249	
	14.	SINGH, Suppl to J Pediatrics (2001) 138(1):S1-S5	
	15.	THIBAUT et al., Cancer (1995) 75(12):2932-2938	
	16.	THIBAUT et al., Cancer Research (1994) 54(7):1690-1694	

Examiner Signature		Date Considered	
--------------------	--	-----------------	--

\*EXAMINER: Initial if reference considered, whether or not citation 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 applicant.

<sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> Applicant is to place a check mark here if English language Translation is attached.

<h1>TRANSMITTAL FORM</h1> <p><i>(to be used for all correspondence after initial filing)</i></p>	Application Number	12/350,111	
	Filing Date	January 7, 2009	
	First Named Inventor	Bruce SCHARSCHMIDT	
	Art Unit	1614	
	Examiner Name	Not Yet Assigned	
Total Number of Pages in This Submission	8	Attorney Docket Number	643982000100

### ENCLOSURES (Check all that apply)

<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input checked="" type="checkbox"/> Information Disclosure Statement (3 pages) <input type="checkbox"/> Certified Copy of Priority Document(s) <input checked="" type="checkbox"/> Reply to Missing Parts/ Incomplete Application <input checked="" type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please Identify below): Declaration for Patent Application (3 pages) PTO/SB/08A/B (1 page) 12 References		
<table border="1" style="width: 100%;"> <tr> <td style="width: 150px;">Remarks</td> <td>CUSTOMER NO.: 25225</td> </tr> </table>			Remarks	CUSTOMER NO.: 25225
Remarks	CUSTOMER NO.: 25225			

### SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	February 24, 2009	Reg. No.	44,422

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DECLARATION FOR PATENT APPLICATION

As the 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 of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

the specification of which was filed on January 7, 2009 as Application No. 12/350,111.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by an amendment, if any, specifically referred to herein.

I acknowledge the duty to disclose all information known to me that is material to patentability as defined in 37 CFR 1.56.

FOREIGN PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

no foreign applications have been filed

foreign application(s) have been filed as follows:

EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing	Priority Claimed Under 35 USC 119
			Yes No

ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS  
(6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Application Number	Country	Date of Filing

CLAIM FOR BENEFIT OF EARLIER U.S. PROVISIONAL APPLICATIONS

I hereby claim priority benefits under Title 35, United States Code §119(e), of any United States provisional patent application(s) listed below:

- no U.S. provisional applications have been filed.
- U.S. provisional application(s) have been filed as follows:

Application Number	Date of Filing	Priority Claimed Under 35 USC 119
61/093,234	August 29, 2008	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
61/048,830	April 29, 2008	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
		<input type="checkbox"/> Yes <input type="checkbox"/> No

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S)

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

- no U.S./PCT applications have been filed.
- U.S./PCT application(s) have been filed as follows:

Application Number	Date of Filing	Status (Patented/Pending/Abandoned)

I hereby 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 were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint:

All practitioners at Customer Number 25225

all of **Morrison & Foerster LLP**, 12531 High Bluff Drive, Suite 100, San Diego, California 92130-2040, jointly, and each of them severally, my attorneys at law/patent agent(s), with full power of substitution, delegation and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, and to transact all business in the U. S. Patent and Trademark Office connected therewith.

Please mail all correspondence to Michael G. Smith, whose address is:

**Morrison & Foerster LLP**  
12531 High Bluff Drive, Suite 100  
San Diego, California 92130-2040

Please direct telephone calls to: Michael G. Smith at (858) 720-5113

Please direct facsimiles to: (858) 720-5125

Full name of sole or first inventor <b>Bruce SCHARSCHMIDT</b>	
Sole or first inventor's signature <i>Bruce F Scharschmidt</i>	Date <i>Feb 20 2009</i>
Residence South San Francisco, California	
Citizenship US	
Mailing Address  601 Gateway Blvd. Ste. 200 South San Francisco, California 94080	

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:  
Bruce SCHARSCHMIDT

Serial No.: 12/350,111

Filing Date: January 7, 2009

For: METHODS OF TREATMENT USING  
AMMONIA-SCAVENGING DRUGS

Examiner: Not Yet Assigned

Group Art Unit: 1614

**INFORMATION DISCLOSURE  
STATEMENT UNDER 37 C.F.R. § 1.97 & 1.98**

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

Dear Madam:

Pursuant to 37 C.F.R. §1.97 and § 1.98, Applicants submit for consideration in the above-identified application the documents listed on the attached Form PTO/SB/08a/b. Copies of foreign documents and non-patent literature are submitted herewith. The Examiner is requested to make these documents of record.

This Information Disclosure Statement is submitted:

- With the application; accordingly, no fee or separate requirements are required.
- Before the mailing of a first Office Action after the filing of a Request for Continued Examination under § 1.114. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.
- Within three months of the application filing date or before mailing of a first Office Action on the merits; accordingly, no fee or separate requirements are required. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.
- After receipt of a first Office Action on the merits but before mailing of a final Office Action or Notice of Allowance.
  - A fee is required. A check in the amount of \_\_ is enclosed.
  - A fee is required. Accordingly, a Fee Transmittal form (PTO/SB/17) is attached to this submission in duplicate.
  - A Certification under 37 C.F.R. § 1.97(e) is provided above; accordingly; no fee is believed to be due.
- After mailing of a final Office Action or Notice of Allowance, but before payment of the issue fee.
  - A Certification under 37 C.F.R. § 1.97(e) is provided above and a check in the amount of \_\_ is enclosed.
  - A Certification under 37 C.F.R. § 1.97(e) is provided above and a Fee Transmittal form (PTO/SB/17 is attached to this submission in duplicate.)

Applicants would appreciate the Examiner initialing and returning the Form PTO/SB/08a/b, indicating that the information has been considered and made of record herein.

The information contained in this Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98 is not to be construed as a representation that: (i) a complete 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 above information constitutes prior art to the subject invention.

In the unlikely event that the transmittal form is separated from this document and the Patent and Trademark Office determines that an extension and/or other relief (such as payment of a fee under 37 C.F.R. § 1.17 (p)) is required, Applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petition and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing 643982000100.

Dated: February 24, 2009

Respectfully submitted,

By: /Michael G. Smith/  
Michael G. Smith  
Registration No.: 44,422  
MORRISON & FOERSTER LLP  
12531 High Bluff Drive, Suite 100  
San Diego, California 92130-2040  
(858) 720-5113



PATENT COOPERATION TREATY

RECEIVED

PCT

MAR 06 2009

INTERNATIONAL SEARCH REPORT

MORRISON & FOERSTER  
SAN DIEGO DOCKETING

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 643982000140	<b>FOR FURTHER ACTION</b>		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 43.6bis(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.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 09/30362

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> 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>B. FIELDS SEARCHED</b> 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)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
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
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 24 February 2009 (24.02.2009)		Date of mailing of the international search report <b>02 MAR 2009</b>
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

Form PCT/ISA/210 (second sheet) (April 2007)

PATENT COOPERATION TREATY

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MAR 06 2009

From the  
INTERNATIONAL SEARCHING AUTHORITY

PCT

MORRISON & FOERSTER  
SAN DIEGO DOCKETING

To:  
MICHAEL G. SMITH  
MORRISON & FOERSTER LLP  
12531 HIGH BLUFF DRIVE, SUITE 100  
SAN DIEGO, CA 92130-2040

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing  
(day/month/year)

02 MAR 2009

Applicant's or agent's file reference  
643982000140

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US 09/30362

International filing date (day/month/year)

07 January 2009 (07.01.2009)

Priority date (day/month/year)

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 43bis.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 66.1bis(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  
Mail Stop PCT, Attn: ISA/US  
Commissioner for Patents  
P.O. Box 1450, Alexandria, Virginia 22313-1450  
Facsimile No. 571-273-3201

Date of completion of this opinion

24 February 2009 (24.02.2009)

Authorized officer:

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:
  - 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 43bis.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, In 26-32; Fig. 3; col 4, In 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, In 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, In 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, In 46-50; col 4, In 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, In 26-32; Fig. 3; col 4, In 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, In 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, In 1-26, "n = 2"; col 5, In 3-15; col 5, In 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, In 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, In 1-26; col 5, In 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, In 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, In 41-45).

Regarding claim 8, Summar (para [0022], [0029], [0035]) and Brusilow-979 (col 4, In 1-26; col 5, In 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 lessen the plasma nitrogen accumulation, as taught in Brusilow-979 (col 1, In 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, In 25-34; col 3, In 42-59, "triglycerides of phenyl alkanolic acid"; col 4, In 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, In 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, In 1-26, "n = 2").

Regarding claims 21 and 22, Brusilow-979 (col 2, In 25-34; col 3, In 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, In 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, In 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, In 42-59, "triglycerides of phenyl alkanolic acid"; col 4, In 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, In 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, In 1-26; col 3, In 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, In 26-32; Fig 3; col 4, In 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% conversion 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, In 27-34; In 41-45; col 5, In 3-15; In 29-35).

Regarding claim 13, Brusilow-979 further teaches the method of claim 12, wherein the PAA prodrug is HPN-100 (col 4, In 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, In 42-55; col 4, In 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, In 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

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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, In 25-34; col 3, In 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, In 26-32; Fig. 3; col 4, In 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, In 26-32).

Regarding claim 16, Brusilow-979 teaches the method of claim 15 (col 2, In 25-34; col 3, In 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, In 26-32; Fig. 3; col 4, In 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, In 3-15; In 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, In 25-34; col 3, In 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, In 26-32; Fig. 3; col 4, In 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, In 26-32).

Regarding claim 18, Brusilow-979 teaches a method of treatment with HPN-100 (col 3, In 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, In 26-32; Fig. 3; col 4, In 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% conversion 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, In 27-34; In 41-45; col 5, In 3-15; In 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, In 26-32; Fig. 3; col 4, In 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 Brusilow-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 Brusilow-979 (col 1, In 27-34; In 41-45; col 5, In 3-15; In 29-35).

Regarding claim 24, Brusilow-979 further teaches the method of claim 23, wherein the nitrogen scavenging drug is HPN-100 (col 4, In 1-26, "n = 2").

Regarding claim 25, Brusilow-647 (col 2, In 26-32; Fig. 3; col 4, In 35-46) and Brusilow-979 (col 1, In 27-34; col 1, In 41-45; col 5, In 3-15) teach the method of claim 24, wherein Brusilow-979 teaches the selected dosage of HPN-100 (col 4, In 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, In 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 Brusilow-647 (col 2, In 26-32; Fig. 3; col 4, In 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, In 42-59, "triglycerides of phenyl alkanolic acid"; col 4, In 1-26; col 4, In 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, In 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 Brusilow-647 (col 2, In 26-32; Fig. 3; col 4, In 35-46, "urinary phenylacetyl glutamine").

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WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
PCT/US 09/30362

Supplemental Box

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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 Brusilow-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% conversion of the prodrug, 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 alkanolic 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.



## Electronic Acknowledgement Receipt

<b>EFS ID:</b>	5439368
<b>Application Number:</b>	12350111
<b>International Application Number:</b>	
<b>Confirmation Number:</b>	6290
<b>Title of Invention:</b>	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
<b>First Named Inventor/Applicant Name:</b>	Bruce SCHARSCHMIDT
<b>Customer Number:</b>	25225
<b>Filer:</b>	Michael Glenn Smith/Jessica Conen
<b>Filer Authorized By:</b>	Michael Glenn Smith
<b>Attorney Docket Number:</b>	643982000100
<b>Receipt Date:</b>	02-JUN-2009
<b>Filing Date:</b>	07-JAN-2009
<b>Time Stamp:</b>	16:56:24
<b>Application Type:</b>	Utility under 35 USC 111(a)

### Payment information:

Submitted with Payment	no
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### File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	643982000100STRANS.pdf	27849 <small>cac1ffe2af36a7265ab93c666063cce625b19a25</small>	no	1

### Warnings:

### Information:

2	Transmittal Letter	643982000100Sids.pdf	24472 87566c525c1138088cbe81f949af3d9736915e	no	3
<b>Warnings:</b>					
<b>Information:</b>					
3	Information Disclosure Statement (IDS) Filed (SB/08)	643982000100SB08.pdf	28269 b075fc5d230ae696b9dfa714a286a820e5548e0	no	1
<b>Warnings:</b>					
<b>Information:</b>					
This is not an USPTO supplied IDS fillable form					
4	NPL Documents	643982000100REF.pdf	523097 546d64503a15f74c6906f200a012fde900dd3871	no	8
<b>Warnings:</b>					
<b>Information:</b>					
<b>Total Files Size (in bytes):</b>			603687		
<p><b>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.</b></p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b>  <b>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.</b></p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b>  <b>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.</b></p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b>  <b>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.</b></p>					

Substitute for form 1449/PTO			<b>Complete if Known</b>	
<b>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</b>  <i>(Use as many sheets as necessary)</i>			Application Number	12/350,111
			Filing Date	January 7, 2009
			First Named Inventor	Bruce SCHARSCHMIDT
			Art Unit	1614
			Examiner Name	Not Yet Assigned
			Attorney Docket Number	643982000100
Sheet	1	of	1	

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. <sup>1</sup>	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code <sup>2</sup> (if known)			
	1.	US-4,284,647	08/1981	Brusilow et al.	

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. <sup>1</sup>	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages Or Relevant Figures Appear	T <sup>6</sup>
		Country Code <sup>3</sup> -Number <sup>4</sup> -Kind Code <sup>5</sup> (if known)				

Examiner Signature		Date Considered	
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\*EXAMINER: Initial if information considered, whether or not citation 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 applicant. <sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> See Kinds Codes of USPTO Patent Documents at [www.uspto.gov](http://www.uspto.gov) or MPEP 901.04. <sup>3</sup> Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). <sup>4</sup> For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. <sup>5</sup> Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. <sup>6</sup> Applicant is to place a check mark here if English language Translation is attached.

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. <sup>1</sup>	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 <sup>2</sup>
	2.	International Search Report and Written Opinion for PCT/US09/30362, mailed 2 March 2009, 8 pages	

Examiner Signature		Date Considered	
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\*EXAMINER: Initial if reference considered, whether or not citation 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 applicant.

<sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> Applicant is to place a check mark here if English language Translation is attached.

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<h1>TRANSMITTAL FORM</h1> <p><i>(to be used for all correspondence after initial filing)</i></p>		Application Number	12/350,111
		Filing Date	January 7, 2009
		First Named Inventor	Bruce SCHARSCHMIDT
		Art Unit	1614
		Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission	5	Attorney Docket Number	643982000100

**ENCLOSURES (Check all that apply)**

<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input checked="" type="checkbox"/> Information Disclosure Statement (3 pages) <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/ Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): PTO form SB/08a/b (1 page) 1 Reference
<input type="text" value="Remarks"/>		CUSTOMER NO.: 25225

**SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT**

Firm Name	MORRISON & FOERSTER LLP		
Signature	/Michael G. Smith/		
Printed name	Michael G. Smith		
Date	June 2, 2009	Reg. No.	44,422

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:  
Bruce SCHARSCHMIDT

Application No.: 12/350,111

Filing Date: January 7, 2009

For: METHODS OF TREATMENT USING  
AMMONIA-SCAVENGING DRUGS

Examiner: Not Yet Assigned

Group Art Unit: 1614

Confirmation No.: 6290

**SUPPLEMENTAL INFORMATION DISCLOSURE  
STATEMENT UNDER 37 C.F.R. § 1.97 & §1.98**

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

Dear Sir:

Pursuant to 37 C.F.R. §1.97 and § 1.98, Applicants submit for consideration in the above-identified application the documents listed on the attached Form PTO/SB/08a/b. A copy of the non-patent document is submitted herewith. The Examiner is requested to make these documents of record.

The documents listed on the attached Form PTO/SB/08a/b were cited in an International Search Report mailed on March 2, 2009, directed to a counterpart international or foreign application and have not been previously cited. A certification under 37 C.F.R. § 1.97(e)(1) follows:

I hereby certify that each item of information was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this Information Disclosure Statement.

This Information Disclosure Statement is submitted:

- With the application; accordingly, no fee or separate requirements are required.
- Before the mailing of a first Office Action after the filing of a Request for Continued Examination under 37 C.F.R. § 1.114. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.
- Within three months of the application filing date or before mailing of a first Office Action on the merits; accordingly, no fee or separate requirements are required. However, if applicable, a certification under 37 C.F.R. § 1.97 (e)(1) has been provided.
- After receipt of a first Office Action on the merits but before mailing of a final Office Action or Notice of Allowance.
  - A fee is required. Accordingly, a Fee Transmittal Form (PTO/SB/17) is attached to this submission.
  - A Certification under 37 C.F.R. § 1.97(e) is provided above; accordingly; no fee is believed to be due.
- After mailing of a final Office Action or Notice of Allowance, but before payment of the Issue Fee.
  - A Certification under 37 C.F.R. § 1.97(e) is provided above and a Fee Transmittal Form (PTO/SB/17) is attached to this submission.)

Applicants would appreciate the Examiner initialing and returning the Form PTO/SB/08a/b, indicating that the information has been considered and made of record herein.

The information contained in this Information Disclosure Statement under 37 C.F.R. § 1.97 and § 1.98 is not to be construed as a representation that: (i) a complete 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 above information constitutes prior art to the subject invention.

In the unlikely event that the transmittal form is separated from this document and the Patent and Trademark Office determines that an extension and/or other relief (such as payment of a fee under 37 C.F.R. § 1.17 (p)) is required, Applicants petition for any required relief including extensions of time and authorize the Commissioner to charge the cost of such petition and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing 643982000100.

Dated: June 2, 2009

Respectfully submitted,

Electronic signature: /Michael G. Smith/  
Michael G. Smith  
Registration No.: 44,422  
MORRISON & FOERSTER LLP  
12531 High Bluff Drive, Suite 100  
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Table with 7 columns: APPLICATION NUMBER, FILING or 371(c) DATE, GRP ART UNIT, FIL FEE REC'D, ATTY,DOCKET.NO, TOT CLAIMS, IND CLAIMS. Row 1: 12/350,111, 01/07/2009, 1614, 1751, 643982000100, 29, 12

CONFIRMATION NO. 6290

UPDATED FILING RECEIPT



25225
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

Date Mailed: 10/02/2009

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

Applicant(s)

Bruce SCHARSCHMIDT, South San Francisco, CA;

Power of Attorney: The patent practitioners associated with Customer Number 25225

Domestic Priority data as claimed by applicant

This appln claims benefit of 61/093,234 08/29/2008
and claims benefit of 61/048,830 04/29/2008

Foreign Applications

If Required, Foreign Filing License Granted: 01/21/2009

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 12/350,111

Projected Publication Date: 01/14/2010

Non-Publication Request: No

Early Publication Request: No

\*\* SMALL ENTITY \*\*



**Title**

METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

**Preliminary Class**

514

**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.

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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).

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Table with 4 columns: APPLICATION NUMBER (12/350,111), FILING OR 371(C) DATE (01/07/2009), FIRST NAMED APPLICANT (Bruce SCHARSCHMIDT), ATTY. DOCKET NO./TITLE (643982000100)

CONFIRMATION NO. 6290

PUBLICATION NOTICE



25225
MORRISON & FOERSTER LLP
12531 HIGH BLUFF DRIVE
SUITE 100
SAN DIEGO, CA 92130-2040

Title:METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS

Publication No.US-2010-0008859-A1

Publication Date:01/14/2010

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.

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## Electronic Acknowledgement Receipt

<b>EFS ID:</b>	6935270
<b>Application Number:</b>	12350111
<b>International Application Number:</b>	
<b>Confirmation Number:</b>	6290
<b>Title of Invention:</b>	METHODS OF TREATMENT USING AMMONIA-SCAVENGING DRUGS
<b>First Named Inventor/Applicant Name:</b>	Bruce SCHARSCHMIDT
<b>Customer Number:</b>	25225
<b>Filer:</b>	Michael Glenn Smith/Jessica Conen
<b>Filer Authorized By:</b>	Michael Glenn Smith
<b>Attorney Docket Number:</b>	643982000100
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<b>Filing Date:</b>	07-JAN-2009
<b>Time Stamp:</b>	18:38:05
<b>Application Type:</b>	Utility under 35 USC 111(a)

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Submitted with Payment	no
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### File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	643982000100TRANS.pdf	27900 <small>087ce55297e08bc53a135556cf76244b8da7ec3d</small>	no	1

### Warnings:

### Information:

2	Transmittal Letter	643982000100SIDS.pdf	23936 1862e5eb9c9cbcf802f846ef0b05a597286a0b63	no	3
<b>Warnings:</b>					
<b>Information:</b>					
3	Information Disclosure Statement (IDS) Filed (SB/08)	643982000100SB08.pdf	31554 fe98b65cefd3a3ae3b989998ca6e7a1c0345bc254	no	1
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4	Foreign Reference	WO05053607A2.pdf	662823 53db1ac7d911f0a5d14213b3472a1cc735ab777f	no	12
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5	Foreign Reference	WO29087474A2.pdf	3777890 9cca4e5b3be5a80b0360dec55d494634306d1c9c	no	67
<b>Warnings:</b>					
<b>Information:</b>					
6	NPL Documents	CLINICALTRIAL2007SNCT00551200.pdf	204266 4ea1586f47c9d9868f32f18593da17c8c253fe07	no	4
<b>Warnings:</b>					
<b>Information:</b>					
7	NPL Documents	COMTE2002JMassSpectrometry.pdf	683712 4aac9f4ff1743204bd225424f985dcd76f5c5	no	10
<b>Warnings:</b>					
<b>Information:</b>					
8	NPL Documents	LEE2008JInheritedMetabolicDis.pdf	121649 da88cf35faecc62aa50b083cfe0aed9190182495	no	1
<b>Warnings:</b>					
<b>Information:</b>					
9	NPL Documents	GB09155458SearchExamReport10082009.pdf	351435 95967ec4e40eb42934326b9eb21f23b9a0df201d	no	5
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**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.

(19) World Intellectual Property  
Organization  
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**WO 2005/053607 A2**

- (51) International Patent Classification<sup>7</sup>: **A61K**
- (21) International Application Number:  
PCT/US2004/038462
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- (25) Filing Language: English
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10/725,064 1 December 2003 (01.12.2003) US
- (71) Applicant: **MEDICIS PHARMACEUTICAL CORPORATION** [US/US]; 8125 N. Hayden Road, Scottsdale, AZ 85258 (US).
- (72) Inventors: **SUMMAR, Marshall, L.**; Brentwood, TN (US). **GARGOSKY, Sharron, Ema**; Scottsdale, AZ (US).
- (74) Agents: **MCNICHOL, William, J.** et al.; Reed Smith LLP, 2500 One Liberty Place, 1650 Market Street, Philadelphia, PA 19103 (US).
- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
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- 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 2005/053607 A2

(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 EPISODESCross 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 (hepatportal sclerosis), schistosomiasis, Budd-



Chlāri 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 11<sup>th</sup> 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

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.

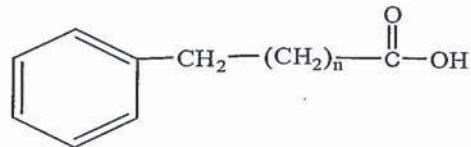
- [17] In another prevention embodiment of the invention, the patient has not had an encephalopathic episode in at least about 12 weeks.
- [18] The risk of hepatic encephalopathic episodes for TIPS patients were noted in the following studies. In one study (Sanyal AJ, Freedman AM, Shiffman ML, et al., 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

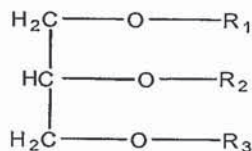
their acids, alcohols, salts, amines, esters, ethers and glycerides, salts, derivatives and metabolites.

- [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:

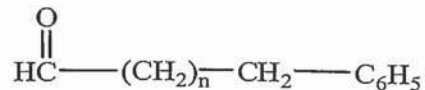


where n is 2, 4, 6 or 8.

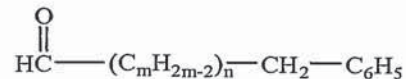
- [24] Another group of compounds useful in the present invention is disclosed in U.S. Pat. No. 5,968,979, which describes phenylalkanoic esters of glycerol according to the following formula:



where R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are independently, H



or



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<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> is not H. Glycerol-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 naphthyl 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

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<sup>2</sup>/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<sup>2</sup> (preferably about 5 to about 6 g/m<sup>2</sup>) 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<sup>2</sup>. More commonly, the daily dose will be between about 6 and about 9 g/m<sup>2</sup>.
- [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

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<sup>2</sup>/day to about 15 g/m<sup>2</sup>/day, preferably about 1 g/m<sup>2</sup>/day to about 8 g/m<sup>2</sup>/day, more preferably about 3 g/m<sup>2</sup>/day to about 8 g/m<sup>2</sup>/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.

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<sup>2</sup>/day.
4. The method of claim 2, wherein the compound is administered in an amount from about 1 to about 8 g/m<sup>2</sup>/ day.
5. The method of claim 2 wherein the compound is administered in an amount from about 3 to about 8 g/m<sup>2</sup>/ 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<sup>2</sup>/day.
13. The method of claim 11, when an initial loading dose of the compound of about 2 to about 13 g/m<sup>2</sup> is additionally administered to the person.



14. The method of claim 11, wherein the compound is administered in an amount of about 1 to about 8 g/m<sup>2</sup>/day.
15. The method of claim 11, wherein the compound is administered in an amount of about 3 to about 8 g/m<sup>2</sup>/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.

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: 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.



WO 2009/087474 A2

- 1 -

**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 AND IMMUNITY, Feb. 2002, vol 70, No 2 p. 953–963*) reported that infection *in vitro* of  
35 HCA-7 cells with *Salmonella enterica* serovar Dublin or enteroinvasive *Escherichia coli*

- 2 -

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 occurring 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

- 3 -

5 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 defined as including 'isoleucine butyrate' though this compound is not described or tested.

10 US7311925 (Zasloff) describes methods of blocking microbial adherence to a eukaryotic cell surface in a mammal by applying a pharmacologically acceptable composition 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 chronic 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)<sub>2</sub> 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.

35 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 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 cathelicidin 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 Ucyclid 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

- 5 -

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.

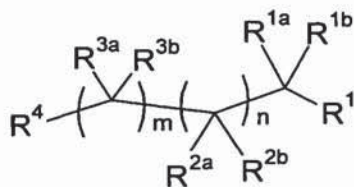
Notwithstanding the above, prior to the present invention, PBA was not known or suggested for the uses claimed herein.

#### 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



- 6 -

R<sup>1</sup> represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, COOR<sup>5</sup>, CONH<sub>2</sub>, CONR<sup>5</sup>R<sup>6</sup>, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>(OOCR<sup>6</sup>) or diglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>OH, or  
5 an amino acid group CONHCR<sup>7</sup>COOH or a salt thereof;

*m* and *n* are each independently 0 or 1;

R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup> and R<sup>3b</sup> each independently represent hydrogen, halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or  
10 nonsaturated alkyl group with 1 to 10 carbon atoms, or a substituted or nonsubstituted aryl group; and/or

R<sup>2a</sup>, together with an adjacent R<sup>3a</sup> or R<sup>1a</sup>, may represent a carbon-carbon  $\pi$  bond; and/or

15 R<sup>2b</sup>, together with an adjacent R<sup>3b</sup> or R<sup>1b</sup>, may represent a carbon-carbon  $\pi$  bond;

R<sup>4</sup> 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<sup>5</sup> 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;

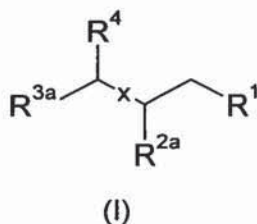
25 R<sup>6</sup> represents hydrogen, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms or a substituted or nonsubstituted aryl group; and

30 R<sup>7</sup> is a side chain of a naturally occurring amino acid or is selected from CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>, or CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(=NH)NHR<sup>8</sup>, where R<sup>8</sup> is hydrogen or a linear or branched acyl group with three to five carbon atoms;

and wherein, if R<sup>1</sup> is carboxyl or a salt thereof, at least one of R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> is selected from halide, amino, hydroxyl, carbonyl, a linear or branched substituted or nonsubstituted saturated or nonsaturated alkyl group with 1 to 10 carbon atoms, or a  
35 substituted or nonsubstituted aryl group.

- 7 -

In some embodiments the compound may be a compound of formula I:



- 5 wherein, preferably, R<sup>1</sup> represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, COOR<sup>5</sup>, CONH<sub>2</sub>, CONR<sup>5</sup>R<sup>6</sup>, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>(OOCR<sup>6</sup>) or diglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>OH, or an amino acid group CONHCR<sup>7</sup>COOH or a salt thereof,
- 10 R<sup>2a</sup> 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<sup>3a</sup> 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<sup>1</sup> is carboxyl or a salt thereof R<sup>3a</sup> is not hydrogen,
- 15 R<sup>4</sup> 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<sup>3a</sup>R<sup>4</sup> together represent hydrogen in which case R<sup>1</sup> is preferably COOR<sup>5</sup>, CONH<sub>2</sub>, CONR<sup>5</sup>R<sup>6</sup>, or a triglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>(OOCR<sup>6</sup>) or diglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>OH,
- R<sup>5</sup> 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<sup>6</sup> 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<sup>7</sup> represents CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(=NH)NHR<sup>8</sup>, where R<sup>8</sup> is hydrogen or a linear or branched acyl group with three to five carbon atoms.

- 8 -

Compounds of formula I are compounds of formula Ia in which R<sup>1a</sup> and R<sup>1b</sup> are both hydrogen, *m* and *n* are both 1, and R<sup>2b</sup> and R<sup>3b</sup> are *either* both hydrogen *or* together form a π bond in position 'x'. If R<sup>2a</sup> and R<sup>3a</sup> also together form a π bond, then position 'x' represents a double bond.

5

Compounds of formula Ia in which R<sup>1a</sup>, R<sup>1b</sup> and R<sup>2b</sup> are all hydrogen, *m* is 0, *n* is 1, and R<sup>4</sup> is hydrogen can also be represented as compounds of formula I where x-R<sup>3a</sup>R<sup>4</sup> together represent hydrogen.

10

In compounds of formula I, 'x' is preferably a single bond.

#### Preferences for R<sup>1</sup>

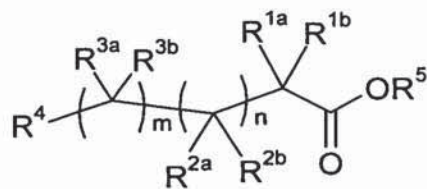
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In certain preferred embodiments, the compound of the invention is a carboxylic acid, in these cases R<sup>1</sup> represents a carboxyl group, or a pharmaceutically acceptable salt thereof. If R<sup>1</sup> is carboxyl or a salt thereof, at least one of R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> is a substituent other than hydrogen. In other preferred embodiments, R<sup>1</sup> is a carboxylic acid derivative, such as an ester or an amide.

20

In some such embodiments, as represented by formula IIa, R<sup>1</sup> is an ester group of formula COOR<sup>5</sup> where R<sup>5</sup> 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<sup>5</sup> groups are methyl and ethyl.

25



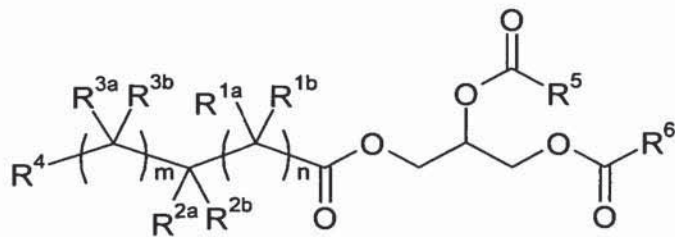
(IIa)

In some preferred embodiments R<sup>1</sup> is an ester selected from a triglyceride ester moiety or diglyceride ester moiety.

30

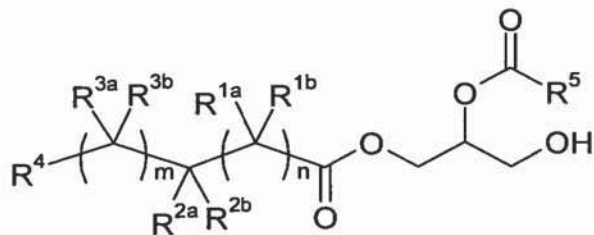
If R<sup>1</sup> is a triglyceride moiety the compounds of the invention are of the following general formula (IIb):

- 9 -



(IIb)

If R<sup>1</sup> is a diglyceride moiety, the compounds of the invention are of the following general formula (IIc):

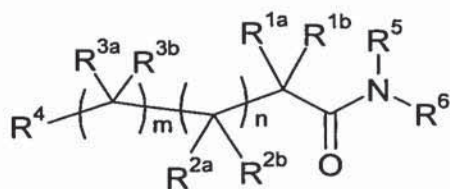


(IIc)

Embodiments of particular interest include glyceryl tributyrate or glyceryl tripropionate. Other preferred embodiments make use of corresponding glycerol esters of one or more 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-phenylbutanoate.

Further embodiments which are carboxylic derivatives embodiments include amides of formula (II d), wherein R<sup>1</sup> is a group of formula CONR<sup>5</sup>R<sup>6</sup>, wherein R<sup>5</sup> represents 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, and R<sup>6</sup> 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.

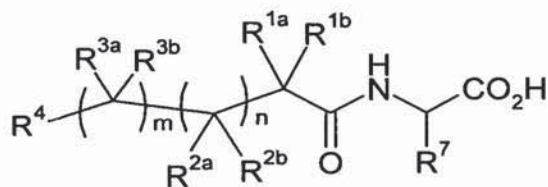
- 10 -



(IId)

In certain embodiments R<sup>1</sup> is an amino acid group, in which case the compounds of the invention may be represented as compounds of the following general formula (IIe):

5



(IIe)

10 or a salt thereof, in which R<sup>7</sup> is an amino acid side chain. In some embodiments R<sup>7</sup> is the side chain of a naturally occurring amino acid.

For example, R<sup>7</sup> may be a side chain of leucine (CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), isoleucine (CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>), methionine (-CH<sub>2</sub>CH<sub>2</sub>SCH<sub>3</sub>), lysine (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), or arginine (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(=NH)NH<sub>2</sub>). In some embodiments, particularly if R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> are all hydrogen and *m* and *n* are 1, R<sup>7</sup> is preferably not an isoleucine side chain (CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>).

20 Alternatively, R<sup>7</sup> may be a derivative or analogue of a naturally occurring amino acid side chain, such as a lysine side chain derivative (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>), an arginine side chain derivative (-CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHC(=NH)NHR<sup>8</sup>), or a group such as -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NHR<sup>8</sup>, wherein R<sup>8</sup> 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.

25 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<sup>2a</sup> and R<sup>4</sup> represent hydrogen. In these embodiments R<sup>3a</sup> is preferably hydrogen, hydroxyl, or a substituted or nonsubstituted aryl group including phenyl, or benzyl, with the above

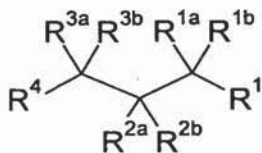
- 11 -

limitation applying to R<sup>3a</sup> in the case where R<sup>1</sup> is carboxyl or a salt thereof. Substituted aryl can be hydroxyl or amino-substituted phenyl, or benzyl.

*Preferred chain lengths*

5

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):



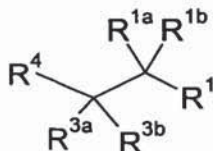
10

(IIIa)

where R<sup>1</sup>, R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> are as previously defined.

15

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):



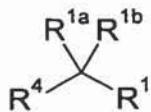
(IIIb)

20

where R<sup>1</sup>, R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> 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.

25

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):



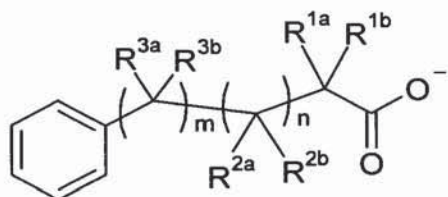
(IIIc)

*Preferred substituents*

- 12 -

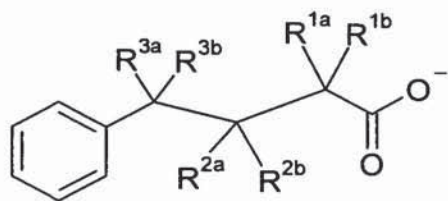
Preferred embodiments of the invention include compounds which are substituted butyric, propionic or acetic acid derivatives of general formulae (IIIa) to (IIIc), wherein R<sup>1</sup> is carboxylate or a derivative thereof as defined above and wherein one or more of R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> 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<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> is an aryl group, most preferably a phenyl or substituted phenyl group. When one of R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup>, R<sup>3b</sup> and R<sup>4</sup> is an aryl group, it is preferred that the others are selected from hydrogen or an alkyl group, the alkyl group being preferably methyl.

Most preferably, R<sup>4</sup> is an aryl group, preferably phenyl or substituted phenyl. Certain preferred compounds according to these embodiments are of general formula (IVa):



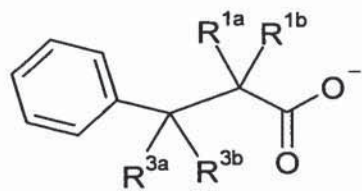
(IVa)

Preferred butyric acid derivatives are therefore of general formula (IVb):



(IVb)

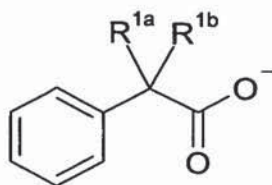
preferred propionic acid derivatives are of general formula (IVc):



(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 $\alpha$ 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 [S77], 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  $\beta$  to the carboxylate (where present)*

In embodiments, one or both of R<sup>2a</sup> and R<sup>2b</sup> may optionally be hydroxyl. This may be 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*

Alkyl:

As used herein the term "alkyl", unless otherwise specified, refers to a C<sub>1-10</sub> 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 saturated, partially unsaturated, or fully unsaturated. In certain instances C<sub>1-4</sub>, C<sub>1-5</sub>, C<sub>1-6</sub> or C<sub>1-7</sub> alkyl groups may be preferred.

Examples of saturated linear C<sub>1-10</sub> alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, n-butyl, n-pentyl (amyl) and n-hexyl.

Examples of saturated branched C<sub>1-10</sub> alkyl groups include, but are not limited to, iso-propyl, iso-butyl, sec-butyl, tert-butyl, and neo-pentyl.

Examples of saturated alicyclic C<sub>1-10</sub> alkyl groups (which may also be referred to as "C<sub>3-10</sub> 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.

Unsaturated alkyl groups contain one or more double or triple bonds i.e. one or more carbon-carbon  $\pi$  bonds. Examples of unsaturated C<sub>1-10</sub> alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C<sub>2-10</sub>alkenyl" groups) include, but are not limited to, ethenyl (vinyl, -CH=CH<sub>2</sub>), 2-propenyl (allyl, -CH-CH=CH<sub>2</sub>), isopropenyl (-C(CH<sub>3</sub>)=CH<sub>2</sub>), butenyl, pentenyl, and hexenyl.

- 15 -

Examples of unsaturated C<sub>1-10</sub> alkyl groups which have one or more carbon-carbon triple bonds (also referred to as "C<sub>2-10</sub> alkynyl" groups) include, but are not limited to, ethynyl (ethynyl) and 2-propynyl (propargyl).

5 Examples of unsaturated alicyclic (carbocyclic) C<sub>1-10</sub> alkyl groups which have one or more carbon-carbon double bonds (also referred to as "C<sub>3-10</sub>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  
10 comprise such groups) such as cyclopropenylmethyl and cyclohexenylmethyl.

Aryl:

As used herein the term "aryl", unless otherwise specified, refers to a C<sub>5-20</sub> aryl group, that is to say a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of a C<sub>5-20</sub> 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<sub>5-20</sub> carboaryl" group.

20 Examples of C<sub>5-20</sub> aryl groups which do not have ring heteroatoms (i.e. C<sub>5-20</sub> carboaryl groups) include, but are not limited to, those derived from benzene (i.e. phenyl) (C<sub>6</sub>), naphthalene (C<sub>10</sub>), anthracene (C<sub>14</sub>), phenanthrene (C<sub>14</sub>), naphthacene (C<sub>18</sub>), and pyrene (C<sub>16</sub>).

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.

30 Alternatively, the ring atoms may include one or more heteroatoms, including but not limited to oxygen, nitrogen, and sulphur, as in "heteroaryl groups". In this case, the group may conveniently be referred to as a "C<sub>5-20</sub> heteroaryl" group, wherein "C<sub>5-20</sub>" 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<sub>5-20</sub> heteroaryl groups include, but are not limited to, C<sub>5</sub> heteroaryl groups derived from furan (oxole), thiophene (thiole), pyrrole (azole), imidazole (1,3-diazole),

- 16 -

pyrazole (1,2-diazole), triazole, oxazole, isoxazole, thiazole, isothiazole, oxadiazole, and oxatriazole; and C<sub>6</sub> 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<sub>5-20</sub> heteroaryl groups which comprise fused rings, include, but are not limited to, C<sub>9</sub> heterocyclic groups derived from benzofuran, isobenzofuran, indole, isoindole, purine (e.g., adenine, guanine), benzothiophene, benzimidazole; C<sub>10</sub> heterocyclic groups derived from quinoline, isoquinoline, benzodiazine, pyridopyridine, quinoxaline; C<sub>13</sub> heterocyclic groups derived from carbazole, dibenzothiophene, dibenzofuran; C<sub>14</sub> heterocyclic groups derived from acridine, xanthen, phenoxathiin, phenazine, phenoxazine, phenothiazine.

10

#### 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<sub>1-7</sub> alkyl group (also referred to as a C<sub>1-7</sub> alkoxy group, discussed below), a C<sub>3-20</sub> heterocyclyl group (also referred to as a C<sub>3-20</sub> heterocyclyloxy group), or a C<sub>5-20</sub> aryl group (also referred to as a C<sub>5-20</sub> aryloxy group), preferably a C<sub>1-7</sub> alkyl group.

25

C<sub>1-7</sub> alkoxy: -OR, wherein R is a C<sub>1-7</sub> alkyl group. Examples of C<sub>1-7</sub> alkoxy groups include, but are not limited to, -OCH<sub>3</sub> (methoxy), -OCH<sub>2</sub>CH<sub>3</sub> (ethoxy) and -OC(CH<sub>3</sub>)<sub>3</sub> (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 imidazolidone; cyclic anhydrides, including but not limited to maleic anhydride and succinic anhydride; cyclic carbonates, such as propylene carbonate; imides, including but

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- 17 -

not limited to, succinimide and maleimide; lactones (cyclic esters, -O-C(=O)- in a ring), including, but not limited to,  $\beta$ -propiolactone,  $\gamma$ -butyrolactone,  $\delta$ -valerolactone, and  $\epsilon$ -caprolactone; and lactams (cyclic amides, -NH-C(=O)- in a ring), including, but not limited to,  $\beta$ -propiolactam,  $\gamma$ -butyrolactam (2-pyrrolidone),  $\delta$ -valerolactam, and  $\epsilon$ -caprolactam.

Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably hydrogen or a C<sub>1-7</sub> alkyl group. Examples of ester groups include, but are not limited to, =NH, =NMe, =NEt, and =NPh.

Formyl (carbaldehyde, carboxaldehyde): -C(=O)H.

Acyl (keto): -C(=O)R, wherein R is an acyl substituent, for example, a C<sub>1-7</sub> alkyl group (also referred to as C<sub>1-7</sub> alkylacyl or C<sub>1-7</sub> alkanoyl), a C<sub>3-20</sub> heterocyclyl group (also referred to as C<sub>3-20</sub> heterocyclylacyl), or a C<sub>5-20</sub> aryl group (also referred to as C<sub>5-20</sub> arylacyl), preferably a C<sub>1-7</sub> alkyl group. Examples of acyl groups include, but are not limited to, -C(=O)CH<sub>3</sub> (acetyl), -C(=O)CH<sub>2</sub>CH<sub>3</sub> (propionyl), -C(=O)C(CH<sub>3</sub>)<sub>3</sub> (butyryl), and -C(=O)Ph (benzoyl, phenone).

Carboxy (carboxylic acid): -COOH.

Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=O)OR, wherein R is an ester substituent, for example, a C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group. Examples of ester groups include, but are not limited to, -C(=O)OCH<sub>3</sub>, -C(=O)OCH<sub>2</sub>CH<sub>3</sub>, -C(=O)OC(CH<sub>3</sub>)<sub>3</sub>, and -C(=O)OPh.

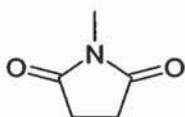
Acyloxy (reverse ester): -OC(=O)R, wherein R is an acyloxy substituent, for example, a C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group. Examples of acyloxy groups include, but are not limited to, -OC(=O)CH<sub>3</sub> (acetoxyl), -OC(=O)CH<sub>2</sub>CH<sub>3</sub>, -OC(=O)C(CH<sub>3</sub>)<sub>3</sub>, -OC(=O)Ph, and -OC(=O)CH<sub>2</sub>Ph.

Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): -C(=O)NR<sup>N1</sup>R<sup>N2</sup>, wherein R<sup>N1</sup> and R<sup>N2</sup> are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, -C(=O)NH<sub>2</sub>, -C(=O)NHCH<sub>3</sub>, -C(=O)N(CH<sub>3</sub>)<sub>2</sub>, -C(=O)NHCH<sub>2</sub>CH<sub>3</sub>, and -C(=O)N(CH<sub>2</sub>CH<sub>3</sub>)<sub>2</sub>, as well as amido groups in which R<sup>N1</sup> and

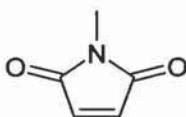
- 18 -

$R^{N2}$ , together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.

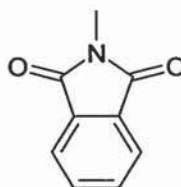
- 5 Acylamido (acylamino):  $-NR^{A1}C(=O)R^{A2}$ , wherein  $R^{A1}$  is an amide substituent, for example, hydrogen, a  $C_{1-7}$  alkyl group, a  $C_{3-20}$  heterocyclyl group, or a  $C_{5-20}$  aryl group, preferably hydrogen or a  $C_{1-7}$  alkyl group, and  $R^{A2}$  is an acyl substituent, for 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

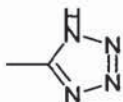
- Acylureido:  $-N(R^{U1})C(O)NR^{U2}C(O)R^{A3}$  wherein  $R^{U1}$  and  $R^{U2}$  are independently ureido substituents, for example, hydrogen, a  $C_{1-7}$  alkyl group, a  $C_{3-20}$  heterocyclyl group, or a  $C_{5-20}$  aryl group, preferably hydrogen or a  $C_{1-7}$  alkyl group.  $R^{A3}$  is an acyl group as defined for acyl groups. Examples of acylureido groups include, but are not limited to,   
 15  $-NHCONHC(O)H$ ,  $-NHCONMeC(O)H$ ,  $-NHCONEtC(O)H$ ,  $-NHCONMeC(O)Me$ ,  $-NHCONEtC(O)Et$ ,  $-NMeCONHC(O)Et$ ,  $-NMeCONHC(O)Me$ ,  $-NMeCONHC(O)Et$ ,   
 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,   
 25  $-NH-C(O)-O-Me$ ,  $-NMe-C(O)-O-Me$ ,  $-NH-C(O)-O-Et$ ,  $-NMe-C(O)-O-t-butyl$ , 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,   
 30  $-C(=S)NH_2$ ,  $-C(=S)NHCH_3$ ,  $-C(=S)N(CH_3)_2$ , and  $-C(=S)NHCH_2CH_3$ .

- 19 -

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, 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  $-C(=NH)NH_2$ .  
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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,  $-C(O)-NN-H$ ,  $-C(O)-NN-Me$ ,  $-C(O)-NN-Et$ ,  $-C(O)-NN-Ph$ , and  $-C(O)-NN-CH_2-Ph$ .  
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Nitro:  $-NO_2$ .

Nitroso:  $-NO$ .

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Azido:  $-N_3$ .

Cyano (nitrile, carbonitrile):  $-CN$ .

- 20 -

Isocyano: -NC.

Cyanato: -OCN.

5 Isocyanato: -NCO.

Thiocyano (thiocyanato): -SCN.

10 Isothiocyano (isothiocyanato): -NCS.

Sulfhydryl (thiol, mercapto): -SH.

Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a C<sub>1-7</sub> alkyl group (also referred to as a C<sub>1-7</sub> alkylthio group), a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group. Examples of C<sub>1-7</sub> alkylthio groups include, but are not limited to, -SCH<sub>3</sub> and -SCH<sub>2</sub>CH<sub>3</sub>.

Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group (also referred to herein as C<sub>1-7</sub> alkyl disulfide). Examples of C<sub>1-7</sub> alkyl disulfide groups include, but are not limited to, -SSCH<sub>3</sub> and -SSCH<sub>2</sub>CH<sub>3</sub>.

Sulfone (sulfonyl): -S(=O)<sub>2</sub>R, wherein R is a sulfone substituent, for example, a C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group. Examples of sulfone groups include, but are not limited to, -S(=O)<sub>2</sub>CH<sub>3</sub> (methanesulfonyl, mesyl), -S(=O)<sub>2</sub>CF<sub>3</sub> (triflyl), -S(=O)<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>, -S(=O)<sub>2</sub>C<sub>4</sub>F<sub>9</sub> (nonaflyl), -S(=O)<sub>2</sub>CH<sub>2</sub>CF<sub>3</sub> (tresyl), -S(=O)<sub>2</sub>Ph (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<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group. Examples of sulfine groups include, but are not limited to, -S(=O)CH<sub>3</sub> and -S(=O)CH<sub>2</sub>CH<sub>3</sub>.

35 Sulfonyloxy: -OS(=O)<sub>2</sub>R, wherein R is a sulfonyloxy substituent, for example, a C<sub>1-7</sub> alkyl group, a C<sub>3-20</sub> heterocyclyl group, or a C<sub>5-20</sub> aryl group, preferably a C<sub>1-7</sub> alkyl group.

- 21 -

Examples of sulfonyloxy groups include, but are not limited to,  $-\text{OS}(=\text{O})_2\text{CH}_3$  and  $-\text{OS}(=\text{O})_2\text{CH}_2\text{CH}_3$ .

5 Sulfonyloxy:  $-\text{OS}(=\text{O})\text{R}$ , wherein R is a sulfonyloxy substituent, for example, a  $\text{C}_{1-7}$  alkyl group, a  $\text{C}_{3-20}$  heterocyclyl group, or a  $\text{C}_{5-20}$  aryl group, preferably a  $\text{C}_{1-7}$  alkyl group. Examples of sulfonyloxy groups include, but are not limited to,  $-\text{OS}(=\text{O})\text{CH}_3$  and  $-\text{OS}(=\text{O})\text{CH}_2\text{CH}_3$ .

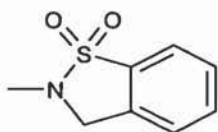
10 Sulfamino:  $-\text{NR}^{\text{N}1}\text{S}(=\text{O})_2\text{OH}$ , wherein  $\text{R}^1$  is an amino substituent, as defined for amino groups. Examples of sulfamino groups include, but are not limited to,  $-\text{NHS}(=\text{O})_2\text{OH}$  and  $-\text{N}(\text{CH}_3)\text{S}(=\text{O})_2\text{OH}$ .

15 Sulfinamino:  $-\text{NR}^{\text{N}1}\text{S}(=\text{O})\text{R}$ , wherein  $\text{R}^{\text{N}1}$  is an amino substituent, as defined for amino groups, and R is a sulfinamino substituent, for example, a  $\text{C}_{1-7}$  alkyl group, a  $\text{C}_{3-20}$  heterocyclyl group, or a  $\text{C}_{5-20}$  aryl group, preferably a  $\text{C}_{1-7}$  alkyl group. Examples of sulfinamino groups include, but are not limited to,  $-\text{NHS}(=\text{O})\text{CH}_3$  and  $-\text{N}(\text{CH}_3)\text{S}(=\text{O})\text{C}_6\text{H}_5$ .

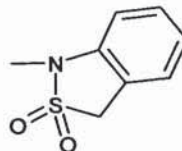
20 Sulfamyl:  $-\text{S}(=\text{O})\text{NR}^{\text{N}1}\text{R}^{\text{N}2}$ , wherein  $\text{R}^{\text{N}1}$  and  $\text{R}^{\text{N}2}$  are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to,  $-\text{S}(=\text{O})\text{NH}_2$ ,  $-\text{S}(=\text{O})\text{NH}(\text{CH}_3)$ ,  $-\text{S}(=\text{O})\text{N}(\text{CH}_3)_2$ ,  $-\text{S}(=\text{O})\text{NH}(\text{CH}_2\text{CH}_3)$ ,  $-\text{S}(=\text{O})\text{N}(\text{CH}_2\text{CH}_3)_2$ , and  $-\text{S}(=\text{O})\text{NHPH}$ .

25 Sulfonamino:  $-\text{NR}^{\text{N}1}\text{S}(=\text{O})_2\text{R}$ , wherein  $\text{R}^{\text{N}1}$  is an amino substituent, as defined for amino groups, and R is a sulfonamino substituent, for example, a  $\text{C}_{1-7}$  alkyl group, a  $\text{C}_{3-20}$  heterocyclyl group, or a  $\text{C}_{5-20}$  aryl group, preferably a  $\text{C}_{1-7}$  alkyl group. Examples of sulfonamino groups include, but are not limited to,  $-\text{NHS}(=\text{O})_2\text{CH}_3$  and  $-\text{N}(\text{CH}_3)\text{S}(=\text{O})_2\text{C}_6\text{H}_5$ . A special class of sulfonamino groups are those derived from sultams – in these groups one of  $\text{R}^1$  and R is a  $\text{C}_{5-20}$  aryl group, preferably phenyl, whilst the other of  $\text{R}^1$  and R is a bidentate group which links to the  $\text{C}_{5-20}$  aryl group, such as a bidentate group derived from a  $\text{C}_{1-7}$  alkyl group. Examples of such groups include, but are not limited to:

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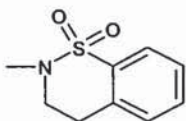
2,3-dihydro-tenzo[d]isothiazole-1,1-dioxide-2-yl



1,3-dihydro-benzo[c]isothiazole-2,2-dioxide-1-yl



- 22 -



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*

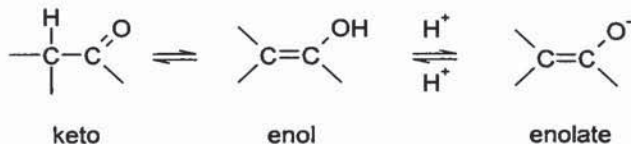
Certain compounds may exist in one or more particular geometric, optical, enantiomeric, diastereomeric, epimeric, stereoisomeric, tautomeric, conformational, or anomeric forms, including but not limited to, cis- and trans-forms; E- and Z-forms; c-, t-, and r-forms; endo- and exo-forms; R-, S-, and meso-forms; D- and L-forms; d- and l-forms; (+) and (-) forms; keto-, enol-, and enolate-forms; syn- and anti-forms; synclinal- and anticlinal-forms;  $\alpha$ - and  $\beta$ -forms; axial and equatorial forms; boat-, chair-, twist-, envelope-, and

- 23 -

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 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<sub>3</sub>, is not to be construed as a reference to its structural isomer, a hydroxymethyl group, -CH<sub>2</sub>OH. Similarly, a reference to ortho-chlorophenyl is not to be construed as a reference to its structural isomer, meta-chlorophenyl. However, a reference to a class of structures may well include structurally isomeric forms falling within that class (e.g., C<sub>1-7</sub> alkyl includes n-propyl and iso-propyl; butyl includes n-, iso-, sec-, and tert-butyl; methoxyphenyl includes ortho-, meta-, and para-methoxyphenyl).

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/amidone, nitroso/oxime, thioketone/enethiol, N-nitroso/hydroxyazo, and nitro/aci-nitro.



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 <sup>1</sup>H, <sup>2</sup>H (D), and <sup>3</sup>H (T); C may be in any isotopic form, including <sup>12</sup>C, <sup>13</sup>C, and <sup>14</sup>C; O may be in any isotopic form, including <sup>16</sup>O and <sup>18</sup>O; and the like.

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, 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.

- 24 -

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<sup>-</sup>), 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<sup>+</sup> and K<sup>+</sup>, alkaline earth cations such as Ca<sup>2+</sup> and Mg<sup>2+</sup>, and other cations such as Al<sup>3+</sup>. Examples of suitable organic cations include, but are not limited to, ammonium ion (i.e., NH<sub>4</sub><sup>+</sup>) and substituted ammonium ions (e.g., NH<sub>3</sub>R<sup>+</sup>, NH<sub>2</sub>R<sub>2</sub><sup>+</sup>, NHR<sub>3</sub><sup>+</sup>, NR<sub>4</sub><sup>+</sup>).

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<sub>3</sub>)<sub>4</sub><sup>+</sup>.

If the compound is cationic, or has a functional group which may be cationic (e.g., -NH<sub>2</sub> may be -NH<sub>3</sub><sup>+</sup>), 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, pantoic, tartaric, citric, gluconic, ascorbic, maleic, hydroxymaleic, phenylacetic, glutamic, aspartic, benzoic, cinnamic, pyruvic, salicylic, sulfanilic, 2-acetoxybenzoic, 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.

- 25 -

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<sub>3</sub>, -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)<sub>2</sub>), 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<sub>3</sub>); a benzyloxy amide (-NHCO-OCH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>, -NH-Cbz); as a t-butoxy amide (-NHCO-OC(CH<sub>3</sub>)<sub>3</sub>, -NH-Boc); a 2-biphenyl-2-propoxy amide (-NHCO-OC(CH<sub>3</sub>)<sub>2</sub>C<sub>6</sub>H<sub>4</sub>C<sub>6</sub>H<sub>5</sub>, -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<sup>+</sup>).

For example, a carboxylic acid group may be protected as an ester for example, as: an C<sub>1-7</sub> alkyl ester (e.g. a methyl ester; a t-butyl ester); a C<sub>1-7</sub> haloalkyl ester (e.g., a C<sub>1-7</sub> trihaloalkyl ester); a triC<sub>1-7</sub> alkylsilyl-C<sub>1-7</sub> alkyl ester; or a C<sub>5-20</sub> aryl-C<sub>1-7</sub> alkyl ester (e.g. a benzyl ester; a nitrobenzyl ester); or as an amide, for example, as a methyl amide.

- 26 -

For example, a thiol group may be protected as a thioether (-SR), for example, as: a benzyl thioether; an acetamidomethyl ether (-S-CH<sub>2</sub>NHC(=O)CH<sub>3</sub>).

### *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.

20

Preferably it comprises at least one aryl substituent, which is preferably at R<sup>4</sup>, such as is defined by any of formula IVb.

25

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.

30

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).

35

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 construed to include a compound which is capable of inhibiting infections, i.e. disease-causing capacity of microbes.

- 27 -

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  
30 daily dosage may be between 250 µg to about 25 g, preferably up to around 5g, more 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  
35 to a tablet, a capsule, a solution, a suspension, a powder, a paste, an elixir, and a syrup. 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

- 28 -

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 acceptable excipient.

15

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*

25

The gastrointestinal tract (GI tract) of mammals is covered by a continuous sheet of 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 intestinal crypts, secrete alpha-defensins into the cryptal well, resulting in concentrations estimated at mg/mL levels, which eventually flush into the gut lumen.

30

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-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

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- 29 -

cryptdins (the murine Paneth cell alpha-defensins) lack functional cryptdins and exhibit increased susceptibility to orally administered Salmonella.

5 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.

10 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.

15

The epithelial cells targeted by the present invention may be any of these. Preferably however the invention is utilised for the treatment of microbial infections of the GI tract.

#### *Microbial infections and diseases*

20

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.

25

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.

30

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  
35 bowel syndrome, dumping syndrome, gluten enteropathy, or food intolerance.



- 30 -

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

- 31 -

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*

15 In the methods and compositions of the present invention, the active compound is 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<sup>2</sup>/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 ICDDR 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.

- 32 -

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.

15

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.

30

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.

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Compositions for oral use may be in the form of hard gelatin capsules in which the active

- 33 -

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  
20 modify their disintegration and the subsequent absorption of the active ingredient within 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*

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- 34 -

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  
5 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  
10 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*

15 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  
20 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  
25 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.  
30

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**

  
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- 35 -

- 5 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<sub>3</sub> or 1,25(OH)<sub>2</sub>D<sub>3</sub>) 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.
- 10 Figure 2: Induction of *CAMP* mRNA expression by butyrate (BA) and PBA derivatives. A) Structures of utilized chemicals butyrate (BA) 4 mM, 4-phenyl butyrate (PBA) 4 mM,  $\alpha$ -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.
- 15 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
- 20 at least three independent experiments.
- 25 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.
- 30 Figure 5: Further demonstrations of synergetic induction of *CAMP* mRNA and pro-LL-37 expression by PBA (4 mM) and 1,25(OH)<sub>2</sub>D<sub>3</sub>. (20 nM) A) VA10 cells were stimulated with PBA (4 mM), 1,25(OH)<sub>2</sub>D<sub>3</sub> (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
- 35 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

- 36 -

cells were stimulated with PBA (4 mM), 1,25(OH)<sub>2</sub>D<sub>3</sub> (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 tributylglycerol.

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  
30 normalized to expression in control samples (solvent) where controls were given the 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

- 37 -

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 Shigella infected epithelia.  
20

## 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<sub>3</sub> or 1,25(OH)<sub>2</sub>D<sub>3</sub>) (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



- 38 -

5 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 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.

10 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  $\alpha$ -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.

#### 20 *Real time PCR*

25 Six-well plates were seeded with  $1.0 \times 10^6$  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 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 -TGTTATCCTTATCACAACCTGAT-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

5 Figure 3 shows the dose-response of *CAMP* mRNA expression in VA10 lung epithelial 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 was dependent on PBA dose and increased over time.

10 In earlier experiments it appeared 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 hours. Total RNA was isolated from the cells and *CAMP* mRNA expression levels analyzed by real time RT-PCR. *CAMP* mRNA expression was significantly 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

- 40 -

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  $1,25(\text{OH})_2\text{D}_3$  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  $1,25(\text{OH})_2\text{D}_3$ , 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  $\mu\text{M}$ ) 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.

- 41 -

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  $\mu$ M SP600125, SB203580 or U0126 to inhibit the  
10 respective kinases. After 24 hours of incubation, total RNA was isolated and analyzed by 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

15

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 tributyrilglycerol, 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-

- 42 -

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  $1 \times 10^9$  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 (Gonosshasthoya 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 inoculum was considered as 0 hr. After development of dysenteric symptoms, rabbits were given glyceryl tributyrates (47  $\mu$ mol/kg body weight, i.e., 140  $\mu$ mol 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  $\mu$ g/ml) at room temperature. After washing, sections were incubated with horse-radish-peroxidase conjugated donkey anti-chicken antibody (1:200; Jackson ImmunoResearch

- 43 -

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)

- 44 -

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 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 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.

#### **Example 9**

##### The effect of PBA on vitamin D co-activator expression

Hypothesizing that the synergistic effect between PBA and  $1,25(\text{OH})_2\text{D}_3$  was due to an induction of VDR co-activator genes by PBA, we analyzed the effect of PBA 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.

#### **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 suggests that PBA has a general effect on mucosal defenses.

#### **Example 11**

##### Synthesis of glyceryl tributyrates

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

- 45 -

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<sub>3</sub> (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<sub>3</sub> (400 ml) and then with HCl (1M in water, 400 ml). The organic layer was collected and dried with Na<sub>2</sub>SO<sub>4</sub> and then concentrated *in vacuo* to afford 29.6 g (98 %) of glyceryl tributyrate <sup>1</sup>H NMR (CDCl<sub>3</sub>), 0.95 (t; *J*=7.4 Hz; 2 X CH<sub>3</sub>), 0.96 (t; *J*=7.4 Hz; CH<sub>3</sub>), 1.60-1.73 (m; 3 X CH<sub>2</sub>), 2.31 (t; *J*=7.4 Hz; 2 X CH<sub>2</sub>), 2.32 (t; *J*=7.35 Hz; CH<sub>2</sub>), 4.16 (dd + AB; *J*=11.9, 6.0 Hz; 2 X CH<sub>a</sub>), 4.31 (dd + AB; *J*=11.9, 4.3 Hz; 2 X CH<sub>b</sub>), 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<sub>3</sub> (*aq.*, 4.2 %, 200 ml) and the water layer extracted once more with DCM (50 ml). The combined organic extracts was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* yielding 16.3 g (94 %) of N-butanoylglycine ethyl ester. <sup>1</sup>H NMR (CDCl<sub>3</sub>), 0.97 (t; *J*=7.4 Hz; CH<sub>3</sub>), 1.30 (t; *J*=7.1 Hz; CH<sub>3</sub>), 1.65-1.74 (m; CH<sub>2</sub>), 2.23 (t; *J*=7.5 Hz; CH<sub>2</sub>), 4.05 (d; 4.9 Hz; CH<sub>2</sub>), 4.23 (q; 7.2 Hz; CH<sub>3</sub>), 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,



- 46 -

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<sub>2</sub>SO<sub>4</sub> and evaporated *in vacuo* yielding 13 g (95%) of N-butanoylglycine. <sup>1</sup>H NMR (CDCl<sub>3</sub>), 0.97 (t; J=7.4 Hz; CH<sub>3</sub>), 1.64-1.74 (m; CH<sub>2</sub>), 2.27 (t; J=7.5 Hz; CH<sub>2</sub>), 4.09 (d; J=5.1 Hz; CH<sub>2</sub>), 6.24 (broad; NH), 8.1 (broad; COOH).

#### Example 14

##### Synthesis of N<sup>α</sup>,N<sup>ε</sup>-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) 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 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. <sup>1</sup>H NMR (CDCl<sub>3</sub>), 0.92-0.98 (m, 6H; 2xCH<sub>3</sub>), 1.3-1.48 (m, 2H; CH<sub>2</sub>), 1.54 (qv, 2H, J=6.8 Hz; CH<sub>2</sub>), 1.62-1.70 (m, 4H; 2xCH<sub>2</sub>), 1.75-1.83 (m, 2H; CH<sub>2</sub>), 1.85-1.95 (m, 2H; CH<sub>2</sub>), 2.18 (t, 2H, J=7.3 Hz; CH<sub>2</sub>), 2.25 (t, 2H, J=6.2 Hz; CH<sub>2</sub>), 3.17-3.22 (m, 1H; ε-CH<sub>2a</sub>), 3.31-3.37 (m, 1H; ε-CH<sub>2b</sub>), 4.52-4.58 (m, 1H; αCH), 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*

- 47 -

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)

- 48 -

*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 immediately after defecation.

15

*Case Management*

After enrolment, the patients are admitted in the study ward of ICDDRDB Dhaka and Matlab hospital. A standard clinical history and clinical examination is performed by the 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 relapse cases.

20  
25*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  $3.2 \pm 1.8$  (mean  $\pm$ 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 size in each group will be 61.

35

- 49 -

*Clinical Parameters measured / recorded*

1. Appetite
2. Abdominal cramps
3. Rectal tenesmus
- 5 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)
- 10 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
- 15 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

20

*Data analysis*

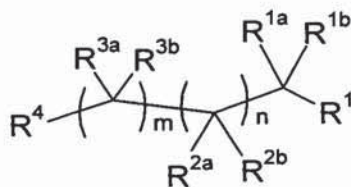
For normally distributed data, it is intended to use appropriate parametric tests (eg. t test) to compare the results between groups. In case the data is skewed, nonparametric tests will be used. Statistical analysis can then be done using two-factor ANOVA to determine significant interactions between 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.

25

30

## 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<sup>1</sup> represents a carboxyl group, phosphate, phosphonate or sulfonate group or pharmaceutically acceptable salt thereof, COOR<sup>5</sup>, CONH<sub>2</sub>, CONR<sup>5</sup>R<sup>6</sup>, or an aldehyde, imine or acetal protected derivative of said compounds, or a triglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>(OOCR<sup>6</sup>) or diglyceride moiety COOCH<sub>2</sub>CH(OOCR<sup>5</sup>)CH<sub>2</sub>OH, or an amino acid group CONHCR<sup>7</sup>COOH or a salt thereof;

*m* and *n* are each independently 0 or 1;

R<sup>1a</sup>, R<sup>1b</sup>, R<sup>2a</sup>, R<sup>2b</sup>, R<sup>3a</sup> and R<sup>3b</sup> 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<sup>2a</sup>, together with an adjacent R<sup>3a</sup> or R<sup>1a</sup>, may represent a carbon-carbon π bond;

and/or

R<sup>2b</sup>, together with an adjacent R<sup>3b</sup> or R<sup>1b</sup>, may represent a carbon-carbon π bond;

R<sup>4</sup> 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;