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UTILITY PATENT APPLICATION TRANSMITTAL <small>(ONLY FOR NEW NONPROVISIONAL APPLICATIONS UNDER 37 CFR 1.53(B))</small>	Attorney Docket No.	40737-509001US
	First Inventor	Stephen Comiskey
	Title	Formulations of Guanylate Cyclase C Agonists and Methods of Use
	Express Mail Label No.	

APPLICATION ELEMENTS <small>See MPEP chapter 600 concerning utility patent application contents.</small>	Commissioner for Patents ADDRESS TO: P.O. Box 1450 Alexandria, VA 22313-1450
ACCOMPANYING APPLICATION PARTS	
1. <input type="checkbox"/> Fee Transmittal Form (e.g., PTO/SB/17) 2. <input type="checkbox"/> Applicant claims small entity status. <small>See 37 CFR 1.27.</small> 3. <input checked="" type="checkbox"/> Specification [Total Pages <u>152</u>] <small>Both the claims and abstract must start on a new page (For information on the preferred arrangement, see MPEP 608.01(a))</small> 4. <input checked="" type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets <u>6</u>] 5. Oath or Declaration [Total Sheets _____] a. <input type="checkbox"/> Newly executed (original or copy) b. <input type="checkbox"/> A copy from a prior application (37 CFR 1.63(d)) <small>(for continuation/divisional with Box 18 completed)</small> i. <input type="checkbox"/> DELETION OF INVENTOR(S) <small>Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</small> 6. <input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76 7. <input type="checkbox"/> CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) <input type="checkbox"/> Landscape Table on CD 8. Nucleotide and/or Amino Acid Sequence Submission <small>(if applicable, items a. – c. are required)</small> a. <input type="checkbox"/> Computer Readable Form (CRF) b. Specification Sequence Listing on: i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or ii. <input type="checkbox"/> Paper c. <input type="checkbox"/> Statements verifying identity of above copies	9. <input type="checkbox"/> Assignment Papers (cover sheet & document(s)) Name of Assignee <div style="border: 1px solid black; height: 20px; width: 100%;"></div> 10. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney <small>(when there is an assignee)</small> 11. <input type="checkbox"/> English Translation Document (if applicable) 12. <input type="checkbox"/> Information Disclosure Statement (PTO/SB/08 or PTO-1449) <input type="checkbox"/> Copies of citations attached 13. <input type="checkbox"/> Preliminary Amendment 14. <input type="checkbox"/> Return Receipt Postcard (MPEP 503) <small>(Should be specifically itemized)</small> 15. <input type="checkbox"/> Certified Copy of Priority Document(s) <small>(if foreign priority is claimed)</small> 16. <input type="checkbox"/> Nonpublication Request under 35 U.S.C.122 (b)(2)(B)(i). <small>Applicant must attach form PTO/SB/35 or equivalent.</small> 17. <input type="checkbox"/> Other: <div style="border: 1px solid black; height: 40px; width: 100%;"></div>
18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76: <input type="checkbox"/> Continuation <input type="checkbox"/> Divisional <input checked="" type="checkbox"/> Continuation-in-part (CIP) of prior application No.: <u>PCT/US2011/051805</u> <small>Prior application information: Examiner <u>Not Yet Assigned</u> Art Unit: <u>N/A</u></small>	
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1		Application.pdf	787899 760dd9aabf278e09e3d827a8e801b7cee9b c6c9e	yes	152

Multipart Description/PDF files in .zip description			
	Document Description	Start	End
	Specification	1	146
	Claims	147	151
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2	Drawings-only black and white line drawings	509001US_Drawings.pdf	203262 6a042335bf71d5516839e4c047dc087b47e e24cd	no	6
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3	Application Data Sheet	ADS.pdf	20179 756610bef3b218d0d1e3c3d2509b5a98a24 14159	no	5
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FORMULATIONS OF GUANYLATE CYCLASE C AGONISTS AND METHODS OF USE

RELATED APPLICATIONS

[01] This application is a continuation-in-part of PCT/US2011/051805 filed on September 15, 2011, which claims the benefit of priority to U.S. Provisional Application No. 61/383,156 filed on September 15, 2010, U.S. Provisional Application No. 61/387,636 filed on September 29, 2010, and U.S. Provisional Application No. 61/392,186 filed on October 12, 2010, the contents of which are incorporated by reference in their entireties.

FIELD OF THE INVENTION

[02] The present invention relates to low-dose formulations of guanylate cyclase C peptide agonists useful for the treatment and prevention of various diseases and disorders.

BACKGROUND OF THE INVENTION

[03] Guanylate cyclase C is a transmembrane form of guanylate cyclase that is expressed on various cells, including gastrointestinal epithelial cells (reviewed in Vaandrager 2002 *Mol. Cell. Biochem.* 230:73-83). It was originally discovered as the intestinal receptor for the heat-stable toxin (ST) peptides secreted by enteric bacteria and which cause diarrhea. The ST peptides share a similar primary amino acid structure with two peptides isolated from intestinal mucosa and urine, guanylin and uroguanylin (Currie, *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:947-951 (1992); Hamra, *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:10464-10468 (1993); Forte, L., *Reg. Pept.* 81:25-39 (1999); Schulz, *et al.*, *Cell* 63:941-948 (1990); Guba, *et al.*, *Gastroenterology* 111:1558-1568 (1996); Joo, *et al.*, *Am. J. Physiol.* 274:G633-G644 (1998)).

[04] In the intestines, guanylin and uroguanylin act as regulators of fluid and electrolyte balance. In response to high oral salt intake, these peptides are released into the intestinal lumen where they bind to guanylate cyclase C localized on the luminal membrane of enterocytes (simple columnar epithelial cells of the small intestines and colon). The binding of the guanylin peptides to guanylate cyclase C induces electrolyte and water excretion into

the intestinal lumen via a complex intracellular signaling cascade that is initiated by an increase in cyclic guanosine monophosphate (cGMP).

[05] The cGMP-mediated signaling that is initiated by the guanylin peptides is critical for the normal functioning of the gut. Any abnormality in this process could lead to gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel diseases. Inflammatory bowel disease is a general name given to a group of disorders that cause the intestines to become inflamed, characterized by red and swollen tissue. Examples include ulcerative colitis and Crohn's disease. Crohn's disease is a serious inflammatory disease that predominantly affects the ileum and colon, but can also occur in other sections of the gastrointestinal tract. Ulcerative colitis is exclusively an inflammatory disease of the colon, the large intestine. Unlike Crohn's disease, in which all layers of the intestine are involved, and in which there can be normal healthy bowel in between patches of diseased bowel, ulcerative colitis affects only the innermost lining (mucosa) of the colon in a continuous manner. Depending on which portion of the gastrointestinal tract is involved, Crohn's disease may be referred to as ileitis, regional enteritis, colitis, etc. Crohn's disease and ulcerative colitis differ from spastic colon or irritable bowel syndrome, which are motility disorders of the gastrointestinal tract. Gastrointestinal inflammation can be a chronic condition. It is estimated that as many as 1,000,000 Americans are afflicted with inflammatory bowel disease, with male and female patients appearing to be equally affected. Most cases are diagnosed before age 30, but the disease can occur in the sixth, seventh, and later decades of life.

[06] IBS and chronic idiopathic constipation are pathological conditions that can cause a great deal of intestinal discomfort and distress but unlike the inflammatory bowel diseases, IBS does not cause the serious inflammation or changes in bowel tissue and it is not thought to increase the risk of colorectal cancer. In the past, inflammatory bowel disease, celiac disease and IBS were regarded as completely separate disorders. Now, with the description of inflammation, albeit low-grade, in IBS, and of symptom overlap between IBS and celiac disease, this contention has come under question. Acute bacterial gastroenteritis is the strongest risk factor identified to date for the subsequent development of postinfective irritable bowel syndrome. Clinical risk factors include prolonged acute illness and the absence of vomiting. A genetically determined susceptibility to inflammatory stimuli may also be a risk factor for irritable bowel syndrome. The underlying pathophysiology indicates

increased intestinal permeability and low-grade inflammation, as well as altered motility and visceral sensitivity. Serotonin (5-hydroxytryptamine [5-HT]) is a key modulator of gut function and is known to play a major role in pathophysiology of IBS. The activity of 5-HT is regulated by cGMP.

[07] While the precise causes of IBS and inflammatory bowel diseases (IBD) are not known, a disruption in the process of continual renewal of the gastrointestinal mucosa may contribute to disease pathology in IBD and aggravate IBS. The renewal process of the gastrointestinal lining is an efficient and dynamic process involving the continual proliferation and replenishment of unwanted damaged cells. Proliferation rates of cells lining the gastrointestinal mucosa are very high, second only to the hematopoietic system. Gastrointestinal homeostasis depends on both the proliferation and programmed cellular death (apoptosis) of epithelial cells lining the gut mucosa. Cells are continually lost from the villus into the lumen of the gut and are replenished at a substantially equal rate by the proliferation of cells in the crypts, followed by their upward movement to the villus. The rates of cell proliferation and apoptosis in the gut epithelium can be increased or decreased in a variety of circumstances, *e.g.*, in response to physiological stimuli such as aging, inflammatory signals, hormones, peptides, growth factors, chemicals and dietary habits. In addition, an enhanced proliferation rate is frequently associated with a reduction in turnover time and an expansion of the proliferative zone. The proliferation index is much higher in pathological states such as ulcerative colitis and other gastrointestinal disorders. Intestinal hyperplasia is a major promoter of gastrointestinal inflammation. Apoptosis and cell proliferation together regulate cell number and determine the proliferation index. Reduced rates of apoptosis are often associated with abnormal growth, inflammation, and neoplastic transformation. Thus, both increased proliferation and/or reduced cell death may increase the proliferation index of intestinal tissue, which may in turn lead to gastrointestinal inflammatory diseases.

[08] In addition to a role for uroguanylin and guanylin as modulators of intestinal fluid and ion secretion, these peptides may also be involved in the continual renewal of gastrointestinal mucosa by maintaining the balance between proliferation and apoptosis. For example, uroguanylin and guanylin peptides appear to promote apoptosis by controlling cellular ion flux. Given the prevalence of inflammatory conditions in Western societies a need exists to

improve the treatment options for inflammatory conditions, particularly of the gastrointestinal tract.

[09] Peptide agonists of guanylate cyclase C agonists (“GCC agonists”) are described in U.S. Patent Nos. 7,041,786, 7,799,897, and U.S. Patent Application Publication Nos. US2009/0048175, US 2010/0069306, US 2010/0120694, US 2010/0093635, and US 2010/0221329. However, the formulation of peptides for pharmaceutical delivery presents a number of special problems. For example, peptides are subject to structural modifications by a variety of degradation mechanisms resulting in problems of chemical and physical instability of the formulation.

SUMMARY OF THE INVENTION

[10] The present invention provides low-dose formulations of peptide agonists of guanylate cyclase C (“GCC”) and methods for their use in the treatment and prevention of human diseases and disorders, such as a gastrointestinal motility disorder, irritable bowel syndrome, a functional gastrointestinal disorder, gastroesophageal reflux disease, functional heartburn, dyspepsia, functional dyspepsia, nonulcer dyspepsia, gastroparesis, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction; Crohn's disease, ulcerative colitis, inflammatory bowel disease, colonic pseudo-obstruction, obesity, congestive heart failure, and benign prostatic hyperplasia. In certain embodiments, the formulations are stabilized against chemical degradation of the peptide. The low-dose formulations of the invention have unexpected efficacy in humans in a dosage range that was not predicted based on studies in primates. The formulations of the invention are particularly useful for the treatment or prevention of chronic idiopathic constipation. In certain embodiments, the GCC agonists are analogs of uroguanylin and bacterial ST peptides. In preferred embodiments, the analogs have superior properties compared to the naturally occurring or “wild-type” peptides. Examples of such superior properties include a high resistance to degradation at the N-terminus and C-terminus from carboxypeptidases, aminopeptidases, and/or by other proteolytic enzymes present in the stimulated human intestinal juices and human gastric juices. Examples of GCC agonists that can be used in the formulations and methods of the invention are described in more detail below and in U.S. Patent Nos. 7,041,786, 7,799,897, and U.S. Patent Application Publication Nos. US2009/0048175, US 2010/0069306, US

2010/0120694, US 2010/0093635, and US 2010/0221329, each of which is incorporated herein by reference in its entirety.

[11] The invention provides an oral dosage formulation comprising one or more pharmaceutically acceptable excipients and at least one GCC agonist peptide, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, and wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1-54 and 56-249. In one embodiment, the GCC agonist peptide has a chromatographic purity of no less than 90%, no less than 90.5%, no less than 91%, no less than 92%, no less than 93%, no less than 94%, no less than 95%, no less than 96%, no less than 97%, no less than 98%, or no less than 99%. The chromatographic purity of the GCC agonist peptide is determined as area percent by HPLC. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1 and 9. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 8 and 9. In one embodiment, the amount of GCC agonist peptide per unit dose is 0.1 mg, 0.3 mg, 0.6 mg, 1.0 mg, 3.0 mg, 6.0 mg, 9.0 mg or 9.5 mg.

[12] In one embodiment, the GCC agonist peptide has a total impurity content of no greater than 10%, no greater than 9.5%, no greater than 9%, no greater than 8%, no greater than 7%, no greater than 6%, no greater than 5%, no greater than 4%, no greater than 3%, no greater than 2%, or no greater than 1%. The total impurity content is determined as total area percentages of impurities by HPLC. The impurities do not include any pharmaceutically acceptable excipient used for the formulation. In one embodiment, the formulation is substantially free of inorganic acids and carboxylic acids, e.g., HCl, phosphoric acid, or acetic acid. In this context, carboxylic acids do not include amino acids or peptides. In this context “substantially” free of acids means that the acid content of the formulation at the time of packaging is preferably less than 0.2%, less than 0.1%, less than 0.05%, less than 0.01%, less than 0.005%, or less than 0.001% of the total weight of the formulation. In one embodiment, the formulation is free of HCl.

[13] In one embodiment, the formulation is a solid formulation. In one embodiment, the formulation is in the form of a powder, granule, sachet, troche, tablet, or capsule. In another embodiment, the formulation is a liquid formulation and the GCC agonist peptide is in

solution or suspension in a lipophilic liquid. In one embodiment, the liquid is a refined specialty oil or a medium chain triglyceride or related ester. In one embodiment, the refined specialty oil is selected from Arachis oil, Castor oil, cottonseed oil, maize (corn) oil, olive oil, sesame oil, soybean oil, and sunflower oil. In one embodiment, the medium chain triglyceride or related ester is AKOMED E, AKOMED R, CAPTEX 355, LABRAFAC CC, LABRAFAC PG, LAUROGLYCOL FCC, MIGLYOL 810, MIGLYOL 812, MIGLYOL 829, MIGLYOL 840, and SOFTISAN 645. In one embodiment, the liquid is selected from the group consisting of medium chain triglycerides, propylene glycol dicaprylocaprate, vitamin E, soybean oil, Cremaphor, PG, and PG 400. In one embodiment, the unit dose is a powder, tablet, or capsule. In one embodiment, the unit dose is a liquid-filled capsule. In one embodiment, the capsule or tablet is in a blister pack or strip. Preferably, the blister pack or strip is made of a material that is impermeable to water vapor and oxygen. In one embodiment the blister pack is comprised of a metal foil. In one embodiment the blister pack is a FOIL/FOIL blister pack. In one embodiment, the container of the blister pack is flushed with an inert gas such as nitrogen or argon. In one embodiment, the container further includes a desiccant. In a preferred embodiment the desiccant is a molecular sieve. In one embodiment, the unit dose is in a high density polyethylene bottle having a seal. In one embodiment, the bottle further comprises a desiccant. In one embodiment, the bottle further comprises an oxygen scavenger or molecular sieve. In one embodiment, the bottle is nearly impermeable to oxygen and water vapor (e.g., much more impermeable than a HDPE bottle), such as an OxyGuard bottle.

[14] In one embodiment, the one or more pharmaceutically acceptable excipients include an inert carrier. In one embodiment, the inert carrier is selected from mannitol, lactose, a microcrystalline cellulose, or starch. In one embodiment, the inert carrier has a particle size of from 50 to 900 microns, from 50 to 800 microns, from 50 to 300 microns, from 50 to 200 microns, from 75 to 150 microns, from 75 to 200 microns, or from 75 to 300 microns.

[15] In one embodiment, the GCC agonist peptide is stabilized against chemical or physical degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.

[16] In one embodiment, the one or more pharmaceutically acceptable excipients include a divalent cation salt such as calcium chloride. In one embodiment, the one or more

pharmaceutically acceptable excipients comprise an amino acid, such as leucine, histidine, or arginine, or an amine such TRIS or TRIS/HCl.

[17] In one embodiment, the oral dosage formulation consists of the GCC agonist peptide described herein, an inert carrier (e.g., Celphere SCP-100, Avicel PH 102, or Avicel PH 112), and a lubricant (e.g., magnesium stearate). In one embodiment, the formulation consists of the GCC agonist peptide, an inert carrier (e.g., Avicel PH 200), a divalent cation salt (e.g., calcium chloride or calcium ascorbate), an amino acid (e.g., leucine, histidine, or arginine) or a protective amine (e.g., TRIS), a coating agent (e.g., Methocel ES Premium LV) and optionally a lubricant (e.g., magnesium stearate) or another additive (e.g., trehalose). In one embodiment, the formulation consists of the GCC agonist peptide, a binder (e.g., Provsolv SMCC 90 LM), and a disintegrant (e.g., Explotab). In one embodiment, the formulation consists of the GCC agonist peptide, a diluent (e.g., Mannogem EZ), a binder (e.g., Provsolv SMCC 90 LM), a disintegrant (e.g., Explotab), a lubricant (e.g., Pruv).

[18] The invention also provides a process for making the oral dosage formulations described herein, wherein the process comprises a step of dry granulation, wet granulation, or spray coating followed by drying. In another embodiment, the process comprises a step of dry mixing. In a preferred embodiment the step of dry mixing includes geometric blending. In one embodiment, the process comprises a step of direct compression. In one embodiment, the process for making the oral dosage formulations described herein is a spray coating-drying process which includes (a) providing an aqueous solution comprising: a GCC agonist peptide selected from the group consisting of SEQ ID NOs: 1-54 and 56-249, and one or more pharmaceutically acceptable excipients, wherein the concentration of the GCC agonist peptide ranges from 10 to 60 mg/mL; and (b) applying the aqueous solution to a pharmaceutically acceptable carrier to generate a GCC agonist peptide-coated carrier.

[19] In one embodiment of the spray coating-drying process above, the one or more pharmaceutically acceptable excipients comprise a divalent cation salt wherein the divalent cation is selected from Ca^{2+} , Mg^{2+} , Zn^{2+} , and Mn^{2+} . In one embodiment, the one or more pharmaceutically acceptable excipients comprise an amino acid selected from leucine, isoleucine, and valine. In one embodiment, the one or more pharmaceutically acceptable excipients comprise a coating agent (such as hypromellose Methocel E5 PremLV). In one embodiment, the aqueous solution has a pH greater than 4 (e.g., 4.5-5.5, 5-6, about 5, or

greater than 5) or even greater than 7. In one embodiment, the aqueous solution is substantially free of inorganic acids and carboxylic acids. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, and 56. In one embodiment, the process further includes drying the GCC agonist peptide-coated carrier.

[20] The invention further provides an oral dosage formulation made by the process described herein. Preferably, the GCC agonist peptide as made is stabilized against chemical or physical degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.

[21] The invention also provides a method for treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject an oral dosage formulation comprising at least one GCC agonist peptide, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, and wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1-54 and 56-249. Preferably, the subject is a human subject. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56. In one embodiment, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1 and 9. In one embodiment, the amount of GCC agonist peptide per unit dose is 0.1 mg, 0.3 mg, 0.6 mg, 1.0 mg, 3.0 mg, 6.0 mg, 9.0 mg, 9.5 mg, or 10 mg.

[22] In one embodiment, the disease or disorder is a gastrointestinal disease or disorder selected from the group consisting of irritable bowel syndrome, non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastro esophageal reflux disease, constipation, gastroparesis, heartburn, gastric cancer, and *H. pylori* infection. In a preferred embodiment, the gastrointestinal disease or disorder is chronic idiopathic constipation.

[23] In one embodiment, the method further comprises administering to the subject an effective amount of an inhibitor of a cGMP-specific phosphodiesterase. In one embodiment, the cGMP-dependent phosphodiesterase inhibitor is selected from the group consisting of sildinac sulfone, zaprinast, and motapizone, vardenafil, and sildenafil.

[24] In one embodiment, the method further comprises administering to the subject an effective amount of at least one laxative. In one embodiment, the at least one laxative is selected from the group consisting of SENNA, MIRALAX, PEG, or calcium polycarbophil.

[25] In one embodiment, the method further comprises administering to the subject an effective amount of at least one anti-inflammatory agent.

[26] The invention also provides pharmaceutical compositions comprising the formulations described herein.

[27] Other features and advantages of the invention will be apparent from and are encompassed by the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[28] Figure 1: Plecanatide (SP-304) treatment reduced time to first BM following daily dose.

[29] Figure 2: Effect of daily treatment with plecanatide on spontaneous bowel movements (SBM) in chronic constipation patients.

[30] Figure 3: Effect of daily treatment with plecanatide on complete spontaneous bowel movements (CSBM) in chronic constipation patients.

[31] Figure 4: Effect of daily treatment with plecanatide on Bristol Stool Form Scores (BSFS) in chronic constipation patients.

[32] Figure 5: Effect of daily treatment with plecanatide on straining scores in chronic constipation patients

[33] Figure 6: Percentage of subjects reporting improvements in abdominal discomfort scores after 14-days of daily treatment with plecanatide.

DETAILED DESCRIPTION

[34] The invention provides pharmaceutical formulations of peptide GCC agonists. It is intended that the formulations of the invention are “pharmaceutical” formulations, meaning

that they are suitable for pharmaceutical use. Accordingly, the term “formulations” as used herein is meant to encompass pharmaceutical formulations even if “pharmaceutical” is not expressly stated. Pharmaceutical compositions comprising the formulations described herein are also provided by the invention. The formulations of the invention preferably provide stability against chemical and physical degradation of the peptide, e.g., plecanatide (i.e., SEQ ID #1).

[35] The invention is based in part upon the discovery that mannitol mixes very effectively with the GCC agonist peptides described herein and provides stability against degradation, allowing the peptides to be formulated at very low doses. The invention is also based in part on the discovery that very low doses of the GCC agonist peptides described herein are effective for the treatment of diseases and disorders in humans. The dosage range found to be effective was not predicted based on animal studies. The invention is also based in part upon the discovery that a divalent cation (e.g., Ca^{2+}) and/or an amino acid (e.g., leucine or arginine) stabilize the GCC agonist peptides described herein during a process (e.g., spray coating-drying process) of manufacturing a formulation of the GCC agonist peptides and provides stability against degradation both during the manufacturing process and storage of the formulation.

[36] Plecanatide is a charged peptide due to the presence of four carboxylic acids and single amine group with a calculated pKa of approximately 3.5. Therefore plecanatide is likely to interact with ions in solution or in the solid state. Plecanatide is a hygroscopic peptide requiring the control of water during manufacture and storage to promote long term stability. Plecanatide is prone to degradation by oxidation in the presence of residual peroxides or formaldehyde contaminants that are formed from peroxide reaction with polymeric excipients. The present invention discloses a manufacturing process and dry solid formulation compositions that minimizes water content. The formulations are comprised of components to minimize levels of residual formaldehyde and peroxides commonly found in many pharmaceutical excipients. The invention also discloses additives (i.e. CaCl_2) that may function as local desiccants in the formulation. Divalent cation salts such as calcium ascorbate, MgCl_2 , ZnCl_2 , MnCl_2 and CaCl_2 bind plecanatide and sterically hinder reactive species such as water or oxygen from causing plecanatide degradation by molecular displacement. The invention further includes scavengers of residual formaldehyde (amines such as TRIS or TRIS/HCl or amino acids such as leucine, isoleucine and valine), and

discloses packaging confirmations to minimize oxygen exposure and water vapor during storage. The invention also discloses a stable manufacturing process comprised of initially dissolving plecanatide in cold water to minimize solution degradation, followed by spray coating the peptide solution on particles and drying to remove moisture.

[37] The formulations of the invention are particularly useful for the treatment or prevention of a gastrointestinal disease or disorder selected from the group consisting of irritable bowel syndrome, non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastro esophageal reflux disease, chronic idiopathic constipation, gastroparesis, heartburn, gastric cancer, and *H. pylori* infection.

[38] In one embodiment, the formulations of the invention are used in a method for the treatment of constipation. Clinically accepted criteria that define constipation range from the frequency of bowel movements, the consistency of feces and the ease of bowel movement. One common definition of constipation is less than three bowel movements per week. Other definitions include abnormally hard stools or defecation that requires excessive straining. Constipation may be idiopathic (functional constipation or slow transit constipation) or secondary to other causes including neurologic, metabolic or endocrine disorders. These disorders include diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple sclerosis, Parkinson's disease, spinal cord lesions, Neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung disease and cystic fibrosis. Constipation may also be the result of surgery or due to the use of drugs such as analgesics (like opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics. In a preferred embodiment, the constipation is chronic idiopathic constipation.

[39] The stabilized formulations of the invention comprise at least one GCC agonist peptide formulated with one or more excipients such that the peptide is stabilized against chemical degradation. Chemical degradation of peptides results from a number of mechanisms including oxidation, water-mediated degradation, and reaction with aldehydes or reducing sugars. The ideal excipient or combination of excipients will be non-hygroscopic, have few or no reducing sugars, and be substantially free of contaminants such as iron, peroxide, and formaldehyde. The formulations of the invention are preferably substantially free of water. In this context “substantially” free of water means that the water content of the

formulation at the time of packaging is preferably less than 7%, less than 5%, less than 1%, or less than 0.5% of the total weight of the formulation. In one embodiment the amount of water is between 0.1 to 5% of the total weight of the formulation. In one embodiment, the amount of water in the formulation of the invention manufactured through a spray-coating process is less than 0.5% (e.g., about 0.47%).

[40] In the context of the present formulations, the term “stable” or “stabilized” refers to the resistance of the peptide to chemical or physical degradation over time. Preferably, a stable formulation of the invention retains an amount of the peptide in the formulation over a period of time that is at least 90%, preferably at least 95%, and most preferably at least 99% the amount of peptide initially present in the formulation. In one embodiment, a stable formulation of the invention, over a period of time (e.g., 18 month), has an increase in the total impurity content not greater than 8%, not greater than 7%, not greater than 6%, not greater than 5%, not greater than 4%, not greater than 3%, not greater than 2%, or not greater than 1%. In one embodiment, the peptide is chemically stable in the formulation for a period of time that is at least 18 months, at least 20 months, or at least 24 months when stored at 25 degrees Celsius (25C) and 60 % relative humidity. In one embodiment, the peptide is chemically stable in the formulation for a period of time that is at least 18 months, at least 20 months, or at least 24 months when stored at 2-8 degrees Celsius (2-8C). In one embodiment, the peptide is chemically stable in the formulation for a period of time that is at least 3 months, 12 months, 18 months and preferably 24 months when stored at 25 degrees Celsius (25C) and 60 % relative humidity. In one embodiment, the peptide is chemically stable in the formulation for a period of time that is at least 3 months, 18 months and preferably 24 months when stored at 30 degrees Celsius (30C).

[41] The low-dose formulations of the invention comprise an amount of at least one GCC agonist peptide per unit dose that is less than 10 mg. It is especially advantageous to formulate oral compositions in unit dosage form for ease of administration and uniformity of dosage. The term “unit dosage form” as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active

compound and the particular therapeutic effect to be achieved. In one embodiment, the unit dosage form is a tablet or a capsule.

[42] In one embodiment of the low-dose formulations of the invention, the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg. In one embodiment, the amount of GCC agonist peptide per unit dose is 0.1 mg, 0.3 mg, 0.6 mg, 1.0 mg, 3.0 mg, 6.0 mg, 9.0 mg, 9.5 mg, or 10 mg.

[43] In one embodiment, the low-dose formulation contains a carrier that is non-hygroscopic. In one embodiment, the carrier is selected from mannitol and maltose (e.g., ADVANTOSE 100).

[44] In one embodiment, the carrier is cellulose, preferably microcrystalline cellulose (e.g., Avicel PH 102, low moisture Avicel PH 112, Avicel PH 200, or Celphere SCP-100). In one embodiment, the carrier is calcium phosphate or calcium sulphate. In another embodiment, the carrier is a saccharide. The term "saccharide" as used herein also refers to polysaccharides. Thus, the term saccharide is meant to include polysaccharides. In one embodiment, the saccharide is selected from mannitol, trehalose, lactose, sucrose, sorbitol, and maltose. In a preferred embodiment, the saccharide is mannitol. Preferably the saccharide has a low water content, a small particle size and a narrow particle-size distribution.

[45] Carriers having small particle sizes, and/or spherical shape, and narrow size distribution are preferred. Particles of less than 20 microns have a relatively high surface area to volume ratio causing inter-particle attractive forces to dominate and resist bulk flow. Larger particles (greater than 100 microns) tend to roll or slide over one another and exhibit superior bulk flow properties compared with small particles. A narrow particle-size distribution reduces particle packing and increases flow. In one embodiment, the particles are between 20 and 500 microns in size (as measured across the largest diameter of the particle, on average). In one embodiment, a small particle size and a narrow particle size range refers to particles having a size range of from 20-300 microns, 50-200 microns, or 75-150 microns. In certain embodiments, the carrier has a substantially spherical shape such as can be obtained with a spray drying process.

[46] In one embodiment, the low-dose formulation is a solid formulation and the unit dose is in the form of a tablet or capsule. In one embodiment, the low-dose formulation is a liquid formulation and the unit dosage form is a liquid-filled capsule. In one embodiment, the liquid formulation is in the form of a solution or suspension of the GCC agonist peptide in an lipophilic liquid. Examples of suitable liquids include medium chain triglycerides (e.g., LABRAFAC Lipophile), propylene glycol dicaprylocaprate (e.g., LABRAFAC PG), vitamin E (e.g., α tocopherol), PEG 400 (e.g., Polyethylene glycol low M.W. (liquid)), propylene glycol, soybean oil, and Castor oil. In one embodiment, the liquid is selected from the group consisting of medium chain triglycerides, propylene glycol dicaprylocaprate, vitamin E, and soybean oil. In one embodiment, the refined specialty oil is selected from Arachis oil, Castor oil, cottonseed oil, maize (corn) oil, olive oil, sesame oil, soybean oil, and sunflower oil. In one embodiment, the medium chain triglyceride or related ester is AKOMED E, AKOMED R, CAPTEX 355, LABRAFAC CC, LABRAFAC PG, LAUROGLYCOL FCC, MIGLYOL 810, MIGLYOL 812, MIGLYOL 829, MIGLYOL 840, and SOFTISAN 645.

[47] A formulation according to the invention may be contained in a blister pack. In a particular embodiment, the powder, tablet, or capsule comprising the formulation is contained in a blister pack. Preferably, the blister pack is made of a material that allows only minimal permeation by water vapor and oxygen. In one embodiment the blister pack is comprised of a metal foil. In one embodiment, the blister pack is comprised of ACLAR. In one embodiment, the container of the blister pack is flushed with an inert gas such as nitrogen or argon. In one embodiment, the container further includes a desiccant. In one embodiment, the desiccant is calcium chloride. In one embodiment the desiccant is a molecular sieve.

[48] While any GCC agonist known in the art can be formulated according to the present invention, analogs of uroguanylin and bacterial ST peptides are preferred. In certain embodiments, the uroguanylin and bacterial ST peptide analogs have superior properties compared to naturally occurring, or “wild-type” peptides. For example, the uroguanylin and bacterial ST peptides for use in the present invention are preferably modified to increase their resistance to degradation at the N-terminus and C-terminus from carboxypeptidases, aminopeptidases, and/or by other proteolytic enzymes present in the stimulated human intestinal juices and human gastric juices. In certain embodiments, the GCC agonist formulation comprises a peptide consisting essentially of an amino acid sequence selected

from SEQ ID NOs: 1-249. In a preferred embodiment, the peptide consists essentially of an amino acid sequence selected from SEQ ID NOs: 1, 8, 9, 55 and 56. The term "consists essentially of" refers to a peptide that is identical to the reference peptide in its amino acid sequence or to a peptide that does not differ substantially in terms of either structure or function from the reference peptide. A peptide differs substantially from the reference peptide if its primary amino acid sequence varies by more than three amino acids from the reference peptide or if its activation of cellular cGMP production is reduced by more than 50% compared to the reference peptide. Preferably, substantially similar peptides differ by no more than two amino acids and not by more than about 25% with respect to activating cGMP production. In preferred embodiments, the GCC agonist is a peptide comprising at least 12 amino acid residues, and most preferably comprising between 12 and 26 amino acids. Non-limiting examples of such analogs of uroguanylin and bacterial ST peptides are described in Section 1.2 below.

[49] The invention provides methods for treating or preventing certain diseases and disorders and methods for increasing gastrointestinal motility in a subject in need thereof by administering an effective amount of a GCC agonist formulation to the subject. The term "treating" as used herein refers to a reduction, a partial improvement, amelioration, or a mitigation of at least one clinical symptom associated with the gastrointestinal disorders being treated. The term "preventing" refers to an inhibition or delay in the onset or progression of at least one clinical symptom associated with the gastrointestinal disorders to be prevented. The term "effective amount" as used herein refers to an amount that provides some improvement or benefit to the subject. In certain embodiments, an effective amount is an amount that provides some alleviation, mitigation, and/or decrease in at least one clinical symptom of the gastrointestinal disorder to be treated. In other embodiments, the effective amount is the amount that provides some inhibition or delay in the onset or progression of at least one clinical symptom associated with the gastrointestinal disorder to be prevented. The therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject. The term "subject" preferably refers to a human subject but may also refer to a non-human primate or other mammal preferably selected from among a mouse, a rat, a dog, a cat, a cow, a horse, or a pig.

[50] In accordance with the methods of the present invention, the GCC agonist formulation can be administered alone or in combination with one or more additional therapeutic agents

to prevent or treat inflammation, cancer and other disorders, particularly of the gastrointestinal tract. In a preferred embodiment, the GCC agonist formulation is administered for the treatment of chronic constipation. In one embodiment, the GCC agonist formulation is administered in combination with one or more additional therapeutic agents selected from the group consisting of phosphodiesterase inhibitors, cyclic nucleotides (such as cGMP and cAMP), a laxative (such as SENNA, METAMUCIL, MIRALAX, PEG, or calcium polycarbophil), a stool softener, an anti-tumor necrosis factor alpha therapy for IBD (such as REMICADE, ENBREL, or HUMAIRA), and anti-inflammatory drugs (such as COX-2 inhibitors, sulfasalazine, 5-ASA derivatives and NSAIDS). In certain embodiments, the GCC agonist formulation is administered in combination with an effective dose of an inhibitor of cGMP-specific phosphodiesterase (cGMP-PDE) either concurrently or sequentially with said GCC agonist. cGMP-PDE inhibitors include, for example, sildinac sulfone, zaprinast, motapizone, vardenafil, and sildenafil. In another embodiment, the GCC agonist formulation is administered in combination with inhibitors of cyclic nucleotide transporters.

1.1 Formulations

[51] The formulations of the invention contain one or more GCC agonist peptides described herein, in combination with one or more pharmaceutically acceptable carriers (also referred to as diluents) and/or excipients. In a preferred embodiment, the formulations of the invention include an inert carrier. The inert carrier is preferably non-hygroscopic. In one embodiment, the carrier in the formulation contains few or no reducing sugars and is substantially free of contaminants including, but not limited to, iron, peroxide, and formaldehyde. In one embodiment, the carrier is selected from the group consisting of sorbitol, mannitol, EMDEX, and starch. In one embodiment, the carrier is mannitol (e.g., MANNOGEM) or microcrystalline cellulose (e.g. PROSOLV, CELPHERE, CELPHERE beads).

[52] The low-dose formulations of the invention contain no greater than 10 mg per unit dose of a GCC agonist peptide. The remainder of the formulation is comprised of the carrier and one or more optional excipients. In one embodiment, the amount of carrier is at least 90% of the total weight of the formulation. In another embodiment, the amount of carrier is

at least 95% or at least 98% of the total weight of the formulation. In one embodiment, the amount of carrier is between 90 and 99.9% of the total weight of the formulation. In one embodiment, the one or more optional excipients comprise a disintegrant which is present at 1 to 5% of the total weight of the formulation. In one embodiment, the one or more optional excipients comprise a lubricant which is present at 0.02 to 5% of the total weight of the formulation. In one embodiment, the one or more optional excipients comprise an amino acid such as arginine, leucine, isoleucine, valine, histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, methionine, asparagine, tyrosine, threonine, tryptophan, or glycine, which is present at 0.1 to 4% (e.g., 0.1-1%) of the total weight of the formulation. In one embodiment, the molar ratio between the amino acid and the GCC agonist peptide is from about 2:1 to about 30:1 or about 2:1 to about 20:1 (e.g., 5:1). In one embodiment, the one or more optional excipients comprise a stabilizer such as a divalent cation salt, more specifically, a water-soluble divalent cation salt (e.g., calcium chloride, magnesium chloride, zinc chloride, manganese chloride, or calcium ascorbate), which is present at 0.1 to 12% (e.g., 0.1-4%) of the total weight of the formulation. In one embodiment, the molar ratio between the salt and the GCC agonist peptide is from about 5:1 to about 20:1 (e.g., 10:1).

[53] The formulations may contain other additives as needed, including for example lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, starch, xylitol, mannitol, myoinositol, and the like, and hydrates thereof, and amino acids, for example alanine, glycine and betaine, and polypeptides and proteins, for example albumen.

[54] Further examples of pharmaceutically acceptable carriers and excipients include, but are not limited to binders, fillers, disintegrants, lubricants, anti-microbial agents, antioxidant, and coating agents such as: BINDERS: corn starch, potato starch, other starches, gelatin, natural and synthetic gums such as acacia, xanthan, sodium alginate, alginic acid, other alginates, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone (e.g., povidone, crospovidone, copovidone, etc), methyl cellulose, Methocel, pre-gelatinized starch (e.g., STARCH 1500® and STARCH 1500 LM®, sold by Colorcon, Ltd.), hydroxypropyl methyl cellulose, microcrystalline cellulose (FMC Corporation, Marcus Hook, PA, USA), Emdex, Plasdone, or mixtures thereof, FILLERS: talc, calcium carbonate (e.g., granules or powder), dibasic calcium phosphate, tribasic

calcium phosphate, calcium sulfate (e.g., granules or powder), microcrystalline cellulose, powdered cellulose, dextrans, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, dextrose, fructose, honey, lactose anhydrate, lactose monohydrate, lactose and aspartame, lactose and cellulose, lactose and microcrystalline cellulose, maltodextrin, maltose, mannitol, microcrystalline cellulose & guar gum, molasses, sucrose, or mixtures thereof, DISINTEGRANTS: agar-agar, alginic acid, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, polacrillin potassium, sodium starch glycolate (such as Explotab), potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algin, other celluloses, gums (like gellan), low-substituted hydroxypropyl cellulose, polyplasdone, or mixtures thereof, LUBRICANTS: calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, Compritol, stearic acid, sodium lauryl sulfate, sodium stearyl fumarate (such as Pruv), vegetable based fatty acids lubricant, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil), zinc stearate, ethyl oleate, ethyl laurate, agar, syloid silica gel (AEROSIL 200, W.R. Grace Co., Baltimore, MD USA), a coagulated aerosol of synthetic silica (Deaussa Co., Piano, TX USA), a pyrogenic silicon dioxide (CAB-O-SIL, Cabot Co., Boston, MA USA), or mixtures thereof, ANTI-CAKING AGENTS: calcium silicate, magnesium silicate, silicon dioxide, colloidal silicon dioxide, talc, or mixtures thereof, ANTIMICROBIAL AGENTS: benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, butyl paraben, cetylpyridinium chloride, cresol, chlorobutanol, dehydroacetic acid, ethylparaben, methylparaben, phenol, phenylethyl alcohol, phenoxyethanol, phenylmercuric acetate, phenylmercuric nitrate, potassium sorbate, propylparaben, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimersol, thymo, or mixtures thereof, ANTOXIDANTS: ascorbic acid, BHA, BHT, EDTA, or mixture thereof, and COATING AGENTS: sodium carboxymethyl cellulose, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose (hypromellose), hydroxypropyl methyl cellulose phthalate, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, gellan gum, maltodextrin, methacrylates, microcrystalline cellulose and carrageenan or mixtures thereof.

[55] The formulation can also include other excipients and categories thereof including but not limited to Pluronic®, Poloxamers (such as Lutrol® and Poloxamer 188), ascorbic acid, glutathione, protease inhibitors (e.g. soybean trypsin inhibitor, organic acids), pH lowering agents, creams and lotions (like maltodextrin and carrageenans); materials for chewable tablets (like dextrose, fructose, lactose monohydrate, lactose and aspartame, lactose and cellulose, maltodextrin, maltose, mannitol, microcrystalline cellulose and guar gum, sorbitol crystalline); parenterals (like mannitol and povidone); plasticizers (like dibutyl sebacate, plasticizers for coatings, polyvinylacetate phthalate); powder lubricants (like glyceryl behenate); soft gelatin capsules (like sorbitol special solution); spheres for coating (like sugar spheres); spherization agents (like glyceryl behenate and microcrystalline cellulose); suspending/gelling agents (like carrageenan, gellan gum, mannitol, microcrystalline cellulose, povidone, sodium starch glycolate, xanthan gum); sweeteners (like aspartame, aspartame and lactose, dextrose, fructose, honey, maltodextrin, maltose, mannitol, molasses, sorbitol crystalline, sorbitol special solution, sucrose); wet granulation agents (like calcium carbonate, lactose anhydrous, lactose monohydrate, maltodextrin, mannitol, microcrystalline cellulose, povidone, starch), caramel, carboxymethylcellulose sodium, cherry cream flavor and cherry flavor, citric acid anhydrous, citric acid, confectioner's sugar, D&C Red No. 33, D&C Yellow #10 Aluminum Lake, disodium edetate, ethyl alcohol 15%, FD&C Yellow No. 6 aluminum lake, FD&C Blue # 1 Aluminum Lake, FD&C Blue No. 1, FD&C blue no. 2 aluminum lake, FD&C Green No.3, FD&C Red No. 40, FD&C Yellow No. 6 Aluminum Lake, FD&C Yellow No. 6, FD&C Yellow No.10, glycerol palmitostearate, glyceryl monostearate, indigo carmine, lecithin, manitol, methyl and propyl parabens, mono ammonium glycyrrhizinate, natural and artificial orange flavor, pharmaceutical glaze, poloxamer 188, Polydextrose, polysorbate 20, polysorbate 80, polyvidone, pregelatinized corn starch, pregelatinized starch, red iron oxide, saccharin sodium, sodium carboxymethyl ether, sodium chloride, sodium citrate, sodium phosphate, strawberry flavor, synthetic black iron oxide, synthetic red iron oxide, titanium dioxide, and white wax.

[56] Solid oral dosage forms may optionally be treated with coating systems (e.g. Opadry® fx film coating system, for example Opadry® blue (OY-LS-20921), Opadry® white (YS-2-7063), Opadry® white (YS- 1-7040), and black ink (S- 1-8 106).

[57] The agents either in their free form or as a salt can be combined with a polymer such as polylactic-glycolic acid (PLGA), poly-(D)-lactic-glycolic-tartaric acid (P(D)LGT) (WO

01/12233), polyglycolic acid (U.S. 3,773,919), polylactic acid (U.S. 4,767,628), poly(ϵ -caprolactone) and poly(alkylene oxide) (U.S. 20030068384) to create a sustained release formulation. Other sustained release formulations and polymers for use in the compositions and methods of the invention are described in EP 0 467 389 A2, WO 93/24150, U.S. 5,612,052, WO 97/40085, WO 03/075887, WO 01/01964A2, U.S. 5,922,356, WO 94/155587, WO 02/074247A2, WO 98/25642, U.S. 5,968,895, U.S. 6,180,608, U.S. 20030171296, U.S. 20020176841, U.S. 5,672,659, U.S. 5,893,985, U.S. 5,134,122, U.S. 5,192,741, U.S. 5,192,741, U.S. 4,668,506, U.S. 4,713,244, U.S. 5,445,832 U.S. 4,931,279, U.S. 5,980,945, WO 02/058672, WO 97/26015, WO 97/04744, and US20020019446. In such sustained release formulations microparticles (Delie and Blanco-Prieto 2005 Molecule 10:65-80) of polypeptide are combined with microparticles of polymer. U.S. 6,011,011 and WO 94/06452 describe a sustained release formulation providing either polyethylene glycols (i.e. PEG 300 and PEG 400) or triacetin. WO 03/053401 describes a formulation which may both enhance bioavailability and provide controlled release of the agent within the GI tract. Additional controlled release formulations are described in WO 02/38129, EP 326151, U.S. 5,236,704, WO 02/30398, WO 98/13029; U.S. 20030064105, U.S. 20030138488A1, U.S. 20030216307A1, U.S. 6,667,060, WO 01/49249, WO 01/49311, WO 01/49249, WO 01/49311, and U.S. 5,877,224 materials which may include those described in WO04041195 (including the seal and enteric coating described therein) and pH-sensitive coatings that achieve delivery in the colon including those described in US4,910,021 and WO9001329. US4910021 describes using a pH-sensitive material to coat a capsule. WO9001329 describes using pH-sensitive coatings on beads containing acid, where the acid in the bead core prolongs dissolution of the pH-sensitive coating. U. S. Patent No. 5,175,003 discloses a dual mechanism polymer mixture composed of pH-sensitive enteric materials and film-forming plasticizers capable of conferring permeability to the enteric material, for use in drug-delivery systems; a matrix pellet composed of a dual mechanism polymer mixture permeated with a drug and sometimes covering a pharmaceutically neutral nucleus; a membrane-coated pellet comprising a matrix pellet coated with a dual mechanism polymer mixture envelope of the same or different composition; and a pharmaceutical dosage form containing matrix pellets. The matrix pellet releases acid-soluble drugs by diffusion in acid pH and by disintegration at pH levels of nominally about 5.0 or higher.

[58] The GCC peptides described herein may be formulated in the pH triggered targeted control release systems described in WO04052339. The agents described herein may be formulated according to the methodology described in any of WO03105812 (extruded hydratable polymers); WO0243767 (enzyme cleavable membrane translocators); WO03007913 and WO03086297 (mucoadhesive systems); WO02072075 (bilayer laminated formulation comprising pH lowering agent and absorption enhancer); WO04064769 (amidated polypeptides); WO05063156 (solid lipid suspension with pseudotropic and/or thixotropic properties upon melting); WO03035029 and WO03035041 (erodible, gastric retentive dosage forms); US5007790 and US5972389 (sustained release dosage forms); WO041 1271 1 (oral extended release compositions); WO05027878, WO02072033, and WO02072034 (delayed release compositions with natural or synthetic gum); WO05030182 (controlled release formulations with an ascending rate of release); WO05048998 (microencapsulation system); US Patent 5,952,314 (biopolymer); US5,108,758 (glassy amylose matrix delivery); US 5,840,860 (modified starch based delivery). JP10324642 (delivery system comprising chitosan and gastric resistant material such as wheat gliadin or zein); US 5,866,619 and US 6,368,629 (saccharide containing polymer); US 6,531,152 (describes a drug delivery system containing a water soluble core (Ca pectinate or other water-insoluble polymers) and outer coat which bursts (e.g. hydrophobic polymer-Eudragit)); US 6,234,464; US 6,403,130 (coating with polymer containing casein and high methoxy pectin; WO0174 175 (Maillard reaction product); WO05063206 (solubility increasing formulation); WO040 19872 (transferring fusion proteins).

[59] The GCC peptides described herein may be formulated using gastrointestinal retention system technology (GIRES; Merrion Pharmaceuticals). GIRES comprises a controlled-release dosage form inside an inflatable pouch, which is placed in a drug capsule for oral administration. The capsule shell can be a HPMC capsule shell or Gelatin capsule shell. Upon dissolution of the capsule, a gas-generating system inflates the pouch in the stomach where it is retained for 16-24 hours, all the time releasing agents described herein.

[60] The GCC peptides described herein can also be formulated using the multi matrix system technology (MMX).

[61] The GCC peptides described herein can be formulated in an osmotic device including the ones disclosed in US 4,503,030, US 5,609,590 and US 5,358,502. US 4,503,030 discloses

an osmotic device for dispensing a drug to certain pH regions of the gastrointestinal tract. More particularly, the invention relates to an osmotic device comprising a wall formed of a semi-permeable pH sensitive composition that surrounds a compartment containing a drug, with a passageway through the wall connecting the exterior of the device with the compartment. The device delivers the drug at a controlled rate in the region of the gastrointestinal tract having a pH of less than 3.5, and the device self- destructs and releases all its drug in the region of the gastrointestinal tract having a pH greater than 3.5, thereby providing total availability for drug absorption. U.S. Patent Nos. 5,609,590 and 5, 358,502 disclose an osmotic bursting device for dispensing a beneficial agent to an aqueous environment. The device comprises a beneficial agent and osmagent surrounded at least in part by a semi-permeable membrane. The beneficial agent may also function as the osmagent. The semi-permeable membrane is permeable to water and substantially impermeable to the beneficial agent and osmagent. A trigger means is attached to the semi-permeable membrane (e.g., joins two capsule halves). The trigger means is activated by a pH of from 3 to 9 and triggers the eventual, but sudden, delivery of the beneficial agent. These devices enable the pH-triggered release of the beneficial agent core as a bolus by osmotic bursting.

[62] In one embodiment the formulation contains a GCC agonist peptide, mannitol, silicified microcrystalline cellulose, sodicum starch glycolate, and sodium stearyl fumarate. The GCC agonist is at a concentration of less than 5% w/w, less than 4%, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.23% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The mannitol is at a concentration of at least 60% w/w, at least 65% w/w, at least 70% w/w, at least 75% w/w, or at least 80% w/w. In some embodiments the mannitol is present at about 79% w/w (e.g., 79.77%). The mannitol is preferably Mannogem EZ. The silicified microcrystalline cellulose is at a concentration of at least 5% w/w, at least 10% w/w, or at least 15% w/w. In some embodiments the concentration of the silicified microcrystalline cellulose is about 15% w/w. The silicified microcrystalline cellulose is preferably Prosolv SMCC 90 LM. The sodicum starch glycolate is at a concentration of at least 1% w/w, at least 2% w/w, at least 3% w/w, or at least 4% w/w. In some embodiments the concentration of the sodicum starch glycolate is about 4% w/w. The sodicum starch glycolate is preferably Explotab. The sodium stearyl fumarate is at

a concentration of at least 0.2% w/w, at least 0.5% w/w, at least 0.7% w/w, at least 0.8% w/w, at least 0.9, or at least 1% w/w. In some embodiments the concentration of the sodium stearyl fumarate is about 1% w/w. The sodium stearyl fumarate is preferably Pruv.

[63] In one embodiment the formulation contains a GCC agonist peptide, silicified microcrystalline cellulose, and sodicum starch glycolate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.3% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The silicified microcrystalline cellulose is at a concentration of at least 10% w/w, at least 20% w/w, at least 30% w/w, at least 40% w/w, at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, or at least 95% w/w. In some embodiments the concentration of the silicified microcrystalline cellulose is about 95.7% w/w. The silicified microcrystalline cellulose is preferably Prosolv SMCC 90 HD. The sodicum starch glycolate is at a concentration of at least 1% w/w, at least 2% w/w, at least 3% w/w, or at least 4% w/w. In some embodiments the concentration of the sodicum starch glycolate is 4% w/w. The sodicum starch glycolate is preferably Explotab.

[64] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, calcium chloride dihydrate, leucine, and hypromellose. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.3246% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 99.10% w/w. The microcrystalline cellulose is preferably Celphere SCP-100. The calcium chloride dihydrate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the calcium chloride dihydrate is about 0.2622% w/w. The leucine is at a concentration of at least 0.05% w/w, at least 0.1% w/w, at least 0.12% w/w, or at least 0.15% w/w. In some embodiments the concentration of leucine is about 0.12% w/w. The hypromellose is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2%

w/w, or at least 0.25% w/w. In some embodiments the concentration of the hypromellose is about 0.2% w/w. The hypromellose is preferably Methocel E5 PremLV.

[65] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, calcium chloride dihydrate, leucine, hypromellose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.36% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 98.75% w/w. The microcrystalline cellulose is preferably Avicel PH 102. The calcium chloride dihydrate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, at least 0.25% w/w, or at least 0.3% w/w. In some embodiments the concentration of the calcium chloride dihydrate is about 0.29% w/w. The leucine is at a concentration of at least 0.05% w/w, at least 0.1% w/w, at least 0.12% w/w, or at least 0.15% w/w. In some embodiments the concentration of leucine is about 0.13% w/w. The hypromellose is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the hypromellose is about 0.22% w/w. The hypromellose is preferably Methocel E5 PremLV. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

[66] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.32% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 99.43% w/w. The microcrystalline cellulose is preferably Avicel PH 102. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or

at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

[67] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 0.32% w/w, about 1.18% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 30% w/w, at least 40% w/w, at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 98.57 % w/w. The microcrystalline cellulose is preferably Avicel PH 102. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

[68] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 1.18% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 30% w/w, at least 40% w/w, at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 97.09 % w/w. The microcrystalline cellulose is preferably Avicel PH 112. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

[69] In one embodiment the formulation contains a GCC agonist peptide, trehalose granules, hypromellose, histidine, calcium ascorbate, trehalose powder, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a

concentration of about 1.18% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The trehalose granules are at a concentration of at least 50% w/w, at least 55% w/w, at least 60% w/w, at least 65% w/w, at least 70% w/w, or at least 75% w/w. In some embodiments the concentration of the trehalose granules is 55-75% w/w. In a particular embodiment, the concentration of the trehalose granules is 70.48% w/w. The hypromellose is at a concentration of at least 0.1% w/w, at least 0.2% w/w, at least 0.3% w/w, at least 0.4% w/w, or at least 0.5% w/w. In some embodiments the concentration of the hypromellose is 0.2-2% w/w. In a particular embodiment the concentration of the hypromellose about 0.5% w/w. The hypromellose is preferably Methocel ES Premium LV. The histine is a concentration of at least 0.6% w/w, at least 0.8% w/w, at least 0.9% w/w, at least 1% w/w, at least 3% w/w, or at least 5% w/w. In some embodiments the concentration of the histidine is 1-6% w/w. In a particular embodiment, the concentration of the arginine is 1.48% w/w. The calcium ascorbate is at a concentration of at least 0.05% w/w, at least 0.07% w/w, at least 0.09% w/w, or at least 0.1% w/w. In some embodiments the concentration of the calcium ascorbate is 0.05-10% w/w. In a particular embodiment, the concentration of the calcium ascorbate is about 0.1% w/w. The trehalose powder is at a concentration of at least 0.5% w/w, at least 0.7% w/w, at least 0.8% w/w, at least 0.9% w/w, at least 1% w/w, or at least 1.2% w/w. In some embodiments the concentration of the trehalose powder is 0.5-4% w/w. In a particular embodiment, the concentration of the trehalose powder is 1.02% w/w. The microcrystalline cellulose is at a concentration of at least 10% w/w, at least 20% w/w, or at least 25% w/w. In some embodiments the concentration of the microcrystalline cellulose is 20-40% w/w. In a particular embodiment, the concentration of the microcrystalline cellulose is 25% w/w. The microcrystalline cellulose is preferably Avicel PH 200. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is 0.2-1% w/w. In a particular embodiment the concentration of the magnesium stearate is about 0.25% w/w.

[70] In one embodiment the formulation contains a GCC agonist peptide, trehalose granules, hypromellose, arginine, calcium ascorbate, trehalose powder, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a

concentration of about 1.17% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The trehalose granules are at a concentration of at least 50% w/w, at least 55% w/w, at least 60% w/w, at least 65% w/w, at least 70% w/w, or at least 75% w/w. In some embodiments the concentration of the trehalose granules is 55-75% w/w. In a particular embodiment, the concentration of the trehalose granules is 70.31% w/w. The hypromellose is at a concentration of at least 0.1% w/w, at least 0.2% w/w, at least 0.3% w/w, at least 0.4% w/w, or at least 0.5% w/w. In some embodiments the concentration of the hypromellose is 0.2-2% w/w. In a particular embodiment the concentration of the hypromellose about 0.5% w/w. The hypromellose is preferably Methocel ES Premium LV. The arginine is a concentration of at least 0.5% w/w, at least 1% w/w, at least 1.5% w/w, or at least 2% w/w. In some embodiments the concentration of the arginine is 1-6% w/w. In a particular embodiment, the concentration of the arginine is 1.66% w/w. The calcium ascorbate is at a concentration of at least 0.05% w/w, at least 0.07% w/w, at least 0.09% w/w, or at least 0.1% w/w. In some embodiments the concentration of the calcium ascorbate is 0.05-10% w/w. In a particular embodiment, the concentration of the calcium ascorbate is about 0.1% w/w. The trehalose powder is at a concentration of at least 0.5% w/w, at least 0.7% w/w, at least 0.8% w/w, at least 0.9% w/w, at least 1% w/w, or at least 1.2% w/w. In some embodiments the concentration of the trehalose powder is 0.5-4% w/w. In a particular embodiment, the concentration of the trehalose powder is 1.02% w/w. The microcrystalline cellulose is at a concentration of at least 10% w/w, at least 20% w/w, or at least 25% w/w. In some embodiments the concentration of the microcrystalline cellulose is 20-40% w/w. In a particular embodiment, the concentration of the microcrystalline cellulose is 25% w/w. The microcrystalline cellulose is preferably Avicel PH 200. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is 0.2-1% w/w. In a particular embodiment the concentration of the magnesium stearate is about 0.25% w/w.

[71] In one embodiment the formulation contains a GCC agonist peptide, trehalose granules, hypromellose, TRIS, calcium ascorbate, trehalose powder, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 1.17% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO:

9. The trehalose granules are at a concentration of at least 50% w/w, at least 55% w/w, at least 60% w/w, at least 65% w/w, at least 70% w/w, or at least 75% w/w. In some embodiments the concentration of the trehalose granules is 55-75% w/w. In a particular embodiment, the concentration of the trehalose granules is 70.81% w/w. The hypromellose is at a concentration of at least 0.1% w/w, at least 0.2% w/w, at least 0.3% w/w, at least 0.4% w/w, or at least 0.5% w/w. In some embodiments the concentration of the hypromellose is 0.2-2% w/w. In a particular embodiment the concentration of the hypromellose about 0.5% w/w. The hypromellose is preferably Methocel ES Premium LV. The TRIS is a concentration of at least 0.6% w/w, at least 0.8% w/w, at least 0.9% w/w, or at least 1% w/w. In some embodiments the concentration of the TRIS is 0.5-6% w/w. In a particular embodiment, the concentration of the arginine is 1.15% w/w. The calcium ascorbate is at a concentration of at least 0.05% w/w, at least 0.07% w/w, at least 0.1% w/w, or at least 1% w/w. In some embodiments the concentration of the calcium ascorbate is 0.05-10% w/w. In a particular embodiment, the concentration of the calcium ascorbate is about 0.1% w/w. The trehalose powder is at a concentration of at least 0.5% w/w, at least 0.7% w/w, at least 0.8% w/w, at least 0.9% w/w, at least 1% w/w, or at least 1.2% w/w. In some embodiments the concentration of the trehalose powder is 0.5-4% w/w. In a particular embodiment, the concentration of the trehalose powder is 1.02% w/w. The microcrystalline cellulose is at a concentration of at least 10% w/w, at least 20% w/w, or at least 25% w/w. In some embodiments the concentration of the microcrystalline cellulose is 20-40% w/w. In a particular embodiment, the concentration of the microcrystalline cellulose is 25% w/w. The microcrystalline cellulose is preferably Avicel PH 200. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is 0.2-1% w/w. In a particular embodiment the concentration of the magnesium stearate is about 0.25% w/w.

[72] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 1.10% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 30% w/w, at least 40% w/w, at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at

least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 98.64 % w/w. The microcrystalline cellulose is preferably Avicel PH 102. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

[73] In one embodiment the formulation contains a GCC agonist peptide, microcrystalline cellulose, and magnesium stearate. The GCC agonist is at a concentration of less than 5% w/w, less than 4% w/w, less than 3% w/w, less than 2% w/w, less than 1% w/w, less than 0.5% w/w, or less than 0.25% w/w. In some embodiments the GCC peptide is at a concentration of about 3.32% w/w. The GCC peptide is preferably SEQ NO: 1 or SEQ NO: 9. The microcrystalline cellulose is at a concentration of at least 30% w/w, at least 40% w/w, at least 50% w/w, at least 60% w/w, at least 70% w/w, at least 80% w/w, at least 90% w/w, at least 95% w/w, or at least 99% w/w. In some embodiments the concentration of the microcrystalline cellulose is about 96.43 % w/w. The microcrystalline cellulose is preferably Avicel PH 102. The magnesium stearate is at a concentration of at least 0.1% w/w, at least 0.15% w/w, at least 0.2% w/w, or at least 0.25% w/w. In some embodiments the concentration of the magnesium stearate is about 0.25% w/w.

1.2 GCC Agonists

[74] The GCC agonists for use in the formulations and methods of the invention bind to guanylate cyclase C and stimulate intracellular production of cGMP. Optionally, the GCC agonists induce apoptosis and inhibit proliferation of epithelial cells. The term, “guanylate cyclase C” refers to a transmembrane form of guanylate cyclase that acts as the intestinal receptor for the heat-stable toxin (ST) peptides secreted by enteric bacteria. Guanylate cyclase C is also the receptor for the naturally occurring peptides guanylin and uroguanylin. The possibility that there may be different receptors for each of these peptides has not been excluded. Hence, the term “guanylate cyclase C” may also encompass a class of transmembrane guanylate cyclase receptors expressed on epithelial cells lining the gastrointestinal mucosa.

[75] The term “GCC agonist” refers to both peptides and non-peptide compounds such as that bind to an intestinal guanylate cyclase C and stimulate the intracellular production of

cGMP. Where the GCC agonist is a peptide, the term encompasses biologically active fragments of such peptides and pro-peptides that bind to guanylate cyclase C and stimulate the intracellular production of cGMP.

[76] Preferably, the GCC agonists for use in the formulations and methods of the invention stimulate intracellular cGMP production at higher levels than naturally occurring GCC agonists such as uroguanylin, guanylin, and ST peptides. In some embodiments, the GCC agonists stimulate intracellular cGMP production at higher levels than the peptide designated SP-304 (SEQ ID NO:1). In specific embodiments, a GCC agonist for use in the formulations and methods of the invention stimulates 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to uroguanylin, guanylin, lymphoguanylin, linacotide, ST peptides, or SP-304. The terms “induce” and “stimulate” are used interchangeably throughout the specification.

[77] Preferably, the GCC agonists for use in the formulations and methods of the invention are more stable than naturally occurring GCC agonists such as uroguanylin, guanylin, and ST peptides. In some embodiments, the GCC agonists are more stable than the peptide designated SP-304. “Stability” in this context refers to resistance to degradation in gastrointestinal fluid and/or intestinal fluid (or simulated gastrointestinal or intestinal fluids) compared to the reference peptide. For example, the GCC agonists for use in the formulations and methods of the invention preferably degrade 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GCC agonists and/or SP-304.

[78] The GCC agonists for use in the formulations and methods of the invention are preferably peptides. In some embodiments, the GCC agonist peptide is less than 30 amino acids in length. In particular embodiments, the GCC agonist peptide is less than or equal to 30, 25, 20, 15, 14, 13, 12, 11, 10, or 5 amino acids in length. Examples of GCC agonist peptides for use in the formulations and methods of the invention include those described in U.S. Serial Nos.: 12/133,344, filed June 4, 2008, 12/478505, filed June 4, 2009; 12/478511, filed June 4, 2009; 12/504288, filed July 16, 2009; and U.S. Provisional Application Serial Nos.: 60/933194, filed June 4, 2007; 61/058,888, filed June 4, 2008; 61/058,892, filed June 4, 2008; and 61/081,289, filed July 16, 2008, each of which is incorporated by reference herein in its entirety.

[79] Specific examples of GCC agonist peptides for use in the formulations and methods of the invention include those described in Tables I-VII below. As used Tables I-VII, the terms “PEG3” or “3PEG” refer to a polyethylene glycol such as aminoethoxy-ethoxy-acetic acid (AeeA), and polymers thereof. The term “X_{aa}” refers to any natural or unnatural amino acid or amino acid analogue. The term “M_{aa}” refers to a cysteine (Cys), penicillamine (Pen) homocysteine, or 3-mercaptoproline. The term “Xaa_{n1}” is meant to denote an amino acid sequence of any natural or unnatural amino acid or amino acid analogue that is one, two or three residues in length; Xaa_{n2} is meant to denote an amino acid sequence that is zero or one residue in length; and Xaa_{n3} is meant to denote an amino acid sequence zero, one, two, three, four, five or six residues in length. Additionally, any amino acid represented by Xaa, Xaa_{n1}, Xaa_{n2}, or Xaa_{n3} may be an L-amino acid, a D-amino acid, a methylated amino acid or any combination of thereof. Optionally, any GCC agonist peptide represented by Formulas I to XX in the tables may contain one or more polyethylene glycol residues at the the N-terminus, C-terminus or both.

[80] In certain embodiments, a GCC agonist formulation of the invention comprises a peptide selected from SEQ ID NOs: 1-249, the sequences of which are set forth below in Tables I to VII below. In one embodiment, a GCC agonist formulation comprises the peptide designated by SEQ ID NOs:1, 8, 9, 55, or 56.

[81] In certain embodiments, a GCC agonist formulation of the invention comprises a peptide that is substantially equivalent to a peptide selected from SEQ ID NOs: 1-249. The term “substantially equivalent” refers to a peptide that has an amino acid sequence equivalent to that of the binding domain where certain residues may be deleted or replaced with other amino acids without impairing the peptide’s ability to bind to an intestinal guanylate cyclase receptor and stimulate fluid and electrolyte transport.

1.2.1 GCC Agonist Peptides

[82] In a preferred embodiment, the GCC agonists for use in the formulations and methods of the invention are GCC agonist peptides. In certain embodiments, the GCC agonist peptides are analogues of uroguanylin or a bacterial ST peptide. Uroguanylin is a circulating peptide hormone with natriuretic activity. An ST peptide is a member of a family of heat stable enterotoxins (ST peptides) secreted by pathogenic strains of *E. coli* and other enteric

bacteria that activate guanylate cyclase receptor and cause secretory diarrhea. Unlike bacterial ST peptides, the binding of uroguanylin to guanylate cyclase receptor is dependent on the physiological pH of the gut. Therefore, uroguanylin is expected to regulate fluid and electrolyte transport in a pH dependent manner and without causing severe diarrhea.

[83] The GCC agonist peptides for use in the formulations and methods of the invention can be polymers of L-amino acids, D-amino acids, or a combination of both. For example, in various embodiments, the peptides are D retro-inverso peptides. The term “retro-inverso isomer” refers to an isomer of a linear peptide in which the direction of the sequence is reversed and the chirality of each amino acid residue is inverted. *See, e.g., Jameson et al., Nature, 368, 744-746 (1994); Brady et al., Nature, 368, 692-693 (1994).* The net result of combining D-enantiomers and reverse synthesis is that the positions of carbonyl and amino groups in each amide bond are exchanged, while the position of the side-chain groups at each alpha carbon is preserved. Unless specifically stated otherwise, it is presumed that any given L-amino acid sequence of the invention may be made into a D retro-inverso peptide by synthesizing a reverse of the sequence for the corresponding native L-amino acid sequence.

[84] The GCC agonist peptides for use in the formulations and methods of the invention are able to induce intracellular cGMP production in cells and tissues expressing guanylate cyclase C. In certain embodiments, the GCC agonist peptide stimulates 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to naturally occurring GCC agonists such as uroguanylin, guanylin, or ST peptides. Optionally, the GCC agonist peptide stimulates 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared SP-304 (SEQ ID NO:1). In further embodiments, the GCC agonist peptide stimulates apoptosis, *e.g.*, programmed cell death, or activate the cystic fibrosis transmembrane conductance regulator (CFTR).

[85] In some embodiments, the GCC agonist peptides for use in the formulations and methods of the invention are more stable than naturally occurring GCC agonists and/or SP-304 (SEQ ID NO:1), SP-339 (linaclotide) (SEQ ID NO: 55) or SP-340 (SEQ ID NO: 56). For example, the GCC agonist peptide degrades 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GCC agonists and/or SP-304, SP-339 (linaclotide) or SP-340. In certain embodiments, the GCC agonist peptides for use in the formulations and methods of the invention are more stable to proteolytic digestion than

naturally occurring GCC agonists and/or SP-304 (SEQ ID NO:1), SP-339 (linaclotide) (SEQ ID NO: 55) or SP-340 (SEQ ID NO: 56). In one embodiment, a GCC agonist peptide is pegylated in order to render the peptides more resistant towards proteolysis by enzymes of the gastrointestinal tract. In a preferred embodiment, the GCC agonist peptide is pegylated with the aminoethoxy-ethoxy-acetic acid (Aeea) group at its C-terminal end, at its N-terminal end, or at both termini.

[86] Specific examples of GCC agonist peptides that can be used in the methods and formulations of the invention include a peptide selected from the group designated by SEQ ID NOs: 1-249.

[87] In one embodiment, the GCC agonist peptide is a peptide having the amino acid sequence of any one of Formulas X- XVII (*e.g.* SEQ ID NO:87-98).

[88] In some embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula I, wherein at least one amino acid of Formula I is a D-amino acid or a methylated amino acid and/or the amino acid at position 16 is a serine. Preferably, the amino acid at position 16 of Formula I is a D-amino acid or a methylated amino acid. For example, the amino acid at position 16 of Formula I is a d-leucine or a d-serine. Optionally, one or more of the amino acids at positions 1-3 of Formula I are D-amino acids or methylated amino acids or a combination of D-amino acids or methylated amino acids. For example, Asn¹, Asp² or Glu³ (or a combination thereof) of Formula I is a D-amino acid or a methylated amino acid. Preferably, the amino acid at position Xaa⁶ of Formula I is a leucine, serine or tyrosine.

[89] In alternative embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula II, wherein at least one amino acid of Formula II is a D-amino acid or a methylated amino acid. Preferably, the amino acid denoted by Xaa_{n2} of Formula II is a D-amino acid or a methylated amino acid. In some embodiments, the amino acid denoted by Xaa_{n2} of Formula II is a leucine, a d-leucine, a serine, or a d-serine. Preferably, the one or more amino acids denoted by Xaa_{n1} of Formula II is a D-amino acid or a methylated amino acid. Preferably, the amino acid at position Xaa⁶ of Formula II is a leucine, a serine, or a tyrosine.

[90] In some embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula III, wherein at least one amino acid of Formula III is a D-amino acid or a methylated amino acid and/or Maa is not a cysteine. Preferably, the amino acid denoted by Xaa_{n2} of Formula III is a D-amino acid or a methylated amino acid. In some embodiments the amino acid denoted by Xaa_{n2} of Formula III is a leucine, a d-leucine, a serine, or a d-serine. Preferably, the one or more amino acids denoted by Xaa_{n1} of Formula III is a D-amino acid or a methylated amino acid. Preferably, the amino acid at position Xaa⁶ of Formula III is a leucine, a serine, or a tyrosine.

[91] In other embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula IV, wherein at least one amino acid of Formula IV is a D-amino acid or a methylated amino acid, and/or Maa is not a cysteine. Preferably, the Xaa_{n2} of Formula IV is a D-amino acid or a methylated amino acid. In some embodiments, the amino acid denoted by Xaa_{n2} of Formula IV is a leucine, a d-leucine, a serine, or a d-serine. Preferably, the one or more of the amino acids denoted by Xaa_{n1} of Formula IV is a D-amino acid or a methylated amino acid. Preferably, the amino acid denoted Xaa⁶ of Formula IV is a leucine, a serine, or a tyrosine.

[92] In further embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula V, wherein at least one amino acid of Formula V is a D-amino acid or a methylated amino acid. Preferably, the amino acid at position 16 of Formula V is a D-amino acid or a methylated amino acid. For example, the amino acid at position 16 (i.e., Xaa¹⁶) of Formula V is a d-leucine or a d-serine. Optionally, one or more of the amino acids at position 1-3 of Formula V are D-amino acids or methylated amino acids or a combination of D-amino acids or methylated amino acids. For example, Asn¹, Asp² or Glu³ (or a combination thereof) of Formula V is a D-amino acid or a methylated amino acid. Preferably, the amino acid denoted at Xaa⁶ of Formula V is a leucine, a serine, or a tyrosine.

[93] In additional embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula VI, VII, VIII, or IX. Preferably, the amino acid at position 6 of Formula VI, VII, VIII, or IX is a leucine, a serine, or a tyrosine. In some aspects the amino acid at position 16 of Formula VI, VII, VIII, or IX is a leucine or a serine. Preferably, the amino acid at position 16 of Formula V is a D-amino acid or a methylated amino acid.

[94] In additional embodiments, GCC agonist peptides include peptides having the amino acid sequence of Formula X, XI, XII, XIII, XIV, XV, XVI or XVII. Optionally, one or more amino acids of Formulas X, XI, XII, XIII, XIV, XV, XVI or XVII is a D-amino acid or a methylated amino acid. Preferably, the amino acid at the carboxy terminus of the peptides according to Formulas X, XI, XII, XIII, XIV, XV, XVI or XVII is a D-amino acid or a methylated amino acid. For example the the amino acid at the carboxy terminus of the peptides according to Formulas X, XI, XII, XIII, XIV, XV, XVI or XVII is a D-tyrosine.

[95] Preferably, the amino acid denoted by Xaa⁶ of Formula XIV is a tyrosine, phenylalanine or a serine. Most preferably the amino acid denoted by Xaa⁶ of Formula XIV is a phenylalanine or a serine. Preferably, the amino acid denoted by Xaa⁴ of Formula XV, XVI or XVII is a tyrosine, a phenylalanine, or a serine. Most preferably, the amino acid position Xaa⁴ of Formula V, XVI or XVII is a phenylalanine or a serine.

[96] In some embodiments, GCRA peptides include peptides containing the amino acid sequence of Formula XVIII. Preferably, the amino acid at position 1 of Formula XVIII is a glutamic acid, aspartic acid, glutamine or lysine. Preferably, the amino acid at position 2 and 3 of Formula XVIII is a glutamic acid, or an aspartic acid. Preferably, the amino acid at position 5 a glutamic acid. Preferably, the amino acid at position 6 of Formula XVIII is an isoleucine, valine, serine, threonine or tyrosine. Preferably, the amino acid at position 8 of Formula XVIII is a valine or isoleucine. Preferably, the amino acid at position 9 of Formula XVIII is a an asparagine. Preferably, the amino acid at position 10 of Formula XVIII is a valine or an methionine. Preferably, the amino acid at position 11 of Formula XVIII is an alanine. Preferably, the amino acid at position 13 of Formula XVIII is a threonine. Preferably, the amino acid at position 14 of Formula XVIII is a glycine. Preferably, the amino acid at position 16 of Formula XVIII is a leucine, serine or threonine

[97] In alternative embodiments, GCRA peptides include peptides containing the amino acid sequence of Formula XIX. Preferably, the amino acid at position 1 of Formula XIX is a serine or asparagine. Preferably, the amino acid at position 2 of Formula XIX is a histidine or an aspartic acid. Preferably, the amino acid at position 3 of Formula XIX is a threonine or a glutamic acid. Preferably, the amino acid at position 5 of Formula XIX is a glutamic acid. Preferably, the amino acid at position 6 of Formula XIX is an isoleucine, leucine, valine or tyrosine. Preferably, the amino acid at position 8, 10, 11, or 13 of Formula XIX is a alanine.

Preferably, the amino acid at position 9 of Formula XIX is an asparagine or a phenylalanine. Preferably, the amino acid at position 14 of Formula XIX is a glycine.

[98] In further embodiments, GCRA peptides include peptides containing the amino acid sequence of Formula XX. Preferably, the amino acid at position 1 of Formula XX is a glutamine. Preferably, the amino acid at position 2 or 3 of Formula XX is a glutamic acid or an aspartic acid. Preferably, the amino acid at position 5 of Formula XX is a glutamic acid. Preferably, the amino acid at position 6 of Formula XX is threonine, glutamine, tyrosine, isoleucine, or leucine. Preferably, the amino acid at position 8 of Formula XX is isoleucine or valine. Preferably, the amino acid at position 9 of Formula XX is asparagine. Preferably, the amino acid at position 10 of Formula XX is methionine or valine. Preferably, the amino acid at position 11 of Formula XX is alanine. Preferably, the amino acid at position 13 of Formula XX is a threonine. Preferably, the amino acid at position 15 of Formula XX is a tyrosine. Optionally, the amino acid at position 15 of Formula XX is two amino acid in length and is Cysteine (Cys), Penicillamine (Pen) homocysteine, or 3-mercaptoproline and serine, leucine or threonine.

[99] In certain embodiments, one or more amino acids of the GCC agonist peptides are replaced by a non-naturally occurring amino acid or a naturally or non-naturally occurring amino acid analog. Such amino acids and amino acid analogs are known in the art. See, for example, Hunt, "The Non-Protein Amino Acids," in *Chemistry and Biochemistry of the Amino Acids*, Barrett, Chapman and Hall, 1985. In some embodiments, an amino acid is replaced by a naturally-occurring, non-essential amino acid, e.g., taurine. Non-limiting examples of naturally occurring amino acids that can be replaced by non-protein amino acids include the following: (1) an aromatic amino acid can be replaced by 3,4-dihydroxy-L-phenylalanine, 3-iodo-L-tyrosine, triiodothyronine, L-thyroxine, phenylglycine (Phg) or nor-tyrosine (norTyr); (2) Phg and norTyr and other amino acids including Phe and Tyr can be substituted by, e.g., a halogen, -CH₃, -OH, -CH₂NH₃, -C(O)H, -CH₂CH₃, -CN, -CH₂CH₂CH₃, -SH, or another group; (3) glutamine residues can be substituted with gamma-Hydroxy-Glu or gamma-Carboxy-Glu; (4) tyrosine residues can be substituted with an alpha substituted amino acid such as L-alpha-methylphenylalanine or by analogues such as: 3-Amino-Tyr; Tyr(CH₃); Tyr(PO₃(CH₃)₂); Tyr(SO₃H); beta-Cyclohexyl-Ala; beta-(1-Cyclopentenyl)-Ala; beta-Cyclopentyl-Ala; beta-Cyclopropyl-Ala; beta-Quinolyl-Ala; beta-

(2-Thiazolyl)-Ala; beta- (Triazole-1-yl)-Ala; beta-(2-Pyridyl)-Ala; beta-(3-Pyridyl)-Ala; Amino-Phe; Fluoro-Phe; Cyclohexyl-Gly; tBu-Gly; beta-(3-benzothienyl)-Ala; beta-(2-thienyl)-Ala; 5-Methyl-Trp; and A- Methyl-Trp; (5) proline residues can be substituted with homopro (L-pipecolic acid); hydroxy-Pro; 3,4-Dehydro-Pro; 4-fluoro-Pro; or alpha-methyl-Pro or an N(alpha)-C(alpha) cyclized amino acid analogues with the structure: n = 0, 1, 2, 3; and (6) alanine residues can be substituted with alpha-substituted or N-methylated amino acid such as alpha-amino isobutyric acid (aib), L/D-alpha-ethylalanine (L/D-isovaline), L/D-methylvaline, or L/D-alpha-methylleucine or a non-natural amino acid such as beta-fluoro-Ala. Alanine can also be substituted with: n = 0, 1, 2, 3 Glycine residues can be substituted with alpha-amino isobutyric acid (aib) or L/D-alpha- ethylalanine (L/D-isovaline).

[100] Further examples of non-natural amino acids include: an unnatural analog of tyrosine; an unnatural analogue of glutamine; an unnatural analogue of phenylalanine; an unnatural analogue of serine; an unnatural analogue of threonine; an alkyl, aryl, acyl, azido, cyano, halo, hydrazine, hydrazide, hydroxyl, alkenyl, alkynyl, ether, thiol, sulfonyl, seleno, ester, thioacid, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, hydroxylamine, keto, or amino substituted amino acid, or any combination thereof; an amino acid with a photoactivatable cross-linker; a spin-labeled amino acid; a fluorescent amino acid; an amino acid with a novel functional group; an amino acid that covalently or noncovalently interacts with another molecule; a metal binding amino acid; an amino acid that is amidated at a site that is not naturally amidated, a metal-containing amino acid; a radioactive amino acid; a photocaged and/or photoisomerizable amino acid; a biotin or biotin-analogue containing amino acid; a glycosylated or carbohydrate modified amino acid; a keto containing amino acid; amino acids comprising polyethylene glycol or polyether; a heavy atom substituted amino acid (*e.g.*, an amino acid containing deuterium, tritium, ¹³C, ¹⁵N, or ¹⁸O); a chemically cleavable or photocleavable amino acid; an amino acid with an elongated side chain; an amino acid containing a toxic group; a sugar substituted amino acid, *e.g.*, a sugar substituted serine or the like; a carbon-linked sugar-containing amino acid; a redox-active amino acid; an α -hydroxy containing acid; an amino thio acid containing amino acid; an α , α disubstituted amino acid; a β - amino acid; a cyclic amino acid other than proline; an O-methyl-L-tyrosine; an L-3-(2- naphthyl)alanine; a 3-methyl-phenylalanine; a p-acetyl-L-phenylalanine; an O-4-allyl-L-tyrosine; a 4-propyl-L-tyrosine; a tri-O-acetyl-GlcNAc β -serine; an L-Dopa; a fluorinated phenylalanine; an isopropyl-L-phenylalanine; a p-azido-L-

phenylalanine; a p-acyl-L-phenylalanine; a p- benzoyl-L-phenylalanine; an L-phosphoserine; a phosphoserine; a phosphotyrosine; a p- iodo-phenylalanine; a 4-fluorophenylglycine; a p-bromophenylalanine; a p-amino-L- phenylalanine; an isopropyl-L-phenylalanine; L-3-(2-naphthyl)alanine; D- 3-(2-naphthyl)alanine (dNal); an amino-, isopropyl-, or O-allyl-containing phenylalanine analogue; a dopa, 0-methyl-L-tyrosine; a glycosylated amino acid; a p-(propargyloxy)phenylalanine; dimethyl-Lysine; hydroxy-proline; mercaptopropionic acid; methyl-lysine; 3-nitro-tyrosine; norleucine; pyro-glutamic acid; Z (Carbobenzoyl); ϵ -Acetyl-Lysine; β -alanine; aminobenzoyl derivative; aminobutyric acid (Abu); citrulline; aminohexanoic acid; aminoisobutyric acid (AIB); cyclohexylalanine; d-cyclohexylalanine; hydroxyproline; nitro-arginine; nitro-phenylalanine; nitro-tyrosine; norvaline; octahydroindole carboxylate; ornithine (Orn); penicillamine (PEN); tetrahydroisoquinoline; acetamidomethyl protected amino acids and pegylated amino acids. Further examples of unnatural amino acids and amino acid analogs can be found in U.S. 20030108885, U.S. 20030082575, US20060019347 (paragraphs 410-418) and the references cited therein. The polypeptides of the invention can include further modifications including those described in US20060019347, paragraph 589. Exemplary GCC agonist peptides which include a non-naturally occurring amino acid include for example SP-368 and SP-369.

[101] In some embodiments, the GCC agonist peptides are cyclic peptides. GCC agonist cyclic peptides can be prepared by methods known in the art. For example, macrocyclization is often accomplished by forming an amide bond between the peptide N- and C-termini, between a side chain and the N- or C-terminus [*e.g.*, with $K_3Fe(CN)_6$ at pH 8.5] (Samson *et al.*, *Endocrinology*, 137: 5182-5185 (1996)), or between two amino acid side chains, such as cysteine. See, *e.g.*, DeGrado, *Adv Protein Chem*, 39: 51-124 (1988). In various embodiments, the GCC agonist peptides are [4,12; 7,15] bicycles.

[102] In certain embodiments, one or both Cys residues which normally form a disulfide bond in a GCC agonist peptide are replaced with homocysteine, penicillamine, 3-mercaptoproline (Kolodziej *et al.* 1996 *Int. J. Pept. Protein Res.* 48:274), β , β dimethylcysteine (Hunt *et al.* 1993 *Int. J. Pept. Protein Res.* 42:249), or diaminopropionic acid (Smith *et al.* 1978 *J. Med. Chem.* 21:117) to form alternative internal cross-links at the positions of the normal disulfide bonds.

[103] In certain embodiments, one or more disulfide bonds in a GCC agonist peptide are replaced by alternative covalent cross-links, *e.g.*, an amide linkage (-CH₂CH(O)NHCH₂- or -CH₂NHCH(O)CH₂-), an ester linkage, a thioester linkage, a lactam bridge, a carbamoyl linkage, a urea linkage, a thiourea linkage, a phosphonate ester linkage, an alkyl linkage (-CH₂CH₂CH₂CH₂-), an alkenyl linkage (-CH₂CH=CHCH₂-), an ether linkage (-CH₂CH₂OCH₂- or -CH₂OCH₂CH₂-), a thioether linkage (-CH₂CH₂SCH₂- or -CH₂SCH₂CH₂-), an amine linkage (-CH₂CH₂NHCH₂- or -CH₂NHCH₂CH₂-) or a thioamide linkage (-CH₂CH(S)HNHCH₂- or -CH₂NHCH(S)CH₂-). For example, Ledu *et al.* (*Proc. Natl. Acad. Sci.* 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links. Exemplary GCC agonist peptides which include a lactam bridge include, for example, SP-370.

[104] In certain embodiments, the GCC agonist peptides have one or more conventional polypeptide bonds replaced by an alternative bond. Such replacements can increase the stability of the polypeptide. For example, replacement of the polypeptide bond between a residue amino terminal to an aromatic residue (*e.g.* Tyr, Phe, Trp) with an alternative bond can reduce cleavage by carboxy peptidases and may increase half-life in the digestive tract. Bonds that can replace polypeptide bonds include: a retro-inverso bond (C(O)-NH instead of NH-C(O)); a reduced amide bond (NH-CH₂); a thiomethylene bond (S-CH₂ or CH₂-S); an oxomethylene bond (O-CH₂ or CH₂-O); an ethylene bond (CH₂-CH₂); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); a fluoro substituted trans-olefine bond (CF=CH); a ketomethylene bond (C(O)-CHR or CHR-C(O) wherein R is H or CH₃); and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH₃).

[105] In certain embodiments, the GCC agonist peptides are modified using standard modifications. Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a combination of any of the preceding. In one aspect described herein, there may be more than one type of modification on the polypeptide. Modifications include but are not limited to: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearylation, succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cys3 or Cys5. The GCC agonist peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methylcoumarin (AMC), fluorescein, NBD (7-Nitrobenz-2-Oxa-1,3-Diazole), p-nitro-anilide,

rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-1- sulfonic acid), dabeyl, dabsyl, dansyl, texas red, Fmoc, and Tamra (Tetramethylrhodamine). The GCC agonist peptides described herein may also be conjugated to, for example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; combinations of PEG, alkyl groups and fatty acid radicals (*See*, U.S. Patent 6,309,633; Soltero et al., 2001 *Innovations in Pharmaceutical Technology* 106-110); BSA and KLH (Keyhole Limpet Hemocyanin). The addition of PEG and other polymers which can be used to modify polypeptides of the invention is described in US20060 19347 section IX.

[106] A GCC agonist peptide can also be a derivatives of a GCC agonist peptide described herein. For example, a derivative includes hybrid and modified forms of GCC agonist peptides in which certain amino acids have been deleted or replaced. A modification may also include glycosylation. Preferrably, where the modification is an amino acid substitution, it is a conservative substitution at one or more positions that are predicted to be non-essential amino acid residues for the biological activity of the peptide. A "conservative substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[107] In one embodiment, a GCC agonist peptide described herein is subjected to random mutagenesis in order to identify mutants having biological activity.

[108] In one embodiment, the GCC agonist peptide is substantially homologous is a GCC agonist peptide described herein. Such substantially homologous peptides can be isolated by virtue of cross-reactivity with antibodies to a GCC agonist peptide described herein.

[109] Further examples of GCC agonist peptides that can be used in the methods and formulations of the invention are found in Tables I - VII below.

1.2.2 Preparation of GCC agonist peptides

[110] GCC agonist peptides can be prepared using art recognized techniques such as molecular cloning, peptide synthesis, or site-directed mutagenesis.

[111] Peptide synthesis can be performed using standard solution phase or solid phase peptide synthesis techniques or a combination of both process where segments are synthesized by solid phase and condensed in solution phase, in which a peptide linkage occurs through the direct condensation of the amino group of one amino acid with the carboxy group of the other amino acid with the elimination of a water molecule. Peptide bond synthesis by direct condensation, as formulated above, requires suppression of the reactive character of the amino group of the first and of the carboxyl group of the second amino acid. The masking substituents must permit their ready removal, without inducing breakdown of the labile peptide molecule.

[112] In solution phase synthesis, a wide variety of coupling methods and protecting groups may be used (*See*, Gross and Meienhofer, eds., "The Peptides: Analysis, Synthesis, Biology," Vol. 1-4 (Academic Press, 1979); Bodansky and Bodansky, "The Practice of Peptide Synthesis," 2d ed. (Springer Verlag, 1994)). In addition, intermediate purification and linear scale up are possible. Those of ordinary skill in the art will appreciate that solution synthesis requires consideration of main chain and side chain protecting groups and activation method. In addition, careful segment selection is necessary to minimize racemization during segment condensation. Solubility considerations are also a factor. Solid phase peptide synthesis uses an insoluble polymer for support during organic synthesis. The polymer-supported peptide chain permits the use of simple washing and filtration steps instead of laborious purifications at intermediate steps. Solid-phase peptide synthesis may generally be performed according to the method of Merrifield et al., J. Am. Chem. Soc., 1963, 85:2149, which involves assembling a linear peptide chain on a resin support using protected amino acids. Solid phase peptide synthesis typically utilizes either the Boc or Fmoc strategy, which are well known in the art.

[113] Those of ordinary skill in the art will recognize that, in solid phase synthesis, deprotection and coupling reactions must go to completion and the side-chain blocking

groups must be stable throughout the synthesis. In addition, solid phase synthesis is generally most suitable when peptides are to be made on a small scale.

[114] Acetylation of the N-terminal can be accomplished by reacting the final peptide with acetic anhydride before cleavage from the resin. C-amidation is accomplished using an appropriate resin such as methylbenzhydrylamine resin using the Boc technology.

[115] Alternatively the GCC agonist peptides are produced by modern cloning techniques. For example, the GCC agonist peptides are produced either in bacteria including, without limitation, *E. coli*, or in other existing systems for polypeptide or protein production (*e.g.*, *Bacillus subtilis*, baculovirus expression systems using *Drosophila* Sf9 cells, yeast or filamentous fungal expression systems, mammalian cell expression systems), or they can be chemically synthesized. If the GCC agonist peptide or variant peptide is to be produced in bacteria, *e.g.*, *E. coli*, the nucleic acid molecule encoding the polypeptide may also encode a leader sequence that permits the secretion of the mature polypeptide from the cell. Thus, the sequence encoding the polypeptide can include the pre sequence and the pro sequence of, for example, a naturally-occurring bacterial ST polypeptide. The secreted, mature polypeptide can be purified from the culture medium.

[116] The sequence encoding a GCC agonist peptide described herein can be inserted into a vector capable of delivering and maintaining the nucleic acid molecule in a bacterial cell. The DNA molecule may be inserted into an autonomously replicating vector (suitable vectors include, for example, pGEM3Z and pcDNA3, and derivatives thereof). The vector nucleic acid may be a bacterial or bacteriophage DNA such as bacteriophage lambda or M13 and derivatives thereof. Construction of a vector containing a nucleic acid described herein can be followed by transformation of a host cell such as a bacterium. Suitable bacterial hosts include but are not limited to, *E. coli*, *B. subtilis*, *Pseudomonas*, *Salmonella*. The genetic construct also includes, in addition to the encoding nucleic acid molecule, elements that allow expression, such as a promoter and regulatory sequences. The expression vectors may contain transcriptional control sequences that control transcriptional initiation, such as promoter, enhancer, operator, and repressor sequences.

[117] A variety of transcriptional control sequences are well known to those in the art. The expression vector can also include a translation regulatory sequence (*e.g.*, an untranslated 5'

sequence, an untranslated 3' sequence, or an internal ribosome entry site). The vector can be capable of autonomous replication or it can integrate into host DNA to ensure stability during polypeptide production.

[118] The protein coding sequence that includes a GCC agonist peptide described herein can also be fused to a nucleic acid encoding a polypeptide affinity tag, *e.g.*, glutathione S-transferase (GST), maltose E binding protein, protein A, FLAG tag, hexa-histidine, myc tag or the influenza HA tag, in order to facilitate purification. The affinity tag or reporter fusion joins the reading frame of the polypeptide of interest to the reading frame of the gene encoding the affinity tag such that a translational fusion is generated. Expression of the fusion gene results in translation of a single polypeptide that includes both the polypeptide of interest and the affinity tag. In some instances where affinity tags are utilized, DNA sequence encoding a protease recognition site will be fused between the reading frames for the affinity tag and the polypeptide of interest.

[119] Genetic constructs and methods suitable for production of immature and mature forms of the GCC agonist peptides and variants described herein in protein expression systems other than bacteria, and well known to those skilled in the art, can also be used to produce polypeptides in a biological system.

[120] The peptides disclosed herein may be modified by attachment of a second molecule that confers a desired property upon the peptide, such as increased half-life in the body, for example, pegylation. Such modifications also fall within the scope of the term "variant" as used herein.

Table I. GCRA Peptides (SP-304 and Derivatives)

Name	Position of Disulfide bonds	Structure	SEQ ID NO
SP-304	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	1
SP-326	C3:C11, C6:C14	Asp ¹ -Glu ² -Cys ³ -Glu ⁴ -Leu ⁵ -Cys ⁶ -Val ⁷ -Asn ⁸ -Val ⁹ -Ala ¹⁰ -Cys ¹¹ -Thr ¹² -Gly ¹³ -Cys ¹⁴ -Leu ¹⁵	2
SP-327	C2:C10, C5:C13	Asp ¹ -Glu ² -Cys ³ -Glu ⁴ -Leu ⁵ -Cys ⁶ -Val ⁷ -Asn ⁸ -Val ⁹ -Ala ¹⁰ -Cys ¹¹ -Thr ¹² -Gly ¹³ -Cys ¹⁴	3
SP-328	C2:C10, C5:C13	Glu ¹ -Cys ² -Glu ³ -Leu ⁴ -Cys ⁵ -Val ⁶ -Asn ⁷ -Val ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Leu ¹⁴	4
SP-329	C2:C10, C5:C13	Glu ¹ -Cys ² -Glu ³ -Leu ⁴ -Cys ⁵ -Val ⁶ -Asn ⁷ -Val ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³	5
SP-330	C1:C9, C4:C12	Cys ¹ -Glu ² -Leu ³ -Cys ⁴ -Val ⁵ -Asn ⁶ -Val ⁷ -Ala ⁸ -Cys ⁹ -Thr ¹⁰ -Gly ¹¹ -Cys ¹² -Leu ¹³	6
SP-331	C1:C9, C4:C12	Cys ¹ -Glu ² -Leu ³ -Cys ⁴ -Val ⁵ -Asn ⁶ -Val ⁷ -Ala ⁸ -Cys ⁹ -Thr ¹⁰ -Gly ¹¹ -Cys ¹²	7
SP332	C4:C12,C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	8
SP-333	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	9
SP-334	C4:C12,C7:C15	dAsn ¹ -dAsp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	10
SP-335	C4:C12,C7:C15	dAsn ¹ -dAsp ² -dGlu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	11
SP-336	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	12
SP-337	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -dLeu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	13
SP-338	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵	14
SP-342	C4:C12, C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	15
SP-343	C4:C12, C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	16
SP-344	C4:C12, C7:C15	PEG3-dAsn ¹ -dAsp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	17
SP-347	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	18
SP-348	C4:C12, C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	19

SP-350	C4:C12, C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	20
SP-352	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	21
SP-358	C4:C12,C7:C15	PEG3-dAsn ¹ -dAsp ² -dGlu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	22
SP-359	C4:C12,C7:C15	PEG3-dAsn ¹ -dAsp ² -dGlu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	23
SP-360	C4:C12, C7:C15	dAsn ¹ -dAsp ² -dGlu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	24
SP-361	C4:C12, C7:C15	dAsn ¹ -dAsp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	25
SP-362	C4:C12, C7:C15	PEG3-dAsn ¹ -dAsp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	26
SP-368	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dNal ¹⁶	27
SP-369	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -AIB ⁸ -Asn ⁹ -AIB ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	28
SP-370	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Asp[Lactam] ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Orn ¹⁵ -dLeu ¹	29
SP-371	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	30
SP-372	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	31
N1	C4:C12,C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	32
N2	C4:C12,C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	33
N3	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ PEG3	34
N4	C4:C12,C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	35
N5	C4:C12,C7:C15	PEG3-dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶	36
N6	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu ¹⁶ -PEG3	37
N7	C4:C12,C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	38
N8	C4:C12,C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶ -PEG3	39
N9	C4:C12,C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	40
N10	C4:C12,C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶ -PEG3	41

N11	C4:C12,C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dSer ¹⁶ -PEG3	42
N12	C4:C12,C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dSer ¹⁶	43
N13	C4:C12,C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dSer ¹⁶ -PEG3	44
Formula I	C4:C12,C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -Xaa ¹⁶	45
Formula II	C4:C12,C7:C15	Xaa _{n1} -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -Xaa _{n2} ¹⁶	46
Formula III	4:12,7:15	Xaa _{n1} -Maa ⁴ -Glu ⁵ -Xaa ⁶ -Maa ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Maa ¹² -Thr ¹³ -Gly ¹⁴ -Maa ¹⁵ -Xaa _{n2}	47
Formula IV	4:12,7:15	Xaa _{n1} -Maa ⁴ -Xaa ⁵ -Xaa ⁶ -Maa ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Maa ¹² -Xaa ¹³ -Xaa ¹⁴ -Maa ¹⁵ -Xaa _{n2}	48
Formula V	C4:C12,C7:C15	Asn ¹ -Asp ² -Asp ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Asn ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -Xaa ¹⁶	49
Formula VI	C4:C12,C7:C15	dAsn ¹ -Glu ² -Glu ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -X ³ ⁸ -Asn ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -d-Xaa ¹⁶	50
Formula VII	C4:C12,C7:C15	dAsn ¹ -dGlu ² -Asp ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Asn ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -d-Xaa ¹⁶	51
Formula VII	C4:C12,C7:C15	dAsn ¹ -dAsp ² -Glu ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Asn ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -d-Xaa ¹⁶	52
Formula VIII	C4:C12,C7:C15	dAsn ¹ -dAsp ² -dGlu ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Tyr ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -d-Xaa ¹⁶	53
Formula IX	C4:C12,C7:C15	dAsn ¹ -dGlu ² -dGlu ³ -Cys ⁴ -Xaa ⁵ -Xaa ⁶ -Cys ⁷ -Xaa ⁸ -Tyr ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -d-Xaa ¹⁶	54

Table II. Linaclotide and Derivatives

Name	Position of Disulfide bonds	Structure	SEQ ID NO:
SP-339 (linaclotide)	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴	55
SP-340	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³	56
SP-349	C1:C6, C2:C10, C5:13	PEG3-Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴ -PEG3	57
SP-353	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	58
SP-354	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	59
SP-355	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -dTyr ¹⁴	60
SP-357	C1:C6, C2:C10, C5:13	PEG3-Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴	61
SP-374	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	62
SP-375	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	63
SP-376	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	64
SP-377	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	65
SP-378	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	66
SP-379	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	67
SP-380	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	68
SP-381	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	69

SP-382	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	70
SP-383	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	71
SP384	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴ -PEG3	72
N14	C1:C6, C2:C10, C5:13	PEG3-Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -PEG3	73
N15	C1:C6, C2:C10, C5:13	PEG3-Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³	74
N16	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Tyr ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -PEG3	75
N17	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	76
N18	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	77
N19	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Ser ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	78
N20	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	79
N21	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	80
N22	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	81
N23	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	82

N24	C3:C8, C4:C12, C7:15	PEG3- Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	83
N25	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	84
N26	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Ser ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴	85
N27	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Phe ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -Tyr ¹⁴	86
N28	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Ser ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³ -	87
N29	C1:C6, C2:C10, C5:13	Cys ¹ -Cys ² -Glu ³ -Phe ⁴ -Cys ⁵ -Cys ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Cys ¹⁰ -Thr ¹¹ -Gly ¹² -Cys ¹³	88
N30	1:6, 2:10, 5:13	Pen ¹ -Pen ² -Glu ³ -Tyr ⁴ -Pen ⁵ -Pen ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Pen ¹⁰ -Thr ¹¹ -Gly ¹² -Pen ¹³ -Tyr ¹⁴	89
N31	1:6, 2:10, 5:13	Pen ¹ -Pen ² -Glu ³ -Tyr ⁴ -Pen ⁵ -Pen ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Pen ¹⁰ -Thr ¹¹ -Gly ¹² -Pen ¹³	90
Formula X	C9:C14, C10:C18, C13:21	Xaa ¹ -Xaa ² -Xaa ³ -Xaa ⁴ -Xaa ⁵ -Xaa ⁶ -Asn ⁷ -Tyr ⁸ -Cys ⁹ -Cys ¹⁰ -Xaa ¹¹ -Tyr ¹² -Cys ¹³ -Cys ¹⁴ -Xaa ¹⁵ -Xaa ¹⁶ -Xaa ¹⁷ -Cys ¹⁸ -Xaa ¹⁹ -Xaa ²⁰ -Cys ²¹ -Xaa ²²	91
Formula XI	C9:C14, C10:C18, C13:21	Xaa ¹ -Xaa ² -Xaa ³ -Xaa ⁴ -Xaa ⁵ -Xaa ⁶ -Asn ⁷ -Phe ⁸ -Cys ⁹ -Cys ¹⁰ -Xaa ¹¹ -Phe ¹² -Cys ¹³ -Cys ¹⁴ -Xaa ¹⁵ -Xaa ¹⁶ -Xaa ¹⁷ -Cys ¹⁸ -Xaa ¹⁹ -Xaa ²⁰ -Cys ²¹ -Xaa ²²	92
Formula XII	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Xaa ⁵ -Phe ⁶ -Cys ⁷ -Cys ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ³ -Xaa ¹⁴ -Cys ¹⁵ -Xaa ¹⁶	93
Formula XIII	3:8, 4:12, C:15	Asn ¹ -Phe ² -Pen ³ -Cys ⁴ -Xaa ⁵ -Phe ⁶ -Cys ⁷ -Pen ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Cys ¹² -Xaa ¹³ -Xaa ¹⁴ -Cys ¹⁵ -Xaa ¹⁶	94
Formula XIV	3:8, 4:12, 7:15	Asn ¹ -Phe ² -Maa ³ -Maa ⁴ -Xaa ⁵ -Xaa ⁶ -Maa ⁷ -Maa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Maa ¹² -Xaa ¹³ -Xaa ¹⁴ -Maa ¹⁵ -Xaa ¹⁶	95
Formula XV	1:6, 2:10, 5:13	Maa ¹ -Maa ² -Glu ³ -Xaa ⁴ -Maa ⁵ -Maa ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Maa ¹⁰ -Thr ¹¹ -Gly ¹² -Maa ¹³ -Tyr ¹⁴	96
Formula XVI	1:6, 2:10, 5:13	Maa ¹ -Maa ² -Glu ³ -Xaa ⁴ -Maa ⁵ -Maa ⁶ -Asn ⁷ -Pro ⁸ -Ala ⁹ -Maa ¹⁰ -Thr ¹¹ -Gly ¹² -Maa ¹³ -	97
Formula XVII	1:6, 2:10, 5:13	Xaa _{n3} ¹ -Maa ¹ -Maa ² -Xaa ³ -Xaa ⁴ -Maa ⁵ -Maa ⁶ -Xaa ⁷ -Xaa ⁸ -Xaa ⁹ -Maa ¹⁰ -Xaa ¹¹ -Xaa ¹² -Maa ¹³ -Xaa _{n2}	98

Table III. GCRA Peptides

Name	Position of Disulfide bonds	Structure	SEQ ID NO:
SP-363	C4:C12,C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu-AMIDE ¹⁶	99
SP-364	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dSer ¹⁶	100
SP-365	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dSer-AMIDE ¹⁶	101
SP-366	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	102
SP-367	C4:C12, C7:C15	dAsn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr-AMIDE ¹⁶	103
SP-373	C4:C12, C7:C15	Pyglu ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dLeu-AMIDE ¹⁶	104
SP-304 di PEG	C4:C12, C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶ -PEG3	105
SP-304 N- PEG	C4:C12, C7:C15	PEG3-Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	106
SP-304 C- PEG	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶ -PEG3	107

Table IV. SP-304 Analogs, Uroguanylin , and Uroguanylin Analogs

Name	Position of Disulfide bonds	Structure	SEQ ID NO
Formula XVIII	C4:C12, C7:C15	Xaa ¹ -Xaa ² -Xaa ³ -Maa ⁴ -Xaa ⁵ -Xaa ⁶ -Maa ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Maa ¹² -Xaa ¹³ -Xaa ¹⁴ -Maa ¹⁵ -Xaa ¹⁶	108
Uroguanylin	C4:C12, C7:C15	Asn ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	109
N32	C4:C12, C7:C15	Glu ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	110
N33	C4:C12, C7:C15	Glu ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	111
N34	C4:C12, C7:C15	Glu ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	112
N35	C4:C12, C7:C15	Glu ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	113
N36	C4:C12, C7:C15	Asp ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	114
N37	C4:C12, C7:C15	Asp ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	115
N38	C4:C12,	Asp ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	116

	C7:C15		
N39	C4:C12, C7:C15	Asp ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	117
N40	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	118
N41	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	119
N42	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	120
N43	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	121
N44	C4:C12, C7:C15	Lys ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	122
N45	C4:C12, C7:C15	Lys ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	123
N46	C4:C12, C7:C15	Lys ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	124
N47	C4:C12, C7:C15	Lys ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	125
N48	C4:C12, C7:C15	Glu ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	126
N49	C4:C12, C7:C15	Glu ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	127

N50	C4:C12, C7:C15	Glu ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	128
N51	C4:C12, C7:C15	Glu ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	129
N52	C4:C12, C7:C15	Asp ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	130
N53	C4:C12, C7:C15	Asp ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	131
N54	C4:C12, C7:C15	Asp ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	132
N55	C4:C12, C7:C15	Asp ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	133
N56	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	134
N57	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	135
N58	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	136
N59	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	137
N60	C4:C12, C7:C15	Lys ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	138
N61	C4:C12,	Lys ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	139

	C7:C15		
N62	C4:C12, C7:C15	Lys ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	140
N63	C4:C12, C7:C15	Lys ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Val ⁸ -Asn ⁹ -Val ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	141
N65	C4:C12, C7:C15	Glu ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	142
N66	C4:C12, C7:C15	Glu ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	143
N67	C4:C12, C7:C15	Glu ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	144
N68	C4:C12, C7:C15	Glu ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	145
N69	C4:C12, C7:C15	Asp ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	146
N70	C4:C12, C7:C15	Asp ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	147
N71	C4:C12, C7:C15	Asp ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	148
N72	C4:C12, C7:C15	Asp ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	149
N73	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	150

N74	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	151
N75	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	152
N76	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	153
N77	C4:C12, C7:C15	Lys ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	154
N78	C4:C12, C7:C15	Lys ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	155
N79	C4:C12, C7:C15	Lys ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	156
N80	C4:C12, C7:C15	Lys ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Leu ¹⁶	157
N81	C4:C12, C7:C15	Glu ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	158
N82	C4:C12, C7:C15	Glu ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	159
N83	C4:C12, C7:C15	Glu ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	160
N84	C4:C12, C7:C15	Glu ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	161
N85	C4:C12,	Asp ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	162

	C7:C15		
N86	C4:C12, C7:C15	Asp ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	163
N87	C4:C12, C7:C15	Asp ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	164
N88	C4:C12, C7:C15	Asp ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	165
N89	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	166
N90	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	167
N91	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	168
N92	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	169
N93	C4:C12, C7:C15	Lys ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	170
N94	C4:C12, C7:C15	Lys ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	171
N95	C4:C12, C7:C15	Lys ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	172
N96	C4:C12, C7:C15	Lys ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	173

Table V. Guanylin and Analogs

Name	Position of Disulfide bonds	Structure	SEQ ID NO
Formula XIX	4:12,7:15	Xaa ¹ -Xaa ² -Xaa ³ -Maa ⁴ -Xaa ⁵ -Xaa ⁶ -Maa ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Maa ¹² -Xaa ¹³ -Xaa ¹⁴ -Maa ¹⁵	174
Guanylin	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Phe ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	175
N97	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	176
N98	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	177
N99	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	178
N100	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	179
N101	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	180
N102	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	181
N103	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	182
N104	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	183
N105	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	184
N106	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	185
N107	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	186

N108	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	187
N109	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	188
N110	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	189
N111	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	190
N112	C4:C12, C7:C15	Ser ¹ -His ² -Thr ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	191
N113	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	192
N114	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	193
N115	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	194
N116	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	195
N117	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	196
N118	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	197
N119	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	198
N120	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	199
N121	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	200
N122	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	201
N123	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	202
N124	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	203

N125	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	204
N126	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	205
N127	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Val ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	206
N128	C4:C12, C7:C15	Asn ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ala ⁸ -Asn ⁹ -Ala ¹⁰ -Ala ¹¹ -Cys ¹² -Ala ¹³ -Gly ¹⁴ -Cys ¹⁵	207

Table VI. Lymphoguanin and Analogs

Name	Position of Disulfide bonds	Structure	SEQ ID NO
Formula XX	4:12,7:15	Xaa ¹ -Xaa ² -Xaa ³ -Maa ⁴ -Xaa ⁵ -Xaa ⁶ -Maa ⁷ -Xaa ⁸ -Xaa ⁹ -Xaa ¹⁰ -Xaa ¹¹ -Maa ¹² -Xaa ¹³ -Xaa ¹⁴ -Xaa _n ¹⁵	208
<u>Lymphoguanin</u>	C4:C12	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Leu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	209
N129	C4:C12	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	210
N130	C4:C12	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	211
N131	C4:C12	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	212
N132	C4:C12	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	213
N133	C4:C12	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	214

N134	C4:C12	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	215
N135	C4:C12	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	216
N136	C4:C12	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	217
N137	C4:C12	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	218
N138	C4:C12	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	219
N139	C4:C12	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	220
N140	C4:C12	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	221
N141	C4:C12	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	222
N142	C4:C12	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	223
N143	C4:C12	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	224
N144	C4:C12	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Tyr ¹⁵	225
N145	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	226
N146	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	227
N147	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	228
N148	C4:C12,	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	229

	C7:C15		
N149	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	230
N150	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser	231
N151	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	232
N152	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Glu ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	233
N153	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	234
N154	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	235
N155	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	236
N156	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	237
N157	C4:C12, C7:C15	Gln ¹ -Glu ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	238

N158	C4:C12, C7:C15	Gln ¹ -Asp ² -Glu ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	239
N159	C4:C12, C7:C15	Gln ¹ -Asp ² -Asp ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	240
N160	C4:C12, C7:C15	Gln ¹ -Glu ² -Asp ³ -Cys ⁴ -Glu ⁵ -Ile ⁶ -Cys ⁷ -Ile ⁸ -Asn ⁹ -Met ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Ser ¹⁶	241

Table VII. ST Peptide and Analogues

Name	Position of Disulfide bonds	Structure	SEQ ID NO
ST Peptide	C3:C8, C4:C12, C7:15	Asn ¹ -Ser ² -Ser ³ -Asn ⁴ -Ser ⁵ -Ser ⁶ -Asn ⁷ -Tyr ⁸ -Cys ⁹ -Cys ¹⁰ -Glu ¹¹ -Lys ¹² -Cys ¹³ -Cys ¹⁴ -Asn ¹⁵ -Pro ¹⁶ -Ala ¹⁷ -Cys ¹⁸ -Thr ¹⁹ -Gly ²⁰ -Cys ²¹ -Tyr ²²	242
N161	C3:C8, C4:C12, C7:15	PEG3-Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	243
N162	C3:C8, C4:C12, C7:15	PEG3-Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	244
N163	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Thr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶ -PEG3	245
N164	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	246

N165	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	247
N166	C3:C8, C4:C12, C7:15	Asn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -dTyr ¹⁶	248
N167	C3:C8, C4:C12, C7:15	dAsn ¹ -Phe ² -Cys ³ -Cys ⁴ -Glu ⁵ -Tyr ⁶ -Cys ⁷ -Cys ⁸ -Asn ⁹ -Pro ¹⁰ -Ala ¹¹ -Cys ¹² -Thr ¹³ -Gly ¹⁴ -Cys ¹⁵ -Tyr ¹⁶	249

1.3 Methods of Use

[121] The invention provides methods for treating or preventing gastrointestinal disorders and increasing gastrointestinal motility in a subject in need thereof by administering an effective amount of a GCC agonist formulation to the subject. Non-limiting examples of gastrointestinal disorders that can be treated or prevented according to the methods of the invention include irritable bowel syndrome (IBS), non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease (GERD), ileus (*e.g.*, post-operative ileus), gastroparesis, heartburn (high acidity in the GI tract), constipation (*e.g.*, constipation associated with use of medications such as opioids, osteoarthritis drugs, or osteoporosis drugs); post surgical constipation, constipation associated with neuropathic disorders, Crohn's disease, and ulcerative colitis.

[122] In one embodiment, the invention provides methods for treating or preventing gastrointestinal motility disorder, irritable bowel syndrome, a functional gastrointestinal disorder, gastroesophageal reflux disease, duodenogastric reflux, functional heartburn, dyspepsia, functional dyspepsia, nonulcer dyspepsia, gastroparesis, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, obesity, congestive heart failure, or benign prostatic hyperplasia.

[123] In one embodiment, the invention provides methods for treating or preventing constipation and/or increasing gastrointestinal motility in a subject in need thereof by administering an effective amount of a GCC agonist formulation to the subject. Clinically accepted criteria that define constipation range from the frequency of bowel movements, the consistency of feces and the ease of bowel movement. One common definition of constipation is less than three bowel movements per week. Other definitions include abnormally hard stools or defecation that requires excessive straining (Schiller 2001 *Aliment Pharmacol Ther* 15:749-763). Constipation may be idiopathic (functional constipation or slow transit constipation) or secondary to other causes including neurologic, metabolic or endocrine disorders. These disorders include diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple sclerosis, Parkinson's disease, spinal cord lesions, Neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung disease and cystic fibrosis. Constipation may also be the result of

surgery or due to the use of drugs such as analgesics (like opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics.

[124] In various embodiments, the constipation is associated with use of a therapeutic agent; the constipation is associated with a neuropathic disorder; the constipation is postsurgical constipation; the constipation is associated with a gastrointestinal disorder; the constipation is idiopathic (functional constipation or slow transit constipation); the constipation is associated with neuropathic, metabolic or endocrine disorder (e.g., diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple Sclerosis, Parkinson's disease, spinal cord lesions, neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung disease or cystic fibrosis). Constipation may also be the result of surgery or due to the use of drugs such as analgesics (e.g., opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics.

[125] In one embodiment, the invention provides methods for treating or preventing chronic idiopathic constipation and increasing gastrointestinal motility in a subject in need thereof by administering an effective amount of a GCC agonist formulation to the subject.

[126] The term "treating" as used herein refers to a reduction, a partial improvement, amelioration, or a mitigation of at least one clinical symptom associated with the gastrointestinal disorders being treated. The term "preventing" refers to an inhibition or delay in the onset or progression of at least one clinical symptom associated with the gastrointestinal disorders to be prevented. The term "effective amount" as used herein refers to an amount that provides some improvement or benefit to the subject. In certain embodiments, an effective amount is an amount that provides some alleviation, mitigation, and/or decrease in at least one clinical symptom of the gastrointestinal disorder to be treated. In other embodiments, the effective amount is the amount that provides some inhibition or delay in the onset or progression of at least one clinical symptom associated with the gastrointestinal disorder to be prevented. The therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject. The term "subject" preferably refers to a human subject but may also refer to a non-human primate or other mammal preferably selected from among a mouse, a rat, a dog, a cat, a cow, a horse, or a pig.

[127] The invention also provides methods for treating gastrointestinal cancer in a subject in need thereof by administering an effective amount of a GCC agonist formulation to the subject. Non-limiting examples of gastrointestinal cancers that can be treated according to the methods of the invention include gastric cancer, esophageal cancer, pancreatic cancer, colorectal cancer, intestinal cancer, anal cancer, liver cancer, gallbladder cancer, or colon cancer.

[128] The invention also provides methods for treating lipid metabolism disorders, biliary disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders including cardiovascular disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, and obesity.

[129] Lipid metabolism disorders include, but are not limited to, dyslipidemia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, sitosterolemia, familial hypercholesterolemia, xanthoma, combined hyperlipidemia, lecithin cholesterol acyltransferase deficiency, tanger disease, abetalipoproteinemia, erectile dysfunction, fatty liver disease, and hepatitis.

[130] Biliary disorders include gallbladder disorders such as for example, gallstones, gall bladder cancer cholangitis, or primary sclerosing cholangitis; or bile duct disorders such as for example, cholecystitis, bile duct cancer or fascioliasis.

[131] Inflammatory disorders include tissue and organ inflammation such as kidney inflammation (e.g., nephritis), gastrointestinal system inflammation (e.g., Crohn's disease and ulcerative colitis); necrotizing enterocolitis (NEC); pancreatic inflammation (e.g., pancreatitis), lung inflammation (e.g., bronchitis or asthma) or skin inflammation (e.g., psoriasis, eczema).

[132] Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis.

[133] Cancer includes tissue and organ carcinogenesis including metastases such as for example gastrointestinal cancer, (e.g., gastric cancer, esophageal cancer, pancreatic cancer colorectal cancer, intestinal cancer, anal cancer, liver cancer, gallbladder cancer, or colon cancer; lung cancer; thyroid cancer; skin cancer (e.g., melanoma); oral cancer; urinary tract cancer (e.g. bladder cancer or kidney cancer); blood cancer (e.g. myeloma or leukemia) or prostate cancer.

[134] Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high triglycerides. Cardiovascular disorders include for example aneurysm, angina, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial infarction (heart attack), or peripheral vascular disease.

[135] Liver disorders include for example cirrhosis and fibrosis. In addition, GC-C agonist may also be useful to facilitate liver regeneration in liver transplant patients. Eye disorders include for example increased intra-ocular pressure, glaucoma, dry eyes retinal degeneration, disorders of tear glands or eye inflammation. Skin disorders include for example xerosis. Oral disorders include for example dry mouth (xerostomia), Sjögren's syndrome, gum diseases (e.g., periodontal disease), or salivary gland duct blockage or malfunction. Prostate disorders include for example benign prostatic hyperplasia (BPH). Endocrine disorders include for example diabetes mellitus, hyperthyroidism, hypothyroidism, and cystic fibrosis.

1.3.1 Therapeutically Effective Dosages

[136] Disorders are treated, prevented or alleviated by administering to a subject, *e.g.*, a mammal such as a human in need thereof, a therapeutically effective dose of a GCC agonist peptide. The present invention is based in part on the unexpected results of clinical trials in humans which demonstrated that the formulations of the invention are therapeutically effective at much lower doses than predicted based on animal studies. In accordance with one aspect of the invention, the therapeutically effective dose is between 0.01 milligrams (mg) and 10 mg per unit dose. The term “unit dose” refers to a single drug delivery entity, *e.g.*, a tablet, capsule, solution or inhalation formulation. In one embodiment, the effective dose is between 0.01 mg and 9 mg. In another embodiment, the effective dose is between 0.01 mg and 5 mg. In another embodiment, the effective dose is between 0.01 mg and 3 mg. In another embodiment, the effective dose is between 0.10 mg and 5 mg. In another embodiment, the effective dose is between 0.10 mg and 3 mg. In one embodiment, the unit dose is .01 mg, .05 mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg, 2.5 mg, 3.0 mg, 5 mg, or 10 mg. In one embodiment, the unit dose is 0.3 mg, 1.0 mg, 3.0 mg, 9.0 mg, or 9.5 mg.

[137] The GCC agonist peptides may be in a pharmaceutical composition in unit dose form, together with one or more pharmaceutically acceptable excipients. The amount of peptide present should be sufficient to have a positive therapeutic effect when administered to a patient. What constitutes a “positive therapeutic effect” will depend upon the particular condition being treated and will include any significant improvement in a condition readily recognized by one of skill in the art.

[138] The GCC agonists for use in the methods described above are preferably administered orally. Dosage forms include solutions, suspensions, emulsions, tablets, and capsules.

[139] The total daily dose can be administered to the patient in a single dose, or in multiple sub-doses. Typically, sub-doses can be administered two to six times per day, preferably two to four times per day, and even more preferably two to three times per day. Preferably, a single daily dose is administered.

[140] The GCC agonists may be administered as either the sole active agent or in combination with one or more additional active agents. In all cases, additional active agents should be administered at a dosage that is therapeutically effective using the existing art as a guide. The GCC agonists may be administered in a single composition or sequentially with the one or more additional active agents. In one embodiment, the GCC agonist is administered in combination with one or more inhibitors of cGMP dependent phosphodiesterase such as suldinac sulfone, zaprinast, motapizone, vardenafil, or sildenafil. In another embodiment, the GCC agonist is administered in combination with one or more chemotherapeutic agents. In another embodiment, the GCC agonist is administered in combination with one or more anti-inflammatory drugs such as steroids or non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin.

[141] Combination therapy can be achieved by administering two or more agents, *e.g.*, a GCC agonist peptide described herein and another compound, each of which is formulated and administered separately, or by administering two or more agents in a single formulation. Other combinations are also encompassed by combination therapy. For example, two agents can be formulated together and administered in conjunction with a separate formulation containing a

third agent. While the two or more agents in the combination therapy can be administered simultaneously, they need not be. For example, administration of a first agent (or combination of agents) can precede administration of a second agent (or combination of agents) by minutes, hours, days, or weeks. Thus, the two or more agents can be administered within minutes of each other or within 1, 2, 3, 6, 9, 12, 15, 18, or 24 hours of each other or within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 days of each other or within 2, 3, 4, 5, 6, 7, 8, 9, or 10 weeks of each other. In some cases even longer intervals are possible. While in many cases it is desirable that the two or more agents used in a combination therapy be present in within the patient's body at the same time, this need not be so.

[142] The GCC agonist peptides described herein may be combined with phosphodiesterase inhibitors, *e.g.*, sulindae sulfone, Zaprinast, sildenafil, vardenafil or tadalafil to further enhance levels of cGMP in the target tissues or organs.

[143] Combination therapy can also include two or more administrations of one or more of the agents used in the combination. For example, if agent X and agent Y are used in a combination, one could administer them sequentially in any combination one or more times, *e.g.*, in the order X-Y- X, X-X-Y, Y-X-Y, Y-Y-X, X-X-Y-Y, etc.

1.3.2 Exemplary Agents for Combination Therapy

[144] The GCC agonist formulations of the invention may be administered alone or in combination with one or more additional therapeutic agents as part of a therapeutic regimen for the treatment or prevention of a gastrointestinal disease or disorder. In some embodiments, the GCC agonist formulation comprises one or more additional therapeutic agents. In other embodiments, the GCC agonist is formulated separately from the one or more additional therapeutic agents. In accordance with this embodiment, the GCC agonist is administered either simultaneously, sequentially, or at a different time than the one or more additional therapeutic agents. In one embodiment, the GCC agonist formulation is administered in combination with one or more additional therapeutic agents selected from the group consisting of phosphodiesterase inhibitors, cyclic nucleotides (such as cGMP and cAMP), a laxative (such as SENNA or METAMUCIL), a stool softner, an anti-tumor necrosis factor alpha therapy for IBD

(such as REMICADE, ENBREL, or HUMIRA), and anti-inflammatory drugs (such as COX-2 inhibitors, sulfasalazine, 5-ASA derivatives and NSAIDS). In certain embodiments, the GCC agonist formulation is administered in combination with an effective dose of an inhibitor of cGMP-specific phosphodiesterase (cGMP-PDE) either concurrently or sequentially with said GCC agonist. cGMP-PDE inhibitors include, for example, suldinac sulfone, zaprinast, motapizone, vardenafil, and sildenafil. In another embodiment, the GCC agonist formulation is administered in combination with inhibitors of cyclic nucleotide transporters. Further examples of therapeutic agents that may be administered in combination with the GCC agonist formulations of the invention are given in the following sections.

1.3.2.1 Agents to Treat Gastrointestinal Cancers

[145] The GCC agonist formulations described herein can be used in combination with one or more antitumor agents including but not limited to alkylating agents, epipodophyllotoxins, nitrosoureas, anti-metabolites, vinca alkaloids, anthracycline antibiotics, nitrogen mustard agents, and the like. Particular antitumor agents include tamoxifen, taxol, etoposide, and 5-fluorouracil. In one embodiment, the GCC agonist formulations are used in combination with an antiviral agent or a monoclonal antibody.

[146] Non-limiting examples of antitumor agents that can be used in combination with the GCC agonist formulations of the invention for the treatment of colon cancer include anti-proliferative agents, agents for DNA modification or repair, DNA synthesis inhibitors, DNA/RNA transcription regulators, RNA processing inhibitors, agents that affect protein expression, synthesis and stability, agents that affect protein localization or their ability to exert their physiological action, agents that interfere with protein-protein or protein-nucleic acid interactions, agents that act by RNA interference, receptor binding molecules of any chemical nature (including small molecules and antibodies), targeted toxins, enzyme activators, enzyme inhibitors, gene regulators, HSP-90 inhibitors, molecules interfering with microtubules or other cytoskeletal components or cell adhesion and motility, agents for phototherapy, and therapy adjuncts.

[147] Representative anti-proliferative agents include N-acetyl-D-sphingosine (C.sub.2 ceramide), apigenin, berberine chloride, dichloromethylenediphosphonic acid disodium salt, loe-emodine, emodin, HA 14-1, N-hexanoyl-D-sphingosine (C.sub.6 ceramide), 7 β -hydroxycholesterol, 25-hydroxycholesterol, hyperforin, parthenolide, and rapamycin.

Representative agents for DNA modification and repair include aphidicolin, bleomycin sulfate, carboplatin, carmustine, chlorambucil, cyclophosphamide monohydrate, cyclophosphamide monohydrate ISOPAC.RTM., cis-diammineplatinum(II) dichloride (Cisplatin), esculetin, melphalan, methoxyamine hydrochloride, mitomycin C, mitoxantrone dihydrochloride, oxaliplatin, and streptozocin.

[148] Representative DNA synthesis inhibitors include (+-)-amethopterin (methotrexate), 3-amino-1,2,4-benzotriazine 1,4-dioxide, aminopterin, cytosine b-D-arabinofurndoside (Ara-C), cytosine b-D-arabinofuranoside (Ara-C) hydrochloride, 2-fluoroadenine-9-b-D-arabinofuranoside (Fludarabine des-phosphate; F-ara-A), 5-fluoro-5'-deoxyuridinc, 5-fluorouracil, ganciclovir, hydroxyurea, 6-mercaptopurine, and 6-thioguanine.

[149] Representative DNA/RNA transcription regulators include actinomycin D, daunorubicin hydrochloride, 5,6-dichlorobenzimidazole 1-b-D-ribofuranoside, doxorubicin hydrochloride, homoharringtonine, and idarubicin hydrochloride.

[150] Representative enzyme activators and inhibitors include forskolin, DL-aminogluthethimide, apicidin, Bowman-Birk Inhibitor, butein, (S)-(+)-camptothecin, curcumin, (-)-deguelin, (-)-depudecin, doxycycline hyclate, etoposide, formestane, fostriecin sodium salt, hispidin, 2-imino-1-imidazolidineacetic acid (Cyclocreatine), oxamflatin, 4-phenylbutyric acid, roscovitine, sodium valproate, trichostatin A, tyrphostin AG 34, tyrphostin AG 879, urinary trypsin inhibitor fragment, valproic acid (2-propylpentanoic acid), and XK469.

[151] Representative gene regulators include 5-aza-2'-deoxycytidine, 5-azacytidine, cholecalciferol (Vitamin D3), ciglitizone, cyproterone acetate, 15-deoxy-D.sup.12,14-prostaglandin J.sub.2, epitestosterone, flutamide, glycyrrhizic acid ammonium salt (glycyrrhizin), 4-hydroxytamoxifen, mifepristone, procainamide hydrochloride, raloxifene hydrochloride, all trans-retinal (vitamin A aldehyde), retinoic acid (vitamin A acid), 9-cis-

retinoic acid, 13-cis-retinoic acid, retinoic acid p-hydroxyanilide, retinol (Vitamin A), tamoxifen, tamoxifen citrate salt, tetradecylthioacetic acid, and troglitazone.

[152] Representative HSP-90 inhibitors include 17-(allylamino)-17-demethoxygeldanamycin and geldanamycin.

[153] Representative microtubule inhibitors include colchicines, dolastatin 15, nocodazole, taxanes and in particular paclitaxel, podophyllotoxin, rhizoxin, vinblastine sulfate salt, vincristine sulfate salt, and vindesine sulfate salt and vinorelbine (Navelbine) ditartrate salt.

[154] Representative agents for performing phototherapy include photoactive porphyrin rings, hypericin, 5-methoxypsoralen, 8-methoxypsoralen, psoralen and ursodeoxycholic acid.

[155] Representative agents used as therapy adjuncts include amifostine, 4-amino-1,8-naphthalimide, brefeldin A, cimetidine, phosphomycin disodium salt, leuprolide (leuprorelin) acetate salt, luteinizing hormone-releasing hormone (LH-RH) acetate salt, lectin, papaverine hydrochloride, pifithrin-a, (-)-scopolamine hydrobromide, and thapsigargin.

[156] The agents can also be anti-VEGF (vascular endothelial growth factor) agents, as such are known in the art. Several antibodies and small molecules are currently in clinical trials or have been approved that function by inhibiting VEGF, such as Avastin (Bevacizumab), SU5416, SU11248 and BAY 43-9006. The agents can also be directed against growth factor receptors such as those of the EGF/Erb-B family such as EGF Receptor (Iressa or Gefitinib, and Tarceva or Erlotinib), Erb-B2, receptor (Herceptin or Trastuzumab), other receptors (such as Rituximab or Rituxan/MabThera), tyrosine kinases, non-receptor tyrosine kinases, cellular serine/threonine kinases (including MAP kinases), and various other proteins whose deregulation contribute to oncogenesis (such as small/Ras family and large/heterotrimeric G proteins). Several antibodies and small molecules targeting those molecules are currently at various stages of development (including approved for treatment or in clinical trials).

[157] In a preferred embodiment, the invention provides a method for treating colon cancer in a subject in need thereof by administering to the subject a GCC agonist formulation in combination with one or more antitumor agent selected from the group consisting of paclitaxel,

docetaxel, tamoxifen, vinorelbine, gemcitabine, cisplatin, etoposide, topotecan, irinotecan, anastrozole, rituximab, trastuzumab, fludarabine, cyclophosphamide, gentuzumab, carboplatin, interferons, and doxorubicin. In a particular embodiment the antitumor agent is paclitaxel. In a further embodiment, the method further comprises an antitumor agent selected from the group consisting of 5-FU, doxorubicin, vinorelbine, cytoxan, and cisplatin.

1.3.2.2 Agents that Treat Crohn's Disease

[158] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of Crohn's disease. Non-limiting examples of the one or more additional therapeutic agents include sulfasalazine and other mesalamine-containing drugs, generally known as 5-ASA agents, such as Asacol, Dipentum, or Pentasa, or infliximab (REMICADE). In certain embodiments, the one or more additional agents is a corticosteroid or an immunosuppressive agent such as 6-mercaptopurine or azathioprine. In another embodiment, the one or more additional agents is an antidiarrheal agent such as diphenoxylate, loperamide, or codeine.

1.3.2.3 Agents that Treat Ulcerative Colitis

[159] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of ulcerative colitis. The agents that are used to treat ulcerative colitis overlap with those used to treat Crohn's Disease. Non-limiting examples of the one or more additional therapeutic agents that can be used in combination with a GCC agonist formulation of the invention include aminosaliclates (drugs that contain 5-aminosalicylic acid (5-ASA)) such as sulfasalazine, olsalazine, mesalamine, and balsalazide. Other therapeutic agents that can be used include corticosteroids, such as prednisone and hydrocortisone, immunomodulators, such as azathioprine, 6-mercapto-purine (6-MP), cytokines, interleukins, and lymphokines, and anti-TNF-alpha agents, including the thiazolidinediones or glitazones such as rosiglitazone and pioglitazone. In one embodiment, the one or more additional therapeutic agents includes both cyclosporine A and 6-MP or azathioprine for the treatment of active, severe ulcerative colitis.

1.3.2.4 Agents that Treat Constipation/Irritable Bowel Syndrome

[160] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of constipation, such as that associated with irritable bowel syndrome. Non-limiting examples of the one or more additional therapeutic agents include laxatives such as SENNA, MIRALAX, LACTULOSE, PEG, or calcium polycarbophil), stool softeners (such as mineral oil or COLACE), bulking agents (such as METAMUCIL or bran), agents such as ZELNORM (also called tegaserod), and anticholinergic medications such as BENTYL and LEVSIN.

1.3.2.5 Agents for the Treatment of Postoperative Ileus

[161] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of postoperative ileus. Non-limiting examples of the one or more additional therapeutic agents include ENTEREG (alvimopan; formerly called ado lor/ ADL 8-2698), conivaptan, and related agents describes in US 6,645,959.

1.3.2.6 Anti-obesity agents

[162] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of obesity. Non-limiting examples of the one or more additional therapeutic agents include 1 β HSD-I (11-beta hydroxy steroid dehydrogenase type 1) inhibitors, such as BVT 3498, BVT 2733, 3-(1-adamantyl)-4-ethyl-5-(ethylthio)- 4H-1,2,4-triazole, 3-(1-adamantyl)-5-(3,4,5-trimethoxyphenyl)-4-methyl-4H-1,2,4-triazole, 3- adamantanyl-4,5,6,7,8,9,10,11,12,3a-decahydro-1,2,4-triazolo[4,3-a][1 l]annulene, and those compounds disclosed in WO01/90091, WOO 1/90090, WOO 1/90092 and WO02/072084; 5HT antagonists such as those in WO03/037871, WO03/037887, and the like; 5HT1a modulators such as carbidopa, benserazide and those disclosed in US6207699, WO03/031439, and the like; 5HT2c (serotonin receptor 2c) agonists, such as BVT933, DPCA37215, IK264, PNU 22394, WAY161503, R-1065, SB 243213 (Glaxo Smith Kline) and YM 348 and those disclosed in US3914250, WO00/77010,

WO02/36596, WO02/48124, WO02/10169, WO01/66548, WO02/44152, WO02/51844, WO02/40456, and WO02/40457; 5HT6 receptor modulators, such as those in WO03/030901, WO03/035061, WO03/039547, and the like; acyl-estrogens, such as oleoyl-estrone, disclosed in del Mar-Grasa, M. et al, Obesity Research, 9:202-9 (2001) and Japanese Patent Application No. JP 2000256190; anorectic bicyclic compounds such as 1426 (Aventis) and 1954 (Aventis), and the compounds disclosed in WO00/18749, WO01/32638, WO01/62746, WO01/62747, and WO03/015769; CB 1 (cannabinoid-1 receptor) antagonist/inverse agonists such as rimonabant (Acomplia; Sanofi), SR-147778 (Sanofi), SR-141716 (Sanofi), BAY 65-2520 (Bayer), and SLV 319 (Solvay), and those disclosed in patent publications US4973587, US5013837, US5081122, US5112820, US5292736, US5532237, US5624941, US6028084, US6509367, US6509367, WO96/33159, WO97/29079, WO98/31227, WO98/33765, WO98/37061, WO98/41519, WO98/43635, WO98/43636, WO99/02499, WO00/10967, WO00/10968, WO01/09120, WO01/58869, WO01/64632, WO01/64633, WO01/64634, WO01/70700, WO01/96330, WO02/076949, WO03/006007, WO03/007887, WO03/020217, WO03/026647, WO03/026648, WO03/027069, WO03/027076, WO03/027114, WO03/037332, WO03/040107, WO03/086940, WO03/084943 and EP658546; CCK-A (cholecystokinin-A) agonists, such as AR-R 15849, GI 181771 (GSK), JMV-180, A- 71378, A-71623 and SR146131 (Sanofi), and those described in US5739106; CNTF (Ciliary neurotrophic factors), such as GI- 181771 (Glaxo-SmithKline), SRI 46131 (Sanofi Synthelabo), butabindide, PD 170,292, and PD 149164 (Pfizer); CNTF derivatives, such as Axokine® (Regeneron), and those disclosed in WO94/09134, WO98/22128, and WO99/43813; dipeptidyl peptidase IV (DP-IV) inhibitors, such as isoleucine thiazolidide, valine pyrrolidide, NVP-DPP728, LAF237, P93/01, P 3298, TSL 225 (tryptophyl-1,2,3,4-tetrahydroisoquinoline-3- carboxylic acid; disclosed by Yamada et al, Bioorg. & Med. Chem. Lett. 8 (1998) 1537-1540), TMC-2A/2B/2C, CD26 inhibitors, FE 999011, P9310/K364, VIP 0177, SDZ 274-444, 2- cyanopyrrolidides and 4-cyanopyrrolidides as disclosed by Ashworth et al, Bioorg. & Med. Chem. Lett., Vol. 6, No. 22, pp 1163-1166 and 2745-2748 (1996) and the compounds disclosed patent publications. WO99/38501, WO99/46272, WO99/67279 (Probiodrug), WO99/67278 (Probiodrug), WO99/61431 (Probiodrug), WO02/083128, WO02/062764, WO03/000180, WO03/000181, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593, WO03/004498, WO03/004496, WO03/017936, WO03/024942,

WO03/024965, WO03/033524, WO03/037327 and EP1258476; growth hormone secretagogue receptor agonists/antagonists, such as NN703, hexarelin, MK- 0677 (Merck), SM-130686, CP-424391 (Pfizer), LY 444,711 (Eli Lilly), L-692,429 and L- 163,255, and such as those disclosed in USSN 09/662448, US provisional application 60/203335, US6358951, US2002049196, US2002/022637, WO01/56592 and WO02/32888; H3 (histamine H3) antagonist/inverse agonists, such as thioperamide, 3-(1H-imidazol-4- yl)propyl N-(4-pentenyl)carbamate), clobenpropit, iodophenpropit, imoproxifan, GT2394 (Gliatech), and A331440, O-[3-(1H-imidazol-4-yl)propanol]carbamates (Kiec-Kononowicz, K. et al., *Pharmazie*, 55:349-55 (2000)), piperidine-containing histamine H3-receptor antagonists (Lazewska, D. et al., *Pharmazie*, 56:927-32 (2001), benzophenone derivatives and related compounds (Sasse, A. et al., *Arch. Pharm.(Weinheim)* 334:45-52 (2001)), substituted N- phenylcarbamates (Reidemeister, S. et al., *Pharmazie*, 55:83-6 (2000)), and proxifan derivatives (Sasse, A. et al., *J. Med. Chem.* 43:3335-43 (2000)) and histamine H3 receptor modulators such as those disclosed in WO02/15905, WO03/024928 and WO03/024929; leptin derivatives, such as those disclosed in US5552524, US5552523, US5552522, US5521283, WO96/23513, WO96/23514, WO96/23515, WO96/23516, WO96/23517, WO96/23518, WO96/23519, and WO96/23520; leptin, including recombinant human leptin (PEG-OB, Hoffman La Roche) and recombinant methionyl human leptin (Amgen); lipase inhibitors, such as tetrahydrolipstatin (orlistat/Xenical®), Triton WRI 339, RHC80267, lipstatin, teasaponin, diethylumbelliferyl phosphate, FL-386, WAY-121898, Bay-N-3176, valilactone, esteracin, ebelactone A, ebelactone B, and RHC 80267, and those disclosed in patent publications WO01/77094, US4598089, US4452813, US5512565, US5391571, US5602151, US4405644, US4189438, and US4242453; lipid metabolism modulators such as maslinic acid, erythrodiol, ursolic acid uvaol, betulinic acid, betulin, and the like and compounds disclosed in WO03/011267; Mc4r (melanocortin 4 receptor) agonists, such as CHIR86036 (Chiron), ME- 10142, ME-10145, and HS-131 (Melacure), and those disclosed in PCT publication Nos. WO99/64002, WO00/74679, WOO 1/991752, WOO 1/25192, WOO 1/52880, WOO 1/74844, WOO 1/70708, WO01/70337, WO01/91752, WO02/059095, WO02/059107, WO02/059108, WO02/059117, WO02/06276, WO02/12166, WO02/11715, WO02/12178, WO02/15909, WO02/38544, WO02/068387, WO02/068388, WO02/067869, WO02/081430, WO03/06604, WO03/007949, WO03/009847, WO03/009850, WO03/013509,

and WO03/031410; Mc5r (melanocortin 5 receptor) modulators, such as those disclosed in WO97/19952, WO00/15826, WO00/15790, US20030092041; melanin-concentrating hormone 1 receptor (MCHR) antagonists, such as T-226296 (Takeda), SB 568849, SNP-7941 (Synaptic), and those disclosed in patent publications WOO 1/21169, WO01/82925, WO01/87834, WO02/051809, WO02/06245, WO02/076929, WO02/076947, WO02/04433, WO02/51809, WO02/083134, WO02/094799, WO03/004027, WO03/13574, WO03/15769, WO03/028641, WO03/035624, WO03/033476, WO03/033480, JP13226269, and JP1437059; mGluR5 modulators such as those disclosed in WO03/029210, WO03/047581, WO03/048137, WO03/051315, WO03/051833, WO03/053922, WO03/059904, and the like; serotonergic agents, such as fenfluramine (such as Pondimin® (Benzeneethanamine, N-ethyl- alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Robbins), dexfenfluramine (such as Redux® (Benzeneethanamine, N-ethyl-alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Interneuron) and sibutramine ((Meridia®, Knoll/Reductil™) including racemic mixtures, as optically pure isomers (+) and (-), and pharmaceutically acceptable salts, solvents, hydrates, clathrates and prodrugs thereof including sibutramine hydrochloride monohydrate salts thereof, and those compounds disclosed in US4746680, US4806570, and US5436272, US20020006964, WOO 1/27068, and WOO 1/62341; NE (norepinephrine) transport inhibitors, such as GW 320659, despiramine, talsupram, and nomifensine; NPY 1 antagonists, such as BIBP3226, J-115814, BIBO 3304, LY-357897, CP-671906, GI- 264879A, and those disclosed in US6001836, WO96/14307, WO01/23387, WO99/51600, WO01/85690, WO01/85098, WO01/85173, and WO01/89528; NPY5 (neuropeptide Y Y5) antagonists, such as 152,804, GW-569180A, GW-594884A, GW- 587081X, GW-548118X, FR235208, FR226928, FR240662, FR252384, 1229U91, GI-264879A, CGP71683A, LY-377897, LY-366377, PD-160170, SR- 120562A, SR-120819A, JCF-104, and H409/22 and those compounds disclosed in patent publications US6140354, US6191160, US6218408, US6258837, US6313298, US6326375, US6329395, US6335345, US6337332, US6329395, US6340683, EP01010691, EP-01044970, WO97/19682, WO97/20820, WO97/20821, WO97/20822, WO97/20823, WO98/27063, WO00/107409, WO00/185714, WO00/185730, WO00/64880, WO00/68197, WO00/69849, WO/0113917, WO01/09120, WO01/14376, WO01/85714, WO01/85730, WO01/07409, WO01/02379, WO01/23388, WO01/23389, WOO 1/44201, WO01/62737, WO01/62738, WO01/09120,

WO02/20488, WO02/22592, WO02/48152, WO02/49648, WO02/051806, WO02/094789, WO03/009845, WO03/014083, WO03/022849, WO03/028726 and Norman et al, J. Med. Chem. 43:4288-4312 (2000); opioid antagonists, such as nalmefene (REVEX ®), 3-methoxynaltrexone, methylnaltrexone, naloxone, and naltrexone (e.g. PT901; Pain Therapeutics, Inc.) and those disclosed in US20050004155 and WO00/21509; orexin antagonists, such as SB-334867-A and those disclosed in patent publications WO01/96302, WO01/68609, WO02/44172, WO02/51232, WO02/51838, WO02/089800, WO02/090355, WO03/023561, WO03/032991, and WO03/037847; PDE inhibitors (e.g. compounds which slow the degradation of cyclic AMP (cAMP) and/or cyclic GMP (cGMP) by inhibition of the phosphodiesterases, which can lead to a relative increase in the intracellular concentration of cAMP and cGMP; possible PDE inhibitors are primarily those substances which are to be numbered among the class consisting of the PDE3 inhibitors, the class consisting of the PDE4 inhibitors and/or the class consisting of the PDE5 inhibitors, in particular those substances which can be designated as mixed types of PDE3/4 inhibitors or as mixed types of PDE3/4/5 inhibitors) such as those disclosed in patent publications DE1470341, DE2108438, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EPOI 12987, EPOI 16948, EP0150937, EP0158380, EP0161632, EP0161918, EP0167121, EP0199127, EP0220044, EP0247725, EP0258191, EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282, EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823, EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474, EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, US4963561, US5141931, WO9117991, WO9200968, WO9212961, WO9307146, WO9315044, WO9315045, WO9318024, WO9319068, WO9319720, WO9319747, WO9319749, WO9319751, WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852, WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045, WO9504046, WO9505386, WO9508534, WO9509623, WO9509624, WO9509627, WO9509836, WO9514667, WO9514680, WO9514681, WO9517392, WO9517399, WO9519362, WO9522520, WO9524381, WO9527692, WO9528926,

WO9535281, WO9535282, WO9600218, WO9601825, WO9602541, WO9611917, DE3142982, DE1 116676, DE2162096, EP0293063, EP0463756, EP0482208, EP0579496, EP0667345 US6331543, US20050004222 (including those disclosed in formulas I- XIII and paragraphs 37-39, 85-0545 and 557-577), WO9307124, EP0163965, EP0393500, EP0510562, EP0553174, WO9501338 and WO9603399, as well as PDE5 inhibitors (such as RX-RA-69, SCH-51866, KT-734, vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702 and sildenafil (Viagra™)), PDE4 inhibitors (such as etazolate, ICI63197, RP73401, imazolidinone (RO-20-1724), MEM 1414 (R1533/R1500; Pharmacia Roche), denbufylline, rolipram, oxagrelate, nitraquazone, Y-590, DH-6471, SKF-94120, motapizone, lixazinone, indolidan, olprinone, atizoram, KS-506-G, dipamfylline, BMY-43351, atizoram, arofylline, filaminast, PDB-093, UCB-29646, CDP-840, SKF-107806, piclamilast, RS-17597, RS-25344- 000, SB-207499, TIBENELAST, SB-210667, SB-211572, SB-211600, SB-212066, SB-212179, GW-3600, CDP-840, mopidamol, anagrelide, ibudilast, amrinone, pimobendan, cilostazol, quazinone and N-(3,5-dichloropyrid-4-yl)-3-cyclopropylmethoxy-4-difluoromethoxybenzamide, PDE3 inhibitors (such as ICI153, 100, bemorandane (RWJ 22867), MCI-154, UD-CG 212, sulmazole, ampizone, cilostamide, carbazeran, piroximone, imazodan, CI-930, siguazodan, adibendan, saterinone, SKF-95654, SDZ-MKS-492, 349-U-85, emoradan, EMD-53998, EMD- 57033, NSP-306, NSP-307, revizinone, NM-702, WIN-62582 and WIN-63291, enoximone and milrinone, PDE3/4 inhibitors (such as benafentrine, trequinsin, ORG-30029, zardaverine, L- 686398, SDZ-ISQ-844, ORG-20241, EMD-54622, and tolafentrine) and other PDE inhibitors (such as vinpocetin, papaverine, enprofylline, cilomilast, fenoximone, pentoxifylline, roflumilast, tadalafil(Cialis®), theophylline, and vardenafil(Levitra®); Neuropeptide Y2 (NPY2) agonists include but are not limited to: polypeptide YY and fragments and variants thereof (e.g. YY3-36 (PYY3-36)(N. Engl. J. Med. 349:941, 2003; IKPEAPGE DASPEELNRY YASLRHYLNL VTRQRY (SEQ ID NO:XXX)) and PYY agonists such as those disclosed in WO02/47712, WO03/026591, WO03/057235, and WO03/027637; serotonin reuptake inhibitors, such as, paroxetine, fluoxetine (Prozac™), fluvoxamine, sertraline, citalopram, and imipramine, and those disclosed in US6162805, US6365633, WO03/00663, WOO 1/27060, and WOO 1/162341; thyroid hormone β agonists, such as KB-2611 (KaroBioBMS), and those disclosed in WO02/15845, WO97/21993, WO99/00353, GB98/284425, U.S. Provisional Application No.

60/183,223, and Japanese Patent Application No. JP 2000256190; UCP-I (uncoupling protein-1), 2, or 3 activators, such as phytaic acid, 4-[(E)-2-(5, 6,7,8- tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), retinoic acid, and those disclosed in WO99/00123; β 3 (beta adrenergic receptor 3) agonists, such as AJ9677/TAK677 (Dainippon/Takeda), L750355 (Merck), CP331648 (Pfizer), CL-316,243, SB 418790, BRL-37344, L-796568, BMS-196085, BRL-35135A, CGP12177A, BTA-243, GW 427353, Trecadrine, Zeneca D7114, N-5984 (Nisshin Kyorin), LY-377604 (Lilly), SR 59119A, and those disclosed in US5541204, US5770615, US5491134, US5776983, US488064, US5705515, US5451677, WO94/18161, WO95/29159, WO97/46556, WO98/04526 and WO98/32753, WO01/74782, WO02/32897, WO03/014113, WO03/016276, WO03/016307, WO03/024948, WO03/024953 and WO03/037881; noradrenergic agents including, but not limited to, diethylpropion (such as Tenuate® (1- propanone, 2-(diethylamino)-1 -phenyl-, hydrochloride), Merrell), dextroamphetamine (also known as dextroamphetamine sulfate, dexamphetamine, dexedrine, Dexampex, Ferndex, Oxydess II, Robese, Spancap #1), mazindol ((or 5-(p-chlorophenyl)-2,5-dihydro-3H- imidazo[2,1-a]isoindol-5-ol) such as Sanorex®, Novartis or Mazanor®, Wyeth Ayerst), phenylpropanolamine (or Benzenemethanol, alpha-(1-aminoethyl)-, hydrochloride), phentermine ((or Phenol, 3-[[4,5-duhydro-1H-imidazol-2-yl)ethyl](4-methylphenyl-amino), monohydrochloride) such as Adipex-P®, Lemmon, FASTIN®, Smith-Kline Beecham and Ionamin®, Medeva), phendimetrazine ((or (2S,3S)-3,4-Dimethyl-2phenylmorpholine L-(+)- tartrate (1 :1)) such as Metra® (Forest) , Plegine® (Wyeth- Ay erst), Prelu-2® (Boehringer Ingelheim), and Statobex® (Lemmon), phendamine tartrate (such as Thephorin® (2,3,4,9- Tetrahydro-2-methyl-9-phenyl-1H-indenol[2,1-c]pyridine L-(+)-tartrate (1 :1)), Hoffmann- LaRoche), methamphetamine (such as Desoxyn®, Abbot ((S)-N, (alpha)-dimethylbenzeneethanamine hydrochloride)), and phendimetrazine tartrate (such as Bontril® Slow-Release Capsules, Amarin (-3,4-Dimethyl-2-phenylmorpholine Tartrate); fatty acid oxidation upregulator/inducers such as Famoxin® (Genset); monamine oxidase inhibitors including but not limited to befloxatone, moclobemide, brofaromine, phenoxathine, esuprone, befol, toloxatone, pirlindol, amiflamine, sercloramine, bazinaprine, lazabemide, milacemide, caroxazone and other certain compounds as disclosed by WO01/12176; and other anti-obesity agents such as 5HT-2 agonists, ACC (acetyl-CoA carboxylase) inhibitors such as those described

in WO03/072197, alpha-lipoic acid (alpha-LA), AOD9604, appetite suppressants such as those in WO03/40107, ATL-962 (Alizyme PLC), benzocaine, benzphetamine hydrochloride (Didrex), bladderwrack (*focus vesiculosus*), BRS3 (bombesin receptor subtype 3) agonists, bupropion, caffeine, CCK agonists, chitosan, chromium, conjugated linoleic acid, corticotropin-releasing hormone agonists, dehydroepiandrosterone, DGAT1 (diacylglycerol acyltransferase 1) inhibitors, DGAT2 (diacylglycerol acyltransferase 2) inhibitors, dicarboxylate transporter inhibitors, ephedra, exendin-4 (an inhibitor of glp-1) FAS (fatty acid synthase) inhibitors (such as Cerulenin and C75), fat resorption inhibitors (such as those in WO03/053451, and the like), fatty acid transporter inhibitors, natural water soluble fibers (such as psyllium, plantago, guar, oat, pectin), galanin antagonists, galega (Goat's Rue, French Lilac), garcinia cambogia, germander (*teucrium chamaedrys*), ghrelin antibodies and ghrelin antagonists (such as those disclosed in WO01/87335, and WO02/08250), polypeptide hormones and variants thereof which affect the islet cell secretion, such as the hormones of the secretin/gastric inhibitory polypeptide (GIP)/vasoactive intestinal polypeptide (VIP)/pituitary adenylate cyclase activating polypeptide (PACAP)/glucagon-like polypeptide II (GLP- II)/glucagon gene family and/or those of the adrenomedullin/amylin/calcitonin gene related polypeptide (CGRP) gene family including GLP-1 (glucagon-like polypeptide 1) agonists (e.g. (1) exendin-4, (2) those GLP-I molecules described in US20050130891 including GLP-1(7-34), GLP-1(7-35), GLP-1(7-36) or GLP-1(7-37) in its C-terminally carboxylated or amidated form or as modified GLP-I polypeptides and modifications thereof including those described in paragraphs 17-44 of US20050130891, and derivatives derived from GLP-1-(7-34)COOH and the corresponding acid amide are employed which have the following general formula: R-NH-HAEGTFTSDVSYLEGQAAKEFIAWLVK-CONH₂ wherein R=H or an organic compound having from 1 to 10 carbon atoms. Preferably, R is the residue of a carboxylic acid. Particularly preferred are the following carboxylic acid residues: formyl, acetyl, propionyl, isopropionyl, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl.) and glp-1 (glucagon-like polypeptide- 1), glucocorticoid antagonists, glucose transporter inhibitors, growth hormone secretagogues (such as those disclosed and specifically described in US5536716), interleukin-6 (IL-6) and modulators thereof (as in WO03/057237, and the like), L- carnitine, Mc3r (melanocortin 3 receptor) agonists, MCH2R (melanin concentrating hormone 2R)

agonist/antagonists, melanin concentrating hormone antagonists, melanocortin agonists (such as Melanotan II or those described in WO 99/64002 and WO 00/74679), nomame herba, phosphate transporter inhibitors, phytopharm compound 57 (CP 644,673), pyruvate, SCD-I (stearoyl-CoA desaturase-1) inhibitors, T71 (Tularik, Inc., Boulder CO), Topiramate (Topimax®, indicated as an anti-convulsant which has been shown to increase weight loss), transcription factor modulators (such as those disclosed in WO03/026576), β -hydroxy steroid dehydrogenase-1 inhibitors (β -HSD-I), β -hydroxy- β -methylbutyrate, p57 (Pfizer), Zonisamide (Zonegran™, indicated as an anti-epileptic which has been shown to lead to weight loss), and the agents disclosed in US20030119428 paragraphs 20-26.

1.3.2.7 Phosphodiesterase inhibitors

[163] In certain embodiments, the regimen of combination therapy includes the administration of one or more phosphodiesterase (“PDE”) inhibitors. PDE inhibitors slow the degradation of cyclic AMP (cAMP) and/or cyclic GMP (cGMP) by inhibiting phosphodiesterases, which can lead to a relative increase in the intracellular concentration of cAMP and/or cGMP. Non-limiting examples of PDE inhibitors that can be used in combination with the GCC agonists of the invention include PDE3 inhibitors, PDE4 inhibitors and/or PDE5 inhibitors, in particular those substances which can be designated as mixed types of PDE3/4 inhibitors or as mixed types of PDE3/4/5 inhibitors. Non-limiting examples of such PDE inhibitors are described in the following patent applications and patents: DE1470341, DE2108438, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EPO1 12987, EPO1 16948, EP0150937, EP0158380, EP0161632, EP0161918, EP0167121, EP0199127, EP0220044, EP0247725, EP0258191, EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282, EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823, EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474, EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, U.S. Pat. Nos. 4,963,561, 5,141,931, WO9117991, WO9200968, WO9212961, WO9307146, WO9315044, WO9315045, WO9318024,

WO9319068, WO9319720, WO9319747, WO9319749, WO9319751, WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852, WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045, WO9504046, WO9505386, WO9508534, WO9509623, WO9509624, WO9509627, WO9509836, WO9514667, WO9514680, WO9514681, WO9517392, WO9517399, WO9519362, WO9522520, WO9524381, WO9527692, WO9528926, WO9535281, WO9535282, WO9600218, WO9601825, WO9602541, WO9611917, DE3142982, DE1 116676, DE2162096, EP0293063, EP0463756, EP0482208, EP0579496, EP0667345 US6,331,543, US20050004222 (including those disclosed in formulas I-XIII and paragraphs 37-39, 85-0545 and 557-577) and WO9307124, EP0163965, EP0393500, EP0510562, EP0553174, WO9501338 and WO9603399. PDE5 inhibitors which may be mentioned by way of example are RX-RA-69, SCH-51866, KT-734, vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702 and sildenafil (Viagra®). PDE4 inhibitors which may be mentioned by way of example are RO-20-1724, MEM 1414 (R1533/R1500; Pharmacia Roche), DENBUFYLLINE, ROLIPRAM, OXAGRELATE, NITRAQUAZONE, Y-590, DH-6471, SKF-94120, MOTAPIZONE, LIXAZINONE, INDOLIDAN, OLPRINONE, ATIZORAM, KS-506-G, DIPAMFYLLINE, BMY-43351, ATIZORAM, AROFYLLINE, FILAMINAST, PDB-093, UCB-29646, CDP-840, SKF- 107806, PICLAMILAST, RS- 17597, RS-25344-000, SB-207499, TIBENELAST, SB-210667, SB-211572, SB-211600, SB-212066, SB-212179, GW-3600, CDP-840, MOPIDAMOL, ANAGRELIDE, IBUDILAST, AMRINONE, PIMOBENDAN, CILOSTAZOL, QUAZINONE and N-(3,5-dichloropyrid-4-yl)-3-cyclopropylmethoxy4-difluoromethoxybenzamide. PDE3 inhibitors which may be mentioned by way of example are SULMAZOLE, AMPIZONE, CILOSTAMIDE, CARBAZERAN, PIROXIMONE, IMAZODAN, CI-930, SIGUAZODAN, ADIBENDAN, SATERINONE, SKF-95654, SDZ-MKS-492, 349-U-85, EMORADAN, EMD-53998, EMD-57033, NSP-306, NSP-307, REVIZINONE, NM-702, WIN-62582 and WIN-63291, ENOXIMONE and MILRINONE. PDE3/4 inhibitors which may be mentioned by way of example are BENAFENTRINE, TREQUINSIN, ORG-30029, ZARDAVERINE, L-686398, SDZ-ISQ-844, ORG-20241, EMD-54622, and TOLAFENTRINE. Other PDE inhibitors include: cilomilast, pentoxifylline, roflumilast, tadalafil(Cialis®), theophylline, and vardenafil(Levitra®), zaprinast (PDE5 specific). GCC AGONIST

1.3.2.8 Analgesic Agents

[164] In certain embodiments, the regimen of combination therapy includes the administration of one or more analgesic agents, *e.g.*, an analgesic compound or an analgesic polypeptide. In some embodiments, the GCC agonist formulation is administered simultaneously or sequentially with one or more analgesic agents. In other embodiments, the GCC agonist is covalently linked or attached to an analgesic agent to create a therapeutic conjugate. Non-limiting examples of analgesic agents that can be used include calcium channel blockers, 5HT receptor antagonists (for example 5HT₃, 5HT₄ and 5HT₁ receptor antagonists), opioid receptor agonists (loperamide, fedotozine, and fentanyl), NK₁ receptor antagonists, CCK receptor agonists (*e.g.*, loxiglumide), NK₁ receptor antagonists, NK₃ receptor antagonists, norepinephrine-serotonin reuptake inhibitors (NSRI), vanilloid and cannaboid receptor agonists, and sialorphin. Further examples of analgesic agents in the various classes are known in the art.

[165] In one embodiment, the analgesic agent is an analgesic polypeptide selected from the group consisting of sialorphin-related polypeptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO: 239), including: VQHNPR (SEQ ID NO: 240); VRQHNPR (SEQ ID NO: 241); VRGQHNPR (SEQ ID NO: 242); VRGPQHNPR (SEQ ID NO: 243); VRGPRQHNPR (SEQ ID NO: 244); VRGPRRQHNPR (SEQ ID NO: 245); and RQHNPR (SEQ ID NO: 246). Sialorphin-related polypeptides bind to neprilysin and inhibit neprilysin-mediated breakdown of substance P and Met-enkephalin. Thus, compounds or polypeptides that are inhibitors of neprilysin are useful analgesic agents which can be administered with the GCC agonists described herein or covalently linked to a GCC agonist to form a therapeutic conjugate. Sialorphin and related polypeptides are described in U.S. Patent 6,589,750; U.S. 20030078200 A1; and WO 02/051435 A2.

[166] In another embodiment, a GCC agonist formulation of the invention is administered as part of a regimen of combination therapy with an opioid receptor antagonist or agonist. In one embodiment, the GCC agonist and the opioid receptor antagonist or agonist are linked via a covalent bond. Non-limiting examples of opioid receptor antagonists include naloxone, naltrexone, methyl naloxone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, nor-binaltorphimine, enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-

homoserine), trimebutine, vasoactive intestinal polypeptide, gastrin, glucagons. Non-limiting examples of opioid receptor agonists include fedotozine, asimadoline, and ketocyclazocine, the compounds described in WO03/097051 and WO05/007626, morphine, diphenyloxyate, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH₂; WO 01/019849 A1), and loperamide.

[167] Further non-limiting examples of analgesic agents that can be used in a regimen of combination therapy along with the GCC agonist formulations of the invention include the dipeptide Tyr-Arg (kyotorphin); the chromogranin-derived polypeptide (CgA 47-66; *See, e.g.*, Ghia et al. 2004 Regulatory polypeptides 119:199); CCK receptor agonists such as caerulein; conotoxin polypeptides; peptide analogs of thymulin (FR Application 2830451); CCK (CCKa or CCKb) receptor antagonists, including loxiglumide and dexloxiglumide (the R- isomer of loxiglumide) (WO 88/05774); 5-HT₄ agonists such as tegaserod (Zelnorm®), mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lorexapride; calcium channel blockers such as ziconotide and related compounds described in, for example, EP625162B1, US 5,364,842, US 5,587,454, US 5,824,645, US 5,859,186, US 5,994,305, US 6,087,091, US 6,136,786, WO 93/13128 A1, EP 1336409 A1, EP 835126 A1, EP 835126 B1, US 5,795,864, US 5,891,849, US 6,054,429, WO 97/01351 A1; NK-1, receptor antagonists such as aprepitant (Merck & Co Inc), vofopitant, ezlopitant (Pfizer, Inc.), R-673 (Hoffmann-La Roche Ltd), SR-48968 (Sanofi Synthelabo), CP-122,721 (Pfizer, Inc.), GW679769 (Glaxo Smith Kline), TAK-637 (Takeda/Abbot), SR-14033, and related compounds described in, for example, EP 873753 A1, US 20010006972 A1, US 20030109417 A1, WO 01/52844 A1 (for a review see Giardina et al. 2003. *Drugs* 6:758); NK-2 receptor antagonists such as nepadutant (Menarini Ricerche SpA), saredutant (Sanofi-Synthelabo), GW597599 (Glaxo Smith Kline), SR-144190 (Sanofi-Synthelabo) and UK-290795 (Pfizer Inc); NK3 receptor antagonists such as osanetant (SR-142801; Sanofi-Synthelabo), SSR-241586, talnetant and related compounds described in, for example, WO 02/094187 A2, EP 876347 A1, WO 97/21680 A1, US 6,277,862, WO 98/1 1090, WO 95/28418, WO 97/19927, and Boden et al. (*J Med Chem.* 39:1664-75, 1996); norepinephrine-serotonin reuptake inhibitors (NSRI) such as milnacipran and related compounds described in WO 03/077897; and vanilloid receptor antagonists such as arvanil and related compounds described in WO 01/64212 A1.

[168] In addition to sialorphin-related polypeptides, analgesic polypeptides include: AspPhe, endomorphin-1, endomorphin-2, nocistatin, dalargin, lupron, ziconotide, and substance P.

1.3.2.9 Insulin and Insulin Modulating Agents

[169] The GCC agonist peptides described herein can be used in combination therapy with insulin and related compounds including primate, rodent, or rabbit insulin including biologically active variants thereof including allelic variants, more preferably human insulin available in recombinant form. Sources of human insulin include pharmaceutically acceptable and sterile formulations such as those available from Eli Lilly (Indianapolis, Ind. 46285) as Humulin™ (human insulin rDNA origin). See, the THE PHYSICIAN'S DESK REFERENCE, 55.sup.th Ed. (2001) Medical Economics, Thomson Healthcare (disclosing other suitable human insulins).

[170] The GCC peptides described herein can also be used in combination therapy with agents that can boost insulin effects or levels of a subject upon administration, e.g. glipizide and/or rosiglitazone. The polypeptides and agonists described herein can be used in combination therapy with SYMLIN® (pramlintide acetate) and Exenatide® (synthetic exendin-4; a 39 aa polypeptide).

1.3.2.10 Anti-Hypertensive Agents

[171] The GCC agonist peptides described herein can be used in combination therapy with an anti-hypertensive agent including but not limited to: (1) diuretics, such as thiazides, including chlorthalidone, chlorthiazide, dichlorophenamide, hydroflumethiazide, indapamide, polythiazide, and hydrochlorothiazide; loop diuretics, such as bumetanide, ethacrynic acid, furosemide, and torsemide; potassium sparing agents, such as amiloride, and triamterene; carbonic anhydrase inhibitors, osmotics (such as glycerin) and aldosterone antagonists, such as spironolactone, eplerenone, and the like; (2) beta-adrenergic blockers such as acebutolol, atenolol, betaxolol, bevantolol, bisoprolol, bopindolol, carteolol, carvedilol, celiprolol, esmolol, indenolol, metoprolol, nadolol, nebivolol, penbutolol, pindolol, propranolol, sotalol, tertatolol, tilisolol, and timolol, and the like; (3) calcium channel blockers such as amlodipine, aranidipine, azelnidipine, barnidipine, benidipine, bepridil, cinaldipine, clevidipine, diltiazem, efonidipine, felodipine, gallopamil, isradipine, lacidipine, lemildipine, lercanidipine, nicardipine, nifedipine, nilvadipine,

nimodipine, nisoldipine, nitrendipine, manidipine, pranidipine, and verapamil, and the like; (4) angiotensin converting enzyme (ACE) inhibitors such as benazepril; captopril; ceranapril; cilazapril; delapril; enalapril; enalapril; fosinopril; imidapril; lisinopril; losinopril; moexipril; quinapril; quinaprilat; ramipril; perindopril; perindopril; quanipril; spirapril; tenocapril; trandolapril, and zofenopril, and the like; (5) neutral endopeptidase inhibitors such as omapatrilat, cadoxatril and ecadotril, fosidotril, sampatrilat, AVE7688, ER4030, and the like; (6) endothelin antagonists such as tezosentan, A308165, and YM62899, and the like; (7) vasodilators such as hydralazine, clonidine, minoxidil, and nicotinyl alcohol, and the like; (8) angiotensin II receptor antagonists such as aprosartan, candesartan, eprosartan, irbesartan, losartan, olmesartan, prazosartan, tasosartan, telmisartan, valsartan, and EXP-3137, FI6828K, and RNH6270, and the like; (9) α/β adrenergic blockers such as nipradilol, arotinolol and amosulalol, and the like; (10) alpha 1 blockers, such as terazosin, urapidil, prazosin, tamsulosin, bunazosin, trimazosin, doxazosin, naftopidil, indoramin, WHP 164, and XENOIO, and the like; (11) alpha 2 agonists such as lofexidine, tiamenidine, moxonidine, rilmenidine and guanobenz, and the like; (12) aldosterone inhibitors, and the like; and (13) angiopoietin-2 -binding agents such as those disclosed in WO03/030833. Specific anti-hypertensive agents that can be used in combination with polypeptides and agonists described herein include, but are not limited to: diuretics, such as thiazides (e.g., chlorthalidone, cyclothiazide (CAS RN 2259-96-3), chlorothiazide (CAS RN 72956-09-3, which may be prepared as disclosed in US2809194), dichlorophenamide, hydroflumethiazide, indapamide, polythiazide, bendroflumethazide, methyclothazide, polythiazide, trichlormethazide, chlorthalidone, indapamide, metolazone, quinethazone, althiazide (CAS RN 5588-16-9, which may be prepared as disclosed in British Patent No. 902,658), benzthiazide (CAS RN 91-33-8, which may be prepared as disclosed in US3108097), buthiazide (which may be prepared as disclosed in British Patent Nos. 861 ,367), and hydrochlorothiazide), loop diuretics (e.g. bumetanide, ethacrynic acid, furosemide, and torasemide), potassium sparing agents (e.g. amiloride, and triamterene (CAS Number 396-01-O)), and aldosterone antagonists (e.g. spironolactone (CAS Number 52-01-7), epi renone, and the like); β -adrenergic blockers such as Amiodarone (Cordarone, Pacerone), bunolol hydrochloride (CAS RN 31969-05-8, Parke-Davis), acebutolol (\pm N-[3-Acetyl-4-[2-hydroxy-3-(1-methylethyl)amino]propoxy]phenyl]-butanamide, or (\pm)-3'-Acetyl-4'-[2-hydroxy -3-

(isopropylamino) propoxy] butyranilide), acebutolol hydrochloride (e.g. Sectral®, Wyeth-Ayerst), alprenolol hydrochloride (CAS RN 13707-88-5 see Netherlands Patent Application No. 6,605,692), atenolol (e.g. Tenormin®, AstraZeneca), carteolol hydrochloride (e.g. Cartrol® Filmtab®, Abbott), Celiprolol hydrochloride (CAS RN 57470-78-7, also see in US4034009), cetamolol hydrochloride (CAS RN 77590-95-5, see also US4059622), labetalol hydrochloride (e.g. Normodyne®, Schering), esmolol hydrochloride (e.g. Brevibloc®, Baxter), levobetaxolol hydrochloride (e.g. Betaxon™ Ophthalmic Suspension, Alcon), levobunolol hydrochloride (e.g. Betagan® Liquifilm® with C CAP® Compliance Cap, Allergan), nadolol (e.g. Nadolol, Mylan), practolol (CAS RN 6673-35-4, see also US3408387), propranolol hydrochloride (CAS RN 318-98-9), sotalol hydrochloride (e.g. Betapace AF™, Berlex), timolol (2-Propanol, 1-[(1,1-dimethylethyl)amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, hemihydrate, (S)-, CAS RN 91524-16-2), timolol maleate (S)-I-[(1,1-dimethylethyl) amino]-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl] oxy]-2-propanol (Z)-2-butenedioate (1 :1) salt, CAS RN 26921-17-5), bisoprolol (2-Propanol, 1-[4-[[2-(1-methylethoxy)ethoxy]-methyl]phenoxy]-3-[(1-methylethyl)amino]-, (±), CAS RN 66722-44-9), bisoprolol fumarate (such as (±)-1-[4-[[2-(1-Methylethoxy) ethoxy]methyl]phenoxy]-3-[(1-methylethyl)amino]-2-propanol (E) -2-butenedioate (2:1) (salt), e.g., Zebeta™, Lederle Consumer), nebivolol (2H-1-Benzopyran-2-methanol, αα'-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-, CAS RN 99200-09-6 see also U.S. Pat. No. 4,654,362), cicloprolol hydrochloride, such 2-Propanol, 1-[4-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-[1-methylethyl)amino]-, hydrochloride, A.A.S. RN 63686-79-3), dexpropranolol hydrochloride (2-Propanol, 1-[1-methylethyl)-amino]-3-(1-naphthalenyloxy)-hydrochloride (CAS RN 13071-11-9), diacetolol hydrochloride (Acetamide, N-[3-acetyl-4-[2-hydroxy-3-[(1-methyl-ethyl)amino]propoxy] [phenyl]-, monohydrochloride CAS RN 69796-04-9), dilevalol hydrochloride (Benzamide, 2-hydroxy-5-[1-hydroxy-2-[1-methyl-3-phenylpropyl)amino]ethyl]-, monohydrochloride, CAS RN 75659-08-4), exaprolol hydrochloride (2-Propanol, 1-(2-cyclohexylphenoxy)-3-[(1-methylethyl)amino]-, hydrochloride CAS RN 59333-90-3), fleistolol sulfate (Benzoic acid, 2-fluoro-, 3-[[2-[aminocarbonyl)amino]-dimethylethyl]amino]-2-hydroxypropyl ester, (+)- sulfate (1 :1) (salt), CAS RN 88844-73-9; metalol hydrochloride (Methanesulfonamide, N-[4-[1-hydroxy-2-(methylamino)propyl]phenyl]-, monohydrochloride CAS RN 7701-65-7), metoprolol 2-

Propanolol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-; CAS RN 37350-58-6), metoprolol tartrate (such as 2-Propanolol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, e.g., Lopressor®, Novartis), pamtolol sulfate (Carbamic acid, [2-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]-ethyl]-, methyl ester, (±) sulfate (salt) (2:1), CAS RN 59954-01-7), penbutolol sulfate (2-Propanolol, 1-(2-cyclopentylphenoxy)-3-[(1,1-dimethyl-ethyl)amino] 1, (S)-, sulfate (2:1) (salt), CAS RN 38363-32-5), practolol (Acetamide, N-[4-[2-hydroxy-3-[(1-methylethyl)amino]-propoxy]phenyl]-, CAS RN 6673-35-4;); tiprenolol hydrochloride (Propanolol, 1-[(1-methylethyl)amino]-3-[2-(methylthio)-phenoxy]-, hydrochloride, (±), CAS RN 39832-43-4), tolamolol (Benzamide, 4-[2-[[2-hydroxy-3-(2-methylphenoxy)-propyl] amino] ethoxyl]-, CAS RN 38103-61-6), bopindolol, indenolol, pindolol, propanolol, tertatolol, and tilisolol, and the like; calcium channel blockers such as besylate salt of amlodipine (such as 3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulphonate, e.g., Norvasc®, Pfizer), clentiazem maleate (1,5-Benzothiazepin-4(5H)-one, 3-(acetyloxy)-8-chloro-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-(2S-cis)-, (Z)-2-butenedioate (1 :1), see also US4567195), isradipine (3,5-Pyridinedicarboxylic acid, 4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-, methyl 1-methylethyl ester, (±)-4-(4-benzofurazanyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate, see also US4466972); nimodipine (such as isopropyl (2-methoxyethyl) 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridine-dicarboxylate, e.g. Nimotop®, Bayer), felodipine (such as ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate-, e.g. Plendil® Extended-Release, AstraZeneca LP), nilvadipine (3,5-Pyridinedicarboxylic acid, 2-cyano-1,4-dihydro-6-methyl-4-(3-nitrophenyl)-,3-methyl 5-(1-methylethyl) ester, also see US3799934), nifedipine (such as 3,5-pyridinedicarboxylic acid,1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester, e.g., Procardia XL® Extended Release Tablets, Pfizer), diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one,3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,-3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis., e.g., Tiazac®, Forest), verapamil hydrochloride (such as benzeneacetonitrile, (alpha)-[[3-[[2-(3,4-dimethoxyphenyl) ethyl]methylamino]propyl] -3,4-dimethoxy-(alpha)-(1-methylethyl) hydrochloride, e.g., Isoptin® SR, Knoll Labs), teludipine hydrochloride (3,5-Pyridinedicarboxylic acid, 2-[(dimethylamino)methyl]4-[2-[(1E)-3-(1,1-dimethylethoxy)-3-oxo-1-

propenyl]phenyl]-1,4-dihydro-6-methyl-, diethyl ester, monohydrochloride) CAS RN 108700-03-4), belfosdil (Phosphonic acid, [2-(2-phenoxy ethyl)- 1,3 -propane- diyl]bis-, tetrabutyl ester CAS RN 103486-79-9), fostedil (Phosphonic acid, [[4-(2-benzothiazolyl)phenyl]methyl]-, diethyl ester CAS RN 75889-62-2), aranidipine, azelnidipine, barnidipine, benidipine, bepridil, cinaldipine, clevidipine, efonidipine, gallopamil, lacidipine, lemildipine, lercanidipine, monatepil maleate (1-Piperazinebutanamide, N-(6, 11 -dihydrodibenzo(b,e)thiepin- 11 -yl)4-(4-fluorophenyl)-, (+)-, (Z)-2-butenedioate (1 :1) (±)-N-(6,11-Dihydrodibenzo(b,e)thiepin-11-yl)-4-(p- fluorophenyl)-1-piperazinebutyramide maleate (1 :1) CAS RN 132046-06-1), nicardipine, nisoldipine, nitrendipine, manidipine, pranidipine, and the like; T-channel calcium antagonists such as mibefradil; angiotensin converting enzyme (ACE) inhibitors such as benazepril, benazepril hydrochloride (such as 3-[[1-(ethoxycarbonyl)-3- phenyl-(1 S)-propyl]amino]-2,3 ,4,5-tetrahydro-2-oxo- 1 H - 1 -(3 S)-benzazepine- 1 -acetic acid monohydrochloride, e.g., Lotrel®, Novartis), captopril (such as 1-[(2S)-3-mercapto-2- methylpropionyl]-L-proline, e.g., Captopril, Mylan, CAS RN 62571-86-2 and others disclosed in US4046889), ceranapril (and others disclosed in US4452790), cetapril (alacepril, Dainippon disclosed in Eur. Therap. Res. 39:671 (1986); 40:543 (1986)), cilazapril (Hoffman-LaRoche) disclosed in J. Cardiovasc. Pharmacol. 9:39 (1987), indalapril (delapril hydrochloride (2H-1,2,4- Benzothiadiazine-7-sulfonamide, 3-bicyclo[2.2.1]hept-5-en-2-yl-6-chloro-3,4-dihydro-, 1,1- dioxide CAS RN 2259-96-3); disclosed in US4385051), enalapril (and others disclosed in US4374829), enalapril, enalaprilat, fosinopril, ((such as L-proline, 4-cyclohexyl-1-[[[2-methyl- 1-(1-oxopropoxy) propoxy](4-phenylbutyl) phosphinyl]acetyl]-, sodium salt, e.g., Monopril, Bristol-Myers Squibb and others disclosed in US4168267), fosinopril sodium (L- Proline, 4-cyclohexyl-1-[[[2-methyl-1-(1-ox- opropoxy)propox), imidapril, indolapril (Schering, disclosed in J. Cardiovasc. Pharmacol. 5:643, 655 (1983)), lisinopril (Merck), losinopril, moexipril, moexipril hydrochloride (3-Isoquinolinecarboxylic acid, 2-[(2S)-2-[[[(1S)- 1 -(ethoxycarbonyl)-3-phenylpropyl]amino]- 1 -oxopropyl]- 1 , -2,3,4-tetrahydro-6,7-dimethoxy-, monohydrochloride, (3S)- CAS RN 82586-52-5), quinapril, quinaprilat, ramipril (Hoechst) disclosed in EP 79022 and Curr. Ther. Res. 40:74 (1986), perindopril erbumine (such as 2S,3aS,7aS- 1 -[(S)-N-[(S)- 1 - Carboxybutyl]alanyl]hexahydro[^]-indolinecarboxylic acid, 1 -ethyl ester, compound with tert-butylamine (1 :1), e.g., Aceon®, Solvay), perindopril (Servier, disclosed in Eur. J. clin.

Pharmacol. 31 :519 (1987)), quanipril (disclosed in US4344949), spirapril (Schering, disclosed in Acta. Pharmacol. Toxicol. 59 (Supp. 5): 173 (1986)), tenocapril, trandolapril, zofenopril (and others disclosed in US4316906), rentiapril (fentiapril, disclosed in Clin. Exp. Pharmacol. Physiol. 10:131 (1983)), pivopril, YS980, teprotide (Bradykinin potentiator BPP9a CAS RN 35115-60-7), BRL 36,378 (Smith Kline Beecham, see EP80822 and EP60668), MC-838 (Chugai, see CA. 102:72588v and Jap. J. Pharmacol. 40:373 (1986)), CGS 14824 (Ciba-Geigy, 3-[[1-ethoxycarbonyl-3-phenyl-(1S)-propyl]amino]-2,3,4,5-tetrahydro-2-oxo-1-(3S)-benzazepine-1-acetic acid HCl, see U.K. Patent No. 2103614), CGS 16,617 (Ciba-Geigy, 3(S)-[[1-(1S)-5-amino-1-carboxypentyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H-1-benzazepine-1-ethanoic acid, see US4473575), Ru 44570 (Hoechst, see Arzneimittelforschung 34:1254 (1985)), R 31-2201 (Hoffman-LaRoche see FEBS Lett. 165:201 (1984)), CI925 (Pharmacologist 26:243, 266 (1984)), WY-44221 (Wyeth, see J. Med. Chem. 26:394 (1983)), and those disclosed in US2003006922 (paragraph 28), US4337201, US4432971 (phosphoramidates); neutral endopeptidase inhibitors such as omapatrilat (Vanlev®), CGS 30440, cadoxatril and ecadotril, fasidotril (also known as aladotril or alatriopril), sampatrilat, mixanpril, and gemopatrilat, AVE7688, ER4030, and those disclosed in US5362727, US5366973, US5225401, US4722810, US5223516, US4749688, US5552397, US5504080, US5612359, US5525723, EP0599444, EP0481522, EP0599444, EP0595610, EP0534363, EP534396, EP534492, EP0629627; endothelin antagonists such as tezosentan, A308165, and YM62899, and the like; vasodilators such as hydralazine (apresoline), clonidine (clonidine hydrochloride (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)4,5-dihydro-, monohydrochloride CAS RN 4205-91-8), catapres, minoxidil (loniten), nicotiny alcohol (roniacol), diltiazem hydrochloride (such as 1,5-Benzothiazepin-4(5H)-one,3-(acetyloxy)-5[2-(dimethylamino)ethyl]-2,3-dihydro-2(4-methoxyphenyl)-, monohydrochloride, (+)-cis, e.g., Tiazac®, Forest), isosorbide dinitrate (such as 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate e.g., Isordil® Titrados®), Wyeth-Ayerst), sosorbide mononitrate (such as 1,4:3,6-dianhydro-D-glucitol-1,5-nitrate, an organic nitrate, e.g., Ismo®, Wyeth-Ayerst), nitroglycerin (such as 2,3 propanetriol trinitrate, e.g., Nitrostat® Parke-Davis), verapamil hydrochloride (such as benzeneacetonitrile, (±)-(alpha)[3-[[2-(3,4 dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy-(alpha)-(1-methylethyl) hydrochloride, e.g., Covera HS® Extended-Release, Searle), chromonar (which may be prepared as disclosed in

US3282938), clonitate (Annalen 1870 155), droprenilamine (which may be prepared as disclosed in DE2521113), lidoflazine (which may be prepared as disclosed in US3267104); prenylamine (which may be prepared as disclosed in US3152173), propatyl nitrate (which may be prepared as disclosed in French Patent No. 1,103,113), mioflazine hydrochloride (1 -Piperazineacetamide, 3-(aminocarbonyl)-4-[4,4-bis(4-fluorophenyl)butyl]-N-(2,6-dichlorophenyl)-, dihydrochloride CAS RN 83898-67-3), mixidine (Benzeneethanamine, 3,4-dimethoxy-N-(1-methyl-2-pyrrolidinylidene)-Pyrrolidine, 2-[(3,4-dimethoxyphenethyl)imino]-1-methyl-1-Methyl-2-[(3,4-dimethoxyphenethyl)imino]pyrrolidine CAS RN 27737-38-8), molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0), isosorbide mononitrate (D-Glucitol, 1,4:3,6-dianhydro-, 5-nitrate CAS RN 16051-77-7), erythrityl tetranitrate (1,2,3,4-Butanetetrol, tetranitrate, (2R,3S)-rel-CAS RN 7297-25-8), clonitrate(1,2-Propanediol, 3-chloro-, dinitrate (7CI, 8CI, 9CI) CAS RN 2612-33-1), dipyridamole Ethanol, 2,2',2'',2'''-[(4,8-di-1-piperidinylpyrimido[5,4-d]pyrimidine-2,6-diyl)dinitrilo]tetrakis- CAS RN 58-32-2), nicorandil (CAS RN 65141-46-0 3-), pyridinecarboxamide (N-[2-(nitrooxy)ethyl]-Nisoldipine-3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester CAS RN 63675-72-9), nifedipine-3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester CAS RN 21829-25-4), perhexiline maleate (Piperidine, 2-(2,2-dicyclohexylethyl)-, (2Z)-2-butenedioate (1 :1) CAS RN 6724-53-4), oxprenolol hydrochloride (2-Propanol, 1-[(1-methylethyl)amino]-3-[2-(2-propenyloxy)phenoxy]-, hydrochloride CAS RN 6452-73-9), pentrinitrol (1,3-Propanediol, 2,2-bis[(nitrooxy)methyl]-, mononitrate (ester) CAS RN 1607-17-6), verapamil (Benzeneacetonitrile, α -[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy- α -(1-methylethyl)- CAS RN 52-53-9) and the like; angiotensin II receptor antagonists such as, aprosartan, zolasartan, olmesartan, prazosartan, FI6828K, RNH6270, candesartan (1 H-Benzimidazole-7-carboxylic acid, 2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]4-yl]methyl]- CAS RN 139481-59-7), candesartan cilexetil ((+/-)-1-(cyclohexylcarbonyloxy)ethyl-2-ethoxy-1-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]-1H-benzimidazole carboxylate, CAS RN 145040-37-5, US5703110 and US5196444), eprosartan (3-[1-4-carboxyphenylmethyl]-2-n-butyl-imidazol-5-yl)-(2-thienylmethyl) propenoic acid, US5185351 and US5650650), irbesartan (2-n-butyl-3-[[2'-(1h-tetrazol-5-yl)biphenyl-4-yl]methyl] 1,3-

diazaspiro[4,4]non-1-en-4-one, US5270317 and US5352788), losartan (2-N-butyl-4-chloro-5-hydroxymethyl-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole, potassium salt, US5138069, US5153197 and US5128355), tasosartan (5,8-dihydro-2,4-dimethyl-8-[(2'-(1H-tetrazol-5-yl)[1,r-biphenyl]4-yl)methyl]-pyrido[2,3-d]pyrimidin-7(6H)-one, US5149699), telmisartan (4'-[(1,4-dimethyl-2'-propyl-(2,6'-bi-1H-benzimidazol)-r-yl)]-[1,1'-biphenyl]-2-carboxylic acid, CAS RN 144701-48-4, US5591762), milfasartan, abitesartan, valsartan (Diovan® (Novartis), (S)-N-valeryl-N-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]valine, US5399578), EXP-3137 (2-N-butyl-4-chloro-1-[(2'-(1H-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid, US5138069, US5153197 and US5128355), 3-(2'-(tetrazol-5-yl)-1,r-biphen-4-yl)methyl-5,7-dimethyl-2-ethyl-3H-imidazo[4,5-b]pyridine, 4'[2-ethyl-4-methyl-6-(5,6,7,8-tetrahydroimidazo[1,2-a]pyridin-2-yl)-benzimidazol-1-yl]-methyl]-1,r-biphenyl]-2-carboxylic acid, 2-butyl-6-(1-methoxy-1-methylethyl)-2-[2'-(1H-tetrazol-5-yl)biphenyl-4-ylmethyl]guinazolin-4(3H)-one, 3-[2'-carboxybiphenyl-4-yl)methyl]-2-cyclopropyl-7-methyl-3H-imidazo[4,5-b]pyridine, 2-butyl-4-chloro-1-[(2'-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-carboxylic acid, 2-butyl-4-chloro-1-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-yl)methyl]-1H-imidazole-5-carboxylic acid-1-(ethoxycarbonyloxy)ethyl ester potassium salt, dipotassium 2-butyl-4-(methylthio)-1-[[2-[[[(propylamino)carbonyl]amino]sulfonyl](1,1'-biphenyl)-4-yl)methyl]-1H-imidazole-5-carboxylate, methyl-2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(1H-tetrazol-5-yl)-[1,1'-biphenyl]-4-yl)methyl]-1-(6H)-pyrimidinyl)methyl]-3-thiophencarboxylate, 5-[(3,5-dibutyl-1H-1,2,4-triazol-1-yl)methyl]-2-[2-(1H-tetrazol-5-ylphenyl)]pyridine, 6-butyl-2-(2-phenylethyl)-5-[[2'-(1H-tetrazol-5-yl)[1,1'-biphenyl]-4-methyl]pyrimidin-4-(3H)-one D,L lysine salt, 5-methyl-7-n-propyl-8-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-[1,2,4]-triazolo[1,5-c]pyrimidin-2(3H)-one, 2,7-diethyl-5-[[2'-(5-tetrazolyl)biphenyl-4-yl)methyl]-5H-pyrazolo[1,5-b][1,2,4]triazole potassium salt, 2-[2-butyl-4,5-dihydro-4-oxo-3-[2'-(1H-tetrazol-5-yl)-4-biphenylmethyl]-3H-imidazol[4,5-c]pyridine-5-ylmethyl]benzoic acid, ethyl ester, potassium salt, 3-methoxy-2,6-dimethyl-4-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl-4-yl]methoxy]pyridine, 2-ethoxy-1-[[2'-(5-oxo-2,5-dihydro-1,2,4-oxadiazol-3-yl)biphenyl-4-yl)methyl]-1H-benzimidazole-7-carboxylic acid, 1-[N-(2'-(1H-tetrazol-5-yl)biphenyl-4-yl-methyl)-N-valerolylaminomethyl]cyclopentane-1-carboxylic acid, 7-methyl-2n-propyl-3-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-3H-imidazo[4,5-b]pyridine, 2-[5-[(2-

ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridine-3-yl)methyl]-2-quinoliny]sodium benzoate, 2-butyl-6-chloro-4-hydroxymethyl-5-methyl-3-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyridine, 2-[[[2-butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl]methyl]amino]benzoic acid tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-6-one, 4(S)-[4-(carboxymethyl)phenoxy]-N-[2(R)-[4-(2-sulfobenzamido)imidazol-1-yl]octanoyl]-L-proline, 1-(2,6-dimethylphenyl)-4-butyl-1,3-dihydro-3-[[6-[2-(1H-tetrazol-5-yl)phenyl]-3-pyridinyl]methyl]-2H-imidazol-2-one, 5,8-ethano-5,8-dimethyl-2-n-propyl-5,6,7,8-tetrahydro-1-[[2'(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H,4H-1,3,4a,8a-tetraazacyclopentanaphthalene-9-one, 4-[1-[2'-(1,2,3,4-tetrazol-5-yl)biphen-4-yl)methylamino]-5,6,7,8-tetrahydro-2-triflylquinazoline, 2-(2-chlorobenzoyl)imino-5-ethyl-3-[2'-(1H-tetrazole-5-yl)biphenyl-4-yl)methyl-1,3,4-thiadiazoline, 2-[5-ethyl-3-[2-(1H-tetrazole-5-yl)biphenyl-4-yl]methyl-1,3,4-thiazoline-2-ylidene]aminocarbonyl-1-cyclopentencarboxylic acid dipotassium salt, and 2-butyl-4-[N-methyl-N-(3-methylcrotonoyl)amino]-1-[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-1H-imidazole-5-carboxylic acid 1-ethoxycarbonyloxyethyl ester, those disclosed in patent publications EP475206, EP497150, EP539086, EP539713, EP535463, EP535465, EP542059, EP497121, EP535420, EP407342, EP415886, EP424317, EP435827, EP433983, EP475898, EP490820, EP528762, EP324377, EP323841, EP420237, EP500297, EP426021, EP480204, EP429257, EP430709, EP434249, EP446062, EP505954, EP524217, EP514197, EP514198, EP514193, EP514192, EP450566, EP468372, EP485929, EP503162, EP533058, EP467207 EP399731, EP399732, EP412848, EP453210, EP456442, EP470794, EP470795, EP495626, EP495627, EP499414, EP499416, EP499415, EP511791, EP516392, EP520723, EP520724, EP539066, EP438869, EP505893, EP530702, EP400835, EP400974, EP401030, EP407102, EP411766, EP409332, EP412594, EP419048, EP480659, EP481614, EP490587, EP467715, EP479479, EP502725, EP503838, EP505098, EP505111 EP513,979 EP507594, EP510812, EP511767, EP512675, EP512676, EP512870, EP517357, EP537937, EP534706, EP527534, EP540356, EP461040, EP540039, EP465368, EP498723, EP498722, EP498721, EP515265, EP503785, EP501892, EP519831, EP532410, EP498361, EP432737, EP504888, EP508393, EP508445, EP403159, EP403158, EP425211, EP427463, EP437103, EP481448, EP488532, EP501269, EP500409, EP540400, EP005528, EP028834, EP028833, EP411507, EP425921, EP430300, EP434038, EP442473, EP443568, EP445811, EP459136, EP483683,

EP518033, EP520423, EP531876, EP531874, EP392317, EP468470, EP470543, EP502314, EP529253, EP543263, EP540209, EP449699, EP465323, EP521768, EP415594, WO92/14468, WO93/08171, WO93/08169, WO91/00277, WO91/00281, WO91/14367, WO92/00067, WO92/00977, WO92/20342, WO93/04045, WO93/04046, WO91/15206, WO92/14714, WO92/09600, WO92/16552, WO93/05025, WO93/03018, WO91/07404, WO92/02508, WO92/13853, WO91/19697, WO91/11909, WO91/12001, WO91/11999, WO91/15209, WO91/15479, WO92/20687, WO92/20662, WO92/20661, WO93/01177, WO91/14679, WO91/13063, WO92/13564, WO91/17148, WO91/18888, WO91/19715, WO92/02257, WO92/04335, WO92/05161, WO92/07852, WO92/15577, WO93/03033, WO91/16313, WO92/00068, WO92/02510, WO92/09278, WO92/10179, WO92/10180, WO92/10186, WO92/10181, WO92/10097, WO92/10183, WO92/10182, WO92/10187, WO92/10184, WO92/10188, WO92/10180, WO92/10185, WO92/20651, WO93/03722, WO93/06828, WO93/03040, WO92/19211, WO92/22533, WO92/06081, WO92/05784, WO93/00341, WO92/04343, WO92/04059, US5104877, US5187168, US5149699, US5185340, US4880804, US5138069, US4916129, US5153197, US5173494, US5137906, US5155126, US5140037, US5137902, US5157026, US5053329, US5132216, US5057522, US5066586, US5089626, US5049565, US5087702, US5124335, US5102880, US5128327, US5151435, US5202322, US5187159, US5198438, US5182288, US5036048, US5140036, US5087634, US5196537, US5153347, US5191086, US5190942, US5177097, US5212177, US5208234, US5208235, US5212195, US5130439, US5045540, US5041152, and US5210204, and pharmaceutically acceptable salts and esters thereof; α/β adrenergic blockers such as nipradilol, arotinolol, amosulalol, bretylium tosylate (CAS RN: 61-75-6), dihydroergtamine mesylate (such as ergotaman-3', 6',18-trione,9,-10-dihydro-12'-hydroxy-2'-methyl-5'-(phenylmethyl)-,(5'(α))-monomethanesulfonate, e.g., DHE 45® Injection, Novartis), carvedilol (such as (\pm)-l-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl] amino] -2-propanol, e.g., Coreg®, SmithKline Beecham), labetalol (such as 5-[l-hydroxy-2-[(l-methyl-3-phenylpropyl) amino] ethyl]salicylamide monohydrochloride, e.g., Normodyne®, Schering), bretylium tosylate (Benzenemethanaminium, 2-bromo-N-ethyl-N,N-dimethyl-, salt with 4-methylbenzenesulfonic acid (1 :1) CAS RN 61-75-6), phentolamine mesylate (Phenol, 3-[[[(4,5-dihydro-1H-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt) CAS RN 65-28-1),

solypertine tartrate (5H-1,3-Dioxolo[4,5-f]indole, 7-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-, (2R,3R)-2,3-dihydroxybutanedioate (1 :1) CAS RN 5591-43-5), zolertine hydrochloride (Piperazine, 1-phenyl4-[2-(1H-tetrazol-5-yl)ethyl]-, monohydrochloride (8Cl, 9Cl) CAS RN 7241-94-3) and the like; α adrenergic receptor blockers, such as alfuzosin (CAS RN: 81403-68-1), terazosin, urapidil, prazosin (Minipress®), tamsulosin, bunazosin, trimazosin, doxazosin, naftopidil, indoramin, WHP 164, XENOIO, fenspiride hydrochloride (which may be prepared as disclosed in US3399192), proroxan (CAS RN 33743-96-3), and labetalol hydrochloride and combinations thereof; α 2 agonists such as methyldopa, methyldopa HCL, lofexidine, tiamenidine, moxonidine, rilmenidine, guanobenz, and the like; aldosterone inhibitors, and the like; renin inhibitors including Aliskiren (SPPIOO; Novartis/Speedel); angiotensin-2-binding agents such as those disclosed in WO03/030833; anti-angina agents such as ranolazine (hydrochloride 1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6), betaxolol hydrochloride (2-Propanol, 1-[4-[2 (cyclopropylmethoxy)ethyl]phenoxy]-3-[(1-methylethyl)amino]-, hydrochloride CAS RN 63659-19-8), butopropazine hydrochloride (Methanone, [4-[3(dibutylamino)propoxy]phenyl](2-ethyl-3-indoliziny)-, monohydrochloride CAS RN 62134-34-3), cinepazet maleate-Piperazineacetic acid, 4-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-, ethyl ester, (2Z)-2-butenedioate (1 :1) CAS RN 50679-07-7), tosisfen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-184), verapamilhydrochloride (Benzeneacetonitrile, α -[3-[[2-(3,4-dimethoxyphenyl)ethyl]methylamino]propyl]-3,4-dimethoxy- α -(1-methylethyl)-, monohydrochloride CAS RN 152-114), molsidomine (1,2,3-Oxadiazolium, 5-[(ethoxycarbonyl)amino]-3-(4-morpholinyl)-, inner salt CAS RN 25717-80-0), and ranolazine hydrochloride (1-Piperazineacetamide, N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); tosisfen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-184); adrenergic stimulants such as guanfacine hydrochloride (such as N-amidino-2-(2,6-dichlorophenyl)acetamide hydrochloride, e.g., Tenex® Tablets available from Robins); methyldopa-hydrochlorothiazide (such as levo-3-(3,4-dihydroxyphenyl)-2-methylalanine) combined with Hydrochlorothiazide (such as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide

1,1-dioxide, e.g., the combination as, e.g., Aldoril® Tablets available from Merck), methyl dopa-chlorothiazide (such as 6-chloro-2H-1, 2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide and methyl dopa as described above, e.g., Aldoclor®, Merck), clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride and chlorthalidone (such as 2-chloro-5-(1-hydroxy-3-oxo-1-isoindolinyl) benzenesulfonamide), e.g., Combipres®, Boehringer Ingelheim), clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, e.g., Catapres®, Boehringer Ingelheim), clonidine (1H-Imidazol-2-amine, N-(2,6-dichlorophenyl)4,5-dihydro-CAS RN 4205-90-7), Hyzaar (Merck; a combination of losartan and hydrochlorothiazide), Co-Diovan (Novartis; a combination of valsartan and hydrochlorothiazide), Lotrel (Novartis; a combination of benazepril and amlodipine) and Caduet (Pfizer; a combination of amlodipine and atorvastatin), and those agents disclosed in US20030069221.

1.3.2.11 Agents for the Treatment of Respiratory Disorders

[172] The GCC agonist peptides described herein can be used in combination therapy with one or more of the following agents useful in the treatment of respiratory and other disorders including but not limited to: (1) β -agonists including but not limited to : albuterol (PRO VENTIL® , S ALBUT AMOI® , VENTOLIN®), bambuterol, bitoterol, clenbuterol, fenoterol, formoterol, isoetharine (BRONKOSOL®, BRONKOMETER®), metaproterenol (ALUPENT®, METAPREL®), pirbuterol (MAXAIR®), reproterol, rimiterol, salmeterol, terbutaline (BRETHAIRE®, BRETHINE®, BRICANYL®), adrenalin, isoproterenol (ISUPREL®), epinephrine bitartrate (PRIMATENE®), ephedrine, orciprenline, fenoterol and isoetharine; (2) steroids, including but not limited to beclomethasone, beclomethasone dipropionate, betamethasone, budesonide, bunedoside, butixocort, dexamethasone, flunisolide, fluocortin, fluticasone, hydrocortisone, methyl prednisone, mometasone, predonisolone, predonisone, tipredane, tixocortal, triamcinolone, and triamcinolone acetonide; (3) β 2-agonist-corticosteroid combinations [e.g., salmeterol-fluticasone (AD V AIR®), formoterol-budesonid (S YMBICORT®)] ; (4) leukotriene D4 receptor antagonists/leukotriene antagonists/LTD4 antagonists (i.e., any compound that is capable of blocking, inhibiting, reducing or otherwise interrupting the interaction between leukotrienes and the Cys LTI receptor) including but not limited to: zafhiukast, montelukast, montelukast sodium (SINGULAIR®), pranlukast, iralukast,

pobilukast, SKB-106,203 and compounds described as having LTD₄ antagonizing activity described in U.S. Patent No. 5,565,473; (5) 5 -lipoxygenase inhibitors and/or leukotriene biosynthesis inhibitors [e.g., zileuton and BAY1005 (CA registry 128253-31-6)]; (6) histamine H₁ receptor antagonists/antihistamines (i.e., any compound that is capable of blocking, inhibiting, reducing or otherwise interrupting the interaction between histamine and its receptor) including but not limited to: astemizole, acrivastine, antazoline, azatadine, azelastine, astemizole, brompheniramine, brompheniramine maleate, carbinoxamine, carebastine, cetirizine, chlorpheniramine, chlorpheniramine maleate, cimetidine clemastine, cyclizine, cyproheptadine, descarboethoxyloratadine, dexchlorpheniramine, dimethindene, diphenhydramine, diphenylpyraline, doxylamine succinate, doxylamine, ebastine, efletirizine, epinastine, famotidine, fexofenadine, hydroxyzine, hydroxyzine, ketotifen, levocabastine, levocetirizine, levocetirizine, loratadine, meclizine, mepyramine, mequitazine, methdilazine, mianserin, mizolastine, noberastine, norastemizole, noraztemizole, phenindamine, pheniramine, picumast, promethazine, pynlamine, pyrillamine, ranitidine, temelastine, terfenadine, trimeprazine, tripelenamine, and triprolidine; (7) an anticholinergic including but not limited to: atropine, benztropine, biperiden, flutropium, hyoscyamine (e.g. Levsin®; Levbid®; Levsin/SL®, Anaspaz®, Levsinex timecaps®, NuLev®), ilutropium, ipratropium, ipratropium bromide, methscopolamine, oxybutinin, rispenzepine, scopolamine, and tiotropium; (8) an anti-tussive including but not limited to: dextromethorphan, codeine, and hydromorphone; (9) a decongestant including but not limited to: pseudoephedrine and phenylpropanolamine; (10) an expectorant including but not limited to: guaifenesin, guaicol sulfate, terpin, ammonium chloride, glycerol guaicolate, and iodinated glycerol; (11) a bronchodilator including but not limited to: theophylline and aminophylline; (12) an anti-inflammatory including but not limited to: fluribiprofen, diclophenac, indomethacin, ketoprofen, S-ketroprophen, tenoxicam; (13) a PDE (phosphodiesterase) inhibitor including but not limited to those disclosed herein; (14) a recombinant humanized monoclonal antibody [e.g. xolair (also called omalizumab), rhuMab, and talizumab]; (15) a humanized lung surfactant including recombinant forms of surfactant proteins SP-B, SP-C or SP-D [e.g. SURFAXIN®, formerly known as dsc-104 (Discovery Laboratories)], (16) agents that inhibit epithelial sodium channels (ENaC) such as amiloride and related compounds; (17) antimicrobial agents used to treat pulmonary infections such as acyclovir,

amikacin, amoxicillin, doxycycline, trimethoprin sulfamethoxazole, amphotericin B, azithromycin, clarithromycin, roxithromycin, clarithromycin, cephalosporins(ceffoxitin, cefmetazole etc), ciprofloxacin, ethambutol, gentimycin, ganciclovir, imipenem, isoniazid, itraconazole, penicillin, ribavirin, rifampin, rifabutin, amantadine, rimantidine, streptomycin, tobramycin, and vancomycin; (18) agents that activate chloride secretion through Ca⁺⁺ dependent chloride channels (such as purinergic receptor (P2Y(2) agonists); (19) agents that decrease sputum viscosity, such as human recombinant DNase 1, (Pulmozyme®); (20) nonsteroidal anti-inflammatory agents (acemetacin, acetaminophen, acetyl salicylic acid, alclofenac, alminoprofen, apazone, aspirin, benoxaprofen, bezpiperylon, bucloxic acid, carprofen, clidanac, diclofenac, diclofenac, diflunisal, diflusinal, etodolac, fenbufen, fenbufen, fenclofenac, fenclozic acid, fenoprofen, fentiazac, feprazone, flufenamic acid, flufenisal, flufenisal, fluprofen, flurbiprofen, flurbiprofen, furofenac, ibufenac, ibuprofen, indomethacin, indomethacin, indoprofen, isoxepac, isoxicam, ketoprofen, ketoprofen, ketorolac, meclofenamic acid, meclofenamic acid, mefenamic acid, mefenamic acid, miroprofen, mofebutazone, nabumetone oxaprozin, naproxen, naproxen, niflumic acid , oxaprozin, oxpinac, oxyphenbutazone, phenacetin, phenylbutazone, phenylbutazone, piroxicam, piroxicam, piroprofen, pranoprofen, sudoxicam, tenoxicam, sulfasalazine, sulindac, sulindac, suprofen, tiaprofenic acid, tiopinac, tioxaprofen, tolfenamic acid, tolmetin, tolmetin, zidometacin, zomepirac, and zomepirac); and (21) aerosolized antioxidant therapeutics such as S-Nitrosoglutathione.

1.3.2.12 Anti-Diabetic Agents

[173] The GCC agonist peptides described herein can be used in therapeutic combination with one or more anti-diabetic agents, including but not limited to: PPAR γ agonists such as glitazones (e.g., WAY-120,744, AD 5075, balaglitazone, ciglitazone, darglitazone (CP-86325, Pfizer), englitazone (CP-68722, Pfizer), isaglitazone (MIT/J&J), MCC- 555 (Mitsubishi disclosed in
5 US5594016), pioglitazone (such as such as Actos™ pioglitazone; Takeda), rosiglitazone (Avandia™;Smith Kline Beecham), rosiglitazone maleate, troglitazone (Rezulin®, disclosed in US4572912), rivoglitazone (CS-Ol 1, Sankyo), GL-262570 (Glaxo Welcome), BRL49653 (disclosed in WO98/05331), CLX-0921, 5-BTZD, GW-0207, LG- 100641, JJT-501

(JPNT/P&U), L-895645 (Merck), R-119702 (Sankyo/Pfizer), NN-2344 (Dr. Reddy/NN), YM-440 (Yamanouchi), LY-300512, LY-519818, R483 (Roche), T131 (Tularik), and the like and compounds disclosed in US4687777, US5002953, US5741803, US5965584, US6150383, US6150384, US6166042, US6166043, US6172090, US6211205, US6271243, US6288095, 5 US6303640, US6329404, US5994554, WO97/10813, WO97/27857, WO97/28115, WO97/28137, WO97/27847, WO00/76488, WO03/000685, WO03/027112, WO03/035602, WO03/048130, WO03/055867, and pharmaceutically acceptable salts thereof; biguanides such as metformin hydrochloride (N,N-dimethylimidodicarbonimidic diamide hydrochloride, such as Glucophage™, Bristol-Myers Squibb); metformin hydrochloride with glyburide, such as 10 Glucovance™, Bristol-Myers Squibb); buformin (Imidodicarbonimidic diamide, N-butyl-); etoformine (l-Butyl-2-ethylbiguanide, Schering A. G.); other metformin salt forms (including where the salt is chosen from the group of, acetate, benzoate, citrate, ftimarate, embonate, chlorophenoxyacetate, glycolate, palmoate, aspartate, methanesulphonate, maleate, parachlorophenoxyisobutyrate, formate, lactate, succinate, sulphate, tartrate, 15 cyclohexanecarboxylate, hexanoate, octanoate, decanoate, hexadecanoate, octodecanoate, benzenesulphonate, trimethoxybenzoate, paratoluenesulphonate, adamantanecarboxylate, glycoxylate, glutarnate, pyrrolidonecarboxylate, naphthalenesulphonate, 1-glucosephosphate, nitrate, sulphite, dithionate and phosphate), and phenformin; protein tyrosine phosphatase- IB (PTP-IB) inhibitors, such as A-401,674, KR 61639, OC- 060062, OC-83839, OC-297962, 20 MC52445, MC52453, ISIS 113715, and those disclosed in WO99/585521, WO99/58518, WO99/58522, WO99/61435, WO03/032916, WO03/032982, WO03/041729, WO03/055883, WO02/26707, WO02/26743, JP2002114768, and pharmaceutically acceptable salts and esters thereof; sulfonylureas such as acetoexamide (e.g. Dymelor, Eli Lilly), carbutamide, chlorpropamide (e.g. Diabinese®, Pfizer), gliamilide (Pfizer), gliclazide (e.g. Diamcron, Servier 25 Canada Inc), glimepiride (e.g. disclosed in US4379785, such as Amaryl , Aventis), glipentide, glipizide (e.g. Glucotrol or Glucotrol XL Extended Release, Pfizer), gliquidone, glisolamide, glyburide/glibenclamide (e.g. Micronase or Glynase Prestab, Pharmacia & Upjohn and Diabeta, Aventis), tolazamide (e.g. Tolinase), and tolbutamide (e.g. Orinase), and pharmaceutically acceptable salts and esters thereof; meglitinides such as repaglinide (e.g. Prandin®, Novo 30 Nordisk), KAD1229 (PF/Kissei), and nateglinide (e.g. Starlix®, Novartis), and pharmaceutically

acceptable salts and esters thereof; α glucoside hydrolase inhibitors (or glucoside inhibitors) such as acarbose (e.g. Precose™, Bayer disclosed in US4904769), miglitol (such as GLYSET™, Pharmacia & Upjohn disclosed in US4639436), camiglibose (Methyl 6-deoxy-6-[(2R,3R,4R,5S)-3,4,5-trihydroxy-2-(hydroxymethyl)piperidino]- α -D-glucopyranoside, Marion Merrell Dow), voglibose (Takeda), adiposine, emiglitate, pradimicin-Q, salbostatin, CKD-711, MDL-25,637, MDL-73,945, and MOR 14, and the compounds disclosed in US4062950, US4174439, US4254256, US4701559, US4639436, US5192772, US4634765, US5157116, US5504078, US5091418, US5217877, US51091 and WOO 1/47528 (polyamines); α -amylase inhibitors such as tendamistat, trestatin, and AI-3688, and the compounds disclosed in US4451455, US4623714, and US4273765; SGLT2 inhibitors including those disclosed in US6414126 and US6515117; an α P2 inhibitor such as disclosed in US6548529; insulin secretagogues such as linogiride, A-4166, forskilin, dibutyl cAMP, isobutylmethylxanthine (IBMX), and pharmaceutically acceptable salts and esters thereof; fatty acid oxidation inhibitors, such as clomoxir, and etomoxir, and pharmaceutically acceptable salts and esters thereof; A2 antagonists, such as midaglizole, isaglidole, derigidole, idazoxan, earoxan, and fluparoxan, and pharmaceutically acceptable salts and esters thereof; insulin and related compounds (e.g. insulin mimetics) such as biota, LP-100, novarapid, insulin detemir, insulin lispro, insulin glargine, insulin zinc suspension (lente and ultralente), Lys-Pro insulin, GLP-I (1-36) amide, GLP-I (73-7) (insulintropin, disclosed in US5614492), LY-315902 (Lilly), GLP-I (7-36)-NH₂), AL-401 (Autoimmune), certain compositions as disclosed in US4579730, US4849405, US4963526, US5642868, US5763396, US5824638, US5843866, US6153632, US6191105, and WO 85/05029, and primate, rodent, or rabbit insulin including biologically active variants thereof including allelic variants, more preferably human insulin available in recombinant form (sources of human insulin include pharmaceutically acceptable and sterile formulations such as those available from Eli Lilly (Indianapolis, Ind. 46285) as Humulin™ (human insulin rDNA origin), also see the THE PHYSICIAN'S DESK REFERENCE, 55.sup.th Ed. (2001) Medical Economics, Thomson Healthcare (disclosing other suitable human insulins); non-thiazolidinediones such as JT-501 and farglitazar (GW-2570/GI-262579), and pharmaceutically acceptable salts and esters thereof; PPAR α/γ dual agonists such as AR-HO39242 (Aztazeneca), GW-409544 (Glaxo-Wellcome), BVT-142, CLX-0940, GW-1536, GW-1929, GW-2433, KRP-

297 (Kyorin Merck; 5-[(2,4-Dioxo thiazolidinyl)methyl] methoxy-N-[[4-(trifluoromethyl)phenyl] methyl]benzamide), L-796449, LR-90, MK-0767 (Merck/Kyorin/Banyu), SB 219994, muraglitazar (BMS), tesaglitazar (Astrazeneca), reglitazar (JTT-501) and those disclosed in WO99/16758, WO99/19313, WO99/20614, WO99/38850, 5 WO00/23415, WO00/23417, WO00/23445, WO00/50414, WO01/00579, WO01/79150, WO02/062799, WO03/004458, WO03/016265, WO03/018010, WO03/033481, WO03/033450, WO03/033453, WO03/043985, WO 031053976, U.S. application Ser. No. 09/664,598, filed Sep. 18, 2000, Murakami et al. Diabetes 47, 1841-1847 (1998), and pharmaceutically acceptable salts and esters thereof; other insulin sensitizing drugs; VPAC2 receptor agonists; GLK modulators, 10 such as those disclosed in WO03/015774; retinoid modulators such as those disclosed in WO03/000249; GSK 3 β /GSK 3 inhibitors such as 4-[2-(2-bromophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl]pyridine and those compounds disclosed in WO03/024447, WO03/037869, WO03/037877, WO03/037891, WO03/068773, EP1295884, EP1295885, and the like; glycogen phosphorylase (HGLPa) inhibitors such as CP-368,296, CP-316,819, BAYR3401, and 15 compounds disclosed in WOO 1/94300, WO02/20530, WO03/037864, and pharmaceutically acceptable salts or esters thereof; ATP consumption promoters such as those disclosed in WO03/007990; TRB3 inhibitors; vanilloid receptor ligands such as those disclosed in WO03/049702; hypoglycemic agents such as those disclosed in WO03/015781 and WO03/040114; glycogen synthase kinase 3 inhibitors such as those disclosed in WO03/035663 20 agents such as those disclosed in WO99/51225, US20030134890, WO01/24786, and WO03/059870; insulin-responsive DNA binding protein-1 (IRDBP-I) as disclosed in WO03/057827, and the like; adenosine A2 antagonists such as those disclosed in WO03/035639, WO03/035640, and the like; PPAR δ agonists such as GW 501516, GW 590735, and compounds disclosed in JP10237049 and WO02/14291; dipeptidyl peptidase IV (DP-IV) inhibitors, such as 25 isoleucine thiazolidide, NVP-DPP728A (1- [[2-(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(S)-pyrrolidine, disclosed by Hughes et al, Biochemistry, 38(36), 11597-11603, 1999), P32/98, NVP-LAF-237, P3298, TSL225 (tryptophyl-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid, disclosed by Yamada et al, Bioorg. & Med. Chem. Lett. 8 (1998) 1537-1540), valine pyrrolidide, TMC-2A/2B/2C, CD- 26 inhibitors, FE999011, 30 P9310/K364, VIP 0177, DPP4, SDZ 274-444, 2-cyanopyrrolidides and 4-cyanopyrrolidides as

disclosed by Ashworth et al, Bioorg. & Med. Chem. Lett., Vol. 6, No. 22, pp 1163-1166 and 2745-2748 (1996) ,and the compounds disclosed in US6395767, US6573287, US6395767 (compounds disclosed include BMS-477118, BMS-471211 and BMS 538,305), WO99/38501, WO99/46272, WO99/67279, WO99/67278, WO99/61431WO03/004498, WO03/004496, 5 EP1258476, WO02/083128, WO02/062764, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593, WO03/000180, and WO03/000181; GLP-I agonists such as exendin-3 and exendin-4 (including the 39 aa polypeptide synthetic exendin-4 called Exenatide®), and compounds disclosed in US2003087821 and NZ 504256, and pharmaceutically acceptable salts and esters thereof; peptides including amlintide and Symlin® 10 (pramlintide acetate); and glycol kinase activators such as those disclosed in US2002103199 (fused heteroaromatic compounds) and WO02/48106 (isoindolin-1-one-substituted propionamide compounds).

EXAMPLES

Example 1: Clinical Study for safety and efficacy in humans for the treatment of chronic idiopathic constipation

[174] A randomized, double-blind, placebo-controlled, 14-day repeat oral, dose ranging study was conducted in patients with chronic idiopathic constipation (CIC). The primary objective of this study was to evaluate the safety of SP-304 (1.0 mg, 3.0 mg, 9.0 mg and 0.3 mg) for 14 days in patients with CIC. One secondary objective was to assess the pharmacokinetic profile of SP-304 in plasma. Other secondary objectives included evaluations of pharmacodynamic effects (efficacy) on parameters such as the time to first bowel movement after daily dosing with SP-304, bowel habits over time – for example, spontaneous bowel movements (SBMs), complete spontaneous bowel movements (CSBMs), and stool consistency [using Bristol Stool Form Scale (BSFS)] – and other patient reported outcomes such as abdominal discomfort.

[175] The study included five arms with assigned interventions as indicated in the table below.

Arms	Interventions
SP-304 1.0 mg: Experimental	Subjects receiving SP-304 1.0 mg for 14 consecutive days
SP-304 3.0 mg: Experimental	Subjects receiving SP-304 3.0 mg for 14 consecutive days
SP-304 9.0 mg: Experimental	Subjects receiving SP-304 9.0 mg for 14 consecutive days
Placebo: Placebo Comparator	Subjects receiving Placebo for 14 consecutive days
SP-304 0.3 mg: Experimental	Subjects receiving SP-304 0.3 mg for 14 consecutive days

[176] Subjects diagnosed with CIC were screened for the anticipated 4 cohorts to yield 80 randomized subjects for enrollment. There were four dose cohorts (1.0 mg, 3.0mg, 9.0 mg and 0.3 mg) with 20 subjects per dose cohort [randomization ratio 3:1 (15 receive SP-304:5 receive placebo)]. Subjects who continued to meet all the entry criteria and complete the pre-treatment bowel movement (BM) diary received, in a double-blind, randomized fashion, SP-304 or matching placebo. The entry criteria included (1) meeting modified ROME III criteria for chronic constipation (CC); (2) no significant finding in colonoscopy within past 5 years; (3) good health as determined by physical examination, medical history, vital signs, ECG, clinical chemistry, hematology, urinalysis, drug screen and serology assessments; and (4) during 14-day pre-treatment period, subjects reporting < 6 SBM and < 3 CSBM in each pre-treatment week. All subjects receiving at least one dose of SP-304 or matching placebo were considered evaluable for the safety endpoints (78 total). If a subject did not have a major protocol deviation, had at least 5 days of study treatment each week and corresponding entries for bowel habits, he/she was considered evaluable for efficacy parameters (54-55 total).

[177] The demographics of the subjects in the study are summarized in the table below.

	Placebo	0.3 mg	1.0 mg	3.0 mg	9.0 mg
Age					
	47.7 (14.6)	51.1 (12.0)	50.5 (10.6)	48.5 (16.1)	47.3 (12.7)
Gender					
Female	18 (90.0%)	12 (85.7%)	14 (100%)	13 (86.7)	12 (80%)
Male	2 (10.0%)	2 (14.3%)	0	2 (13.3%)	3 (20%)
Race					
White	17 (85.0%)	13 (92.9%)	12 (85.7%)	14 (93.3%)	12 (80.0%)

African American	1 (5.0%)	0	1 (7.1%)	0	2 (13.3%)
Asian	1 (5.0%)	1 (7.1%)	1 (7.1%)	0	1 (6.7%)
American Indian	1 (5.0%)	0	0	0	0
Other	0	0	0	1 (6.7%)	0

Values for age are the mean (standard deviation); values for gender and race are the number (percentage of experimental arm).

Results

[178] Pharmacokinetics and Safety:

[179] There was no detectable systemic absorption of plecanatide (assay sensitivity ≥ 10 ng/mL). No serious adverse events (SAE) were reported in subjects receiving plecanatide and no deaths reported in this study. 10% (2/20) subjects who received placebo and 17.2% (10/58) subjects who received SP-304 reported adverse events considered as related to the treatment. The majority of adverse events were mild / moderate and transient in nature. 10% (2/20) subjects who received placebo and 5.2% (3/58) subjects who received SP-304 reported GI-related adverse events considered as related to treatment. There was no diarrhea reported for any subject receiving SP-304. The table below is a GI-related adverse event (AE) summary.

	Placebo n=20	0.3 mg n=14	1.0 mg n=14	3.0 mg n=15	9.0 mg n=15
Abdominal Cramping	1 (5.0%)	0	0	0	0
Abdominal Pain	1 (5.0%)	0	0	0	0
Bloating	0	0	0	0	1 (6.7%)
Diarrhea	1 (5.0%)	0	0	0	0
Flatulence	2 (10.0%)	0	0	0	0
Nausea	0	1 (7.1%)		0	0
Upset Stomach	0	0	0	1 (6.7%)	0

Values are the number (percentage of experimental arm).

[180] Efficacy:

[181] SP-304 (plecanatide) treatment decreased the time to first bowel movement, increased stool frequency (SBM and CSBM), improved stool consistency, and reduced straining and abdominal discomfort. See Figures 1-6.

Example 2: Composition of Wet Granulation batch 10005

Item No.	Ingredient	Use	Concentration % w/w
1	SP304		0.23
2	Mannogem EZ, USP/EP (Mannitol)	Diluent	79.77
3	PROSOLV SMCC 90 LM (silicified microcrystalline cellulose)	Binder	15.0
4	Purified Water (chilled to 5°C), USP	vehicle	n/a
5	Purified Water (chilled to 5°C), USP		n/a
6	Explotab (Sodium Starch Glycolate)	Disintregant	4.0
7	Pruv (sodium stearyl fumarate)	Lubricant	1.0
	Total		100

Example 3: Composition of Wet Granulation batch 10007

Item No.	Ingredient	Use	Concentration % w/w
1	SP304		0.3
3	PROSOLV SMCC 90 HD (silicified microcrystalline cellulose)	Binder	95.7
4	Purified Water (chilled to 5°C), USP	vehicle	n/a
5	Purified Water (chilled to 5°C), USP		n/a

6	Explotab (Sodium Starch Glycolate)	Disintregant	4.0
	Total		100

Example 4: EXCIPIENT COMPATIBILITY

[182] Binary mixtures of SP-304 were prepared and stored in glass vials. For solid excipients the binary mixtures were comprised of 9.1% or 50% excipient. Glass vials were stored at 40C/75RH open or closed. The percent purity (measured by HPLC) of the GCC agonist peptide (SP-304) after storage for the time indicated in each column (i.e., 1, 2, or 3 months for the closed vial and 0.5, 1, 2, or 3 months for the open vials) is indicated by numerical values.

PURPOSE	EXCIPIENT	Closed			Open			
		1M	2M	3M	0.5M	1M	2M	3M
None	None	91.4	88.2	84.1	93.7	91.2	88.2	84.8
Diluent	Sorbitol	92.4	90.1	87.2	92.2	90.8	87.1	80.9
	Mannitol	91.9	88.4	85.1	92.6	90.5	87.9	83.8
	Prosolv	92.2	89.6	86.3	93	90.5	87.8	83.7
	Starch	91.4	88.7	85.4	92.5	90.5	87.9	83.7
Binder	Emdex	91.3	88.7	85.2	91.8	90.7	87.9	81.9
	Plasdone	92.8	90.6	85.6	93.1	90.4	87.3	83
Disintegrant	Explotab	91.9	89.4	87.1	92.2	90.3	84.7	78.3
	Polyplasdone	92	89	85.6	93.5	90.3	87.4	83.1
Glidant	Cabosil	92.1	88.3	85.6	92.6	90.5	87.3	84
Lubricant	Mg stearte	91.5	87.7	84.6	92.6	90.6	87.6	83.8
	PRUV	92	88.3	85.7	92.2	90.5	87.5	83.8
	compritol	90.8	87.1	84.4	92	90.5	86.7	84.1
Excipient	PEG 3350	90.9	87	83.3	91.5	89.4	84.4	77.5
Antioxidant	Ascorbic acid	91.3	86.9	83	92.8	90	85.7	83.8
	BHA	91.9	88.9	85.9	93.5	90.8	87.4	85.8
	BHT	90.8	87.2	84.6	92.4	90.3	86.6	83.6
	EDTA	90.9	87.5	84.1	92.3	90.4	86.7	84.6
Capsule	HPMC capsule	92.2	89	85.2	92.3	90.2	86.4	83.5
	Gelatin capsule	91.5	88.3	84.3	84.3	90.5	86.7	83.6

Liquid for liquid filled capsule	Medium chain trig		90.4					
	PG dicaprylocaprate		89.3					
	Vit E		90					
	Soybean oil		89.6					
	Cremaphor		79.7					
	PG		3.4					
	PG 400		0.7					

Example 5: Geometric dry mix for 0.3mg capsule

[183] Place 12g mannitol in mortar. Add 4g SP-304 and gently mix until a visually uniform powder is obtained. Transfer to Turbula mixer. Rinse mortar with mannitol and transfer to Turbula mixer and mix at high speed for 10 minutes. Add about 150g of mannitol to 4 quart V-shell mixer. Transfer the contents of the Turbula mixer to the V-shell and add 150g of mannitol mix. Discharge v-shell contents and screen through 40 mesh and return to mixer. Add 586g of mannitol to mixer and mix for 20 minutes.

Example 6: Wet granulation process:

[184] Batch 017-10005 comprised of mannitol and low-moisture (2.4%) PROSOLV LM90 (0.33 g/mL) was sprayed with SP-304 solution and fluid bed dried resulted in granulation water content of 0.35%. The final blend contained 1% water, flowed well, and filled capsules well. The 2nd prototype 017-1006 comprised of the same components was adjusted to obtain a target capsule fill weight of 100 mg based on the results of the 1st batch. Water was sprayed onto powder blend with SP-304. The inlet temperature was 50C and the granulation was dried for 1.5 hours and stopped when the product temperature reached 36C. The 3rd (batch017-10006) and 4th (batch 017-10007) capsule prototypes will use PROSOLV HD90, which is a higher density material with superior flow properties and higher moisture content of 5.5% than the PROSOLV LM90. The moisture content of the PROSOLV HD90 is readily removed by fluid bed drying.

The density of PROSOLV HD90 is about 0.55 g/mL. The PRUV lubricant will be removed for these batches.

Example 7: Wet granulation stability

[185] SP-304 was extracted from the capsules by sonication at either at room temperature (RT) or cold temperature and the amount of peptide was determined by HPLC. Initial percentages are based on the amount stated on the label.

Batch	% peptide (initial)	% peptide (1 mos at RT)
017-10006	101.1 (sonicated RT)	97.6 (sonicated cold)
017-10008	97.5 (sonicated RT)	108.2 (sonicated cold)

Example 8: 1M capsule stability in HDPE Bottles

[186] Capsules contained 0.3 mg SP-304 with the remainder of the fill weight (up to 5 mg) made up by mannitol (Perlitol 300 DC). Each capsule contained 1.5% by weight SP-304 and 98.5% mannitol. The capsule shell was composed of HPMC. Amounts are relative to the amount specified on the label (i.e., 0.30 mg peptide). The indicated number of capsules was placed in a high density polyethylene bottle with an induction seal and molecular sieve desiccant for 1 month at either 2-8C (first two columns) or 25C and 60% relative humidity (last two columns). The initial amount of peptide present was 101% of the label claim. The last row gives the amount of peptide remaining after 1 month storage at the indicated temperature as determined by HPLC.

2-8C	2-8C	25C/60RH	25C/60RH
1-capsule per bottle	6-capsules per bottle	1-capsule per bottle	6-capsules per bottle
100%	92%	92%	98%

Example 9: Composition of batch 1528-2855-RD (capsules) and spray coating and drying process

Item No.	Ingredient	Amount per unit (mg)	Concentration % w/w
1	SP-304	0.3246	0.3246
2	Microcrystalline cellulose (Cephene SCP-100)	99.10	99.10
3	Calcium chloride dihydrate	0.2622	0.2622
4	Leucine USP	0.1171	0.1171
5	Hypromellose (Methocel E5 PremLV)	0.2000	0.2000
6	Purified Water, USP	7.2 mL*	n/a
	Total	100	100

*: The amount of water is calculated based on use of 119.0 mL purified water for the whole batch containing 5.356 g SP-304.

[187] The spray drying process of making the batch 2855-RD is described below.

Preparation of Coating Dispersion:

[188] Purified water was added to a glass container and stirred such that a liquid vortex was produced without introducing air. Then calcium chloride dihydrate was slowly added into the water. The mixture was stirred until the salt was dissolved or well dispersed. Next, leucine was slowly added and the resulting mixture was stirred until the amino acid was dissolved or well dispersed. Afterward, methocel was slowly added and the mixture was stirred until methocel was completely dissolved. The solution could be warmed up to dissolve methocel, if necessary. The resulting excipient solution was allowed to cool to room temperature and pass through 80 mesh screen. Then, 127.9g of screened excipient solution was added to a glass container and placed in an ice bath for 0.5 to 1 hour until the solution reached 0 °C. Next, SP-304 was added into the cold excipient solution. The mixture was stir vigorously to allow the peptide to dissolve

in the cold solution. The resulting peptide solution was kept cold in the ice bath as a spraying/coating solution.

Drug Layering

[189] A Glatt GPCG-2 fluid bed processor (with top spray tower) with a Wurster insert was set up for drug layering onto Celphere SCP-100 beads. After loading the Wurster column with Celphere SCP-100 beads, bed temperature was raised to 35 °C and maintained for 30 minutes with minimum fluidization of the beads. The bed temperature was reduced until an exhaust temperature of 35 °C was achieved. The pump tubing of the peristaltic pump used was primed by circulating the spraying solution mentioned above. After the spraying apparatus was adjusted to obtain a satisfactory spray pattern, the coating solution was sprayed onto Celphere SCP-100 beads until all coating solution was sprayed. Operating parameters were recorded. The bed temperature and fluidization were maintained until the beads were sufficiently dry. The fluidization was then reduced while the bed temperature was maintained at 35 °C for 10 minutes. 2g of beads were sampled for moisture analysis when the bed temperature was kept at 35 °C. When the moisture of the sampled beads reached < 5% moisture, the coated beads were discharged and loaded into a dry container. LOD (loss on drying) 2.399%.

Example 10: Composition of batch 1528-2851-RD (tablets) and spray coating and drying process

Item No.	Ingredient	Amount per unit (mg)	Concentration % w/w
1	SP-304	0.3246	0.3607
2	Microcrystalline cellulose (Avicel PH 102)	88.88	98.75
3	Calcium chloride dihydrate	0.2622	0.2913
4	Leucine USP	0.1171	0.1301
5	Hypromellose (Methocel E5 PremLV)	0.2000	0.2222

6	Magnesium stearate	0.225	0.2500
7	Purified Water, USP	7.2 mL*	n/a
	Total	90.0	100

*: The amount of water is calculated based on use of 119.0 mL purified water for the whole batch containing 5.356 g SP-304.

[190] The spray coating and drying process of making the batch 2851-RD is described below.

Preparation of Coating Dispersion:

[191] Purified water was added to a glass container and stirred such that a liquid vortex was produced without introducing air. Then calcium chloride dihydrate was slowly added into the water. The mixture was stirred until the salt was dissolved or well dispersed. Next, leucine was slowly added and the resulting mixture was stirred until the amino acid was dissolved or well dispersed. Afterward, methocel was slowly added and the mixture was stirred until methocel was completely dissolved. The solution could be warmed up to dissolve methocel, if necessary. The resulting excipient solution was allowed to cool to room temperature and pass through 80 mesh screen. Then, 127.9g of screened excipient solution was added to a glass container and placed in an ice bath for 0.5 to 1 hour until the solution reached 0 °C. Next, SP-304 was added into the cold excipient solution. The mixture was stir vigorously to allow the peptide to dissolve in the cold solution. The resulting peptide solution was kept cold in the ice bath as a spraying/coating solution.

Drug Layering

[192] A Glatt GPCG-2 fluid bed processor (with top spray tower) with a Wurster insert was set up for drug layering onto Avicel PH 102 beads. After loading the Wurster column with Avicel PH 102 beads, temperature was raised to 35 °C and maintained for 30 minutes with minimum fluidization of the beads. The bed temperature was reduced until an exhaust temperature of 35 °C was achieved. The pump tubing of the peristaltic pump used was primed by circulating the spraying solution mentioned above. After the spraying apparatus was adjusted to obtain a satisfactory spray pattern, the coating solution was sprayed onto Avicel PH 102 beads until all coating solution was sprayed. Operating parameters were recorded. The bed temperature and

fluidization were maintained until the beads were sufficiently dry. The fluidization was then reduced while the bed temperature was maintained at 35 °C for 10 minutes. 2g of beads were sampled for moisture analysis when the bed temperature was kept at 35 °C. When the moisture of the sampled beads reached < 5% moisture, the coated beads were discharged and loaded into a dry container. LOD (loss on drying) <5%.

[193] The net weight of the coated blend was determined for calculation of the amount of magnesium stearate needed to lubricate the blend. Then the magnesium stearate was added to the coated blend and the mixture was blended for 1 minute.

Compression

[194] A Fette tablet press was set up. Then the blend mixture was loaded into the powder hopper and tooling was installed. The weight of each tablet was set to be 90 mg±5% and hardness to be 4-6 Kp. The weight, hardness and thickness of tablets were measured and recorded every 5 to 10 minutes. Friability measurement was also performed to ensure satisfactory product.

Example 11: Composition of batch 1528-2850-RD (capsules) and process

Item No.	Ingredient	Concentration % w/w
1	SP-304	0.3246
2	Microcrystalline cellulose (Avicel PH 102)	99.43
3	Magnesium stearate	0.2500
4	HPMC capsule shells	n/a
	Total	100

[195] The dry blend process of making the batch 2850-RD is described below.

Blending:

[196] Avicel PH 102 was screened through a 60 mesh screen. V-blenders (1 Qt, 4Qt, and 16 Qt) were then dusted by the screened Avicel PH 102. SP-304 was screened through a 200 mesh screen and loaded into the 1-Qt V-blender. Then, about 80 g Avicel PH 102 was added into the 1-Qt blender and the mixture was blended for 10 minutes at 25 rpm. The mixture was then transferred to the 4-Qt V-blender which was pre-dusted by the screened Avicel PH 102. The 1-Qt blender was rinsed with Avicel and the rinse material was transferred to the 4-Qt blender. The rinsing was repeated until all SP-304 was transferred to the 4-Qt blender. About 200g Avicel was added to the 4-Qt V-blender and the mixture was blended for 10 minutes. The resulting blend was then screened through a 60 mesh screen and then transferred into the pre-dusted 16-Qt blender (dusted with 1500g Avicel). The 4-Qt blender was rinsed with Avicel and the rinse material was transferred to the 16-Qt blender. The remaining Avicel was added to the 16-Qt blender and the mixture was blended for 10 minutes. The resulting blend was passed through Comil and then returned to the 16-Qt blender and was further blended for 5 minutes. Proper amount of magnesium stearate was weighed, screened through a 60 mesh screen, and added into the 16-Qt blender. The resulting mixture was blended for 2 minutes.

Encapsulation

[197] A MG2 Planeta capsule filler was set up. Average weight of the empty capsule shells was determined and target capsule fill weight was calculated ($\pm 5\%$). The blend from the above process was added into the hopper of the capsule filler and encapsulation was started. Run weight parameters were manually adjusted. Resulting capsules were then sorted according to the target fill weight.

Example 12: Composition of batch 1528-2850B-RD (tablets) and process

Item No.	Ingredient	Concentration % w/w
1	SP-304	0.3246
2	Microcrystalline cellulose (Avicel PH	99.43

	102)	
3	Magnesium stearate	0.2500
	Total	100

[198] The dry blend process of making the batch 2850B-RD is described below.

Blending:

[199] Avicel PH 102 was screened through a 60 mesh screen. V-blenders (1 Qt, 4Qt, and 16 Qt) were then dusted by the screened Avicel PH 102. SP-304 was screened through a 200 mesh screen and loaded into the 1-Qt V-blender. Then, about 80 g Avicel PH 102 was added into the 1-Qt blender and the mixture was blended for 10 minutes at 25 rpm. The mixture was then transferred to the 4-Qt V-blender which was pre-dusted by the screened Avicel PH 102. The 1-Qt blender was rinsed with Avicel and the rinse material was transferred to the 4-Qt blender. The rinsing was repeated until all SP-304 was transferred to the 4-Qt blender. About 200g Avicel was added to 4-Qt V-blender and the mixture was blended for 10 minutes. The resulting blend was then screened through a 60 mesh screen and then transferred into the pre-dusted 16-Qt blender (dusted with 1500g Avicel). The 4-Qt blender was rinsed with Avicel and the rinse material was transferred to the 16-Qt blender. The remaining Avicel was added to the 16-Qt blender and the mixture was blended for 10 minutes. The resulting blend was passed through Comil and then returned to the 16-Qt blender and was further blended for 5 minutes. Proper amount of magnesium stearate was weighed, screened through a 60 mesh screen, and added into the 16-Qt blender. The resulting mixture was blended for 2 minutes.

Compression

[200] A Fette tablet press was set up. Then the blend mixture was loaded into the powder hopper and tooling was installed. The weight of each tablet was set to be 90 mg±5% and hardness to be 4-6 Kp. The weight, hardness, and thickness of tablets were measured and recorded every 5 to 10 minutes. Friability measurement was also performed to ensure satisfactory product.

Example 13: Composition of dry blend tablet formulation 1528-3161-RD, 1mg for vacuum drying

Item No.	Ingredient	Concentration % w/w
1	SP-304	1.176
2	Microcrystalline cellulose (Avicel PH 102)	98.57
3	Magnesium stearate	0.2500
	Total	100

Example 14: Composition of dry blend tablet formulation 1528-3162-RD, 1mg with low-moisture cellulose

Item No.	Ingredient	Concentration % w/w
1	SP-304	1.176
2	Microcrystalline cellulose (Avicel PH 112)	97.09
3	Magnesium stearate	0.2500
	Total	100

5 Example 15: Composition of spray coated trehalose granules tablet formulation 1528-3170-RD, 1mg

Item No.	Ingredient	Concentration % w/w
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1	SP-304	1.176
2	Trehalose granules	70.48
3	Methocel ES Premium LV	0.50
4	Histidine (in coating solution)	0.9225
5	Calcium ascorbate	0.100
6	Purified water	N/A
7	Trehalose powder (in coating solution)	1.0176
8	Microcrystalline cellulose (Avicel PH 200)	25.00
9	Histidine	0.5535
10	Magnesium stearate	0.2500
	Total	100

The process for making spray coated trehalose Granules tablet formulation 1528-3170-RD is described below.

Preparation of the Coating Dispersion

- 5 [201] Add purified water to labeled container and begin stirring. Stir such that a liquid vortex is produced without introducing air into liquid. Slowly add Methocel to solution. Stir until methocel is completely dissolved. Warm the solution if necessary to dissolve Methocel (≤ 50 °C). Solution must be cooled before adding other materials. Add Trehalose to solution. Stir until materials are dissolved. Add Calcium Ascorbate to solution. Stir until materials are

dissolved. Adjust pH to 7.0 with 1N NaOH solution if pH >7.0. Record adjusted pH. Place the Coating Solution in an ice bath and allow it stay in the batch for 0.5 to 1 hour until it reaches the ice temperature. Check with a thermometer to ensure at ice temperature. Weigh portions of required amount of API on a weighing boat and add each portion carefully to the cold Excipient Solution. Stir vigorously to allow peptide wetting and dissolving in the cold solution. Total amount of peptide must equal 14.107 g. Continue stirring solution such that a liquid vortex is produced without introducing air into liquid. Stir until PLECANATIDE is completely dissolved. Keep peptide solution cold all the time in the ice bath. Add Histidine to solution. Stir not more than 10min to dissolve the material. Obtain final pH of the Coating Solution. Obtain net weight of the Coating Solution. Coating Solution must be used within 30min to avoid coloration.

Drug Layering

[202] Setup Glatt GPCG2 with Wurster insert according to SOP EQP-OCM-064 for drug layering onto Trehalose Granules with coating dispersion. Use Glatt GPCG2 In-process form, "EQP-OCM-064-F1," to record in-process information. Turn unit on and preheat column. Fluid Bed Processor: Glatt GPCG-2. Filter: 200 micron screen. Product Container: 4" wurster, stainless steel. Insert height from bottom: 1". Spray direction: Top Spray. Fluid Nozzle Size/Type: 1mm. Pump: Peristaltic, Master Flex LS. Tubing: Nalge #14 Silicon. Bed Temperature: $\leq 40^{\circ}\text{C}$. Inlet air temperature: Adjust to meet bed temperature target. Outlet air temperature: Monitor & record. Spray rate: initial rate 4-6g/min, adjust as required. Atomizing air pressure: 20 psi. Air flow: 60cmh and adjust for fluidization. Prepare double polyethylene bags large enough to hold drug layered Granules. Load column with Trehalose. Increase bed temperature to 35°C and maintain for 30 minutes with minimum fluidization of the Granules. Reduce bed temperature until an exhaust temperature of 35°C is achieved. Prime pump tubing by circulating spraying solution; must not use more than 40g for tubing priming. Adjust the spraying apparatus to obtain satisfactory spray pattern. Coating Solution Weight after priming should > 317g. Record initial weight below before spraying onto trehalose. Start spraying the coating solution onto Trehalose Granules. Record operating parameters on fluid bed processing form. Stop spraying when 297.2 g of coating solution has been sprayed. Maintain bed temperature and continue fluidization until Granules are sufficiently dry. Reduce fluidization

and maintain bed temperature at 35°C for 10 minutes. Do not cool down the Granules. Sample 2g for moisture analysis until moisture is below 1%. Discharge coated Granules into pre-prepared and labeled container (with tare weight) lined with double polyethylene bag. Calculate net weight of drug layered Granules. Setup Lyophilizer per SOP EQP-OCM-00002. Load drug layered granules into a Lyoguard tray (Save bags). Use recipe 3 to dry blend overnight. Discharge dried blend into saved polyethylene bags. Obtain final moisture of the dried granules. Record final Moisture (<1%). Calculate net weight of dried Granules.

Blending

[203] Screen required Avicel and pass through 60 mesh screen. Setup 4 qt V-blender per SOP EQP-OCM-00056. Weigh amount of Histidine needed and blend with small amount of Avicel weighed. Charge into 4 qt. V-blender. Transfer Plecanatide Dried Granules into the V-Blender. Rinse 2-3 times the Lyoguard tray from Step 24 with adequate amount of Weighed Avicel. Transfer rinses into 4 qt. V-blender. Transfer all remaining Pre-weighed/screened Avicel into the V-Blender. Mix for 15 minutes. Weigh and screen Magnesium Stearate through a 60 mesh screen. Charge Magnesium Stearate to the 4 qt V-Blender. Ensure the cover is securely closed with no potential powder leakage during blending. Blend for 2 minutes.

Compression

[204] Set-up Korsch press per SOP EQP-OCM-00087. Install 0.250" Standard Concave Round Plain tolling. Obtain blend Assay results and calculate Target Tablet Weight. Acceptable weight range of tablets is $\pm 5.0\%$. Load the Final Blend into the powder hopper. Refill as necessary. Adjust fill weight to obtain tablets in the range of 95.0 - 105.0mg and hardness in the range of 4-6kP. Verify friability is NMT 1.0%. Check 5 tablet weights periodically every 5-10min to ensure tablet weight is within the range and record on form QRA-DOC-00011-F6. After tablet weights are recorded, obtain and record 3 tablet hardness and thickness during the periodic weight check. Continue to compress acceptable tablets until the blend is used up. Once press is running properly to achieve specifications above, perform final Friability test and record results (Spec: NMT 1.0%).

Example 16: Composition of spray coated trehalose granules tablet formulation 1528-3171-RD, 1mg

Item No.	Ingredient	Concentration % w/w
1	SP-304	1.167
2	Trehalose granules	70.31
3	Methocel ES Premium LV	0.50
4	Arginine	1.657
5	Calcium ascorbate	0.100
6	Water for injection	N/A
7	Trehalose powder (in coating solution)	1.0176
8	Microcrystalline cellulose (Avicel PH 200)	25.00
9	Magnesium stearate	0.2500
	Total	100

[205] The process for making spray coated trehalose Granules tablet formulation 1528-3171-RD is described below.

5 Preparation of Coating Solution

Add purified water (Item 6) to labeled container and begin stirring. Stir such that a liquid vortex is produced without introducing air into liquid. Slowly add Methocel to solution. Stir until methocel is completely dissolved. Warm the solution if necessary to dissolve Methocel (≤ 50 °C). Record appearance of solution. Solution must be cooled before adding other materials.

Add Trehalose to solution. Stir until materials are dissolved. Record appearance of solution.
Add Arginine to solution. Stir until materials are dissolved. Record appearance of solution.
Add Calcium Ascorbate to solution. Stir until materials are dissolved. Record appearance of
solution. Adjust solution pH to pH 8.5 - 8.6 with concentrated HCl followed by adjust pH to 8.3
5 – 8.4 with 10N HCl. Record final adjusted pH. Place the Coating Solution in an ice bath and
allow it stay in the batch for 0.5 to 1 hour until it reaches the ice temperature. Check with a
thermometer to ensure at ice temperature. Weigh portions of required amount of API on a
weighing boat and add each portion carefully to the cold Excipient Solution. Stir vigorously to
allow peptide wetting and dissolving in the cold solution. Total amount of peptide must equal
10 14.006 g. Continue stirring solution such that a liquid vortex is produced without introducing air
into liquid. Stir until PLECANATIDE is completely dissolved. Keep peptide solution cold all
the time in the ice bath. Weigh 5.0g of WFI to rinse API container. Carefully rinse the side of
coating solution container and completely transfer the rinse back to the coating solution
container. Obtain final pH of the Coating Solution. Obtain net weight of the Coating Solution
15 (~360.3 g). Coating Solution must be used within as soon as possible.

Drug Layering

[206] Setup Glatt GPCG2 with Wurster insert according to SOP EQP-OCM-064 for drug
layering onto Trehalose Granules with coating dispersion. Use Glatt GPCG2 In-process form,
"EQP-OCM-064-F1," to record in-process information. Turn unit on and preheat column.
20 Fluid Bed Processor: Glatt GPCG-2. Filter: 200 micron screen. Product Container: 4"
wurster, stainless steel. Insert height from bottom: 1". Spray direction: Top Spray. Fluid
Nozzle Size/ Type: 1mm. Pump: Peristaltic, Master Flex LS. Tubing: Nalge #14 Silicon. Bed
Temperature: $\leq 40^{\circ}\text{C}$. Inlet air temperature: Adjust to meet bed temperature target. Outlet air
temperature: Monitor & record. Spray rate: initial rate 4-6g/min, adjust as required. Atomizing
25 air pressure: 20psi. Air flow: 60cmh and adjust for fluidization. Load column with Trehalose G.
Increase bed temperature to 35°C and maintain for 30 minutes with minimum fluidization of the
Granules. Reduce bed temperature until an exhaust temperature of 35°C is achieved. Prime
pump tubing with coating solution. Must not use more than 40g for tubing priming. Adjust the
spraying apparatus to obtain satisfactory spray pattern. Record initial weight below before

spraying onto trehalose. Start spraying the coating solution onto Trehalose Granules. Record operating parameters on fluid bed processing form. Stop spraying when 300.3 g of coating solution has been sprayed. Maintain bed temperature and continue fluidization until Granules are sufficiently dry. Reduce fluidization and maintain bed temperature at 35°C for 10 minutes.

5 Do not cool down the Granules. Sample 2g for moisture analysis until moisture is below 1%. Discharge coated Granules into pre-prepared and labeled container (with tare weight) lined with double polyethylene bag. Calculate net weight of drug layered Granules. If moisture is > 1%, vacuum dry blend as follows: Setup Lyophilizer per SOP EQP-OCM-00002. Load drug layered granules into a Lyoguard tray. Use recipe 3 to dry blend overnight. Discharge dried blend into

10 saved polyethylene bags. Obtain final moisture of the dried granules. Calculate net weight of dried Granules.

Blending

[207] Screen required Avicel and pass through 60 mesh screen. Setup 4 qt V-blender. Transfer Plecanatide Dried Granules into the V-Blender. Save bag for discharging final blend. Rinse 2-3

15 times the Lyoguard tray and bag with adequate amount of Weighed Avicel. Transfer rinses into 4 qt. V-b;ender. Transfer all remaining Pre-weighed/screened Avicel into the V-Blender. Mix for 20 minutes. Weigh and screen Magnesium Stearate through a 60 mesh screen. Charge Magnesium Stearate to the 4 qt V-Blender. Ensure the cover is securely closed with no potential powder leakage during blending. Blend for 2 minutes. Sample 3 x 350 mg of blend at three

20 locations. Obtain exact weight of each sample that has been transferred into the sampling bottle.

Compression

Set-up Korsch press per SOP EQP-OCM-00087. Install 0.250" Standard Concave Round Plain tolling. Obtain blend Assay results and calculate Target Tablet Weight. Acceptable weight range of tablets is $\pm 5.0\%$. Load the Final Blend into the powder hopper. Refill as necessary. Adjust

25 fill weight to obtain tablets in the range of 95.0 - 105.0mg and hardness in the range of 4-6kP. Verify friability is NMT 1.0%. Check 5 tablet weights periodically every 5-10min to ensure tablet weight is within the range. After tablet weights are recorded, obtain and record 3 tablet hardness and thickness during the periodic weight check. Continue to compress acceptable

tablets until the blend is used up. Once press is running properly to achieve specifications above, perform final Friability test and record results (Spec: NMT 1.0%).

Example 17: Composition of spray coated trehalose granules tablet formulation 1528-3172, 1mg

Item No.	Ingredient	Concentration % w/w
1	SP-304	1.167
2	Trehalose granules	70.81
3	Methocel ES Premium LV	0.50
4	TRIS	1.1524
5	Calcium ascorbate	0.100
6	Water for injection	N/A
7	Trehalose powder (in coating solution)	1.0176
8	Microcrystalline cellulose (Avicel PH 200)	25.00
9	Magnesium stearate	0.2500
	Total	100

- 5 [208] The process for making spray coated trehalose granules tablet formulation 1528-3172-RD is described below.

Preparation of Coating Solution

[209] Add purified water to labeled container and begin stirring. Stir such that a liquid vortex is produced without introducing air into liquid. Slowly add Methocel to solution. Stir until

methocel is completely dissolved. Warm the solution if necessary to dissolve Methocel ($\leq 50^{\circ}\text{C}$). Record appearance of solution.

[210] Solution must be cooled before adding other materials. Add Trehalose to solution. Stir until materials are dissolved. Record appearance of solution. Add TRIS to solution. Stir until materials are dissolved. Record appearance of solution. Add Calcium Ascorbate to solution. Stir until materials are dissolved. Record appearance of solution. Obtain solution pH: Adjust pH to pH 7.8 – 7.9 with concentrated HCl followed by adjust pH to 7.7 – 7.6 with 10N HCl. Record final adjusted pH. Place the Coating Solution in an ice bath and allow it stay in the batch for 0.5 to 1 hour until it reaches the ice temperature. Check with a thermometer to ensure at ice temperature. Weigh portions of required amount of API on a weighing boat and add each portion carefully to the cold Excipient Solution. Stir vigorously to allow peptide wetting and dissolving in the cold solution. Total amount of peptide must equal 14.006 g. Continue stirring solution such that a liquid vortex is produced without introducing air into liquid. Stir until PLECANATIDE is completely dissolved. Keep peptide solution cold all the time in the ice bath. Weigh 5.0g of WFI to rinse API container. Carefully rinse the side of coating solution container and completely transfer the rinse back to the coating solution container. Obtain final pH of the Coating Solution. Obtain net weight of the Coating Solution (~354.2 g). Coating Solution must be used as soon as possible.

The blending and compression processes for batch 1528-3172-RD are similar to that described above for batch 1528-3171-RD.

Example 18: Composition of 1mg dry blend tablet formulation 1528-2925-RD

Item No.	Ingredient	Concentration % w/w
1	SP-304	1.106
2	Microcrystalline cellulose (Avicel PH 102)	98.64

3	Magnesium stearate	0.2500
	Total	100

Example 19: Composition of 3mg dry blend tablet formulation 1528-2926-RD

Item No.	Ingredient	Concentration % w/w
1	SP-304	3.318
2	Microcrystalline cellulose (Avicel PH 102)	96.43
3	Magnesium stearate	0.2500
	Total	100

[211] Other batches were prepared by the processes similar to those described in Examples 9-12. Their compositions are listed below.

[212] Batch 500-55: 0.33% plecanatide, 95.17% microcrystalline cellulose, 4.0% sodium starch glycolate, and 0.5% magnesium stearate.

[213] Batches 1528-2907-RD and 2010F100A: 3.318% plecanatide, 96.43% Avicel, and 0.25% Mg stearate.

[214] Batches 1528-2906-RD and 2010F099A: 1.106% plecanatide, 98.65% Avicel, and 0.25% Mg stearate.

[215] Batches 1528-2890-RD and 2010F101A: 0.3246% plecanatide, 99.43% Avicel, and 0.25% Mg stearate.

[216] Formula compositions for batches 11H141, 11H152, and 11H140 in this table below (not previously disclosed) are the same as the formula compositions for GMP stability batches 2010F101A, 2010F099A, and 2010F100A, respectively.

Example 20: Plecanatide tablet and capsule stability

[217] Capsules and tablets of different batches were tested for their stability and the results were provided. Unless otherwise specified, 1M, 2M, 3M, or 4M in the tables below denotes that the measurements were carried out at the end of 1, 2, 3, or 4 month(s) of the storage period.

Potency Summary: This test was performed by taking a composite sample of about 5 units to determine the average potency of the sample. The table below shows the stability of capsules or tablets in terms of potency (% of label claim).

Lot (description)	Potency (% Label Claim)																				
	Bulk*	Package	Storage Condition																		
		Package	Initial	40C/75RH			30C/65RH			25C/60RH					5C						
				1M	2M	3M	1M	2M	3M	1M	2M	3M	7M	10M	1M	2M	3M	4M	7M	8.5M	
1528-2850- RD (0.3mg dry blend capsules)	88	HDPE bottle		89		87			89			91		80				89.3		89	
		Oxyguard bottle		91		91			92			91		79					88.9		90
		Blister strip	90	90		85			88			91		79							90
1528-2855- RD (0.3mg coated bead capsule)	94	HDPE bottle		101		100			96			102		88						98	
		Oxyguard bottle		101		96			99			104		87							100
		Blister strip		97		103			99			98		87							97
500-55 (0.3mg dry blend capsule)	97	HDPE bottle		97		94			95			96		84						98	
		Oxyguard bottle		98		96			96			102		83							97
		Blister strip	93	97		93			95			106		83							96
1528-2850B- RD (0.3mg dry blend tablet)	76	HDPE bottle		85		88			94			83		67						70	
		Oxyguard bottle		84		84			88			74		74							80
1528-2851- RD (0.3mg coated particle tablet)	96	HDPE bottle		115		72			90			99		99						78	
		Oxyguard bottle		81		88			83			111		85							96
2010F100A (3mg dry blend capsule)	101	Blister strip	97	95	94	91	95	95	92	97	95	93			97	94	94				
2010F101A (0.3mg dry blend capsule)	97	Blister strip	92	91	91	86	94	92	85	95	93	88			95	95	92				
2010F099A (1mg dry blend capsule)	98	Blister strip	94	92	91	89	93	94	89	94	94	91			95	94	92				
11H141 (0.3mg dry)	103	Blister strip	101	95	92	87	98	93	92	96	92	95			100	97	97				

blend capsule)																			
11H152 (1mg dry blend capsule)	102	Blister strip	97	91	91	93	94	95	96	96	95	96			97	95	97		
11H140 (3mg dry blend capsule)	105	Blister strip	99	94	95	94	95	94	97	99	95	97			99	97	97		
1528-2925-RD (1mg dry blend tablet)	99	Oxyguard 40cc with PharnaKeep												99					103
1528-2926-RD (3mg dry blend tablet)	100	Oxyguard 40cc with PharnaKeep												94					93
1528-2907-RD (3mg dry blend capsule)	98																		
1528-2906-RD (1mg dry blend capsule)	98																		
1528-2890-RD (0.3mg dry blend capsule)	93																		

*Blend

[218] As demonstrated by the table above, there was little or no appreciable loss in potency after storage under accelerated conditions (40C/75RH or 30C/65RH), which suggests that these capsules or tablets could be stable at room temperature for 18 months or for longer times if refrigerated or stored at 25C.

[219] Water content summary: The table below shows that the water content was stable over the testing period in the packages evaluated for various capsule/tablet compositions. This further demonstrated that products were stable.

5 [220]

Lot	Water (in-process)	Packaging	Water packaged product																	
			Initial	40C/75RH			30C/65RH			25C/60RH					5C					
				1M	2M	3M	1M	2M	3M	1M	2M	3M	7M	10M	1M	2M	3M	4M	7M	8.5M
1528-2850-RD 0.3mg dry blend capsule		32-count, HDPE bottle, 60cc, N2, 2g mol. sieve		5.03		5.64			3.00			2.22		2.39				5.48		1.8
		32-count, Oxyguard bottle, 40cc, PharmaKeep KD-20		5.07		5.24			4.28			5.33		4.08				5.31		3.7
		Blister, N2	4.21	4.87		5.80			4.76			4.31		4.09						
1528-2855-RD 0.3mg coated bead capsule	2.40	32-count, HDPE bottle, 60cc, N2, 2g mol. sieve		0.57		0.47			1.63			0.68		0.42						0.2
		32-count, Oxyguard bottle, 40cc, PharmaKeep KD-20		2.10		1.05			1.29			2.07		0.30						0.8
		Blister strip		0.73		2.11			0.54			0.58		0.32						0.3
500-55 0.3mg dry blend capsule		HDPE bottle		5.63		4.19			5.51			5.79		2.98						2.7
		Oxyguard bottle		5.78		4.69			5.90			5.66		2.99						2.8
		Blister strip	4.09	5.78		4.17			5.53			6.16		3.12						2.9
1528-2850B-RD 0.3mg dry blend tablet		32-count, HDPE bottle, 60cc, N2, 2g mol. sieve		4.09		4.03			6.28			6.10		2.86						2.1
		32-count, Oxyguard bottle, 40cc, PharmaKeep KD-20		4.81		4.91			6.15			6.30		4.05						3.4
1528-2851-RD 0.3mg coated particle tablet	3.32	32-count, HDPE bottle, 60cc, N2, 2g mol. sieve		4.33		4.50			5.09			5.90		2.55						1.5
		32-count, Oxyguard bottle, 40cc, PharmaKeep KD-20		5.15		4.88			5.82			6.02		4.34						3.0
2010F100A (3mg dry blend capsule)		Blister strip	4.7	4.5	4.6	4.4	4.5	4.7	4.4	4.5	4.8	4.4			4.5	4.8	4.5			
2010F101A (0.3mg dry blend capsule)		Blister strip	4.5	4.8	4.7	4.7	4.5	4.7	4.3	4.4	4.7	4.3			4.5	4.7	4.2			
2010F099A (1mg dry blend capsule)		Blister strip	4.6	4.4	4.6	4.4	4.5	4.5	4.3	4.4	4.6	4.4			4.2	4.7	4.3			

11H141 (0.3mg dry blend capsule)	Blister strip	5	4.8	4.9	4.9	5.1	4.9	4.8	5.0	5.0	4.9			5.0	4.9	4.9			
11H152 (1mg dry blend capsule)	Blister strip	5.2	4.8	4.9	4.8	4.8	4.8	4.9	4.8	4.8	4.9			5.0	4.9	4.8			
11H140 (3mg dry blend capsule)	Blister strip	5.2	5.0	5.0	5.0	4.9	5.0	5.0	4.9	5.0	4.9			4.9	4.9	4.8			
1528-2925- RD (1mg dry blend tablet)	Oxyguard 40cc with PharnaKeep												4.9						4.0
1528-2926- RD (3mg dry blend capsule)	Oxyguard 40cc with PharnaKeep												4.0						4.0
1528-2907- RD 3mg dry blend capsule	Bulk capsule	4.78																	
1528-2906- RD 1m dry blend capsule	Bulk capsule	4.84																	
1528-2890- RD	Bulk capsule	4.8																	

[221]

5 Impurity summary: The table below shows the product stability in terms of HPLC or UPLC of total impurities as a function of time and storage condition. The data in the table suggest that the increase in total impurities in tested batches except batch 500-55 be no greater than 7% at room temperature after 18 months. It also suggest that the increase in total impurities in all tested 1528-2855-RD batche in different packages be no greater than 7% at 30 °C for 18 months. It was also observed that the 1528-2855-RD batch had less impurity increase than the 1528- 2850-RD batch or was more stable than the 1528-2850-RD batch.

Batch	Package	Initial	Total impurities % area																			
			40C/75RH			30C/65RH			25C/60RH					5C								
			1M	2M	3M	1M	2M	3M	1M	2M	3M	7M	10M	1M	2M	3M	4M	7M	8.5M			
1528-2850-RD	HDPE bottle	3.2	5.1		5.9			4.4			3.8		4.8				3.1		3.7			
	Oxyguard bottle		5.7		7.4			5.3			4.3		5.3				3.1		3.5			
	Blister strip		5.5		7.0			5.0			4.3		5.5							3.7		
1528-2855-RD	HDPE bottle	3.5	3.6		5.1			3.8			3.4		4.4							3.4		
	Oxyguard bottle		3.9		4.4			4.1			3.7		4.0								3.7	
	Blister strip		4.0		5.2			4.0			3.6		4.2								3.8	
500-55	HDPE bottle	3.2	5.7		8.4			5.4			4.4		6.0								3.5	
	Oxyguard bottle		5.6		7.0			5.1			4.3		5.6									3.5
	Blister strip		6.5		8.0			5.7			4.8		6.5									3.6
1528-2850B-RD	HDPE bottle	3.6	5.0		6.5			4.5			3.9		4.7								3.7	
	Oxyguard bottle		5.6		7.3			4.7			4.1		4.9									3.6
1528-2851-RD	HDPE bottle	3.7	4.2		5.1			4.0			3.8		3.9								3.7	
	Oxyguard bottle		4.9		6.8			4.7			4.4		4.3									3.9
2010F101A (0.3mg dry blend capsule)	Blister strip	2.1	4.4	3.9	4.7	2.9	3.2	3.4	3.1	2.7	3.2			2.0	1.3	2.0						
2010F099A (1mg dry blend capsule)	Blister strip	2.9	3.7	3.8	4.3	3.1	3.1	3.6	2.7	2.9	3.2			2.4	2.4	2.4						
2010F100A (3mg dry blend)	Blister strip	2.4	3.2	3.6	4.2	2.8	2.8	3.0	2.6	2.7	2.9			2.4	2.5	2.7						

capsule)																		
11H141 (0.3mg dry blend capsule)	Blister strip	1.3	3.3	4.2	4.5	2.5	3.6	3.3	2.0	2.8	2.9			1.4	1.5	1.8		
11H152 (1mg dry blend capsule)	Blister strip	2.4	3.6	4.2	4.1	2.6	3.2	3.1	2.6	3.1	2.9			2.3	2.3	2.1		
11H140 (3mg dry blend capsule)	Blister strip	2.1	3.5	3.7	4.5	2.6	2.7	3.3	2.5	2.7	2.9			2.3	2.2	1.8		
1528-2925- RD (1mg dry blend tablet)	Oxyguard 40cc with PharnaKeep												2.7					1.7
1528-2926- RD (3mg dry blend capsule)	Oxyguard 40cc with PharnaKeep												2.6					
1528-2906- RD	HDPE bottle	1.83		5.18														
1528-2907- RD	HDPE bottle	1.85		4.58														
1528-2890- RD	Bulk	1.9																

Content uniformity: This test was performed by placing 10 individual capsule/tablet units in 10 individual bottles and potency of each unit was measured to show whether individual capsules or tablets have uniform potency (% label claim or %LC).

0.3mg Dry blend tablet 1528-2850B-RD	
Sample	%LC
	1528-2850B- RD (dry tabs)
1	78.62
2	91.43
3	86.52
4	90.9
5	84.83
6	95.29
7	75.69
8	76.87
9	84.92
10	86.9
Mean	85.2
std. dev	6.51
% RSD	7.64

0.3mg Coated particle tablet 1528-2851-RD		
Sample	Weight (mg)	% Label Claim
1	88.86	69.55
2	89	94.41
3	88.89	94.34
4	88.6	72.18
5	88.37	142.52

6	88.76	149.44
7	89.42	78.8
8	88.56	131.08
9	89.08	102.55
10	88.78	99.13
Mean		103.4
St. Dev		28.53
%RSD		27.59

0.3mg Dry blend capsule 1528-2890		3mg Dry blend capsule 1528-2907-RD		1mg Dry blend capsule 1528-2906-RD	
Sample	%LC	Sample	%LC	Sample	%LC
1	87.2	1	94.5	1	98.1
2	94.6	2	101.2	2	101.8
3	92.6	3	97.9	3	93.1
4	94.2	4	94.5	4	97.5
5	93.5	5	95.9	5	97.9
6	91.7	6	95.2	6	97.1
7	91.6	7	96.1	7	94.5
8	99	8	99	8	100.1
9	91.8	9	93.8	9	98.1
10	92.1	10	93.4	10	97.9
Mean	92.8	Mean	96.2	Mean	97.6
RSD	3.20%	RSD	2.60%	RSD	2.50%
AV(10)***	12.8	AV(10)	8.4	AV(10)	6.8

***AV = acceptance value used for UPS <905> content uniformity. Ideally AV should be less than 15 to pass USP <905> content uniformity.

0.3mg dry blend capsule 1528-2850-RD		
Sample	Original %LC	Re -preparation %LC
1	82.73	85.87
2	84.57	89.45
3	80.29	91.39
4	84.88	88.45
5	85.2	86.96
6	82.9	84.84
7	84.75	86.21
8	86.58	91.37
9	84.34	88.79
10	88.82	84.75
Mean	84.51	87.81
std. dev	2.288445	2.467121
% RSD	2.7	2.8

Conte1528-2855-RD Sample	%LC	1528-2850B-RD Sample	%LC
1	88.82	1	78.62
2	93.73	2	91.43
3	89.06	3	86.52
4	84.94	4	90.9
5	89.93	5	84.83
6	88.7	6	95.29
7	88.71	7	75.69
8	86.85	8	76.87
9	86.92	9	84.92
10	91.33	10	86.9
Mean	88.9	Mean	85.2
std. dev	2.45	std. dev	6.51
% RSD	2.76	% RSD	7.64

500-55	
Sample	% label claim
1	96.90%
2	99.40%
3	103.20%
4	96.90%
5	100.00%
6	99.60%
7	96.90%
8	102.80%
9	96.80%
10	93.90%
Mean	98.60%
SD	2.91
RSD	3.00%
AV	7.1 (PASS)

[222] The data in the tables above show that all of the batches yield very good content uniformity acceptable for commercial product.

[223] Dissolution 50-rpm summary: The tables below are summaries of the dissolution of drug from capsules or tablets in an unconventional small-volume apparatus needed to measure the small amount of drug in the units using slow stirring to look for changes in dissolution over time. The test was performed by placing one unit into a very small volume of water at 37C with a paddle stirring at 50-rpm (which is slow) and data were collected at 15, 30 45, and 60 minutes to show the drug release rate over time. These tested products are “immediate release” oral solid dosage forms and a conventional requirement is to have about 75% released in about 45 minutes. The tables summarize the results at 45 minutes and indicate that dissolution was stable over time.

		Dissolution (% label claim at 45 minutes)						
		Initial		40C/75RH	30C/65RH		25C	5C
Lot (description)		bulk	0M	1M	2M	3M	3M	4M
1528-2850-RD (dry blend V- Cap capsule HDPE bottle)	Vessel 1	85		78	84	81	86	83
	Vessel 2	87		73	90	82	84	85
	Vessel 3	88		79	85	79	91	87
	Vessel 4	84		86	87	78	83	85
	Vessel 5	89		72	89	80	79	90
	Vessel 6	88		81	85	82	88	83
	Average	87		78	87	80	85	85
	RSD	2		6.4	2.7	2.1	5.0	2.9
1528-2850-RD (dry blend Vcap capsule OxyGuard bottle)	Vessel 1	85		69	89	79	88	82
	Vessel 2	87		75	89	87	81	85
	Vessel 3	88		77	87	86	84	86
	Vessel 4	84		80	87	83	83	80
	Vessel 5	89		71	88	89	84	84
	Vessel 6	88		76	88	79	86	89
	Average	87		75	88	84	84	84
	RSD	2		5.3	1.2	5.2	3.1	3.6
1528-2850-RD (dry blend V- cap capsule blister strip)	Vessel 1	85	75	59	86	73	83	
	Vessel 2	87	89	77	79	81	81	
	Vessel 3	88	88	83	87	74	84	
	Vessel 4	84	89	67	93	85	83	
	Vessel 5	89	93	75	82	82	84	
	Vessel 6	88	90	82	90	67	87	
	Average	87	87	74	86	77	84	
	RSD	2	7	12.5	6.3	8.6	2.4	

		Dissolution (% label claim at 45 minutes)				
		Initial	40C/75RH	30C/65RH		25C
Lot (description)		bulk	1M	2M	3M	3M
1528-2855-RD (coated bead V-Cap capsule HDPE bottle)	Vessel 1	104	85	100	79	83
	Vessel 2	89	90	97	83	88
	Vessel 3	91	84	71	91	50

	Vessel 4	88	64	73	94	88
	Vessel 5	94	75	72	75	92
	Vessel 6	93	80	39	96	94
	Average	93	80	75	86	83
	RSD	6	12	29	9.7	20
1528-2855RD (coated bead V-cap capsule OxyGuard bottle)	Vessel 1	104	88	80	87	78
	Vessel 2	89	79	91	86	94
	Vessel 3	91	84	63	92	74
	Vessel 4	88	92	98	90	98
	Vessel 5	94	89	81	81	93
	Vessel 6	93	44	99	81	78
	Average	93	79	85	86	86
	RSD	6	23	16	5.3	12.1
1528-2855-RD (coated bead V-cap capsule blister strip)	Vessel 1	104	85	98	100	81
	Vessel 2	89	84	94	63	80
	Vessel 3	91	97	96	82	87
	Vessel 4	88	94	96	55	74
	Vessel 5	94	64	75	95	66
	Vessel 6	93	96	102	89	82
	Average	93	87	93	81	78
	RSD	6	14	10	22.4	9.2

Lot (description)	Dissolution (% label claim at 45 minutes)			
	Initial	40C/75RH	30C/65RH	
	bulk	1M	2M	3M

1528-2851- RD (coated particle tablet HDPE bottle)	Vessel 1	58%	67	68	89
	Vessel 2	77%	84	78	124
	Vessel 3	57%	62	68	70
	Vessel 4	96%	110	84	105
	Vessel 5	95%	65	107	61
	Vessel 6	64%	103	76	51
	Average	74%	82	80	83
	RSD	24%	26	18	33
1528-2851- RD (coated particle tablet OxyGuard bottle)	Vessel 1	58%	89	54	118
	Vessel 2	77%	73	101	69
	Vessel 3	57%	75	82	80
	Vessel 4	96%	68	67	73
	Vessel 5	95%	76	162	96
	Vessel 6	64%	97	82	95
	Average	74%	80	91	89
	RSD	24%	14	42	21

Lot (description)	Dissolution (% label claim at 45 minutes)				
		Initial	40C/75RH	30C/65RH	
		bulk	1M	2M	3M
1528-2850B- RD (dry blend tablet HDPE bottle)	Vessel 1	90%	88	96	92
	Vessel 2	69%	79	82	92
	Vessel 3	83%	76	100	85
	Vessel 4	94%	96	86	94
	Vessel 5	88%	89	89	83
	Vessel 6	92%	83	97	83
	Average	86%	85	92	88
	RSD	11%	8.2	8	5.6
1528-2850B- RD (dry blend tablet OxyGuard bottle)	Vessel 1	90%	74	80	91
	Vessel 2	69%	97	87	95
	Vessel 3	83%	91	86	90
	Vessel 4	94%	94	91	90
	Vessel 5	88%	83	91	89
	Vessel 6	92%	91	76	84
	Average	86%	88	85	90

	RSD	11%	9.6	7	4.0
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Lot (description)	Dissolution (% label claim at 45 minutes)						
		Initial		40C/75RH	30C/65RH		25C
		bulk	0M	1M	2M	3M	3M
500-55 (dry blend V-Cap Plus capsule HDPE bottle)	Vessel 1	95		90	92	91	89
	Vessel 2	98		85	98	97	98
	Vessel 3	69		85	96	94	76
	Vessel 4	94		89	95	100	97
	Vessel 5	99		89	97	98	86
	Vessel 6	104		100	99	94	92
	Average	93		89	96	96	90
	RSD	13.1		6.2	2.4	3.6	9.1
500-55 (dry blend V-Cap Plus capsule OxyGuard bottle)	Vessel 1	95		84	103	99	94
	Vessel 2	98		97	101	95	103
	Vessel 3	69		97	99	98	97
	Vessel 4	94		92	97	92	96
	Vessel 5	99		91	100	95	101
	Vessel 6	104		96	95	93	91
	Average	93		93	99	95	97
	RSD	13.1		5.3	2.7	2.7	4.3
500-55 (dry blend V-Cap Plus capsule foil blister)	Vessel 1	95	98	99		89	98
	Vessel 2	98	101	88		94	87
	Vessel 3	69	107	90		89	96
	Vessel 4	94	96	90		86	87
	Vessel 5	99	99	68		89	94
	Vessel 6	104	99	90		82	89
	Average	93	100	87		88	92
	RSD	13.1	3.8	11.8		4.3	5.5

Dry blend 3mg lot 1528-2907-RD 500-mL				
	15 min	30 min	45 min	60 min
Vessel 1	91	96	97	96
Vessel 2	96	95	97	96

Vessel 3	96	97	97	97
Vessel 4	95	102	100	100
Vessel 5	97	96	96	97
Vessel 6	92	99	98	98
Average	94	97	98	97
RSD	2.7	2.5	1.1	1.4

Dry blend 1mg lot 1528-2906-RD 150-mL				
	15 min	30 min	45 min	60 min
Vessel 1	65	92	96	99
Vessel 2	49	91	95	96
Vessel 3	46	88	96	97
Vessel 4	44	96	101	102
Vessel 5	39	78	93	99
Vessel 6	57	90	95	96
Average	50	89	96	98
RSD	18.8	7	2.8	2.4

Dry blend 0.3mg lot 1528-2890-RD 50-mL				
	15 min	30 min	45 min	60 min
Vessel 1	57	94	100	105
Vessel 2	60	96	100	105
Vessel 3	86	93	94	95
Vessel 4	76	90	91	101
Vessel 5	69	90	97	106
Vessel 6	68	95	97	97
Average	69	93	97	102
RSD	15.6	2.8	3.4	4.5

Lot (strength)	COA	Capsule Dissolution at 45 minutes											
		5C			25C			30C			40C		
		1M	2M	3M	1M	2M	3M	1M	2M	3M	1M	2M	3M
2011F101 A (0.3mg)	98%	99%	95%	95%	95%	92%	95%	94%	93%	97%	93%	90%	92%
2011F099 A (1mg)	96%	95%	95%	95%	91%	93%	94%	93%	90%	95%	95%	92%	93%
2011F100 A (3mg)	99%	101%	97%	97%	100%	95%	95%	98%	95%	95%	96%	93%	95%
11H141 (0.3mg)	101%	102%	101%	101%	105%	96%	106%	102%	97%	103%	99%	96%	98%

11H152 (1mg)	96%	96%	99%	97%	96%	99%	97%	96%	96%	98%	96%	96%	98%
11H140 (3mg)	102%	102%	102%	101%	105%	100%	97%	102%	99%	102%	101%	99%	96%

[224] Dissolution 75-rpm: The tables below show a few examples where the stirring rate was increased slightly to 75-rpm to give more consistent results and indicates stable dissolution after accelerated storage of 1 or 2 months at 40C 75% relative humidity.

Dry blend 0.3mg lot 1528-2850-RD 1M 40C/75RH 75-rpm 50-mL				
	15 min	30 min	45 min	60 min
Vessel 1	75	80	80	81
Vessel 2	61	75	80	82
Vessel 3	65	81	83	84
Vessel 4	78	86	84	85
Vessel 5	66	79	83	84
Vessel 6	62	79	84	86
Average	68	80	82	84
RSD	10.3	4.5	2.3	2.2

Dry blend 1mg lot 1528-2906A-RD 2M 40C/75RH 75-rpm 50-mL				
	15 min	30 min	45 min	60 min
Vessel 1	69	84	88	88
Vessel 2	62	82	84	85
Vessel 3	65	82	85	85
Vessel 4	58	70	80	79
Vessel 5	59	77	82	81
Vessel 6	68	80	83	84
Average	64	79	84	84
RSD	7.2	6.4	3.3	3.8

[225] 2855-RD dissolution: The tables below are all the dissolution profiles of batch 1528-2850-RD and indicate stable drug release over time.

	Initial Percent Dissolved
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Vessel	15	30	45	60
1	84%	99%	104%	104%
2	28%	80%	89%	92%
3	68%	83%	91%	95%
4	56%	79%	88%	98%
5	29%	83%	94%	98%
6	74%	85%	93%	96%
Mean	57%	85%	93%	97%
RSD	41.20%	8.50%	6.00%	4.20%

1M 40C/75RH OxyGuard Packaging					2M 30C/65RH OxyGuard				3M 30C/65RH OxyGuard				3M 25C/60RH OxyGuard			
Vessel	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min
1	35	74	88	93	47	67	80	90	76	83	87	88	44	62	78	85
2	46	74	79	85	57	80	91	95	65	79	86	91	70	89	94	97
3	39	78	84	88	43	55	63	71	64	84	92	97	48	62	74	79
4	59	82	92	94	75	92	98	101	71	85	90	94	65	92	98	103
5	22	82	89	92	38	64	81	92	60	75	81	87	72	86	93	96
6	4	20	44	61	54	94	99	101	55	74	81	87	53	74	78	84
Average	34	68	79	86	52	75	85	92	65	80	86	91	59	78	86	91
RSD	57	35	23	14	25	21	16	12	11.7	5.7	5.3	4.6	20.1	17.4	12.1	10.4

1M 40C/75RH HDPE Bottle					2M 30C/65RH HDPE				3M 30C/65RH HDPE				3M 25C/60RH HDPE			
Vessel	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min
1	61	78	85	89	78	97	100	103	58	72	79	85	54	70	83	92
2	63	83	90	92	77	93	97	98	51	72	83	90	66	81	88	92
3	66	79	84	91	41	59	71	78	53	84	91	94	10	29	50	66
4	25	44	64	77	50	65	73	78	66	89	94	95	69	81	88	92
5	47	67	75	80	37	59	72	83	48	66	75	81	68	83	92	97
6	57	71	80	85	6	21	39	52	85	94	96	99	82	91	94	97
Average	53	70	80	86	48	66	75	82	60	80	86	91	58	73	83	89
RSD	28	20	12	7	56	42	29	22	22.6	14	9.7	7.3	43	30.6	19.6	13.3

1M 40C/75RH Blister Packaging					2M 30C/65RH Blister				3M 30C/65RH Blister				3M 25C/60RH Blister			
Vessel	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min
1	36	69	85	90	61	91	98	100	82	95	100	102	53	71	81	90
2	41	69	84	88	57	82	94	100	31	48	63	74	27	57	80	87

3	67	96	97	98	63	87	96	100	69	77	82	85	70	78	87	92
4	54	83	94	104	36	80	96	100	29	41	55	69	52	66	74	87
5	10	46	64	79	45	61	75	83	84	94	95	97	25	48	66	80
6	70	91	96	100	87	100	102	104	74	84	89	82	50	74	82	84
Average	47	76	87	93	58	83	93	98	62	73	81	85	46	66	78	87
RSD	48	25	14	10	30	16	10	8	40.5	32.1	22.4	14.9	37.0	17.0	9.2	5.3

[226] Bathes 2850-RD, 2850B-RD, 2851-RD, and 500-55 were also tested in the similar fashion and all showed stable drug release over time.

We claim:

1. An oral dosage formulation comprising at least one GCC agonist peptide and one or more pharmaceutically acceptable excipients, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, and the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 9 and 8.
2. An oral dosage formulation comprising at least one GCC agonist peptide and one or more pharmaceutically acceptable excipients, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1-54 and 56-249, and the GCC agonist peptide has a chromatographic purity of no less than 91%.
3. The oral dosage formulation of claim 2, wherein the GCC agonist peptide has a chromatographic purity of no less than 92% or no less than 95%.
4. The oral dosage formulation of claim 2, wherein the GCC agonist peptide has a total impurity content of no greater than 9%, 7%, 6%, or 5%.
5. The oral dosage formulation of claim 2, wherein the formulation is substantially free of inorganic acids and carboxylic acids.
6. The oral dosage formulation of claim 2, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56.
7. The oral dosage formulation of claim 2, wherein the amount of GCC agonist peptide per unit dose is 0.1 mg, 0.3 mg, 1.0 mg, 3.0 mg, 6.0 mg, 9.0 mg or 9.5 mg.
8. The oral dosage formulation of claim 2, wherein the formulation is a solid formulation and the unit dose is a powder, granule, sachet, troche, tablet, or capsule.
9. The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise an inert carrier.

10. The oral dosage formulation of claim 9, wherein the inert carrier is a selected from mannitol, lactose, a microcrystalline cellulose, or starch.
11. The oral dosage formulation of claim 10, wherein the inert carrier has a particle size of from 50 to 900 microns.
12. The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise a divalent cation salt.
13. The oral dosage formulation of claim 12, wherein the salt is calcium chloride or calcium ascorbate.
14. The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise an amino acid or amine, and the molar ratio between the amino acid and GCC agonist peptide is 2:1 to 30:1.
15. The oral dosage formulation of claim 14, wherein the amino acid is leucine, histidine, or arginine.
16. The oral dosage formulation of claim 2, wherein the formulation consists of the GCC agonist peptide, an inert carrier, and a lubricant.
17. The oral dosage formulation of claim 2, wherein the formulation consists of the GCC agonist peptide, an inert carrier, a divalent cation salt, an amino acid, a coating agent and optionally a lubricant.
18. The oral dosage of formulation of claim 17, wherein the inert carrier is microcrystalline cellulose and the lubricant is magnesium stearate.
19. The oral dosage of formulation of claim 18, wherein the divalent cation salt is calcium chloride or calcium ascorbate, the amino acid is leucine, histidine, or arginine, and the coating agent is hypromellose.

20. The oral dosage formulation of claim 2, wherein the GCC agonist peptide is stabilized against degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.
21. The oral dosage formulation of claim 2, wherein the formulation is in the form of a capsule or tablet.
22. The oral dosage formulation of claim 21, wherein the capsule or tablet is in a blister pack or strip.
23. The oral dosage formulation of claim 22, wherein the GCC agonist peptide is in solution or suspension in a lipophilic liquid.
24. The oral dosage formulation of claim 23, wherein the unit dosage form is a liquid-filled capsule.
25. The oral dosage formulation of claim 2, wherein the liquid is a refined specialty oil or a medium chain triglyceride or related ester.
26. A process for making an oral dosage formulation comprising at least one GCC agonist peptide, the method comprising:
 - a) providing an aqueous solution comprising: a GCC agonist peptide selected from the group consisting of SEQ ID NOs: 1-54 and 56-249, and one or more pharmaceutically acceptable excipients, wherein the concentration of the GCC agonist peptide ranges from 10 to 60 mg/mL; and
 - b) applying the aqueous solution to a pharmaceutically acceptable carrier to generate a GCC agonist peptide-coated carrier.
27. The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise a divalent cation salt wherein the divalent cation is selected from Ca^{2+} , Mg^{2+} , Zn^{2+} , and Mn^{2+}
28. The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise an amino acid selected from leucine, histidine, and arginine.

29. The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise a coating agent.
30. The process of claim 29, wherein the coating agent is hypromellose.
31. The process of claim 26, wherein the aqueous solution has a pH greater than 4 or 5.
32. The process of claim 26, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, and 56.
33. The process of claim 26, wherein the aqueous solution is substantially free of inorganic acids and carboxylic acids.
34. The process of claim 26, further comprising drying the GCC agonist peptide-coated carrier.
35. An oral dosage formulation made by the process of claim 26, wherein the GCC agonist peptide is stabilized against degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.
36. A method for treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject an oral dosage formulation of claim 2.
37. The method of claim 36, wherein the disease or disorder is a gastrointestinal disease or disorder selected from the group consisting of irritable bowel syndrome, chronic idiopathic constipation, non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease, constipation, gastroparesis, heartburn, gastric cancer, and H. pylori infection.
38. The method of claim 36, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56.

39. The method of claim 36, further comprising administering to the subject an effective amount of an inhibitor of a cGMP-specific phosphodiesterase.
40. The method of claim 36, further comprising administering to the subject an effective amount of at least one laxative.
41. The method of claim 36, further comprising administering to the subject an effective amount of at least one anti-inflammatory agent.
42. A pharmaceutical composition comprising the oral dosage formulation of claim 2.

ABSTRACT OF THE DISCLOSURE

The invention provides low-dose formulations of guanylate cyclase-C (“GCC”) agonist peptides and methods for their use. The formulations of the invention can be administered either alone or in combination with one or more additional therapeutic agents, preferably an inhibitor of cGMP-dependent phosphodiesterase or a laxative.

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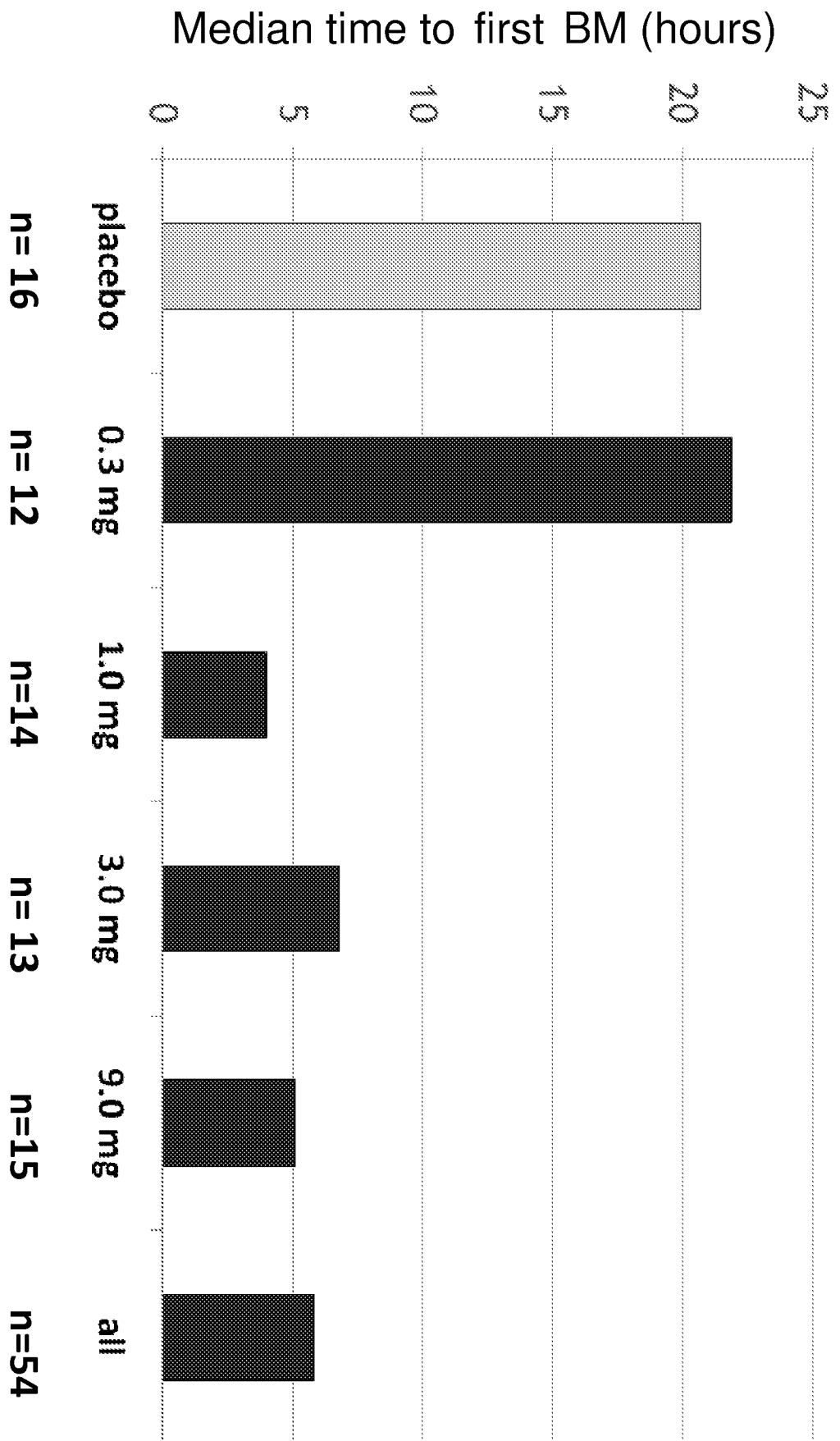


Fig. 1

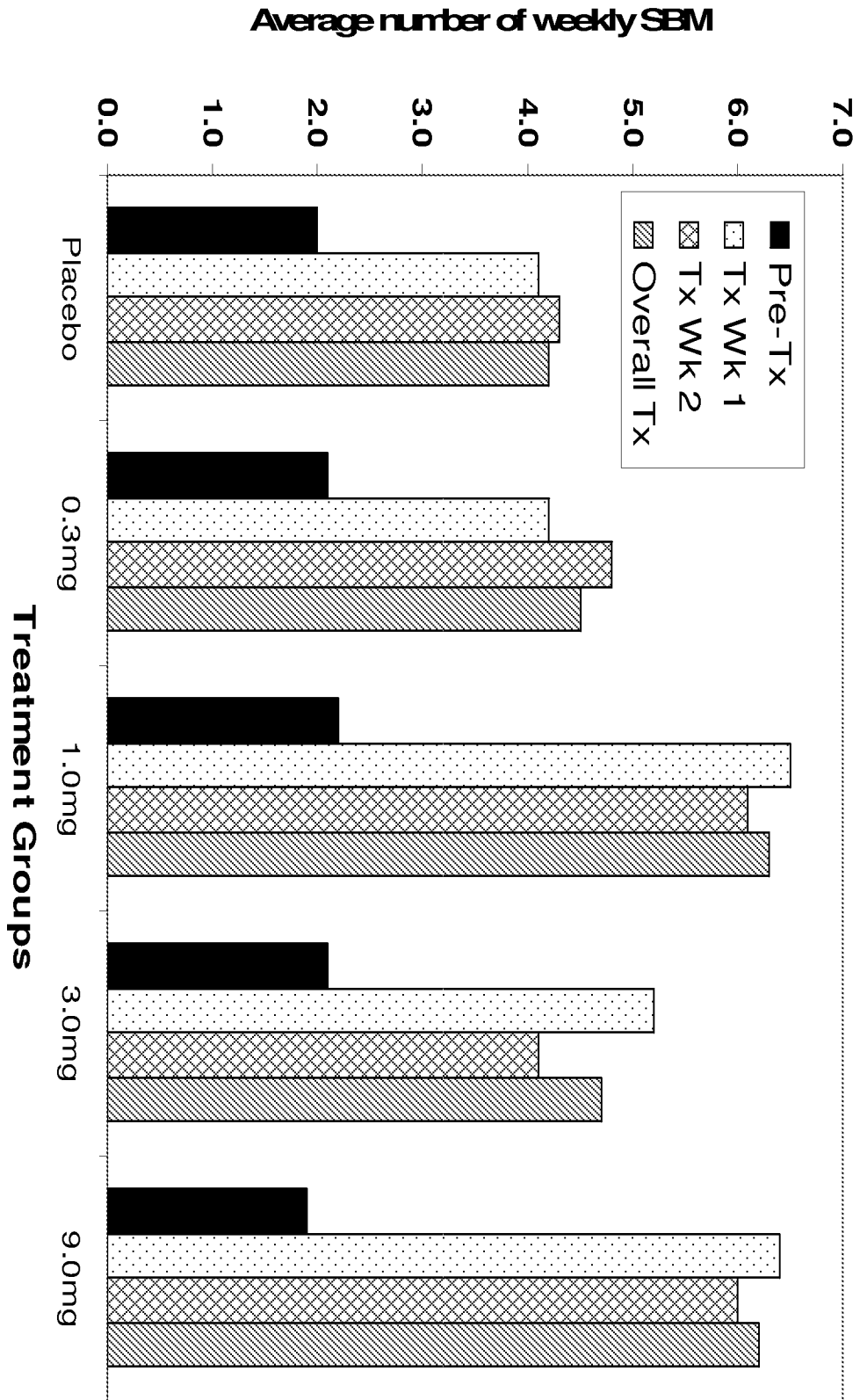


Fig. 2

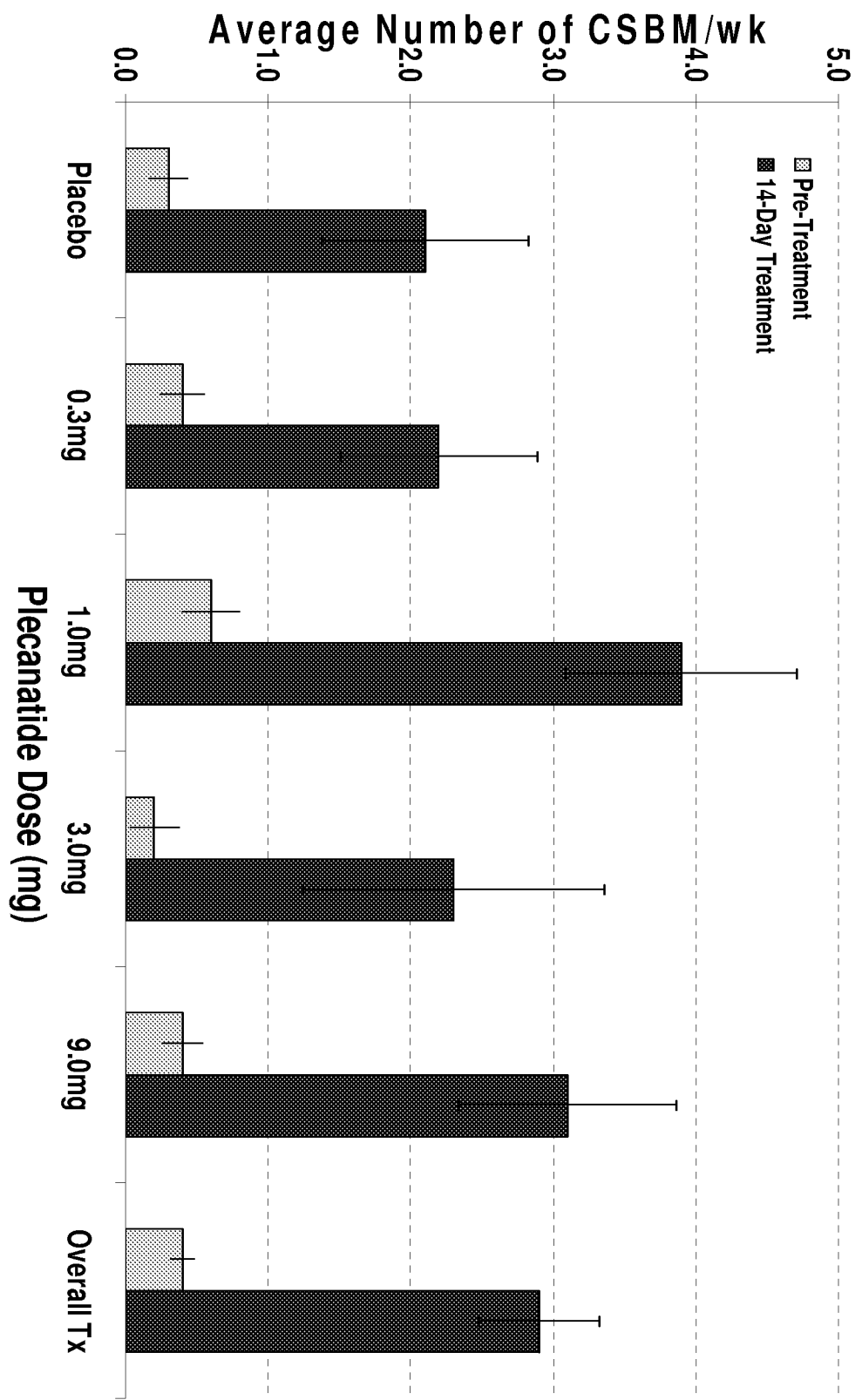


Fig. 3

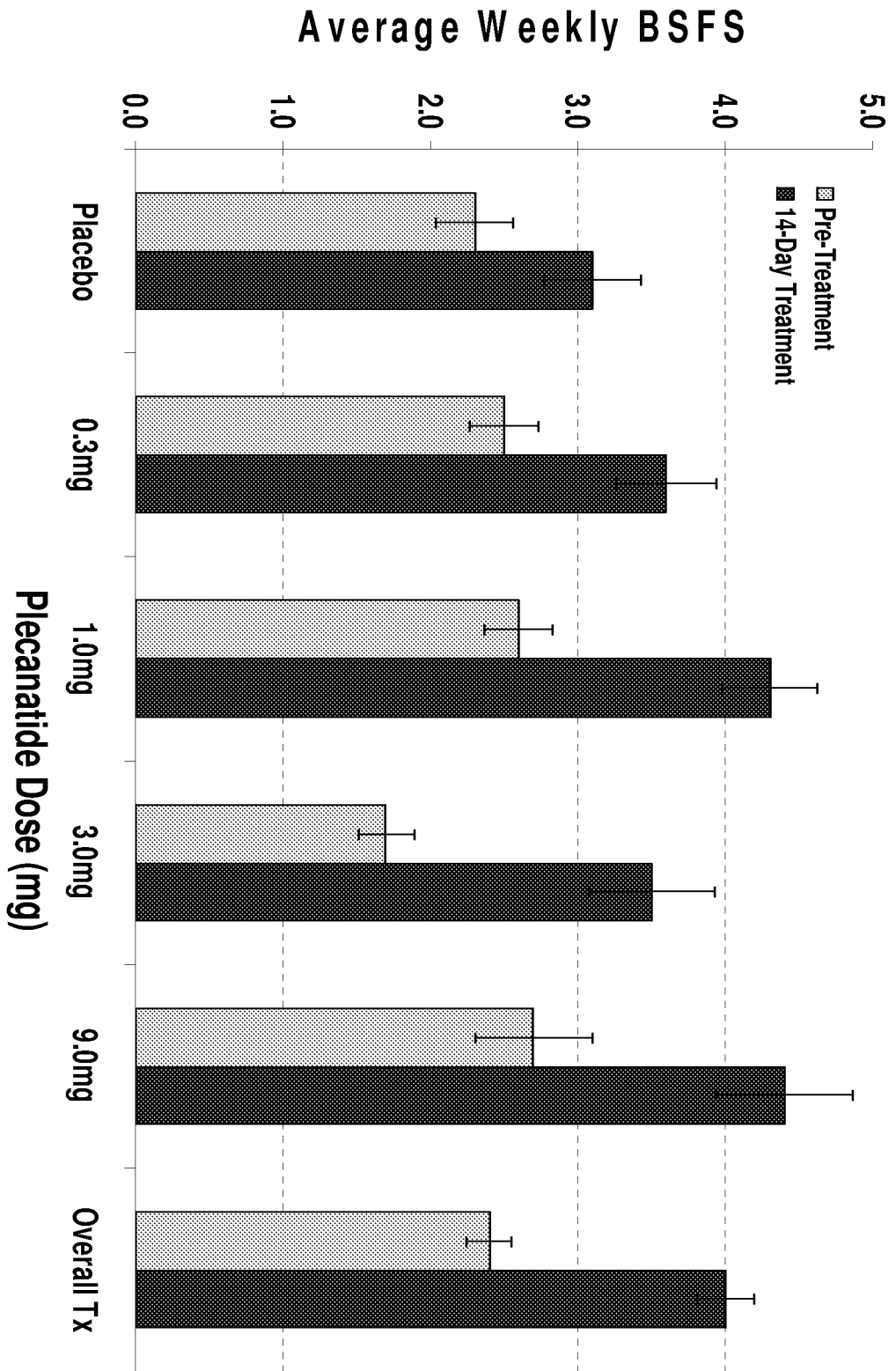


Fig. 4

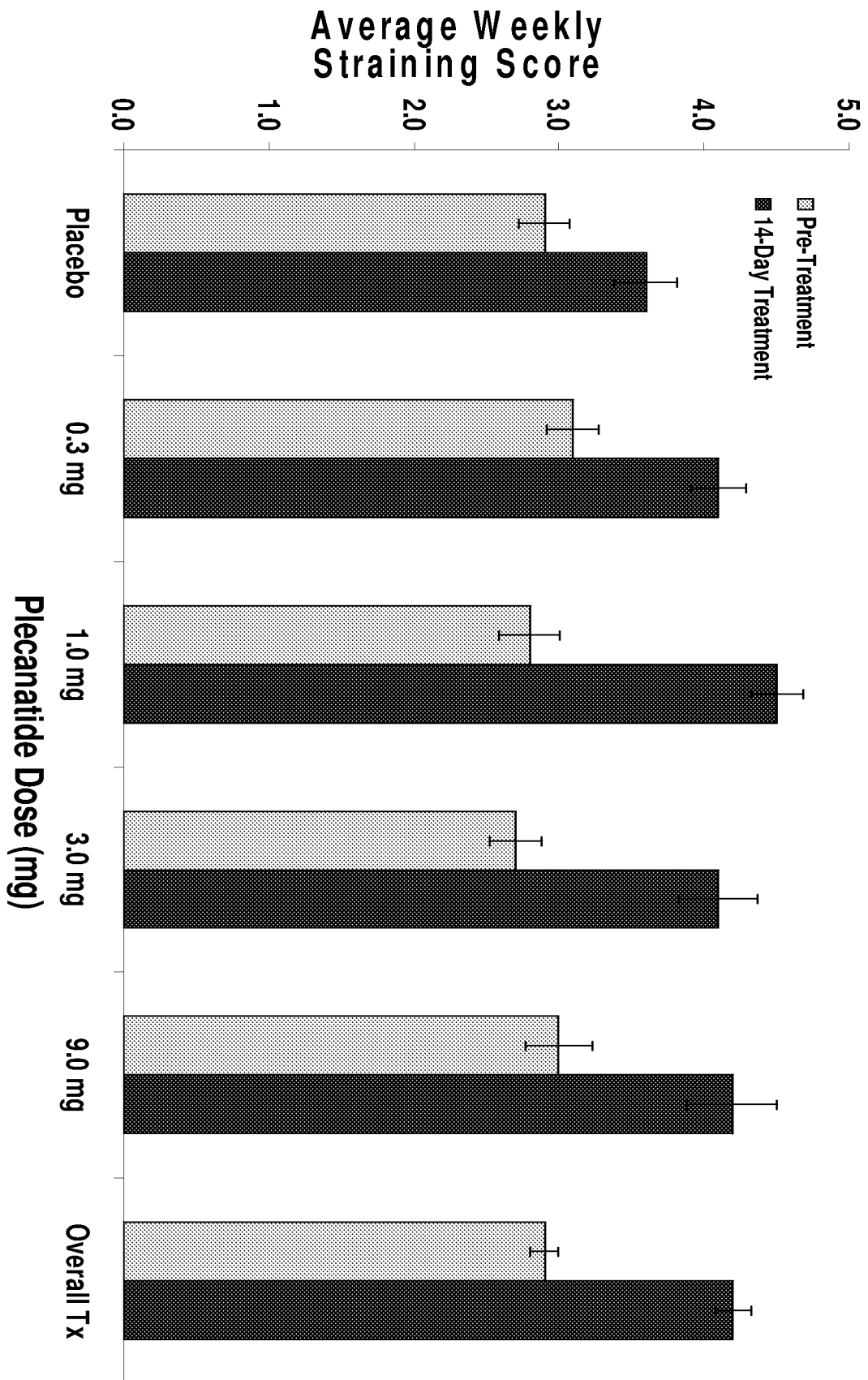


Fig. 5

% of Subjects Reporting Improvement in Abdominal Discomfort

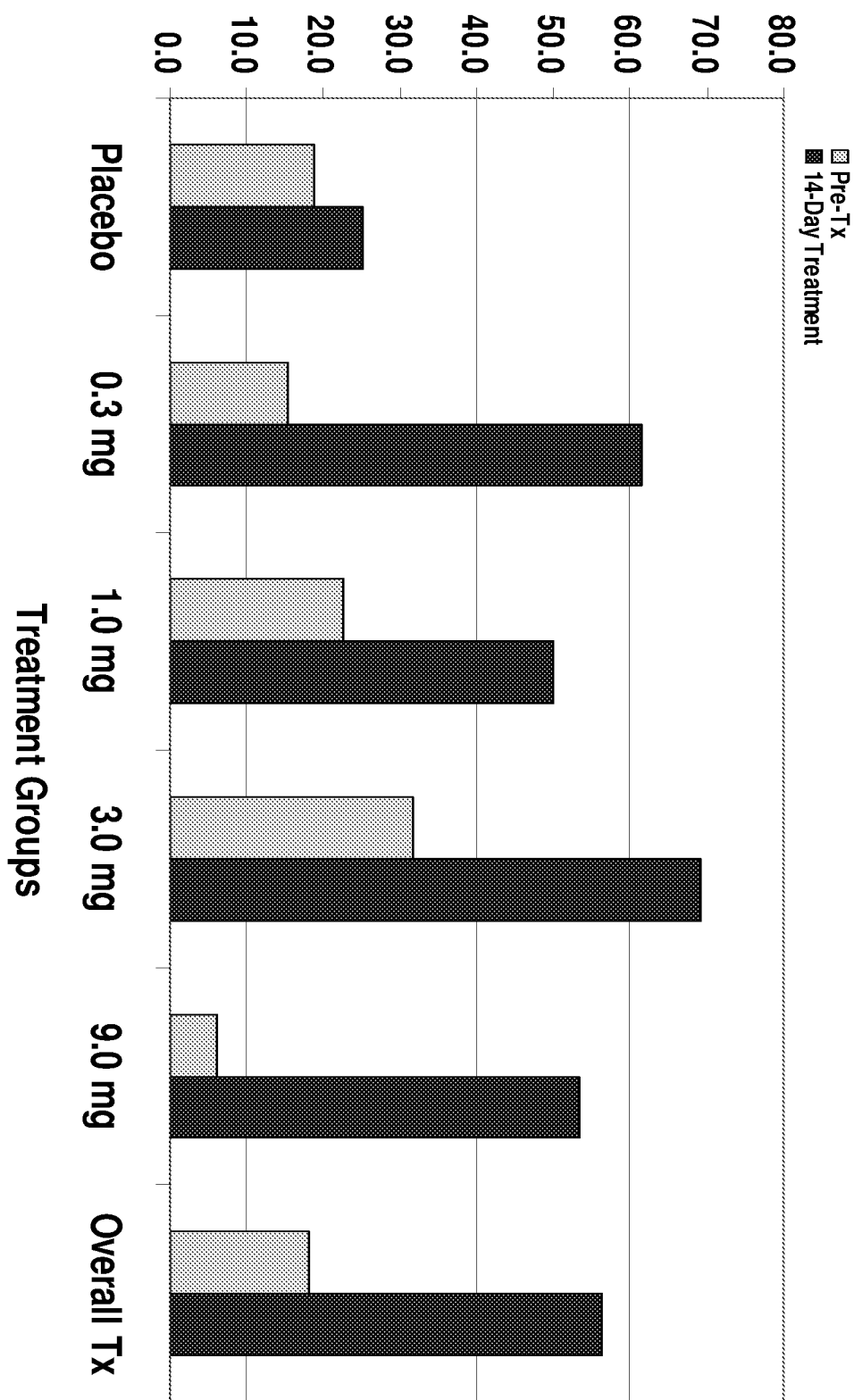


Fig. 6

Application Data Sheet

Application Information

Application Type::	Regular
Subject Matter::	Utility
Suggested Group Art Unit::	N/A
CD-ROM or CD-R?::	None
Sequence submission?::	None
Computer Readable Form (CRF)?::	No
Title::	Formulations of Guanylate Cyclase C Agonists and Methods of Use
Attorney Docket Number::	40737-509001US
Request for Early Publication?::	No
Request for Non-Publication?::	No
Total Drawing Sheets::	6
Small Entity?::	No
Petition included?::	No
Secrecy Order in Parent Appl.?::	No

Applicant Information

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City of mailing address:: Audubon
State or Province of mailing address:: PA

Postal or Zip Code of mailing address:: 19403

Correspondence Information

Correspondence Customer Number:: 30623

Representative Information

Representative Customer Number:: 30623

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Continuation-in-part of	PCT/US2011/051805	09/15/11
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/383156	09/15/10
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/387636	09/29/10
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/392186	10/12/10

Foreign Priority Information

Assignee Information

Assignee name:: Synergy Pharmaceuticals Inc.

Street of mailing address:: 420 Lexington Avenue
Suite 1609

City of mailing address:: New York

State or Province of mailing address:: NY

Postal or Zip Code of mailing address:: 10170

SCORE Placeholder Sheet for IFW Content

Application Number: 13421769

Document Date: 3/15/2012

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- Drawings – Other than Black and White Line Drawings

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
13/421,769

APPLICATION AS FILED - PART I

(Column 1) (Column 2)

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A
TOTAL CLAIMS (37 CFR 1.16(j))	42 minus 20 = *	22
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 = *	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))		

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

SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	
N/A	
N/A	
TOTAL	

OR OTHER THAN SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	380
N/A	620
N/A	250
x 60 =	1320
x 250 =	0.00
	310
	0.00
TOTAL	2880

APPLICATION AS AMENDED - PART II

(Column 1) (Column 2) (Column 3)

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	* Minus **	=
Independent (37 CFR 1.16(h))	* Minus ***	=	
Application Size Fee (37 CFR 1.16(s))			
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))			

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

(Column 1) (Column 2) (Column 3)

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	* Minus **	=
Independent (37 CFR 1.16(h))	* Minus ***	=	
Application Size Fee (37 CFR 1.16(s))			
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))			

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

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



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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: 13/421,769, 03/15/2012, 1629, 0.00, 40737-509001US, 42, 3

CONFIRMATION NO. 3135

30623
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C
ONE FINANCIAL CENTER
BOSTON, MA 02111

FILING RECEIPT



Date Mailed: 04/03/2012

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

Applicant(s)

Stephen Comiskey, Doylestown, PA;
Rong Feng, Langhorne, PA;
John Foss, Doylestown, PA;
Kunwar Shailubhai, Audubon, PA;

Assignment For Published Patent Application

Synergy Pharmaceuticals Inc., New York, NY

Power of Attorney: None

Domestic Priority data as claimed by applicant

This application is a CIP of PCT/US2011/051805 09/15/2011
which claims benefit of 61/383,156 09/15/2010
and claims benefit of 61/387,636 09/29/2010
and claims benefit of 61/392,186 10/12/2010

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

If Required, Foreign Filing License Granted: 04/02/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 13/421,769

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

Non-Publication Request: No

Early Publication Request: No

Title

Formulations of Guanylate Cyclase C Agonists and Methods of Use

Preliminary Class

514

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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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Alexandria, Virginia 22313-1450
www.uspto.gov

Table with 4 columns: APPLICATION NUMBER (13/421,769), FILING OR 371(C) DATE (03/15/2012), FIRST NAMED APPLICANT (Stephen Comiskey), ATTY. DOCKET NO./TITLE (40737-509001US)

CONFIRMATION NO. 3135

FORMALITIES LETTER

30623
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C
ONE FINANCIAL CENTER
BOSTON, MA 02111



Date Mailed: 04/03/2012

NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(b)

Filing Date Granted

Items Required To Avoid Abandonment:

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

- The statutory basic filing fee is missing. Applicant must submit \$380 to complete the basic filing fee for a non-small entity. If appropriate, applicant may make a written assertion of entitlement to small entity status and pay the small entity filing fee (37 CFR 1.27).
The oath or declaration is missing. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required. Note: If a petition under 37 CFR 1.47 is being filed, an oath or declaration in compliance with 37 CFR 1.63 signed by all available joint inventors, or if no inventor is available by a party with sufficient proprietary interest, is required.
This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 CFR 1.821(c) Applicant must provide an initial paper or compact disc copy of the "Sequence Listing", as well as an amendment specifically directing its entry into the application and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000).
A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 CFR 1.821(e). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000). Applicant must provide an initial computer readable form (CRF) copy of the "Sequence Listing" and a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If applicant desires the sequence

listing in the instant application to be identical with that of another application on file in the U.S. Patent and Trademark Office, such request in accordance with 37 CFR 1.821(e) may be submitted in lieu of a new CRF.

Applicant is cautioned that correction of the above items may cause the specification and drawings page count to exceed 100 pages. If the specification and drawings exceed 100 pages, applicant will need to submit the required application size fee.

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For questions regarding compliance to these requirements, please contact:

- **For Rules Interpretation, call (571) 272-0623**
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- **Send e-mail correspondence for Patentin Software Program Help to ebc@uspto.gov**

The applicant needs to satisfy supplemental fees problems indicated below.

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

- Additional claim fees of **\$1320** as a non-small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- A surcharge (for late submission of filing fee, search fee, examination fee or oath or declaration) as set forth in 37 CFR 1.16(f) of **\$130** for a non-small entity, must be submitted.

SUMMARY OF FEES DUE:

Total fee(s) required within **TWO MONTHS** from the date of this Notice is **\$3010** for a non-small entity

- **\$380** Statutory basic filing fee.
- **\$130** Surcharge.
- The application search fee has not been paid. Applicant must submit **\$620** to complete the search fee.
- The application examination fee has not been paid. Applicant must submit **\$250** to complete the examination fee for a non-small entity.
- The specification and drawings submitted electronically contain the equivalent of more than 100 pages. Applicant owes **\$310** for **16** pages in excess of **100** pages for a non-small entity.
- Total additional claim fee(s) for this application is **\$1320**
 - **\$1320** for **22** total claims over 20.

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POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Title	Formulations of Guanylate Cyclase C Agonists And Methods Of Use
	Art Unit	1629
	Examiner Name	Not Yet Assigned
	Attorney Docket No.	40737-509001US

I hereby revoke all previous powers of attorney given in the above-identified application.

A Power of Attorney is submitted herewith.
 OR

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30623

OR

I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number	Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

The address associated with the above-mentioned Customer Number:
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OR

Firm or Individual Name

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Country	Telephone	Email	

I am the:

Applicant/Inventor.
 OR

Assignee of record of the entire interest. See 37 CFR 3.71.
 Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____

SIGNATURE of Applicant or Assignee of Record

Signature	Date	
Name	Telephone	
Title and Company		

Signature: *[Handwritten Signature]* Date: 5/8/12
 Name: Gary S. Jacob, Ph.D. Telephone: 912-497-7560
 Title and Company: Chief Executive Officer and Chief Scientific Officer, Synergy Pharmaceuticals, Inc.

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

*Total of 1 forms are submitted.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Stephen Comiskey et al.

Application No./Patent No.: 13/421,769 Filed/Issue Date: March 15, 2012

Titled: Formulations Of Guanylate Cyclase C Agonists And Methods Of Use

Synergy Pharmaceuticals, Inc., a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

- 1. the assignee of the entire right, title, and interest in;
- 2. an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or
- 3. an assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made) the patent application/patent identified above by virtue of either:

A. An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 028079, Frame 0873, or for which a copy thereof is attached.

OR

B. A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

- 1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
- 2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
- 3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

Additional documents in the chain of title are listed on a supplemental sheet(s).

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

GARY S. JACOB
Signature
GARY S. JACOB
Printed or Typed Name

5/8/12
Date
CEO
Title

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

<h1>TRANSMITTAL FORM</h1> <p><i>(to be used for all correspondence after initial filing)</i></p>		Application Number	13/421,769
		Filing Date	March 15, 2012
		First Named Inventor	Stephen Comiskey
		Art Unit	1629
		Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission	3	Attorney Docket Number	40737-509001US

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 type="checkbox"/> Information Disclosure Statement <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 checked="" 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): Statement under 37 CFR 3.73(b)
<input type="text"/> Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MINTZ LEVIN COHN FERRIS GLOVSKY AND POPEO, P.C.		
Signature	/Cynthia Kozakiewicz/		
Printed name	Cynthia A. Kozakiewicz, J.D., Ph.D.		
Date	May 23, 2012	Reg. No.	42,764

Electronic Acknowledgement Receipt

EFS ID:	12849493
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	30623
Filer:	Cynthia A. Kozakiewicz/Victoria Hughes
Filer Authorized By:	Cynthia A. Kozakiewicz
Attorney Docket Number:	40737-509001US
Receipt Date:	23-MAY-2012
Filing Date:	15-MAR-2012
Time Stamp:	16:47:21
Application Type:	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	Power of Attorney	40737-509001US_- _40737-509001US-POA_1.pdf	60266 <small>b015aa707e891181e638f78104c7ec01ec7d c1b6</small>	no	1

Warnings:

Information:

2	Assignee showing of ownership per 37 CFR 3.73(b).	40737-509001US_- _40737-509001US-Stmt_2.pdf	56722	no	1
			eb9ff2969bc05e3a65b0d47aed5a467bb168be83		

Warnings:

Information:

3	Miscellaneous Incoming Letter	40737-509001US_- _40737-509001US-Trans_3.pdf	116065	no	1
			ced5ea7a5c77af7bcd440a4e3f490d7d2b580b35		

Warnings:

Information:

Total Files Size (in bytes):			233053		
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

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

National Stage of an International Application under 35 U.S.C. 371

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

New International Application Filed with the USPTO as a Receiving Office

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Comiskey et al.

Application No.: 13/421,769

Confirmation No.: 3135

Filed: March 15, 2012

Art Unit: 1629

For: Formulations Of Guanylate Cyclase C Agonists
And Methods Of Use

Examiner: Not Yet Assigned

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

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

In response to the Notice to File Missing Parts of Nonprovisional Application mailed April 3, 2012, Applicants submit herein an Executed Declaration for Utility Application, a Supplemental Application Data Sheet, a Computer Readable Form of Sequence Listing (TXT. File), a Statement in Support of Computer Readable Form and electronic payment of the surcharge of \$65.00 as set forth in 37 C.F.R. 1.16(f). Applicants are entitled to small entity status.

Although Applicants believe no additional fees are due with this submission, the Commissioner is hereby authorized to charge payment of any additional fees required in connection with the papers transmitted herewith, or credit any overpayment of same, to Deposit Account No. 50-0311, (Reference No. 40737-509001US).

Dated: June 4, 2012

Respectfully submitted,

/Cynthia Kozakiewicz/
Cynthia A. Kozakiewicz, J.D., Ph.D.
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Attorneys for Applicant

VIA EFS
Date of Deposit: June 4, 2012

Attorney Docket No.: 40737-509001US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Comiskey et al.

Application No.: 13/421,769

Confirmation No.: 3135

Filed: March 15, 2012

Art Unit: 1629

For: Formulations Of Guanylate Cyclase C Agonists
And Methods Of Use

Examiner: Not Yet Assigned

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

PRELIMINARY AMENDMENT

Prior to examination of this application, please amend the application as follows.

Amendments to the Specification begin on page 2 of this paper.

Remarks begin on page 12 of this paper.

Amendments to the Specification:

Please insert the following paragraph at page 1, before the “Field of Invention” section:

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING

The contents of the text file named “40737509001US_SequenceListing.txt”, which was created on May 25, 2012 and is 112 KB in size, are hereby incorporated by reference in their entirety.

Please amend paragraph [162], starting on page 74, line 16 as follows:

[162] In one embodiment, a GCC agonist formulation of the invention is administered as part of a combination therapy with one or more additional therapeutic agents for the treatment of obesity. Non-limiting examples of the one or more additional therapeutic agents include 1 β HSD-I (11-beta hydroxy steroid dehydrogenase type 1) inhibitors, such as BVT 3498, BVT 2733, 3-(1-adamantyl)-4-ethyl-5-(ethylthio)-4H-1,2,4-triazole, 3-(1-adamantyl)-5-(3,4,5-trimethoxyphenyl)-4-methyl-4H-1,2,4-triazole, 3-adamantanyl-4,5,6,7,8,9,10,11,12,3a-decahydro-1,2,4-triazolo[4,3-a][1,4]annulene, and those compounds disclosed in WO01/90091, WOO 1/90090, WOO 1/90092 and WO02/072084; 5HT antagonists such as those in WO03/037871, WO03/037887, and the like; 5HT1a modulators such as carbidopa, benserazide and those disclosed in US6207699, WO03/031439, and the like; 5HT2c (serotonin receptor 2c) agonists, such as BVT933, DPCA37215, IK264, PNU 22394, WAY161503, R-1065, SB 243213 (Glaxo Smith Kline) and YM 348 and those disclosed in US3914250, WO00/77010, WO02/36596, WO02/48124, WO02/10169, WO01/66548, WO02/44152, WO02/51844, WO02/40456, and WO02/40457; 5HT6 receptor modulators, such as those in WO03/030901, WO03/035061, WO03/039547, and the like; acyl-estrogens, such as oleoyl-estrone, disclosed in del Mar-Grasa, M. et al, Obesity Research, 9:202-9 (2001) and Japanese Patent Application No. JP 2000256190; anorectic bicyclic compounds such as 1426 (Aventis) and 1954 (Aventis), and the compounds disclosed in WO00/18749, WO01/32638,

WO01/62746, WO01/62747, and WO03/015769; CB 1 (cannabinoid-1 receptor) antagonist/inverse agonists such as rimonabant (Acomplia; Sanofi), SR-147778 (Sanofi), SR-141716 (Sanofi), BAY 65-2520 (Bayer), and SLV 319 (Solvay), and those disclosed in patent publications US4973587, US5013837, US5081122, US5112820, US5292736, US5532237, US5624941, US6028084, US6509367, US6509367, WO96/33159, WO97/29079, WO98/31227, WO98/33765, WO98/37061, WO98/41519, WO98/43635, WO98/43636, WO99/02499, WO00/10967, WO00/10968, WO01/09120, WO01/58869, WO01/64632, WO01/64633, WO01/64634, WO01/70700, WO01/96330, WO02/076949, WO03/006007, WO03/007887, WO03/020217, WO03/026647, WO03/026648, WO03/027069, WO03/027076, WO03/027114, WO03/037332, WO03/040107, WO03/086940, WO03/084943 and EP658546; CCK-A (cholecystokinin-A) agonists, such as AR-R 15849, GI 181771 (GSK), JMV-180, A- 71378, A-71623 and SR146131 (Sanofi), and those described in US5739106; CNTF (Ciliary neurotrophic factors), such as GI- 181771 (Glaxo-SmithKline), SRI 46131 (Sanofi Synthelabo), butabindide, PD 170,292, and PD 149164 (Pfizer); CNTF derivatives, such as Axokine® (Regeneron), and those disclosed in WO94/09134, WO98/22128, and WO99/43813; dipeptidyl peptidase IV (DP-IV) inhibitors, such as isoleucine thiazolidide, valine pyrrolidide, NVP-DPP728, LAF237, P93/01, P 3298, TSL 225 (tryptophyl-1,2,3,4-tetrahydroisoquinoline-3- carboxylic acid; disclosed by Yamada et al, Bioorg. & Med. Chem. Lett. 8 (1998) 1537-1540), TMC-2A/2B/2C, CD26 inhibitors, FE 999011, P9310/K364, VIP 0177, SDZ 274-444, 2- cyanopyrrolidides and 4-cyanopyrrolidides as disclosed by Ashworth et al, Bioorg. & Med. Chem. Lett., Vol. 6, No. 22, pp 1163-1166 and 2745-2748 (1996) and the compounds disclosed patent publications. WO99/38501, WO99/46272, WO99/67279 (Probiodrug), WO99/67278 (Probiodrug), WO99/61431 (Probiodrug), WO02/083128, WO02/062764, WO03/000180, WO03/000181, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593, WO03/004498, WO03/004496, WO03/017936, WO03/024942, WO03/024965, WO03/033524, WO03/037327 and EP1258476; growth hormone secretagogue receptor agonists/antagonists, such as NN703, hexarelin, MK- 0677 (Merck), SM-130686, CP-424391 (Pfizer), LY 444,711 (Eli Lilly), L-692,429 and L- 163,255, and such as those disclosed in USSN 09/662448, US provisional application 60/203335, US6358951, US2002049196, US2002/022637, WO01/56592 and WO02/32888; H3 (histamine H3) antagonist/inverse agonists, such as

thioperamide, 3-(1H-imidazol-4-yl)propyl N-(4-pentenyl)carbamate), clobenpropit, iodophenpropit, imoproxifan, GT2394 (Gliatech), and A331440, O-[3-(1H-imidazol-4-yl)propanol]carbamates (Kiec-Kononowicz, K. et al., *Pharmazie*, 55:349-55 (2000)), piperidine-containing histamine H₃-receptor antagonists (Lazewska, D. et al., *Pharmazie*, 56:927-32 (2001), benzophenone derivatives and related compounds (Sasse, A. et al., *Arch. Pharm.(Weinheim)* 334:45-52 (2001)), substituted N-phenylcarbamates (Reidemeister, S. et al., *Pharmazie*, 55:83-6 (2000)), and proxifan derivatives (Sasse, A. et al., *J. Med. Chem.* 43:3335-43 (2000)) and histamine H₃ receptor modulators such as those disclosed in WO02/15905, WO03/024928 and WO03/024929; leptin derivatives, such as those disclosed in US5552524, US5552523, US5552522, US5521283, WO96/23513, WO96/23514, WO96/23515, WO96/23516, WO96/23517, WO96/23518, WO96/23519, and WO96/23520; leptin, including recombinant human leptin (PEG-OB, Hoffman La Roche) and recombinant methionyl human leptin (Amgen); lipase inhibitors, such as tetrahydrolipstatin (orlistat/Xenical®), Triton WR1 339, RHC80267, lipstatin, teasaponin, diethylumbelliferyl phosphate, FL-386, WAY-121898, Bay-N-3176, valilactone, esteracin, ebelactone A, ebelactone B, and RHC 80267, and those disclosed in patent publications WO01/77094, US4598089, US4452813, US5512565, US5391571, US5602151, US4405644, US4189438, and US4242453; lipid metabolism modulators such as maslinic acid, erythrodiol, ursolic acid uvaol, betulinic acid, betulin, and the like and compounds disclosed in WO03/011267; Mc4r (melanocortin 4 receptor) agonists, such as CHIR86036 (Chiron), ME-10142, ME-10145, and HS-131 (Melacure), and those disclosed in PCT publication Nos. WO99/64002, WO00/74679, WOO 1/991752, WOO 1/25192, WOO 1/52880, WOO 1/74844, WOO 1/70708, WO01/70337, WO01/91752, WO02/059095, WO02/059107, WO02/059108, WO02/059117, WO02/06276, WO02/12166, WO02/11715, WO02/12178, WO02/15909, WO02/38544, WO02/068387, WO02/068388, WO02/067869, WO02/081430, WO03/06604, WO03/007949, WO03/009847, WO03/009850, WO03/013509, and WO03/031410; Mc5r (melanocortin 5 receptor) modulators, such as those disclosed in WO97/19952, WO00/15826, WO00/15790, US20030092041; melanin-concentrating hormone 1 receptor (MCHR) antagonists, such as T-226296 (Takeda), SB 568849, SNP-7941 (Synaptic), and those disclosed in patent publications WOO 1/21169, WO01/82925, WO01/87834, WO02/051809, WO02/06245, WO02/076929, WO02/076947, WO02/04433, WO02/51809, WO02/083134, WO02/094799,

WO03/004027, WO03/13574, WO03/15769, WO03/028641, WO03/035624, WO03/033476, WO03/033480, JP13226269, and JP1437059; mGluR5 modulators such as those disclosed in WO03/029210, WO03/047581, WO03/048137, WO03/051315, WO03/051833, WO03/053922, WO03/059904, and the like; serotonergic agents, such as fenfluramine (such as Pondimin® (Benzeneethanamine, N-ethyl- alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Robbins), dexfenfluramine (such as Redux® (Benzeneethanamine, N-ethyl-alpha-methyl-3-(trifluoromethyl)-, hydrochloride), Interneuron) and sibutramine ((Meridia®, Knoll/Reductil™) including racemic mixtures, as optically pure isomers (+) and (-), and pharmaceutically acceptable salts, solvents, hydrates, clathrates and prodrugs thereof including sibutramine hydrochloride monohydrate salts thereof, and those compounds disclosed in US4746680, US4806570, and US5436272, US20020006964, WOO 1/27068, and WOO 1/62341; NE (norepinephrine) transport inhibitors, such as GW 320659, despiramine, talsupram, and nomifensine; NPY 1 antagonists, such as BIBP3226, J-115814, BIBO 3304, LY-357897, CP-671906, GI- 264879A, and those disclosed in US6001836, WO96/14307, WO01/23387, WO99/51600, WO01/85690, WO01/85098, WO01/85173, and WO01/89528; NPY5 (neuropeptide Y Y5) antagonists, such as 152,804, GW-569180A, GW-594884A, GW- 587081X, GW-548118X, FR235208, FR226928, FR240662, FR252384, 1229U91, GI-264879A, CGP71683A, LY-377897, LY-366377, PD-160170, SR-120562A, SR-120819A, JCF-104, and H409/22 and those compounds disclosed in patent publications US6140354, US6191160, US6218408, US6258837, US6313298, US6326375, US6329395, US6335345, US6337332, US6329395, US6340683, EP01010691, EP-01044970, WO97/19682, WO97/20820, WO97/20821, WO97/20822, WO97/20823, WO98/27063, WO00/107409, WO00/185714, WO00/185730, WO00/64880, WO00/68197, WO00/69849, WO/0113917, WO01/09120, WO01/14376, WO01/85714, WO01/85730, WO01/07409, WO01/02379, WO01/23388, WO01/23389, WOO 1/44201, WO01/62737, WO01/62738, WO01/09120, WO02/20488, WO02/22592, WO02/48152, WO02/49648, WO02/051806, WO02/094789, WO03/009845, WO03/014083, WO03/022849, WO03/028726 and Norman et al, J. Med. Chem. 43:4288-4312 (2000); opioid antagonists, such as nalmefene (REVEX ®), 3-methoxynaltrexone, methylnaltrexone, naloxone, and naltrexone (e.g. PT901; Pain Therapeutics, Inc.) and those disclosed in US20050004155 and WO00/21509; orexin antagonists, such as SB-

334867-A and those disclosed in patent publications WO01/96302, WO01/68609, WO02/44172, WO02/51232, WO02/51838, WO02/089800, WO02/090355, WO03/023561, WO03/032991, and WO03/037847; PDE inhibitors (e.g. compounds which slow the degradation of cyclic AMP (cAMP) and/or cyclic GMP (cGMP) by inhibition of the phosphodiesterases, which can lead to a relative increase in the intracellular concentration of cAMP and cGMP; possible PDE inhibitors are primarily those substances which are to be numbered among the class consisting of the PDE3 inhibitors, the class consisting of the PDE4 inhibitors and/or the class consisting of the PDE5 inhibitors, in particular those substances which can be designated as mixed types of PDE3/4 inhibitors or as mixed types of PDE3/4/5 inhibitors) such as those disclosed in patent publications DE1470341, DE2108438, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EPO1 12987, EPO1 16948, EP0150937, EP0158380, EP0161632, EP0161918, EP0167121, EP0199127, EP0220044, EP0247725, EP0258191, EP0272910, EP0272914, EP0294647, EP0300726, EP0335386, EP0357788, EP0389282, EP0406958, EP0426180, EP0428302, EP0435811, EP0470805, EP0482208, EP0490823, EP0506194, EP0511865, EP0527117, EP0626939, EP0664289, EP0671389, EP0685474, EP0685475, EP0685479, JP92234389, JP94329652, JP95010875, US4963561, US5141931, WO9117991, WO9200968, WO9212961, WO9307146, WO9315044, WO9315045, WO9318024, WO9319068, WO9319720, WO9319747, WO9319749, WO9319751, WO9325517, WO9402465, WO9406423, WO9412461, WO9420455, WO9422852, WO9425437, WO9427947, WO9500516, WO9501980, WO9503794, WO9504045, WO9504046, WO9505386, WO9508534, WO9509623, WO9509624, WO9509627, WO9509836, WO9514667, WO9514680, WO9514681, WO9517392, WO9517399, WO9519362, WO9522520, WO9524381, WO9527692, WO9528926, WO9535281, WO9535282, WO9600218, WO9601825, WO9602541, WO9611917, DE3142982, DE1 116676, DE2162096, EP0293063, EP0463756, EP0482208, EP0579496, EP0667345 US6331543, US20050004222 (including those disclosed in formulas I- XIII and paragraphs 37-39, 85-0545 and 557-577), WO9307124, EP0163965, EP0393500, EP0510562, EP0553174, WO9501338 and WO9603399, as well as PDE5 inhibitors (such as RX-RA-69, SCH-

51866, KT-734, vesnarinone, zaprinast, SKF-96231, ER-21355, BF/GP-385, NM-702 and sildenafil (Viagra™), PDE4 inhibitors (such as etazolate, ICI63197, RP73401, imazolidinone (RO-20-1724), MEM 1414 (R1533/R1500; Pharmacia Roche), denbufylline, rolipram, oxagrelate, nitraquazone, Y-590, DH-6471, SKF-94120, motapizone, lixazinone, indolidan, olprinone, atizoram, KS-506-G, dipamfylline, BMY-43351, atizoram, arofylline, filaminast, PDB-093, UCB-29646, CDP-840, SKF-107806, piclamilast, RS-17597, RS-25344-000, SB-207499, TIBENELAST, SB-210667, SB-211572, SB-211600, SB-212066, SB-212179, GW-3600, CDP-840, mopidamol, anagrelide, ibudilast, amrinone, pimobendan, cilostazol, quazinone and N-(3,5-dichloropyrid-4-yl)-3-cyclopropylmethoxy-4-difluoromethoxybenzamide, PDE3 inhibitors (such as ICI153, 100, bemorandane (RWJ 22867), MCI-154, UD-CG 212, sulmazole, ampizone, cilostamide, carbazeran, piroximone, imazodan, CI-930, siguazodan, adibendan, saterinone, SKF-95654, SDZ-MKS-492, 349-U-85, emoradan, EMD-53998, EMD-57033, NSP-306, NSP-307, revizinone, NM-702, WIN-62582 and WIN-63291, enoximone and milrinone, PDE3/4 inhibitors (such as benafentrine, trequinsin, ORG-30029, zardaverine, L-686398, SDZ-ISQ-844, ORG-20241, EMD-54622, and tolafentrine) and other PDE inhibitors (such as vinpocetin, papaverine, enprofylline, cilomilast, fenoximone, pentoxifylline, roflumilast, tadalafil(Cialis®), theophylline, and vardenafil(Levitra®); Neuropeptide Y2 (NPY2) agonists include but are not limited to: polypeptide YY and fragments and variants thereof (e.g. YY3-36 (PYY3-36) (N. Engl. J. Med. 349:941, 2003; IKPEAPGE DASPEELNRY YASLRHYLNL VTRQRY (SEQ ID NO: ~~XXX~~ 250)) and PYY agonists such as those disclosed in WO02/47712, WO03/026591, WO03/057235, and WO03/027637; serotonin reuptake inhibitors, such as, paroxetine, fluoxetine (Prozac™), fluvoxamine, sertraline, citalopram, and imipramine, and those disclosed in US6162805, US6365633, WO03/00663, WOO 1/27060, and WOO 1/162341; thyroid hormone β agonists, such as KB-2611 (KaroBioBMS), and those disclosed in WO02/15845, WO97/21993, WO99/00353, GB98/284425, U.S. Provisional Application No. 60/183,223, and Japanese Patent Application No. JP 2000256190; UCP-I (uncoupling protein-1), 2, or 3 activators, such as phytanic acid, 4-[(E)-2-(5, 6,7,8- tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNPB), retinoic acid, and those disclosed in WO99/00123; β_3 (beta adrenergic receptor 3) agonists, such as AJ9677/TAK677 (Dainippon/Takeda), L750355 (Merck), CP331648 (Pfizer), CL-316,243, SB 418790, BRL-37344, L-796568, BMS-196085, BRL-

35135A, CGP12177A, BTA-243, GW 427353, Trecadrine, Zeneca D7114, N-5984 (Nisshin Kyorin), LY-377604 (Lilly), SR 59119A, and those disclosed in US5541204, US5770615, US5491134, US5776983, US488064, US5705515, US5451677, WO94/18161, WO95/29159, WO97/46556, WO98/04526 and WO98/32753, WO01/74782, WO02/32897, WO03/014113, WO03/016276, WO03/016307, WO03/024948, WO03/024953 and WO03/037881; noradrenergic agents including, but not limited to, diethylpropion (such as Tenuate® (1- propanone, 2-(diethylamino)-1 -phenyl-, hydrochloride), Merrell), dextroamphetamine (also known as dextroamphetamine sulfate, dexamphetamine, dexedrine, Dexampex, Ferndex, Oxydess II, Robese, Spancap #1), mazindol ((or 5-(p-chlorophenyl)-2,5-dihydro-3H- imidazo[2,1-a]isoindol-5-ol) such as Sanorex®, Novartis or Mazanor®, Wyeth Ayerst), phenylpropanolamine (or Benzenemethanol, alpha-(1-aminoethyl)-, hydrochloride), phentermine ((or Phenol, 3-[[4,5-duhydro-1H-imidazol-2-yl)ethyl](4-methylpheny-l)amino], monohydrochloride) such as Adipex-P®, Lemmon, FASTIN®, Smith-Kline Beecham and Ionamin®, Medeva), phendimetrazine ((or (2S,3S)-3,4-Dimethyl-2phenylmorpholine L-(+)- tartrate (1 :1)) such as Metra® (Forest) , Plegine® (Wyeth- Ay erst), Prelu-2® (Boehringer Ingelheim), and Statobex® (Lemmon), phendamine tartrate (such as Thephorin® (2,3,4,9- Tetrahydro-2-methyl-9-phenyl-1H-indenol[2,1-c]pyridine L-(+)-tartrate (1 :1)), Hoffmann- LaRoche), methamphetamine (such as Desoxyn®, Abbot ((S)-N, (alpha)-dimethylbenzeneethanamine hydrochloride)), and phendimetrazine tartrate (such as Bontril® Slow-Release Capsules, Amarin (-3,4-Dimethyl-2-phenylmorpholine Tartrate); fatty acid oxidation upregulator/inducers such as Famoxin® (Genset); monamine oxidase inhibitors including but not limited to befloxatone, moclobemide, brofaromine, phenoxathine, esuprone, befol, toloxatone, pirlindol, amiflamine, serclorephine, bazinapriline, lazabemide, milacemide, caroxazone and other certain compounds as disclosed by WO01/12176; and other anti-obesity agents such as 5HT-2 agonists, ACC (acetyl-CoA carboxylase) inhibitors such as those described in WO03/072197, alpha-lipoic acid (alpha-LA), AOD9604, appetite suppressants such as those in WO03/40107, ATL-962 (Alizyme PLC), benzocaine, benzphetamine hydrochloride (Didrex), bladderwrack (focus vesiculosus), BRS3 (bombesin receptor subtype 3) agonists, bupropion, caffeine, CCK agonists, chitosan, chromium, conjugated linoleic acid, corticotropin-releasing hormone agonists, dehydroepiandrosterone, DGAT1 (diacylglycerol acyltransferase 1) inhibitors, DGAT2

(diacylglycerol acyltransferase 2) inhibitors, dicarboxylate transporter inhibitors, ephedra, exendin-4 (an inhibitor of glp-1) FAS (fatty acid synthase) inhibitors (such as Cerulenin and C75), fat resorption inhibitors (such as those in WO03/053451, and the like), fatty acid transporter inhibitors, natural water soluble fibers (such as psyllium, plantago, guar, oat, pectin), galanin antagonists, galega (Goat's Rue, French Lilac), garcinia cambogia, germander (teucrium chamaedrys), ghrelin antibodies and ghrelin antagonists (such as those disclosed in WO01/87335, and WO02/08250), polypeptide hormones and variants thereof which affect the islet cell secretion, such as the hormones of the secretin/gastric inhibitory polypeptide (GIP)/vasoactive intestinal polypeptide (VIP)/pituitary adenylate cyclase activating polypeptide (PACAP)/glucagon-like polypeptide II (GLP- II)/glucagon gene family and/or those of the adrenomedullin/amylin/calcitonin gene related polypeptide (CGRP) gene family including GLP-1 (glucagon-like polypeptide 1) agonists (e.g. (1) exendin-4, (2) those GLP-I molecules described in US20050130891 including GLP- 1(7-34), GLP-1(7-35), GLP-1(7-36) or GLP-1(7-37) in its C-terminally carboxylated or amidated form or as modified GLP-I polypeptides and modifications thereof including those described in paragraphs 17-44 of US20050130891, and derivatives derived from GLP-1-(7- 34)COOH and the corresponding acid amide are employed which have the following general formula: R-NH-HAEGTFTSDVSYLEGQAAKEFIAWLVK-CONH₂ (SEQ ID NO: 251) wherein R=H or an organic compound having from 1 to 10 carbon atoms. Preferably, R is the residue of a carboxylic acid. Particularly preferred are the following carboxylic acid residues: formyl, acetyl, propionyl, isopropionyl, methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, tert-butyl.) and glp-1 (glucagon-like polypeptide- 1), glucocorticoid antagonists, glucose transporter inhibitors, growth hormone secretagogues (such as those disclosed and specifically described in US5536716), interleukin-6 (IL-6) and modulators thereof (as in WO03/057237, and the like), L- carnitine, Mc3r (melanocortin 3 receptor) agonists, MCH2R (melanin concentrating hormone 2R) agonist/antagonists, melanin concentrating hormone antagonists, melanocortin agonists (such as Melanotan II or those described in WO 99/64002 and WO 00/74679), nomame herba, phosphate transporter inhibitors, phytopharm compound 57 (CP 644,673), pyruvate, SCD-I (stearoyl-CoA desaturase-1) inhibitors, T71 (Tularik, Inc., Boulder CO), Topiramate (Topimax®, indicated as an anti-convulsant which has been shown to increase weight loss), transcription factor modulators (such as those disclosed in WO03/026576),

β -hydroxy steroid dehydrogenase- 1 inhibitors (β -HSD-I), β -hydroxy- β -methylbutyrate, p57 (Pfizer), Zonisamide (Zonegran™, indicated as an anti-epileptic which has been shown to lead to weight loss), and the agents disclosed in US20030119428 paragraphs 20-26.

Please amend paragraph [165], starting on page 84, line 13 as follows:

[165] In one embodiment, the analgesic agent is an analgesic polypeptide selected from the group consisting of sialorphin-related polypeptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO: 239252), including: VQHNPR (SEQ ID NO: 240253); VRQHNPR (SEQ ID NO: 241254); VRGQHNPR (SEQ ID NO: 242255); VRGPQHNPR (SEQ ID NO: 243256); VRGPRQHNPR (SEQ ID NO: 244257); VRGPRRQHNPR (SEQ ID NO: 245258); and RQHNPR (SEQ ID NO: 246259). Sialorphin-related polypeptides bind to neprilysin and inhibit neprilysin-mediated breakdown of substance P and Met-enkephalin. Thus, compounds or polypeptides that are inhibitors of neprilysin are useful analgesic agents which can be administered with the GCC agonists described herein or covalently linked to a GCC agonist to form a therapeutic conjugate. Sialorphin and related polypeptides are described in U.S. Patent 6,589,750; U.S. 20030078200 A1; and WO 02/051435 A2.

Please amend paragraph [166], starting on page 84, line 24 as follows:

[166] In another embodiment, a GCC agonist formulation of the invention is administered as part of a regimen of combination therapy with an opioid receptor antagonist or agonist. In one embodiment, the GCC agonist and the opioid receptor antagonist or agonist are linked via a covalent bond. Non-limiting examples of opioid receptor antagonists include naloxone, naltrexone, methyl naloxone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, nor-binaltorphimine, enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-homoserine (SEQ ID NO; 260)), trimebutine, vasoactive intestinal polypeptide, gastrin, glucagons. Non-limiting examples of opioid receptor agonists include fedotozine, asimadoline, and ketocyclazocine, the compounds described in WO03/097051 and WO05/007626, morphine, diphenyloxylate,

frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH₂ (SEQ ID NO; 261); WO 01/019849 A1), and
loperamide.

REMARKS

Applicants have amended the specification to incorporate the contents of the text file containing the sequence listing for this application in accordance with the provisions of 37 C.F.R. §§ 1.52 and 1.77. The sequence listing in the text file does not include any new matter that goes beyond the disclosure of the application as filed. Applicants have also corrected some typographical errors in the specification. No new matter has been added.

If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Dated: June 4, 2012

Respectfully submitted,

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Supplemental Application Data Sheet

Application Information

Application number:: 13/421,769
Filing Date:: 03/15/12
Application Type:: Regular
Subject Matter:: Utility
Suggested Group Art Unit:: 1629
CD-ROM or CD-R?:: None
Sequence submission?:: ~~None~~ TXT
Computer Readable Form (CRF)?:: ~~No~~ Yes
Title:: Formulations of Guanylate Cyclase C Agonists
and Methods of Use
Attorney Docket Number:: 40737-509001US
Request for Early Publication?:: No
Request for Non-Publication?:: No
Total Drawing Sheets:: 6
Small Entity?:: ~~No~~ Yes
Petition included?:: No
Secrecy Order in Parent Appl.?:: No

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Correspondence Information

Correspondence Customer Number:: 30623

Representative Information

Representative Customer Number:: 30623

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::
This Application	Continuation-in-part of	PCT/US2011/051805	09/15/11
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/383156	09/15/10
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/387636	09/29/10
PCT/US2011/051805	An application claiming the benefit under 35 USC 119(e)	61/392186	10/12/10

Foreign Priority Information

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Signature:

A signature of the applicant or representative is required in accordance with 37 CFR 1.33 and 10.18. Please see 37 CFR 1.4(d) for the form of the signature.			
Signature	/Cynthia Kozakiewicz/	Date	June 4, 2012
Name (Print/Type)	Cynthia Kozakiewicz, J.D., Ph.D.	Registration No. (Attorney/Agent)	42,764

Electronic Patent Application Fee Transmittal

Application Number:	13421769
Filing Date:	15-Mar-2012
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Filer:	Cynthia A. Kozakiewicz/Donna Doyle
Attorney Docket Number:	40737-509001US

Filed as Small Entity

Utility under 35 USC 111(a) Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late filing fee for oath or declaration	2051	1	65	65

Petition:

Patent-Appeals-and-Interference:

Post-Allowance-and-Post-Issuance:

Extension-of-Time:

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				65

Electronic Acknowledgement Receipt

EFS ID:	12931002
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	30623
Filer:	Cynthia A. Kozakiewicz/Donna Doyle
Filer Authorized By:	Cynthia A. Kozakiewicz
Attorney Docket Number:	40737-509001US
Receipt Date:	04-JUN-2012
Filing Date:	15-MAR-2012
Time Stamp:	18:57:39
Application Type:	Utility under 35 USC 111(a)

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Payment Type	Credit Card
Payment was successfully received in RAM	\$65
RAM confirmation Number	6283
Deposit Account	
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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1	Oath or Declaration filed	40737-509001US_-_Declaration_1.pdf	202483 bce9ee536b843f05c4361a3d0c63456581414c4c	no	2
Warnings:					
Information:					
2	Miscellaneous Incoming Letter	40737-509001US_-_Transmittal_4.pdf	28078 62f5c92511fa55b1a9e8b795debc068c280f8091	no	1
Warnings:					
Information:					
3	CRF Statement Paper and CRF are the same	40737-509001US_-_Statement_5.pdf	22875 0f6967e8be0e5c98d4d4f54787d674222b732a1db	no	1
Warnings:					
Information:					
4	Applicant Response to Pre-Exam Formalities Notice	40737-509001US_-_Response_6.pdf	20078 858434d44dcb5c3352fb6af2bbc103ec9594fd	no	1
Warnings:					
Information:					
5		PreliminaryAmendment.pdf	80898 182094d7ab0e43f7b31c3749b71e00540df64d2f	yes	12
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Preliminary Amendment		1	1	
	Specification		2	11	
Applicant Arguments/Remarks Made in an Amendment		12	12		
Warnings:					
Information:					
6	Sequence Listing (Text File)	Sequence.TXT	115674	no	0
Warnings:					
Information:					
7	Application Data Sheet	ADS.pdf	22216 fc073d6ef1a235228bf748f2d46d0de7b0e73739	no	5
Warnings:					
Information:					
This is not an USPTO supplied ADS fillable form					

8	Fee Worksheet (SB06)	fee-info.pdf	29876 b74f4b1d9838e51d988b722fecf84ed09ee2cb46	no	2
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Warnings:

Information:

Total Files Size (in bytes):	522178
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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.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

DECLARATION (37 CFR 1.63) FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET (37 CFR 1.76)

Title of Invention	Formulations of Guanylate Cyclase C Agonists and Methods of Use
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As the below named inventor(s), I/we declare that:

This declaration is directed to:

The attached application, or

United States application or PCT international application number 13/421,769
filed on 03/15/2012

As amended on _____ (if applicable);

I/we believe that I/we am/are the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought;

I/we have reviewed and understand the contents of the above-identified application, including the claims, as amended by any amendment specifically referred to above;

I/we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me/us to be material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT International filing date of the continuation-in-part application.

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

All statements made herein of my/our own knowledge are true, all statements made herein 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 are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF INVENTOR(S)	
Inventor one: <u>Stephen Comiskey</u>	Date: <u>04/16/2012</u>
Signature: <u><i>Stephen Comiskey</i></u>	Citizen of: <u>US</u>
Inventor two: <u>Rong Feng</u>	Date: <u>04/16/2012</u>
Signature: <u><i>Rong Feng</i></u>	Citizen of: <u>Canada</u>
<input checked="" type="checkbox"/> Additional inventors or a legal representative are being named on <u>1</u> additional form(s) attached hereto.	

DECLARATION FOR UTILITY OR DESIGN APPLICATION USING AN APPLICATION DATA SHEET		ADDITIONAL INVENTOR(S) Supplemental Sheet Page 1 of 1	
Inventor three:	<u>John Foss</u>	Date:	<u>04/16/2012</u>
Signature:	<u><i>John Foss</i></u>	Citizen of:	<u>US</u>
Inventor four:	<u>Kunwar Shailubhai</u>	Date:	<u>04/16/2012</u>
Signature:	<u><i>Kunwar Shailubhai</i></u>	Citizen of:	<u>US</u>

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<h1>TRANSMITTAL FORM</h1> <p><i>(to be used for all correspondence after initial filing)</i></p>	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Art Unit	1629
	Examiner Name	Not Yet Assigned
Total Number of Pages in This Submission	Attorney Docket Number	40737-509001US

ENCLOSURES (Check all that apply)

<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input checked="" 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 type="checkbox"/> Information Disclosure Statement <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 Utility Application; Supplemental Application Data Sheet; CRF of Sequence Listing (TXT.) and Statement in Support of CRF
<input type="text"/> Remarks		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	MINTZ LEVIN COHN FERRIS GLOVSKY AND POPEO, P.C.		
Signature	/Cynthia Kozakiewicz/		
Printed name	Cynthia Kozakiewicz		
Date	June 4, 2012	Reg. No.	42,764

SCORE Placeholder Sheet for IFW Content

Application Number: 13421769

Document Date: 06/04/2012

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

- **Sequence Listing**

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 13/421,769	Filing Date 03/15/2012	<input type="checkbox"/> To be Mailed
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APPLICATION AS FILED – PART I			OTHER THAN SMALL ENTITY				
	(Column 1)	(Column 2)	SMALL ENTITY <input checked="" type="checkbox"/>	OR			
FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)	OR	RATE (\$)	FEE (\$)
<input type="checkbox"/> BASIC FEE <small>(37 CFR 1.16(a), (b), or (c))</small>	N/A	N/A	N/A			N/A	
<input type="checkbox"/> SEARCH FEE <small>(37 CFR 1.16(k), (j), or (m))</small>	N/A	N/A	N/A			N/A	
<input type="checkbox"/> EXAMINATION FEE <small>(37 CFR 1.16(o), (p), or (q))</small>	N/A	N/A	N/A			N/A	
TOTAL CLAIMS <small>(37 CFR 1.16(j))</small>	minus 20 =	*	X \$ =		OR	X \$ =	
INDEPENDENT CLAIMS <small>(37 CFR 1.16(h))</small>	minus 3 =	*	X \$ =			X \$ =	
<input type="checkbox"/> APPLICATION SIZE FEE <small>(37 CFR 1.16(s))</small>	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						
<input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT <small>(37 CFR 1.16(j))</small>							
			TOTAL			TOTAL	

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

APPLICATION AS AMENDED – PART II					OTHER THAN SMALL ENTITY				
	(Column 1)	(Column 2)	(Column 3)						
AMENDMENT	06/04/2012	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)
	Total <small>(37 CFR 1.16(i))</small>	* 42	Minus ** 42	= 0	X \$30 =	0	OR	X \$ =	
	Independent <small>(37 CFR 1.16(h))</small>	* 3	Minus *** 3	= 0	X \$125 =	0	OR	X \$ =	
	<input type="checkbox"/> Application Size Fee <small>(37 CFR 1.16(s))</small>								
	<input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <small>(37 CFR 1.16(j))</small>						OR		
					TOTAL ADD'L FEE	0	OR	TOTAL ADD'L FEE	

	(Column 1)	(Column 2)	(Column 3)						
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)
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					TOTAL ADD'L FEE		OR	TOTAL ADD'L FEE	

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

Legal Instrument Examiner:
 /ANDREW j1339 JAMES JR/

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

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

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Sequence Listing was accepted.

See attached Validation Report.

If you need help call the Patent Electronic Business Center at (866) 217-9197 (toll free).

Reviewer: Durreshwar Anjum

Timestamp: [year=2012; month=6; day=12; hr=14; min=50; sec=44; ms=507;]

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Application No: 13421769 Version No: 1.0

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Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu
1 5 10 15

<210> 25
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<220>
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<223> wherein ASN is a D-amino acid

<220>
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<222> (2)..(2)
<223> wherein ASP is a D-amino acid

<220>
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<223> wherein LEU at position 16 is attached to polyethylene glycol

<220>
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<223> wherein LEU is a D-amino acid

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<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> wherein ASN is a D-amino acid

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<222> (2)..(2)

<223> wherein ASP is a D-amino acid

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<220>
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<223> wherein the x is a 2-aminoisobutyric acid, Aib

<220>
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<222> (10)..(10)
<223> wherein the x is a 2-aminoisobutyric acid, Aib

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<400> 28

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<220>
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<220>

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<223> wherein x at position 15 is ornithine

<220>
<221> MOD_RES
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<223> wherein x is an ornithine, Orn

<220>
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<223> wherein LEU is a D-amino acid

<400> 29

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<220>

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<223> wherein LEU is a D-amino acid

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<223> wherein ASN is a D-amino acid

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<222> (16)..(16)

<223> wherein LEU is a D-amino acid

<220>

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<223> wherein LEU at position 16 is attached to polyethylene glycol

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Asn Asp Glu Cys Glu Tyr Cys Val Asn Val Ala Cys Thr Gly Cys Leu

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<220>

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<222> (1)..(1)

<223> wherein ASN is a D-amino acid

<220>

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<223> wherein LEU is a D-amino acid

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<212> PRT

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<223> wher

VIA EFS
Date of Deposit: June 4, 2012

Attorney Docket No.: 40737-509001US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Stephen Comiskey et al.

Application No.: 13/421,769

Confirmation No.: 3135

Filed: March 15, 2012

Art Unit: 1629

For: Formulations Of Guanylate Cyclase C Agonists
And Methods Of Use

Examiner: Not Yet Assigned

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

RESPONSE TO NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

In response to the Notice to File Missing Parts of Nonprovisional Application mailed April 3, 2012, Applicants submit herein an Executed Declaration for Utility Application, a Supplemental Application Data Sheet, a Computer Readable Form of Sequence Listing (TXT. File), a Statement in Support of Computer Readable Form and electronic payment of the surcharge of \$65.00 as set forth in 37 C.F.R. 1.16(f). Applicants are entitled to small entity status.

Although Applicants believe no additional fees are due with this submission, the Commissioner is hereby authorized to charge payment of any additional fees required in connection with the papers transmitted herewith, or credit any overpayment of same, to Deposit Account No. 50-0311, (Reference No. 40737-509001US).

Dated: June 4, 2012

Respectfully submitted,

06/13/2012 HTEKLEMI 00000019 500311 13421769

01 FC:4011	95.00 DA
02 FC:2111	310.00 DA
03 FC:2311	125.00 DA
04 FC:2081	155.00 DA
05 FC:2202	660.00 DA

/Cynthia Kozakiewicz/
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Registration No.: 42,764
Lian Ouyang, Ph.D.
Registration No.: 69,254
MINTZ LEVIN COHN FERRIS GLOVSKY AND
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One Financial Center
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(617) 542-6000
Attorneys for Applicant

PATENT APPLICATION FEE DETERMINATION RECORD

Substitute for Form PTO-875

Application or Docket Number
13/421,769

APPLICATION AS FILED - PART I

(Column 1) (Column 2)

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A
SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A
TOTAL CLAIMS (37 CFR 1.16(j))	42 minus 20 = *	22
INDEPENDENT CLAIMS (37 CFR 1.16(h))	3 minus 3 = *	
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).	
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))		

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

SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	95
N/A	310
N/A	125
x 30 =	660
x 125 =	0.00
	155
	0.00
TOTAL	1345

OR OTHER THAN SMALL ENTITY

RATE(\$)	FEE(\$)
N/A	
N/A	
N/A	
TOTAL	

APPLICATION AS AMENDED - PART II

(Column 1) (Column 2) (Column 3)

AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	*	Minus	**	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=
	Application Size Fee (37 CFR 1.16(s))				
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

(Column 1) (Column 2) (Column 3)

AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total (37 CFR 1.16(i))	*	Minus	**	=
	Independent (37 CFR 1.16(h))	*	Minus	***	=
	Application Size Fee (37 CFR 1.16(s))				
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))					

SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

OR OTHER THAN SMALL ENTITY

RATE(\$)	ADDITIONAL FEE(\$)
x =	
x =	
TOTAL ADD'L FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.



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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: 13/421,769, 03/15/2012, 1629, 1410, 40737-509001US, 42, 3

CONFIRMATION NO. 3135

UPDATED FILING RECEIPT

30623
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C
ONE FINANCIAL CENTER
BOSTON, MA 02111



Date Mailed: 06/14/2012

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

Applicant(s)

Stephen Comiskey, Doylestown, PA;
Rong Feng, Langhorne, PA;
John Foss, Doylestown, PA;
Kunwar Shailubhai, Audubon, PA;

Assignment For Published Patent Application

Synergy Pharmaceuticals Inc., New York, NY

Power of Attorney: The patent practitioners associated with Customer Number 30623

Domestic Priority data as claimed by applicant

This application is a CIP of PCT/US2011/051805 09/15/2011
which claims benefit of 61/383,156 09/15/2010
and claims benefit of 61/387,636 09/29/2010
and claims benefit of 61/392,186 10/12/2010

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

If Required, Foreign Filing License Granted: 04/02/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 13/421,769

Projected Publication Date: 09/20/2012

Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **

Title

Formulations of Guanylate Cyclase C Agonists and Methods of Use

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.

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

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

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

LICENSE FOR FOREIGN FILING UNDER**Title 35, United States Code, Section 184****Title 37, Code of Federal Regulations, 5.11 & 5.15****GRANTED**

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

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

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

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

NOT GRANTED

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

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/421,769	03/15/2012	Stephen Comiskey	40737-509001US

CONFIRMATION NO. 3135

POA ACCEPTANCE LETTER

30623
MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C
ONE FINANCIAL CENTER
BOSTON, MA 02111



Date Mailed: 06/14/2012

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 05/23/2012.

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

/tha/

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



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Table with 4 columns: APPLICATION NUMBER (13/421,769), FILING OR 371(C) DATE (03/15/2012), FIRST NAMED APPLICANT (Stephen Comiskey), ATTY. DOCKET NO./TITLE (40737-509001US)

CONFIRMATION NO. 3135

PUBLICATION NOTICE



30623
Mintz Levin/Boston Office
One Financial Center
Boston, MA 02111

Title:Formulations of Guanylate Cyclase C Agonists and Methods of Use

Publication No.US-2012-0237593-A1
Publication Date:09/20/2012

NOTICE OF PUBLICATION OF APPLICATION

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

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

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

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

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

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



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
13/421,769	03/15/2012	Stephen Comiskey	40737-509001US	3135
30623	7590	06/02/2014	EXAMINER	
Mintz Levin/Boston Office One Financial Center Boston, MA 02111			LEE, JIA-HAI	
			ART UNIT	PAPER NUMBER
			1676	
			NOTIFICATION DATE	DELIVERY MODE
			06/02/2014	ELECTRONIC

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

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

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

IPDocketingBOS@mintz.com
IPFileroombos@mintz.com

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-25, 35, and 42 are drawn to an oral dosage formulation comprising at least one GCC agonist peptide and one or more pharmaceutically acceptable excipients, classified in 424/780, 424/451, 424/463, and 514/13.2.

II. Claims 26-34 are drawn to a process of making an oral dosage formulation of claim 1, classified in 424/400, 514/13.2, 514/21.4-21.5, and 514/530.

III. Claims 36-41 are drawn to a method for treating or preventing a disease or disorder in a subject in need by administering the oral dosage formulation of claim 2, classified in 424/93.4 and 514/4.9.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make another and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case, the process of making an oral dosage formulation of GCC agonist peptide can be used to make insulin, which is a materially different product. In addition, a GCC agonist peptide can be formulated in a composition

Art Unit: 1676

for systemic administration or inhalation, which is made by a materially different process. Thus, invention I and II are distinct.

Inventions I and III are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product. See MPEP § 806.05(h). In the instant case, the process for using a GCC agonist peptide can be practiced with a small molecule compound, which is a materially different product. In addition, the process of using oral administration of a GCC agonist peptide can be administered by inhalation, which is a materially different process of using that product. Thus, invention I and III are distinct.

Inventions II and III are directed to related methods. The related inventions are distinct if: (1) the inventions as claimed are either not capable of use together or can have a materially different design, mode of operation, function, or effect; (2) the inventions do not overlap in scope, i.e., are mutually exclusive; and (3) the inventions as claimed are not obvious variants. See MPEP § 806.05(j). In the instant case, the invention II (a process of making an oral dosage formulation of a GCC agonist peptide) and III (a method for treating a disease or disorder in a subject in need by administering the oral dosage formulation of a GCC agonist peptide) as claimed have a materially different design, mode of operation, function, or effect. Furthermore, the inventions as claimed do not encompass overlapping subject matter and there is nothing of record to show them to be obvious variants. Thus, inventions I-III are distinct from one another.

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Restriction for examination purposes as indicated is proper because all these inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

Different invention groups require different fields of prior art search in a separate class and subclass for GCC agonist peptides, the components in the oral dosage formulation comprising one of more GCC agonist peptides as well as the CPC database, resulting in a serious search and examination burden.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) and (ii) identification of the claims encompassing the elected invention.

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

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record

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showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

This application contains claims directed to the following patentably distinct species of GCC agonist peptide sequences. The species are independent or distinct because each peptide sequence has its unique amino acid sequence and affinity for binding to its target.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claims 1-2, 6, 16-17, 26, 32, and 38 are generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply: Difference polypeptide sequence SEQ ID NOs: 1-54 and 56-249 as claimed, and GCC agonist peptide formulations are a search and examination burden without this species election requirement.

Applicant is required to make species election as follows:

1) If any of the group I-III is elected, Applicant is further required to elect a GCC agonist peptide SEQ ID NO.

2) If group I is elected, Applicant is further required to elect the formulation without a divalent cation salt (claim 16) or to elect the formulation comprising a divalent

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cation salt (claim 17) in addition to the SEQ ID NO of a GCC agonist peptide.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

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

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

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

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

The examiner has required restriction between product or apparatus claims and process claims. Where applicant elects claims directed to the product/apparatus, and all product/apparatus claims are subsequently found allowable, withdrawn process claims that include all the limitations of the allowable product/apparatus claims should be considered for rejoinder. All claims directed to a nonelected process invention must include all the limitations of an allowable product/apparatus claim for that process invention to be rejoined.

In the event of rejoinder, the requirement for restriction between the product/apparatus claims and the rejoined process claims will be withdrawn, and the rejoined process claims will be fully examined for patentability in accordance with 37 CFR 1.104. Thus, to be allowable, the rejoined claims must meet all criteria for patentability including the requirements of 35 U.S.C. 101, 102, 103 and 112. Until all

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claims to the elected product/apparatus are found allowable, an otherwise proper restriction requirement between product/apparatus claims and process claims may be maintained. Withdrawn process claims that are not commensurate in scope with an allowable product/apparatus claim will not be rejoined. See MPEP § 821.04.

Additionally, in order for rejoinder to occur, applicant is advised that the process claims should be amended during prosecution to require the limitations of the product/apparatus claims. **Failure to do so may result in no rejoinder.** Further, note that the prohibition against double patenting rejections of 35 U.S.C. 121 does not apply where the restriction requirement is withdrawn by the examiner before the patent issues. See MPEP § 804.01.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JIA-HAI LEE whose telephone number is (571)270-1691. The examiner can normally be reached on Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Karlheinz R. Skowronek can be reached on 571-272-9047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should

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you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/J. L./
Examiner, Art Unit 1676

/KARLHEINZ R SKOWRONEK/
Supervisory Patent Examiner, Art
Unit 1676

26-May-2014

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: COMISKEY, Stephen Confirmation No.: 3135
Application No.: 13/421,769 Group Art Unit: 1676
Filed: March 15, 2012 Examiner: LEE, Jia-Hai
FOR: **FORMULATIONS OF GUANYLATE CYCLASE C AGONISTS AND METHODS OF USE**

Mail Stop Amendment

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

RESPONSE TO RESTRICTION AND ELECTION OF SPECIES REQUIRMENTS

In response to the Restriction Requirement mailed June 2nd, 2014, please enter the following remarks. Because August 2nd falls on a Saturday, this response is timely filed on August 4th, 2014, according to the next business day rule.

Remarks begin on page 2.

REMARKS

Claims 1-42 are pending.

RESTRICTION REQUIREMENT

In the Restriction Requirement, the Examiner required Applicants elect one of Groups I – III. Applicants herein elect the invention of Group I, drawn to an oral dosage formulation comprising at least one GCC agonist peptide and one or more pharmaceutically acceptable excipients. Claims 1-25, 35, and 42 encompass this elected invention.

The Examiner has also required two species elections if Group I is elected: (1) a GCC agonist peptide, and (2) a formulation without a divalent cation salt (claim 16) or a formulation comprising a divalent cation salt (claim 17). Applicants herein elect the species of GCC agonist peptide of SEQ ID NO: 1, and a formulation without a divalent cation salt. Claims 1-16, 18-25, 35, and 42 encompass these elected species.

Applicants reserve the right to request rejoinder of claims to non-elected subject matter, upon the allowance of a claim directed to the elected invention. Applicants further reserve the right to file one or more divisional applications directed to the non-elected subject matter in this application.

CONCLUSION

In view of the foregoing, Applicants respectfully submit that no further impediments exist to the examination of this application. The Examiner is requested to call the undersigned if any questions or comments arise.

The Director is hereby authorized to charge any appropriate fees under 37 C.F.R. §§1.16, 1.17, and 1.21 that may be required by this paper, and to credit any overpayment, to Deposit Account No. 50-1283.

Dated: August 4th, 2014

Respectfully submitted,
COOLEY LLP

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Electronic Acknowledgement Receipt

EFS ID:	19764888
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	30623
Filer:	Anne Elizabeth Fleckenstein
Filer Authorized By:	
Attorney Docket Number:	40737-509001US
Receipt Date:	04-AUG-2014
Filing Date:	15-MAR-2012
Time Stamp:	16:56:41
Application Type:	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	Response to Election / Restriction Filed	SYPA_009_X001US_Response.pdf	80181 <small>da77744d449406c7a7211852d40d3702c34f2d82</small>	no	3

Warnings:

Information:

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

New Applications Under 35 U.S.C. 111

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

National Stage of an International Application under 35 U.S.C. 371

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

New International Application Filed with the USPTO as a Receiving Office

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



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Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Includes details for application 13/421,769, inventor Stephen Comiskey, and examiner LEE, JIA-HAI.

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

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

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

IPDocketingBOS@mintz.com
IPFileroombos@mintz.com

DETAILED ACTION

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Applicant's election of group I, claims 1-25, 35, and 42 in the reply filed on 08/04/2014 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

In response to the species election, Applicant elect the species of SEQ ID NO: 1 as a guanylate cyclase C (GCC) agonist peptide and a formulation without a divalent cation salt, Reading on claims 2-11, 14-16, 20-25, and 42. To correct an administrative error in the prior restriction requirement, claim 35 is withdrawn as depending from the nonelected invention of claim 26 in group II.

Claims 1, 12-13, 17-19, and 26-41 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention and species, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 08/04/2014.

Claim Status

Claims 1-42 are pending.

Claims 1, 12-13, 17-19, and 26-41 were withdrawn as being directed to a non-elected invention and species, the election having been made on 08/04/2014.

Claims 2-11, 14-16, 20-25, and 42 have been examined.

Priority

This application is a CIP of PCT/US2011/051805 filed on 09/15/2011, which claims benefit of 61/383,156 filed on 09/15/2010, claims benefit of 61/387,636 filed on 09/29/2010, and claims benefit of 61/392,186 filed on 10/12/2010.

Claim Objections

Claims 2-4, 6-7, 14, 16-17, and 20 are objected to because of the following informalities: Abbreviation of GCC. The objection may be overcome by replacing the first GCC used in the claim set (e.g., claim 1) with its full name guanylate cyclase C.

Claim Rejections - 35 USC § 112

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 3-4, 7, and 25 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor, or for pre-AIA the applicant regards as the invention.

A broad range or limitation together with a narrow range or limitation that falls within the broad range or limitation (in the same claim) is considered indefinite, since the resulting claim does not clearly set forth the metes and bounds of the patent

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protection desired. See MPEP § 2173.05(c). Note the explanation given by the Board of Patent Appeals and Interferences in *Ex parte Wu*, 10 USPQ2d 2031, 2033 (Bd. Pat. App. & Inter. 1989), as to where broad language is followed by "such as" and then narrow language. The Board stated that this can render a claim indefinite by raising a question or doubt as to whether the feature introduced by such language is (a) merely exemplary of the remainder of the claim, and therefore not required, or (b) a required feature of the claims. Note also, for example, the decisions of *Ex parte Steigewald*, 131 USPQ 74 (Bd. App. 1961); *Ex parte Hall*, 83 USPQ 38 (Bd. App. 1948); and *Ex parte Hasche*, 86 USPQ 481 (Bd. App. 1949). In the present instance,

Claim 3 recites the broad recitation no less than 92%, and the claim also recites no less than 95% which is the narrower statement of the range/limitation. The rejection may be overcome to amend the claim to be no less than 92%.

Claim 4 recites the broad recitation no greater than 9%, and the claim also recites no greater than 5% which is the narrower statement of the range/limitation. The rejection may be overcome to amend the claim to be no greater than 9%.

Claim 7 recites the broad recitation per unit dose 9.5 mg, and the claim also recites per unit dose 0.1 mg which is the narrower statement of the range/limitation. The rejection may be overcome to amend the claim to be per unit dose 0.1-9.5 mg.

Claim 20 recites the limitation "temperature, relative humidity, and a period of 18 months" in claim 2. Claim 2 does not refer to any of the recited terminology.

Claim 25 recites the limitation "the liquid" in claim 2. There is insufficient

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antecedent basis for this limitation in the claim. Claim 2 does not refer to liquid.

Appropriate correction is required.

The following is a quotation of 35 U.S.C. 112(d):

(d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), fourth paragraph:

Subject to the [fifth paragraph of 35 U.S.C. 112 (pre-AIA)], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

Claim 20 is rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Claim 20 is drawn to an oral dosage formulation comprising at least one GCC agonist peptide and excipient having the same scope of claim 2. Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

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(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 2-11, 14-16, 20-22, 25, and 42 are rejected under pre-AIA 35 U.S.C.

102(b) as anticipated by Shailubhai et al. (WO 02/078683 A1) or, in the alternative, under pre-AIA 35 U.S.C. 103(a) as obvious over Shailubhai et al. (WO 02/078683 A1) in view of Fretzen et al. (WO 2010/027404 A2).

Claim 2 is drawn to an oral dosage formulation comprising a GCC agonist peptide of SEQ ID NO: 1 in a unit dose and pharmaceutically acceptable excipients. Claim 3 is drawn to the GCC agonist peptide has a chromatography purity no less than 92%. Claim 4 is drawn to the GCC agonist peptide has a total impurity content of no greater than 9%. Claim 5 is drawn to the oral dosage formulation is substantially free of inorganic acids and carboxylic acids. Claim 6 is drawn to the GCC agonist peptide is SEQ ID NO: 1. Claim 7 is drawn to the amount of GCC agonist peptide per unit dose is 1 mg. Claim 8 is drawn to the peptide composition is a solid formulation and the unit dose is a tablet or capsule. Claims 9-10 are drawn to the pharmaceutically acceptable excipients comprise an inert carrier of cellulose. Claim 11 is drawn to the inert carrier has a particle size of 50 to 900 microns. Claims 14-15 are drawn to the excipient comprises an amino acid of leucine and the molar ratio of leucine to GCC agonist peptide is from 5:1 to 50:1. Claim 16 is drawn to the formulation consists of the GCC agonist peptide, an inert carrier of cellulose, and a lubricant of leucine. Claim 20 is drawn to the formulated peptide is stabilized against degradation after 18-24 months of storage at 25°C and 60% relative humidity. Claim 21 is drawn to the formulation is in the

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form of a tablet or a capsule. Claim 22 is drawn to the tablet is in a blister pack. Claim 25 is drawn to the liquid in the formulation is a vegetable oil. Claim 42 is drawn to a pharmaceutical composition comprising the oral dosage formula of claim 2.

Shailubhai et al. teach a pharmaceutical composition comprising a guanylate cyclase C (GCC) agonist peptide having the sequence of Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu with 100% homology to SEQ ID NO: 1 of this instant application (p6, lin 32) and formulated with pharmaceutically acceptable excipients for oral administration (p17, lin 45-49). Shailubhai et al. show the unit dosage of the GCC agonist peptide (p27, claim 22) is between 100 µg - 3 g (p4, lin 20-24) or 1 µg -10 mg (p7, lin 14) and the purity of the GCC agonist peptide is >95% (p21, lin 6).

With respect to claim 3, Shailubhai et al. show the purity of the GCC agonist peptide is >95% (p21, lin 6) in compliance with cGMP level (p21, Table 4).

With respect to claim 4, Shailubhai et al. show the impurity of the GCC agonist peptide is < 5%, calculated as impurity < 100%-95% (p21, lin 6) in compliance with cGMP level (p21, Table 4).

With respect to claim 6, Shailubhai et al. show the GCC agonist peptide having the sequence of Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu with 100% homology to SEQ ID NO: 1 of this instant application (p6, lin 32).

With respect to claim 7, Shailubhai et al. show the GCC agonist peptide has the dosage of once-a-day unit dose between 10 µg - 2 mg (p20, lin 1-8).

With respect to claim 8, Shailubhai et al. show the solid formulation of GCC agonist peptide in a unit dose is powders, tablets, and capsules (p17, lin 44-49).

With respect to claims 9-10, Shailubhai et al. show the pharmaceutically acceptable excipients comprise an inert carrier of cellulose (p18, lin 11-19).

With respect to claim 21, Shailubhai et al. show the oral dosage formulation of GCC agonist peptide is in the form of a capsule or tablet (p17, lin 44-49).

With respect to claim 42, Shailubhai et al. show pharmaceutical composition comprising a GCC agonist peptide of SEQ ID NO: 20 (100% homology to SEQ ID NO: 1), which is formulated with pharmaceutically acceptable excipients for oral administration (p17, lin 45-49).

In the alternative, Shailubhai et al. teach an oral dosage formulation comprising a GCC agonist peptide sequence of SEQ ID NO: 1 and excipients as applied to claims 2-4, 6-10, 21, and 42 above.

Shailubhai et al. do not specify the intrinsic stability of the GCC agonist peptide and specified excipients in the formulated peptide composition for oral administration.

Fretzen et al. teach a method and composition comprising stable solid formulation of therapeutic polypeptides suitable for oral administration (Title, Abstract). Fretzen et al. teach a peptide formulation for oral administration comprises (a) an aqueous coating solution, the peptide, a sterically hindered primary amine (e.g., leucine) and (b) a pharmaceutically acceptable carrier of filler (p6, lin 10-18) to form the tablets or to be placed into capsules (p6, lin 24-25). Fretzen et al. suggest the molar ratio of leucine to the peptide is ranged from 5:1 to 50:1 (p7, lin 32-34 bridging to p8, lin 1-5). Fretzen et al. teach the pharmaceutically acceptable carrier of filler is a microcrystalline cellulose or a sugar alcohol of mannitol (p9, lin 1-6), and the average diameter of the

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carrier particles size is between 50 μm and 1000 μm (p9, lin 7). Fretzen et al. suggest the lubricant of the peptide composition can be the amino acid leucine (p9, lin 8).

Fretzen et al. suggest the use of a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule (p16, lin 2-3). Fretzen et al. further suggest the formulated peptide is stabilized against degradation, less than 2% degradation of the therapeutic peptide, after 18-24 months of storage at 25°C and 60% relative humidity (p2, lin 15-21) based on the liquid chromatography analysis (p2, lin 1-3).

With respect to claim 3, Fretzen et al. teach a peptide formulation for oral administration has chromatography purity $\geq 95\%$ (p2, lin 1-3).

With respect to claim 5, Fretzen et al. teach a peptide formulation for oral administration comprises (a) an aqueous coating solution, the peptide, a sterically hindered primary amine (e.g., leucine) and (b) a pharmaceutically acceptable carrier of filler (p6, lin 10-18) to form the tablets or to be placed into capsules (p6, lin 24-25). The composition described is substantially free of inorganic acids and carboxylic acids as no components contain inorganic acids and/or carboxylic acids, reading on the limitation of claim 5.

With respect to claim 8, Fretzen et al. teach the therapeutic peptide composition is provided in a unit dosage form of a tablet, a capsule or a sachet for oral administration (p10, lin 28-30)

With respect to claims 10-11, Fretzen et al. teach the pharmaceutically acceptable carrier of filler is a microcrystalline cellulose or a sugar alcohol of mannitol

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(p9, lin 1-6), and the average diameter of the carrier particles size is between 50 μm and 1000 μm (p9, lin 7), reading on the limitation of claims 10-11.

With respect to claims 14-15, Fretzen et al. suggest the molar ratio of an amino acid of leucine (comprising a primary amine) to the therapeutic peptide is ranged from 5:1 to 50:1 (p7, lin 32-34 bridging to p8, lin 1-5).

With respect to claim 16, Fretzen et al. suggest a formulation consists of a therapeutic peptide, an inert carrier of a microcrystalline cellulose (p9, lin 1-6) and a lubricant of an amino acid leucine (p9, lin 8).

With respect to claim 20, Fretzen et al. further suggest the formulated peptide is stabilized against degradation, less than 2% degradation, after 18-24 months of storage at 25°C and 60% relative humidity (p2, lin 15-21).

With respect to claim 21, Fretzen et al. suggest final pharmaceutical composition is in the form of tablets or to be placed into capsules (p6, lin 24-25).

With respect to claim 22, Fretzen et al. suggest the use of a blister pack with individual doses of a tablet for pressing out of the pack according to a therapeutic schedule (p16, lin 2-3).

With respect to claim 25, Fretzen et al. suggest the use of special oil, e.g., mineral oil or vegetable oil, as a lubricant and/or glidant in the oral dosage composition (p9, lin 9).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine Shailubhai's oral dosage formulation comprising a GCC agonist peptide with Fretzen's method and composition for manufacturing stable peptide

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formulation because both Shailubhai's composition is formulated peptide in unit dose for oral administration and Fretzen's method and composition can stabilize Shailubhai's GCC agonist peptide in the pharmaceutical composition, after 18-24 months of storage at 25°C and 60% relative humidity (Fretzen et al. p2, lin 15-21). The combination with no change in their respective functions would have yielded nothing more than predictable results to one of ordinary skill in the art at the time of the invention.

Claim Rejections - 35 USC § 103

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under pre-AIA 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 23-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over

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Shailubhai et al. and Fretzen et al. as applied to claims 2-11, 14-16, 20-22, 25, 42 and further in view of Currier et al. (US 2009/0253634 A1).

Claim 23 is drawn to the GCC agonist peptide is in solution. Claim 24 is drawn to the unit dosage form is a liquid-filled capsule.

Shailubhai et al. in view of Fretzen et al. teach an oral dosage formulation comprising a GCC agonist peptide and one or more excipients has a chromatography purity $\geq 95\%$ (Fretzen et al. p2, lin 1-3) as applied to claims 2-11, 14-16, 20-22, 25, and 42 described above.

Shailubhai et al. in view of Fretzen et al. suggest the unit dosage of GCC peptide composition in the form of a solution, but do not specify in a liquid-filled capsule.

Currie et al. teach methods and compositions comprising a GCC agonist peptide to treat gastrointestinal disorders (Title and Abstract). Currie et al. teach blister packs are well known for the packaging of pharmaceutical unit dosage forms of tablets and capsules [0325-0326]. Currie et al. teach a GCC agonist peptide is formulated in various forms such as a solution or a suspension in an aqueous or a non-aqueous liquid [0264]. Currie et al. suggest a capsule can be filled by a powder or a liquid comprising a GCC agonist peptide [0314, 0316].

With respect to claim 23, Currie et al. teach a GCC agonist peptide is formulated in various forms such as a solution or a suspension in an aqueous or a non-aqueous liquid [0264].

With respect to claim 24, Currie et al. suggest a capsule can be filled by a powder or a liquid comprising a unit dosage of GCC agonist peptide [0314, 0316, 0325-

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0326]. “ [A] person of ordinary skill has good reason to pursue the known options within his or her technical grasp of unit dosage form of a pharmaceutical composition in liquid. If this leads to the anticipated success, it is likely that product [was] not of innovation but of ordinary skill and common sense. In that instance the fact that a combination of the unit dosage of GCC peptide composition (Shailubhai et al. in view of Fretzen et al.) and Currie’s GCC agonist peptide in a liquid-filled capsule was obvious to try might show that it was obvious under § 103.” *KSR International Co. v. Teleflex Inc.*, 550 U.S. ___, ___, 82 USPQ2d 1385, 1397 (2007). Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings (Shailubhai et al. in view of Fretzen et al.) with Currie’s teaching of liquid-filled capsule because Shailubhai et al. in view of Fretzen et al. suggest the unit dosage of GCC peptide composition in the form of a solution (but do not specify in a liquid-filled capsule) and Currie et al. suggest a unit dosage form of GCC agonist peptide in a liquid for can be filled into a capsule [0314, 0316]. All the claimed elements were known, in the prior art, and one of ordinary skill in the art could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art at the time of the invention.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the claims at issue are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the reference application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO internet Web site contains terminal disclaimer forms which may be used. Please visit <http://www.uspto.gov/forms/>. The filing date of the application will determine what form should be used. A web-based eTerminal Disclaimer may be filled

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out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to <http://www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp>.

Claims 2 and 8 are provisionally rejected on the ground of nonstatutory double patenting as being unpatentable over claims 2-3 of copending Application No. 14/228,843 ('843 application). Although the claims at issue are not identical, they are not patentably distinct from each other because SEQ ID NO: 16 claimed in both applications is identical peptide sequence comprising the identical modifications. Claim 2 of the '843 application is directed to a pharmaceutical composition in unit dose form comprising a GCC agonist peptide of SEQ ID NO: 16 in a therapeutically effective amount and a pharmaceutical carrier or excipient, reading on the limitation of claims 2 and 8 of this instant application.

This is a provisional nonstatutory double patenting rejection because the patentably indistinct claims have not in fact been patented.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JIA-HAI LEE whose telephone number is (571)270-1691. The examiner can normally be reached on Mon-Fri.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's

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supervisor, Karlheinz R. Skowronek can be reached on 571-272-9047. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/J. L./
Examiner, Art Unit 1676

13-August-2014

/David Lukton/
Primary Examiner, Art Unit 1676

Notice of References Cited	Application/Control No. 13/421,769	Applicant(s)/Patent Under Reexamination COMISKEY ET AL.	
	Examiner JIA-HAI LEE	Art Unit 1676	Page 1 of 1

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*	Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A US-2009/0253634	10-2009	Currie et al.	514/14
	B US-			
	C US-			
	D US-			
	E US-			
	F US-			
	G US-			
	H US-			
	I US-			
	J US-			
	K US-			
	L US-			
	M US-			

FOREIGN PATENT DOCUMENTS

*	Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N WO 2010/027404 A2	03-2010	US	Fretzen et al.	A61K38/17, A61K9/14
	O WO 02/078683 A1	10-2002	US	Shailubhai et al.	A61K31/00, C07K2/00
	P				
	Q				
	R				
	S				
	T				

NON-PATENT DOCUMENTS

*	Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
U	
V	
W	
X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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(54) Title: STABLE SOLID FORMULATION OF THERAPEUTIC POLYPEPTIDES SUITABLE FOR ORAL ADMINISTRATION

(57) Abstract: Solid, stable formulations of therapeutic polypeptide suitable for oral administration are described herein as are methods for preparing such formulations. The therapeutic polypeptide formulations described herein are stable and have a sufficient shelf life for manufacturing, storing and distributing the drug.

**TABLE SOLID FORMULATION OF THERAPEUTIC POLYPEPTIDES SUITABLE
FOR ORAL ADMINISTRATION**

FIELD

5 This disclosure concerns solid formulations of therapeutic polypeptides suitable for oral administration and methods for preparing such formulations.

PRIORITY CLAIM

10 This application claims priority to United States Application Serial No. 61/094,370, filed September 04, 2008. The entire contents of the aforementioned application are incorporated herein by reference.

BACKGROUND

15 Many therapeutic polypeptides are formulated in aqueous solution because they are most active in this form. However, most polypeptides are not particularly stable in aqueous solution, such that the formulations often have a short half-life and require refrigeration. Although aqueous solutions of polypeptides can be dried by freeze-drying, spray-drying or other methods, such dried formulations may also be unstable and have reduced activity relative to an aqueous solution of the polypeptide. Typical break-down mechanisms that
20 occur both in aqueous solution and in dried formulations include aggregation and oxidative or hydrolytic degradation. Thus, the majority of therapeutic polypeptides, whether in aqueous solution or dried, are stored under refrigerated conditions due to their limited stability.

SUMMARY

25 Solid, stable formulations of therapeutic polypeptides are described herein as are methods for preparing such formulations. The formulations described herein contain a therapeutic polypeptide.

30 The therapeutic polypeptide formulations described herein can be stable and can have a sufficient shelf life for manufacturing, storing and distributing the drug. For example, formulations described herein are expected to have a shelf life of at least 12 months at room temperature storage conditions (e.g., 25°C/60% relative humidity (RH)). In further embodiments, the formulations described herein are expected to have a shelf life of at least 18 months or at least 24 months at room temperature storage conditions (e.g., 25°C/60% RH). Thus, when assessed in an assay on a weight/weight basis as determined by high pressure

liquid chromatography (HPLC) against a therapeutic polypeptide reference standard, $\geq 95\%$ of the original amount of therapeutic polypeptide in the composition remains after three months when packaged samples are stored at accelerated conditions (40°C/75% RH). In further embodiments, $\geq 90\%$ of the original amount of therapeutic polypeptide in the composition remains after at least 6 months when packaged samples are stored at accelerated conditions (40°C/75% RH). In addition, chromatographic purity of the therapeutic polypeptide as determined as area percent by HPLC remains at $\geq 95\%$ over the course of at least three months when packaged samples are stored at accelerated conditions (40°C/75% RH). In further embodiments, the chromatographic purity of the therapeutic polypeptide as determined by area percent by HPLC remains at $\geq 90\%$ over the course of at least 6 months when packaged samples are stored at accelerated conditions (40 °C/75% RH). Thus, for example, no more than about 10% of the therapeutic polypeptide undergoes degradation to other products.

In one embodiment, the invention comprises a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the invention comprises a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the invention comprises a unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24

months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the invention comprises a unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the invention comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In another embodiment, the invention comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4% or 2% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

In one embodiment, the invention comprises a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the invention comprises a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic

polypeptide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the invention comprises a unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant. In another embodiment, the invention comprises a unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

In one embodiment, the invention comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after 18 months or 24 months of storage of the sealed container at 25°C at 60% relative humidity in a sealed container containing a desiccant. In a further embodiment, the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 18 months or 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity. In another embodiment, the invention comprises a sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after 3 months or 6 months of storage of the

sealed container containing a desiccant at 40°C at 75% relative humidity. In a further embodiment, the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after 3 months or 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

5 The assay value on a weight/weight basis (“weight/weight assay”) may be determined by comparing, e.g., by HPLC, the amount of therapeutic polypeptide in a sample, to a therapeutic polypeptide reference standard. As used herein, the weight of therapeutic polypeptide in a composition after storage at room temperature or accelerated conditions at a specified time point (e.g., three or six months of storage under accelerated conditions
10 [40°C/75% RH] or 12, 18 or 24 months of storage under room temperature conditions [25 °C/60% RH]) is compared to the weight of therapeutic polypeptide in a composition at an initial time (e.g., the time when the pharmaceutical composition is released for clinical or patient use (“the release date”)) to provide the weight/weight assay value. For example, the weight of therapeutic polypeptide in a composition is measured after storage for a specified
15 time at accelerated conditions (40°C/75% RH) and compared to the weight of therapeutic polypeptide that was present in the sample at the release date. In another example, the weight of therapeutic polypeptide in a composition is measured after storage for a specified time at room temperature conditions (25°C/60% RH) and compared to the weight of therapeutic polypeptide that was present in the sample at the release date. Thus, the phrase “≥ 90% of the
20 original amount of therapeutic polypeptide in the composition remains after at least 6 months when packaged samples are stored at accelerated conditions (40°C/75% RH)” means the weight of therapeutic polypeptide in the composition measured in an assay on a weight/weight basis as determined by HPLC after at least 6 months storage at accelerated conditions is ≥ 90% of the amount of therapeutic polypeptide in the composition present at
25 the initial time (e.g., the release date of the therapeutic polypeptide composition).

 Chromatographic purity of therapeutic polypeptide may be assessed by performing HPLC under the conditions described herein. The area under the therapeutic polypeptide peak is measured and compared to the total area under all peaks excluding the solvent peak and any non-polypeptide related peaks (i.e., peaks associated with excipients that may be
30 observed in a placebo). As used herein, the chromatographic purity of therapeutic polypeptide in a composition after storage at room temperature or accelerated conditions at a specified time point (e.g., three or six months of storage under accelerated conditions [40°C/75% RH] or 12, 18 or 24 months of storage under room temperature conditions [25 °C/60% RH]) is compared to the chromatographic purity of therapeutic polypeptide in a composition at an

initial time (e.g., the time when the pharmaceutical composition is released for clinical or patient use (“the release date”)) to provide the chromatographic purity value. For example, the chromatographic purity of therapeutic polypeptide in a composition is measured after storage for a specified time at accelerated conditions (40°C/75% RH) and compared to the chromatographic purity of therapeutic polypeptide in the composition at the release date. In another example, the chromatographic purity of therapeutic polypeptide in a composition is measured after storage for a specified time at room temperature conditions (25°C/60% RH) and compared to the chromatographic purity of therapeutic polypeptide in the composition at the release date.

10 This disclosure features a method for preparing a pharmaceutical composition comprising therapeutic polypeptide or a pharmaceutically acceptable salt thereof, the method comprising: (a) providing a solution, e.g., an aqueous solution (“the coating solution”), comprising: (i) purified therapeutic polypeptide or a pharmaceutically acceptable salt thereof; (ii) a cation selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} and/or a sterically hindered primary amine (e.g., leucine) and, optionally, (iii) a pharmaceutically acceptable binder; and (b) applying the coating solution to a pharmaceutically acceptable filler to generate polypeptide-coated filler (e.g., by spraying, mixing or coating the pharmaceutically acceptable filler with the coating solution). The method can optionally include one or more of: (i) blending the polypeptide-coated filler with a pharmaceutically acceptable glidant, a pharmaceutically acceptable lubricant or a pharmaceutically acceptable additive that acts as both a glidant and lubricant; (ii) blending the polypeptide-coated filler with filler that is not polypeptide-coated, (iii) blending the polypeptide-coated filler with other additives; (iii) applying a pharmaceutically acceptable coating additive to the polypeptide-coated filler. The final pharmaceutical composition can be placed into capsules (e.g., gelatin capsule) or used to form tablets.

In some embodiments, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier, therapeutic polypeptide and one or more agents selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} and a sterically hindered primary amine, wherein the agent improves at least one attribute of the composition, relative to a pharmaceutical composition without the agent. In further embodiments, the agent is Mg^{2+} , Ca^{2+} or Zn^{2+} . In a further embodiment, the agent is Ca^{2+} . In another embodiment, the agent is a sterically hindered primary amine. In a further embodiment, the sterically hindered primary amine is an amino acid. In yet a further embodiment, the amino acid is a naturally-occurring amino acid. In a still further embodiment, the naturally-occurring amino acid is

selected from the group consisting of: histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine, glycine, and valine; yet further, the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine; in another embodiment, the naturally-occurring amino acid is leucine or methionine; still further, the naturally-occurring amino acid is leucine. In another embodiment, the sterically hindered primary amine is a non-naturally occurring amino acid (e.g., 1-aminocyclohexane carboxylic acid). In a further embodiment, the sterically hindered primary amine is cyclohexylamine, 2-methylbutylamine or chitosan. In another embodiment, the sterically hindered primary amine can be a mixture of more than one sterically hindered primary amine. For example, the sterically hindered primary amine may be a mixture of two or more amino acids. In further embodiments, the pharmaceutical composition comprising a therapeutic polypeptide is a mixture of two or more therapeutic polypeptides.

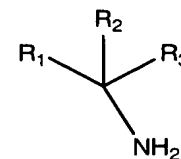
In other embodiments, there is provided a pharmaceutical composition comprising a pharmaceutically acceptable carrier, therapeutic polypeptide, a cation selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} and a sterically hindered primary amine. In one embodiment, the cation is Ca^{2+} . In another embodiment, the cation is a mixture of two or three of Mg^{2+} , Ca^{2+} and Zn^{2+} . In a further embodiment, the pharmaceutical composition further comprises a pharmaceutically acceptable binder and/or a pharmaceutically acceptable glidant, lubricant or additive that acts as both a glidant and lubricant and/or an antioxidant. In a further embodiment, the sterically hindered primary amine is an amino acid. In yet a further embodiment, the amino acid is a naturally-occurring amino acid. In a still further embodiment, the naturally-occurring amino acid is selected from the group consisting of: histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine, glycine, and valine; yet further, the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine; in another embodiment, the naturally-occurring amino acid is leucine or methionine; still further, the naturally-occurring amino acid is leucine. In another embodiment, the sterically hindered primary amine can be a mixture of more than one sterically hindered primary amines. For example, the sterically hindered primary amine may be a mixture of two or more amino acids.

In some cases the molar ratio of cation:sterically hindered primary amine:therapeutic polypeptide (e.g., Ca^{2+} :leucine:therapeutic polypeptide) in the aqueous solution applied to the carrier is 5-100:5-50:1. It can be desirable for the molar ratio of cation:sterically hindered

primary amine (e.g., Ca^{2+} :leucine) to be equal to or greater than 2:1 (e.g., between 5:1 and 2:1). Thus, in some cases the molar ratio of cation:sterically hindered primary amine:therapeutic polypeptide (e.g., Ca^{2+} :leucine:therapeutic polypeptide) applied to the carrier is 100:50:1, 100:30:1, 80:40:1, 80:30:1, 80:20:1, 60:30:1, 60:20:1, 50:30:1, 50:20:1, 40:20:1, 20:20:1, 10:10:1, 10:5:1 or 5:10:1. When binder, e.g., methylcellulose, is present in the therapeutic polypeptide solution applied to the carrier it can be present at 0.5% - 2.5% by weight (e.g., 0.7%-1.7% or 0.7% - 1% or 1.5% or 0.7%).

The weight of therapeutic polypeptide applied to a given weight of filler (e.g., microcrystalline cellulose) can vary from about 0.02:100 to about 2.67:100. Thus, about 0.05 mg to about 6.0 mg of therapeutic polypeptide can be applied to 225 mg of filler. In a further embodiment, the weight of therapeutic polypeptide applied to a given weight of filler is about 0.05 mg to about 2.0 mg of therapeutic polypeptide (e.g., 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg peptide for 225 mg of filler).

In various embodiments: the sterically hindered primary amine is an amino acid (e.g., a naturally-occurring amino acid or a naturally-occurring amino acid selected from histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, methionine, asparagine, tyrosine, threonine, leucine, isoleucine, tryptophan, glycine or valine). In other cases the sterically hindered primary amine is a non-naturally occurring amino acid (e.g., lanthionine, theanine or 1-aminocyclohexane carboxylic acid). In a further embodiment, the sterically hindered primary amine is cyclohexylamine or 2-methylbutylamine. In other cases, the sterically hindered primary amine is an amino sugar (e.g., chitosan or glucosamine).



In some cases, the sterically hindered primary amine has the formula: , wherein R_1 , R_2 and R_3 are independently selected from: H; $-\text{C}(\text{O})\text{OH}$; C_1 - C_6 alkyl, optionally substituted by $-\text{CO}_2\text{H}$, $-\text{CONH}_2$, or a 5-10 membered aryl or heteroaryl; C_1 - C_6 alkoxyalkyl; or C_1 - C_6 thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or $-\text{NH}_2$, and provided that no more than two of R_1 , R_2 and R_3 are H. In a further embodiment, no more than one of R_1 , R_2 and R_3 is H.

In various cases: the antioxidant is selected from BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), vitamin E, propyl gallate, ascorbic acid and salts or esters thereof, tocopherol and esters thereof, alpha-lipoic acid, beta-carotene; the pharmaceutically acceptable binder is polyvinyl alcohol; the pharmaceutically acceptable binder is selected

from: a starch (e.g., corn starch, pre-gelatinized potato starch, rice starch, wheat starch, and sodium starch glycolate), maltodextrin and a cellulose ether (e.g., methyl cellulose, hydroxyethyl cellulose, hydroxyethyl methyl cellulose and hydroxypropyl methyl cellulose); the pharmaceutically acceptable filler is cellulose (e.g., microfine cellulose or
5 microcrystalline cellulose); the pharmaceutically acceptable filler is a sugar or a sugar alcohol (e.g., mannitol, isomalt, sorbitol, dextrose, xylitol, sucrose and lactose); the filler comprises particles having an average diameter between 50 μm and 1000 μm ; the lubricant and/or glidant is selected from: talc, leucine, magnesium stearate, stearic acid and polyvinyl alcohol; and the lubricant and/or glidant is selected from: calcium stearate, mineral oil, vegetable oil,
10 PEG (e.g., PEG that is liquid or solid at room temperature), sodium benzoate, and sodium lauryl sulfate.

In some cases, the therapeutic polypeptide solution used in a method for preparing the formulation has a pH below 7 (e.g., a pH between 1 and 3 or a pH between about 1.5 and about 2.5). The pH can be adjusted with, e.g., phosphoric acid. In some cases, the solution is
15 buffered. Various pharmaceutically acceptable buffers can be used (e.g., phosphate buffer).

In some cases, the therapeutic polypeptide solution used in a method for preparing the formulation comprises both a cation (e.g., CaCl_2) and a sterically hindered primary amine (e.g., leucine).

In some cases the therapeutic polypeptide solution comprises CaCl_2 and leucine; the
20 binder is methylcellulose; the filler is microcrystalline cellulose; the glidant and/or lubricant comprises talc or leucine.

In certain embodiments the therapeutic polypeptide does not comprise or consist of the amino acid sequence CCEYCCNPACTGCY. In certain embodiments, the therapeutic polypeptide does not comprise or consist of a GC-C receptors agonist polypeptide.

25 Also featured is a pharmaceutical composition prepared by any of the methods described herein.

DETAILED DESCRIPTION

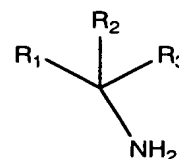
30 Compositions containing a therapeutic polypeptide can include any therapeutic polypeptide, for example, which include, but are not limited to, bone morphogenic proteins, insulin, colchicine, glucagon, thyroid stimulating hormone, parathyroid and pituitary hormones, calcitonin, renin, prolactin, corticotrophin, thyrotropic hormone, follicle stimulating hormone, chorionic gonadotropin, gonadotropin releasing hormone, bovine somatotropin, porcine somatotropin, oxytocin, vasopressin, GRF, somatostatin, lyppressin, pancreozymin, luteinizing

hormone, LHRH, LHRH agonists and antagonists, leuprolide, interferons such as interferon alpha-2a, interferon alpha-2b, and consensus interferon, interleukins, growth hormones such as human growth hormone and its derivatives such as methionine-human growth hormone and des-phenylalanine human growth hormone, parathyroid hormone, bovine growth hormone and porcine growth hormone, fertility inhibitors such as the prostaglandins, fertility promoters, growth factors such as epidermal growth factors (EGF), platelet-derived growth factors (PDGF), fibro-blast growth factors (FGF), transforming growth factors-alpha (TGF- α), transforming growth factors-beta (TGF- β), erythropoietin (EPO), insulin-like growth factor-I (IGF-I), insulin-like growth factor-II (IGF-II), interleukin-1, interleukin-2, interleukin-6, interleukin-8, tumor necrosis factor-alpha (TNF- α), tumor necrosis factor-beta (TNF β), Interferon-alpha (INF- α), Interferon-beta (INF- β), Interferon-gamma (INF- γ), Interferon-omega (INF- Ω), colony stimulating factors (CSF), vascular cell growth factor (VEGF), thrombopoietin (TPO), stromal cell-derived factors (SDF), placenta growth factor (PIGF), hepatocyte growth factor (HGF), granulocyte macrophage colony stimulating factor (GM-CSF), glial-derived neurotrophin factor (GDNF), granulocyte colony stimulating factor (G-CSF), ciliary neurotropic factor (CNTF), bone growth factor, transforming growth factor, bone morphogeneic proteins (BMP), coagulation factors, human pancreas hormone releasing factor, analogs and derivatives of these polypeptides, and pharmaceutically acceptable salts of these compounds, or their analogs or derivatives. In certain embodiments, the therapeutic polypeptide may be a mixture of two or more therapeutic polypeptides described herein.

In some embodiments, the solid, stable formulation of the therapeutic polypeptide is administered orally. In other embodiments, the solid, stable formulation is solubilized in an appropriate excipient for administration by other routes. For example, the formulation may be solubilized and the therapeutic polypeptide may be administered, e.g., by intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, topical, sublingual, intraarticular (in the joints), intradermal, buccal, ophthalmic (including intraocular), intranasally (including using a cannula), intraspinally or intrathecally. In one embodiment, the therapeutic polypeptide composition is provided in a discrete unit, a unit dosage form, (e.g., a tablet, a capsule, a sachet) that is effective at such dosage either for administration orally or for solubilization and subsequent administration by other routes. In another embodiment, the therapeutic polypeptide is provided in a unit dosage form either for administration orally or for solubilization for subsequent administration by other routes, wherein the unit dosage form provides multiple effective dosages (i.e., each unit dosage form provides more than one effective dosages of the therapeutic polypeptide). In another

embodiment, the therapeutic polypeptide is provided in a unit dosage form that provides an effective dosage with multiple unit dosage forms either for administration orally or for solubilization and subsequent administration by other routes. In certain embodiments, the unit dosage form and daily dose are equivalent. In various embodiments, the unit dosage form is administered orally with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast). In various embodiments, the unit dosage form is administered once a day, twice a day or three times a day either orally or via another route. In various embodiments, the unit dosage form is administered once a week, twice a week, three times a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every two months, once every three months, or once every six months either orally or via another route. The unit dosage form can optionally comprise other additives. In some embodiments, one, two or three unit dosage forms will contain the dose of therapeutic polypeptide. The precise amount of compound administered to a patient will be the responsibility of the attendant physician. However, the dose employed will depend on a number of factors, including the age and sex of the patient, the precise disorder being treated, and its severity.

A cation of the invention may be provided as a pharmaceutically acceptable salt i.e., a cation with an appropriate counterion. Examples of pharmaceutically acceptable salts that may be used in the invention include, without limitation, magnesium acetate, magnesium chloride, magnesium phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium phosphate, calcium carbonate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate, manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum phosphate or aluminum sulfate. In one embodiment, a pharmaceutically acceptable salt that may be used is calcium chloride, magnesium chloride and zinc acetate.



As used herein, the sterically hindered primary amine has the formula: ,
 wherein R₁, R₂ and R₃ are independently selected from: H; -C(O)OH; C1-C6 alkyl,
 optionally substituted by -CO₂H, -CONH₂, or a 5-10 membered aryl or heteroaryl; C1-C6
 alkoxyalkyl; or C1-C6 thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be

singly or multiply substituted with halogen or $-\text{NH}_2$, and provided that no more than two of R_1 , R_2 and R_3 are H. In a further embodiment, no more than one of R_1 , R_2 and R_3 is H.

The term "alkyl", as used herein, refers to a saturated linear or branched-chain monovalent hydrocarbon radical. Unless otherwise specified, an alkyl group contains 1-20
5 carbon atoms (e.g., 1-20 carbon atoms, 1-10 carbon atoms, 1-8 carbon atoms, 1-6 carbon atoms, 1-4 carbon atoms or 1-3 carbon atoms). Examples of alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, s-butyl, t-butyl, pentyl, hexyl, heptyl, octyl and the like.

The terms C_{n-m} "alkoxyalkyl" and C_{n-m} "thioalkoxyalkyl" mean alkyl, substituted with
10 one or more alkoxy or thioalkoxy groups, as the case may be, wherein the combined total number of carbons of the alkyl and alkoxy groups, or alkyl and thioalkoxy groups, combined, as the case may be, is between the values of n and m. For example, a C_{4-6} alkoxyalkyl has a total of 4-6 carbons divided between the alkyl and alkoxy portion; e.g. it can be $-\text{CH}_2\text{OCH}_2\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{OCH}_2\text{CH}_3$ or $-\text{CH}_2\text{CH}_2\text{CH}_2\text{OCH}_3$.

15 As used herein, the term "aryl" (as in "aryl ring" or "aryl group"), used alone or as part of a larger moiety, refers to a carbocyclic ring system wherein at least one ring in the system is aromatic and has a single point of attachment to the rest of the molecule. Unless otherwise specified, an aryl group may be monocyclic, bicyclic or tricyclic and contain 6-18 ring members. Examples of aryl rings include, but are not limited to, phenyl, naphthyl,
20 indanyl, indenyl, tetralin, fluorenyl, and anthracenyl.

The term "heteroaryl" (or "heteroaromatic" or "heteroaryl group" or "aromatic heterocycle") used alone or as part of a larger moiety as in "heteroaralkyl" or "heteroarylalkoxy" refers to a ring system wherein at least one ring in the system is aromatic and contains one or more heteroatoms, wherein each ring in the system contains 3 to 7 ring
25 members and which has a single point of attachment to the rest of the molecule. Unless otherwise specified, a heteroaryl ring system may be monocyclic, bicyclic or tricyclic and have a total of five to fourteen ring members. In one embodiment, all rings in a heteroaryl system are aromatic. Also included in this definition are heteroaryl radicals where the heteroaryl ring is fused with one or more aromatic or non-aromatic carbocyclic or
30 heterocyclic rings, or combinations thereof, as long as the radical or point of attachment is in the heteroaryl ring. Bicyclic 6,5 heteroaromatic system, as used herein, for example, is a six membered heteroaromatic ring fused to a second five membered ring wherein the radical or point of attachment is on the six membered ring.

Heteroaryl rings include, but are not limited to the following monocycles: 2-furanyl, 3-furanyl, N-imidazolyl, 2-imidazolyl, 4-imidazolyl, 5-imidazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-oxazolyl, 4-oxazolyl, 5-oxazolyl, N-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, pyridazinyl (e.g., 3-pyridazinyl), 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, tetrazolyl (e.g., 5-tetrazolyl), triazolyl (e.g., 2-triazolyl and 5-triazolyl), 2-thienyl, 3-thienyl, pyrazolyl (e.g., 2-pyrazolyl), isothiazolyl, 1,2,3-oxadiazolyl, 1,2,5-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,3-triazolyl, 1,2,3-thiadiazolyl, 1,3,4-thiadiazolyl, 1,2,5-thiadiazolyl, pyrazinyl, 1,3,5-triazinyl, and the following bicycles: benzimidazolyl, benzofuryl, benzothiophenyl, benzopyrazinyl, benzopyranonyl, indolyl (e.g., 2-indolyl), purinyl, quinolinyl (e.g., 2-quinolinyl, 3-quinolinyl, 4-quinolinyl), and isoquinolinyl (e.g., 1-isoquinolinyl, 3-isoquinolinyl, or 4-isoquinolinyl).

As used herein, the term “binder” refers to any pharmaceutically acceptable binder that may be used in the practice of the invention. Examples of pharmaceutically acceptable binders include, without limitation, corn starch, potato starch, other starches, gelatin, natural and synthetic gums such as acacia, powdered tragacanth, guar gum, cellulose and its derivatives (e.g., ethyl cellulose, cellulose acetate, carboxymethyl cellulose calcium, sodium carboxymethyl cellulose), polyvinyl pyrrolidone (e.g., polyvinyl pyrrolidone K30), methyl cellulose, pre-gelatinized starch (e.g., STARCH 1500® and STARCH 1500 LM®, sold by Colorcon, Ltd.), hypromellose (hydroxypropyl methylcellulose), microcrystalline cellulose (e.g. AVICEL™, such as, AVICEL-PH-101™, -103™ and -105™, sold by FMC Corporation, Marcus Hook, PA, USA), and mixtures thereof.

As used herein, the term “filler” refers to any pharmaceutically acceptable filler that may be used in the practice of the invention. Examples of pharmaceutically acceptable fillers include, without limitation, talc, calcium carbonate (e.g., granules or powder), dibasic calcium phosphate, tribasic calcium phosphate, calcium sulfate (e.g., granules or powder), microcrystalline cellulose (e.g., Avicel PH101), powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, xylitol, mannitol, myoinositol, and mixtures thereof.

Examples of pharmaceutically acceptable fillers that may be particularly used for coating with therapeutic polypeptide include, without limitation, talc, microcrystalline cellulose (e.g., Avicel PH101), powdered cellulose, dextrates, kaolin, mannitol, silicic acid, sorbitol, starch, pre-gelatinized starch, lactose, glucose, fructose, galactose, trehalose,

sucrose, maltose, isomalt, dibasic calcium phosphate, raffinose, maltitol, melezitose, stachyose, lactitol, palatinite, xylitol, mannitol, myoinositol, and mixtures thereof.

As used herein, the term “additives” refers to any pharmaceutically acceptable additive. Pharmaceutically acceptable additives include, without limitation, disintegrants, dispersing additives, lubricants, glidants, antioxidants, coating additives, diluents, surfactants, 5 flavoring additives, humectants, absorption promoting additives, controlled release additives, anti-caking additives, anti-microbial agents (e.g., preservatives), colorants, desiccants, plasticizers and dyes.

As used herein, an “excipient” is any pharmaceutically acceptable additive, filler, 10 binder or agent.

As used herein, “purified therapeutic polypeptide” is therapeutic polypeptide or a pharmaceutically acceptable salt thereof that is greater than or equal to 95 percent pure. therapeutic polypeptide purity can be measured, for example, by chromatographic purity of therapeutic polypeptide using HPLC.

15 In some embodiments, the therapeutic polypeptide composition is provided in a solid form for oral administration. Examples of such forms include, without limitation, a tablet, a sachet, a pellet, a capsule or a powder. In some embodiments, the compositions can be used to create unit dosage forms, e.g., tablets, capsules, sachets or pellets. Orally administered compositions can include, for example, binders, lubricants, inert diluents, lubricating, surface 20 active or dispersing additives, flavoring additives, and humectants. Orally administered formulations such as tablets may optionally be coated or scored and may be formulated so as to provide sustained, delayed or controlled release of the therapeutic polypeptide therein. The therapeutic polypeptide can be co-administered or co-formulated with other medications.

The compositions can include, for example, various additional solvents, dispersants, 25 coatings, absorption promoting additives, controlled release additives, and one or more inert additives (which include, for example, starches, polyols, granulating additives, microcrystalline cellulose, diluents, lubricants, binders, disintegrating additives, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or non-aqueous techniques. Compositions can also include, for example, anti-caking 30 additives, preservatives, sweetening additives, colorants, flavors, desiccants, plasticizers, dyes, and the like.

Suitable disintegrants include, for example, agar-agar, calcium carbonate, microcrystalline cellulose, croscarmellose sodium, crospovidone, povidone, polacrillin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized

starch, clays, other algin, other celluloses, gums, and mixtures thereof.

Suitable lubricants include, for example, calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, talc, hydrogenated vegetable oil (e.g., peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil), zinc stearate, ethyl oleate, ethyl laurate, agar, syloid silica gel (AEROSIL 200, W.R. Grace Co., Baltimore, MD USA), a coagulated aerosol of synthetic silica (Evonik Degussa Co., Plano, TX USA), a pyrogenic silicon dioxide (CAB-O-SIL, Cabot Co., Boston, MA USA), and mixtures thereof.

Suitable anti-caking additives include, for example, calcium silicate, magnesium silicate, silicon dioxide, colloidal silicon dioxide, talc, and mixtures thereof.

Suitable anti-microbial additives that may be used, e.g., as a preservative for the therapeutic polypeptide compositions, include, for example, benzalkonium chloride, benzethonium chloride, benzoic acid, benzyl alcohol, butyl paraben, cetylpyridinium chloride, cresol, chlorobutanol, dehydroacetic acid, ethylparaben, methylparaben, phenol, phenylethyl alcohol, phenoxyethanol, phenylmercuric acetate, phenylmercuric nitrate, potassium sorbate, propylparaben, sodium benzoate, sodium dehydroacetate, sodium propionate, sorbic acid, thimersol, thymo, and mixtures thereof.

Suitable coating additives include, for example, sodium carboxymethyl cellulose, cellulose acetate phthalate, ethylcellulose, gelatin, pharmaceutical glaze, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methyl cellulose phthalate, methylcellulose, polyethylene glycol, polyvinyl acetate phthalate, shellac, sucrose, titanium dioxide, carnauba wax, microcrystalline wax, and mixtures thereof.

In certain embodiments, suitable additives for the therapeutic polypeptide composition include one or more of sucrose, talc, magnesium stearate, crospovidone or BHA.

In certain embodiments, the term "95%" may be 95.0%, the term "90%" may be 90.0%, the term "10%" may be 10.0%, the term "9%" may be 9.0%, the term "8%" may be 8.0%, the term "7%" may be 7.0%, the term "6%" may be 6.0%, the term "5%" may be 5.0%, the term "4%" may be 4.0%, the term "3%" may be 3.0%, the term "2%" may be 2.0%, and the term "1%" may be 1.0%.

In certain embodiments, the therapeutic polypeptide composition is provided in a unit dosage form. In some embodiments, the unit dosage form is a capsule, a tablet, a sachet, a pellet or a powder. In one such embodiment, the unit dosage form is a capsule or tablet. Such unit dosage forms may be contained in a container such as, without limitation, a paper

or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. It is feasible that more than one container can be used together in a single package to provide a single dosage form.

5 For example, tablets or capsules may be contained in a bottle which is in turn contained within a box. In some embodiments, the unit dosage forms are provided in a container further comprising a desiccant. In a further embodiment, the unit dosage forms, e.g., a quantity of tablets or capsules, are provided in a container, e.g., a bottle, jar or re-sealable bag, containing a desiccant. In a further embodiment, the container containing the unit
10 dosage forms is packaged with administration or dosage instructions. In certain embodiments, the therapeutic polypeptide composition is provided in a kit. The therapeutic polypeptide composition described herein and combination therapy agents can be packaged as a kit that includes single or multiple doses of two or more agents, each packaged or formulated individually, or single or multiple doses of two or more agents packaged or
15 formulated in combination. Thus, the therapeutic polypeptide composition can be present in first container, and the kit can optionally include one or more agents in a second container. The container or containers are placed within a package, and the package can optionally include administration or dosage instructions.

20

EXAMPLES

A therapeutic polypeptide or a pharmaceutically acceptable salt thereof may be produced and purified using standard techniques known in the art, e.g., chemical synthesis or recombinant expression followed by and purification using standard techniques.

25 **Example 1: Formulation Method A**

Preparation of the Coating Solution: Approximately 32 g to 42 g of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The cation, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. The sterically hindered
30 primary amine, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. Other additives, such as antioxidants, are then added, if desired. The pH of the solution is tested, and hydrochloric acid is added, if necessary, to produce a solution having a pH between 1.5 and 2.0. The binder is then added to the solution and the mixture is then stirred for sufficient

time to achieve a clear solution. The desired amount of therapeutic polypeptide is added to the solution and mixed for 30-100 minutes to provide the coating solution.

Preparation of the Active Beads: Approximately 30-36 g of dried microcrystalline cellulose beads are added to a Mini Column Fluid Bed Coater. The microcrystalline cellulose beads are fluidized and heated prior to layering. Next, the coating solution is layered to the beads. The spraying temperature is controlled between 24°C and 55°C by controlling inlet temperature, spray rate, atomization pressure, and air volume. After the entire coating solution is layered to the beads, the beads are dried. The product of this process is referred to as active beads.

10

Example 2: Formulation Method B

Preparation of the Coating Solution: Approximately 8.3 kg of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The cation, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. The sterically hindered primary amine, if used, is added to the solution in a quantity to provide the desired concentration, and the solution is mixed for sufficient time to produce a clear solution. Other additives, such as antioxidants, are then added, if desired. The binder is then added to the solution and the solution is mixed for sufficient time to achieve a clear solution. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is Solution 1. Approximately 8.3 kg of purified water is mixed with hydrochloric acid to create a solution with a pH between 1.5 and 2.0. The desired amount of therapeutic polypeptide is added to the solution and mixed for 10 to 30 minutes. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is Solution 2. Solution 1 and Solution 2 are then mixed together. The pH of the solution is tested, and hydrochloric acid is added if necessary to produce a solution having a pH between 1.5 and 2.0. This is the coating solution.

20

Preparation of the Active Beads: Approximately 24.19 kg of microcrystalline cellulose beads are added to a Wurster Column of a Glatt GPCG-30 Fluid Bed. The microcrystalline cellulose beads are fluidized and heated to product temperature of 45-47°C. Next, the coating solution is layered to the beads. The product spraying temperature is controlled between 37°C and 47°C by controlling inlet temperature, spray rate, atomization pressure, and air volume. After the entire coating solution is layered to the beads, the beads

30

are dried with a product drying temperature of 37°C to 47°C. The product of this process is referred to as active beads.

Example 3: Preparation of capsules containing a therapeutic polypeptide formulation

5 The therapeutic polypeptide content on active beads may be measured as described below or by other equivalent methods.

 To form capsules suitable for oral administration, an appropriate amount of active beads is used to fill gelatin capsules (e.g., Size 2 gelatin capsules). An appropriate amount of active beads may contain 50 µg to 2 mg therapeutic polypeptide per capsule with a range of ±
10 5%. In another embodiment, an appropriate amount of active beads to fill a desired number of gelatin capsules is placed in a container. One or more pharmaceutically acceptable fillers or other pharmaceutically acceptable additives may be added, if desired, to the container. In some embodiments, a filler or additive is talc, leucine, microcrystalline cellulose or mannitol. The contents of the container are blended and the mixture is used to fill gelatin capsules with
15 an appropriate amount of active beads containing therapeutic polypeptide (e.g., 50 µg to 2 mg therapeutic polypeptide per capsule with a range of ± 5%).

 In an alternative embodiment, an appropriate amount of active beads is used to fill gelatin capsules and one or more pharmaceutically acceptable fillers or other pharmaceutically acceptable additives are added to the gelatin capsules.

20

Example 5: Measurement of therapeutic polypeptide content and purity

 Therapeutic polypeptide content and purity may be determined by reverse phase gradient liquid chromatography. The therapeutic polypeptide content is measured by determining the therapeutic polypeptide concentration in the prepared sample against a
25 similarly prepared external therapeutic polypeptide standard.

Claims

1. A pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
2. The pharmaceutical composition according to claim 1, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, or 4% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
3. A unit dosage form of a pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.
4. The unit dosage form according to claim 3, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5% or 4% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.
5. A sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

6. The sealed container according to claim 5, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5% or 4% after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.
7. A pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis decreases by less than 10% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
8. The pharmaceutical composition according to claim 7, wherein the assay value for the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
9. A unit dosage form of a pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the assay value for the receptor agonist polypeptide determined on a weight/weight basis decreases by less than 10% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.
10. The unit dosage form according to claim 9, wherein the assay value for the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

11. A sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising a therapeutic polypeptide and a pharmaceutically acceptable excipient wherein the assay value for therapeutic polypeptide in the unit dosage forms determined on a weight/weight basis decreases by less than 10% after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage the sealed container containing a desiccant at 40°C at 75% relative humidity.
12. The sealed container according to claim 11, wherein the assay value for the therapeutic polypeptide decreases by less than 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% after (a) 18 months of storage the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage the sealed container containing a desiccant at 40°C at 75% relative humidity.
13. The unit dosage form according to any one of claims 3-4 or 9-10, wherein each unit dosage form contains from 25 µg to 1 g therapeutic polypeptide.
14. The sealed container according to any one of claims 5-6 or 11-12, wherein each unit dosage form contains from 25 µg to 1 g therapeutic polypeptide.
15. The pharmaceutical composition according to either of claims 1 or 2, wherein the chromatographic purity of the therapeutic polypeptide (a) 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
16. The unit dosage form according to either of claims 3 or 4, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after (a) 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.
17. The sealed container according to either of claims 5 or 6, wherein the chromatographic purity of the therapeutic polypeptide decreases by less than 10% after (a) 24

months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

5 18. The pharmaceutical composition according to either of claims 7 or 8, wherein the assay value of the therapeutic polypeptide decreases by less than 10% after (a) 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

10 19. The unit dosage form according to either of claims 9 or 10, wherein the assay value of the therapeutic polypeptide decreases by less than 10% after (a) 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity
15 in a sealed container containing a desiccant.

20 20. The sealed container according to either of claims 11 or 12, wherein the assay value of the therapeutic polypeptide decreases by less than 10% after (a) a first 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b)
20 a first 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

25 21. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, a therapeutic polypeptide and one or more agents selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ ,
30 Na^+ or Al^{3+} or a sterically hindered primary amine, wherein the agent improves at least one attribute of the composition, relative to a pharmaceutical composition without the agent, after (a) a first 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) a first 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container
30 containing a desiccant, wherein the attribute is selected from: a decrease in the rate of degradation of therapeutic polypeptide as measured by therapeutic polypeptide content, a decrease in the rate of degradation of therapeutic polypeptide as measured by chromatographic purity of therapeutic polypeptide, a decrease in the amount of a therapeutic polypeptide oxidation product relative to the amount of therapeutic polypeptide, and a

decrease in the amount of a therapeutic polypeptide hydrolysis product relative to the amount of therapeutic polypeptide.

22. The pharmaceutical composition according to claim 21, wherein the agent is Mg^{2+} , Ca^{2+} ,
5 Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} .

23. The pharmaceutical composition according to claim 22, wherein the agent is Mg^{2+} ,
 Ca^{2+} or Zn^{2+} .

10 24. The pharmaceutical composition according to claim 22, wherein the Mg^{2+} , Ca^{2+} , Zn^{2+} ,
 Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium acetate, magnesium chloride, magnesium
phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium phosphate, calcium
carbonate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate,
manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium
15 acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium
chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum
phosphate or aluminum sulfate.

25. The pharmaceutical composition according to claim 24, wherein Mg^{2+} , Ca^{2+} , Zn^{2+} ,
20 Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium chloride, calcium chloride, calcium
phosphate, calcium sulfate, zinc acetate, manganese chloride, potassium chloride, sodium
chloride or aluminum chloride.

26. The pharmaceutical composition according to claim 21, wherein the agent is Mg^{2+} ,
25 Ca^{2+} or Zn^{2+} .

27. The pharmaceutical composition according to claim 26, wherein the Mg^{2+} , Ca^{2+} or
 Zn^{2+} is provided as magnesium chloride, calcium chloride or zinc acetate.

30 28. The pharmaceutical composition according to claim 26, wherein the agent is Ca^{2+} .

29. The pharmaceutical composition according to claim 27, wherein the Ca^{2+} is provided
as calcium chloride.

30. The pharmaceutical composition according to claim 21, wherein the agent is a sterically hindered primary amine.

31. The pharmaceutical composition according to claim 30, wherein the sterically hindered primary amine is an amino acid.

32. The pharmaceutical composition according to claim 31, wherein the amino acid is a naturally-occurring amino acid.

33. The pharmaceutical composition according to claim 32, wherein the naturally-occurring amino acid is histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine, glycine or valine.

34. The pharmaceutical composition according to claim 33, wherein the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine.

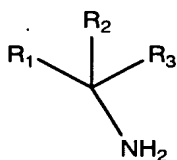
35. The pharmaceutical composition according to claim 34, wherein the naturally-occurring amino acid is leucine or methionine.

36. The pharmaceutical composition according to claim 35, wherein the naturally-occurring amino acid is leucine.

37. The pharmaceutical composition according to claim 30, wherein the sterically hindered primary amine is a non-naturally occurring amino acid.

38. The pharmaceutical composition according to claim 37, wherein the non-naturally occurring amino acid is 1-aminocyclohexane carboxylic acid.

39. The pharmaceutical composition according to claim 30, wherein the sterically



hindered primary amine has the formula: $\text{C}(\text{R}_1)(\text{R}_2)(\text{R}_3)\text{NH}_2$, wherein R₁, R₂ and R₃ are

independently selected from: H; $-C(O)OH$; C_1-C_6 alkyl, optionally substituted by $-CO_2H$, $-CONH_2$, or a 5-10 membered aryl or heteroaryl; C_1-C_6 alkoxyalkyl; or C_1-C_6 thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or $-NH_2$, and provided that no more than two of R_1 , R_2 and R_3 are H.

5

40. The pharmaceutical composition according to claim 39, wherein the sterically hindered primary amine is cyclohexylamine or 2-methylbutylamine.

41. The pharmaceutical composition according to claim 30, wherein the sterically hindered primary amine is chitosan.

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42. The pharmaceutical composition according to any one of claims 30-41, wherein the pharmaceutical composition further comprises Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} .

43. The pharmaceutical composition according to claim 42, wherein the Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium acetate, magnesium chloride, magnesium phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium phosphate, calcium carbonate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate, manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum phosphate or aluminum sulfate.

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44. The pharmaceutical composition according to claim 43, wherein Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium chloride, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, manganese chloride, potassium chloride, sodium chloride or aluminum chloride.

25

45. The pharmaceutical composition according to claim 42, wherein the Mg^{2+} , Ca^{2+} or Zn^{2+} is provided as magnesium chloride, calcium chloride or zinc acetate.

30

46. The pharmaceutical composition according to claim 42, wherein the pharmaceutical composition further comprises Ca^{2+} .

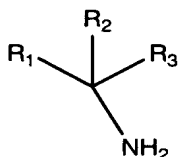
47. The pharmaceutical composition according to claim 46, wherein the Ca^{2+} is provided as calcium chloride.
48. The pharmaceutical composition according to any one of claims 21-47, further
5 comprising an antioxidant.
49. The pharmaceutical composition according to claim 48, wherein the antioxidant is BHA, vitamin E or propyl gallate.
- 10 50. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, therapeutic polypeptide, a cation selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} and a sterically hindered primary amine.
- 15 51. The pharmaceutical composition according to claim 50 further comprising a pharmaceutically acceptable binder.
52. The pharmaceutical composition according to claim 50 or 51 further comprising a pharmaceutically acceptable glidant, lubricant or additive that acts as both a glidant and
20 lubricant.
53. The pharmaceutical composition according to any one of claims 50-52 wherein the sterically hindered primary amine is an amino acid.
54. The pharmaceutical composition according to claim 53 wherein the amino acid is a
25 naturally-occurring amino acid.
55. The pharmaceutical composition according to claim 54 wherein the naturally-occurring amino acid is histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, asparagine, tyrosine, threonine, isoleucine, tryptophan,
30 methionine, glycine or valine.
56. The pharmaceutical composition according to claim 55 wherein the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine.

57. The pharmaceutical composition of claim 56 wherein the naturally-occurring amino acid is leucine.

58. The pharmaceutical composition according to claim 50, wherein the sterically hindered primary amine is a non-naturally occurring amino acid.

59. The pharmaceutical composition according to claim 58, wherein the non-naturally occurring amino acid is 1-aminocyclohexane carboxylic acid.

60. The pharmaceutical composition according to claim 50, wherein the sterically



hindered primary amine has the formula: $\text{C}(\text{R}_1)(\text{R}_2)(\text{R}_3)\text{NH}_2$, wherein R₁, R₂ and R₃ are independently selected from: H; -C(O)OH; C₁-C₆ alkyl, optionally substituted by -CO₂H, -CONH₂, or a 5-10 membered aryl or heteroaryl; C₁-C₆ alkoxyalkyl; or C₁-C₆ thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or -NH₂, and provided that no more than two of R₁, R₂ and R₃ are H.

61. The pharmaceutical composition according to claim 60, wherein the sterically hindered primary amine is cyclohexylamine or 2-methylbutylamine.

62. The pharmaceutical composition according to claim 50, wherein the sterically hindered primary amine is chitosan.

63. The pharmaceutical composition according to any one of claims 50-62 further comprising an antioxidant.

64. The pharmaceutical composition according to claim 63, wherein the antioxidant is BHA, vitamin E or propyl gallate.

65. The pharmaceutical composition according to any of claims 51-64 wherein the pharmaceutically acceptable binder is selected from polyvinyl alcohol, povidone, a starch, maltodextrin or a cellulose ether.

66. The pharmaceutical composition of claim 65 wherein the pharmaceutically acceptable binder is a cellulose ether.
- 5 67. The pharmaceutical composition of claim 66 wherein the cellulose ether is selected from: methyl cellulose, hydroxyethyl cellulose, hydroxyethyl methyl cellulose and hydroxypropyl methyl cellulose.
68. The pharmaceutical composition of any of claims 50-67, further comprising a
10 pharmaceutically acceptable filler.
69. The pharmaceutical composition according to claim 68, wherein the pharmaceutically acceptable filler is cellulose, isomalt, mannitol or dibasic calcium phosphate.
- 15 70. The pharmaceutical composition of claim 69 wherein the cellulose is selected from microfine cellulose and microcrystalline cellulose.
71. The pharmaceutical composition of any of claims 68-70, wherein the pharmaceutically acceptable filler comprises particles having an average diameter between
20 150 μm and 1000 μm .
72. The pharmaceutical composition according to any of claims 50-71, wherein the Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium acetate, magnesium chloride, magnesium phosphate, magnesium sulfate, calcium acetate, calcium chloride, calcium
25 phosphate, calcium carbonate, calcium sulfate, zinc acetate, zinc chloride, zinc phosphate, zinc sulfate, manganese acetate, manganese chloride, manganese phosphate, manganese sulfate, potassium acetate, potassium chloride, potassium phosphate, potassium sulfate, sodium acetate, sodium chloride, sodium phosphate, sodium sulfate, aluminum acetate, aluminum chloride, aluminum phosphate or aluminum sulfate.
30
73. The pharmaceutical composition according to claim 72, wherein Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+ or Al^{3+} is provided as magnesium chloride, calcium chloride, calcium phosphate, calcium sulfate, zinc acetate, manganese chloride, potassium chloride, sodium chloride or aluminum chloride.

74. The pharmaceutical composition of claim 73, wherein Mg^{2+} , Ca^{2+} , or Zn^{2+} is provided as magnesium chloride, calcium chloride or zinc acetate.
- 5
75. The pharmaceutical composition of any of claims 50-71 wherein the cation is Ca^{2+} .
76. The pharmaceutical composition according to claim 75, wherein the cation is provided as calcium chloride.
- 10
77. The pharmaceutical composition of either of claims 75 or 76 wherein the sterically hindered primary amine is leucine.
78. The pharmaceutical composition of claim 77 wherein the molar ratio of Ca^{2+} to leucine is at least 1:1.
- 15
79. The pharmaceutical composition of claim 78 wherein the molar ratio of Ca^{2+} to leucine is at least 1.5:1.
- 20
80. The pharmaceutical composition of claim 79, wherein the molar ratio of Ca^{2+} to leucine is at least 2:1.
81. The pharmaceutical composition of any of claims 50-80 wherein the sterically hindered amine is leucine and the molar ratio of leucine to therapeutic polypeptide is at least
- 25 10:1.
82. The pharmaceutical composition of claim 81 wherein the molar ratio of leucine to therapeutic polypeptide is at least 20:1.
- 30
83. The pharmaceutical composition of claim 82 wherein the molar ratio of leucine to therapeutic polypeptide is at least 30:1.

84. The pharmaceutical composition of any of claims 50-83, wherein pharmaceutical composition comprises a filler and the weight ratio of therapeutic polypeptide to pharmaceutically acceptable filler is between 1:25 and 1:2,500.
- 5 85. The pharmaceutical composition according to claim 84, wherein the weight ratio of therapeutic polypeptide to pharmaceutically acceptable filler is between 1:100 and 1:2000.
86. The pharmaceutical composition according to claim 85, wherein the weight ratio of therapeutic polypeptide to pharmaceutically acceptable filler is between 1:100 and 1:1000.
- 10 87. The pharmaceutical composition according to any one of claims 50-86, wherein the molar ratio of cation:sterically hindered primary amine:therapeutic polypeptide is 40-100:20-50:1.
- 15 88. The pharmaceutical composition according to claim 87, wherein the cation is Ca^{2+} .
89. The pharmaceutical composition according to claim 88, wherein the sterically hindered primary amine is leucine.
- 20 90. The pharmaceutical composition according to claim 89, wherein the molar ratio of Ca^{2+} :leucine:therapeutic polypeptide is 100:30:1, 80:40:1, 80:30:1, 80:20:1, 60:30:1, 60:20:1, 50:30:1, 50:20:1, 40:20:1, 20:20:1, 10:10:1, 10:5:1, 5:10:1 or 5:5:1.
- 25 91. The pharmaceutical composition according to claim 90, wherein the molar ratio of Ca^{2+} :leucine:therapeutic polypeptide is 60:30:1.
92. The pharmaceutical composition according to any one of claims 88-91, wherein the cation is provided as CaCl_2 .
- 30 93. A capsule or tablet comprising the pharmaceutical composition according to any one of claims 50-92.
94. The capsule or tablet according to claim 93, wherein each capsule or tablet comprises 25 μg to 1 g therapeutic polypeptide.

95. The capsule or tablet according to claim 94, wherein each capsule or tablet comprises 100 μg to 500 mg therapeutic polypeptide.
- 5 96. A method for preparing a pharmaceutical composition comprising therapeutic polypeptide or a salt thereof, the method comprising:
- (a) providing an aqueous solution comprising:
 - (i) a therapeutic polypeptide or a pharmaceutically acceptable salt thereof
 - (ii) one or more of a cation selected from Mg^{2+} , Ca^{2+} , Zn^{2+} , Mn^{2+} , K^+ , Na^+
10 or Al^{3+} and a sterically hindered primary amine; and
 - (iii) a pharmaceutically acceptable binder; and
 - (b) applying the aqueous solution to a pharmaceutically acceptable filler to generate therapeutic polypeptide-coated filler.
- 15 97. The method of claim 96, wherein the aqueous solution comprises a cation.
98. The method of claim 96, wherein the aqueous solution comprises a sterically hindered primary amine.
- 20 99. The method of claim 96, wherein the aqueous solution comprises a cation and a sterically hindered primary amine.
100. The method of any one of claims 96-99, wherein the aqueous solution further comprises an antioxidant.
- 25 101. The method of claim 100 wherein the antioxidant is BHA, BHT, vitamin E, propyl gallate, ascorbic acid and salts or esters thereof, tocopherol and esters thereof, alpha-lipoic acid or beta-carotene.
- 30 102. The method of claim 101 wherein the antioxidant is BHA.
103. The method of claim 98, wherein the sterically hindered primary amine is an amino acid.

104. The method of claim 103, wherein the amino acid is a naturally-occurring amino acid.

105. The method of claim 104, wherein the naturally-occurring amino acid is selected from histidine, phenylalanine, alanine, glutamic acid, aspartic acid, glutamine, leucine, methionine, 5 asparagine, tyrosine, threonine, isoleucine, tryptophan, methionine, glycine, or valine.

106. The method of claim 105, wherein the naturally-occurring amino acid is leucine, isoleucine, alanine or methionine.

107. The method of claim 106, wherein the naturally-occurring amino acid is leucine or methionine.

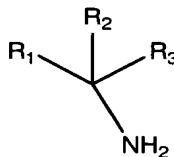
108. The method of claim 107, wherein the naturally-occurring amino acid is leucine.

109. The pharmaceutical composition according to claim 98, wherein the sterically hindered primary amine is a non-naturally occurring amino acid.

110. The pharmaceutical composition according to claim 109, wherein the non-naturally occurring amino acid is 1-aminocyclohexane carboxylic acid.

20

111. The pharmaceutical composition according to claim 98, wherein the sterically



hindered primary amine has the formula: $\begin{array}{c} R_2 \\ | \\ R_1 - C - R_3 \\ | \\ NH_2 \end{array}$, wherein R_1 , R_2 and R_3 are independently selected from: H; $-C(O)OH$; C_1-C_6 alkyl, optionally substituted by $-CO_2H$, $-CONH_2$, or a 5-10 membered aryl or heteroaryl; C_1-C_6 alkoxyalkyl; or C_1-C_6 25 thioalkoxyalkyl, wherein any of the alkyl or aryl groups above can be singly or multiply substituted with halogen or $-NH_2$, and provided that no more than two of R_1 , R_2 and R_3 are H.

112. The pharmaceutical composition according to claim 111, wherein the sterically hindered primary amine is cyclohexylamine or 2-methylbutylamine.

30

113. The pharmaceutical composition according to claim 98, wherein the sterically hindered primary amine is chitosan.

5 114. The method of any one of claims 96-113, wherein the aqueous solution further comprises Ca^{2+} .

115. The method of claim 114, wherein the Ca^{2+} is provided as CaCl_2 .

10 116. The method of any one of claims 103-115, wherein the aqueous solution further comprises an antioxidant.

117. The method of claim 116, wherein the antioxidant is BHA.

15 118. The method of any one of claims 96-117, wherein the binder is selected from polyvinyl alcohol, a starch, maltodextrin or a cellulose ether.

20 119. The method of claim 118, wherein the binder is a cellulose ether selected from methyl cellulose, hydroxyethyl cellulose, hydroxyethyl methyl cellulose or hydroxypropyl methyl cellulose.

120. The method of any one of claims 96-119, wherein the filler is selected from cellulose, isomalt, mannitol or dibasic calcium phosphate.

25 121. The method of claim 120, wherein the filler is microfine cellulose or microcrystalline cellulose.

122. The method of any one of claims 96-121, wherein the aqueous solution is applied to the filler by spraying.

30 123. The method of any one of claims 96-122, wherein the weight ratio of therapeutic polypeptide to pharmaceutically acceptable filler is between 1:100 and 1:2500.

124. The method according to claim 123, wherein the weight ratio of therapeutic polypeptide to pharmaceutically acceptable filler is between 1:100 and 1:1000.

125. The method according to any one of claims 96-124, wherein the aqueous solution comprises a cation and a sterically hindered primary amine, and the molar ratio of cation:sterically hindered primary amine:therapeutic polypeptide is 40-100:20-30:1.

5

126. The method according to claim 125, wherein the cation is Ca^{2+} .

127. The method according to claim 125, wherein the sterically hindered primary amine is leucine.

10

128. The method according to claim 125, wherein the cation is Ca^{2+} and the sterically hindered primary amine is leucine, and the molar ratio of Ca^{2+} :leucine:therapeutic polypeptide is 100:30:1, 80:40:1, 80:30:1, 80:20:1, 60:30:1, 60:20:1, 50:30:1, 50:20:1, 40:20:1, 20:20:1, 10:10:1, 10:5:1 or 5:5:1.

15

129. The method according to claim 128, wherein the molar ratio of Ca^{2+} :leucine:therapeutic polypeptide is 60:30:1.

130. The method according to any one of claims 123-129, wherein the pharmaceutically acceptable filler is selected from cellulose, isomalt, mannitol or dibasic calcium phosphate.

20

131. The method according to claim 130, wherein the pharmaceutically acceptable filler is microfine cellulose or microcrystalline cellulose.

132. The method according to any one of claims 123-131, wherein the pharmaceutically acceptable binder is polyvinyl alcohol, a starch, maltodextrin or a cellulose ether.

25

133. The method according to claim 132, wherein the pharmaceutically acceptable binder is a cellulose ether selected from methyl cellulose, hydroxyethyl cellulose, hydroxyethyl methyl cellulose or hydroxypropyl methyl cellulose.

30

134. The method according to any one of claims 96-133, wherein the therapeutic polypeptide-coated filler is mixed with one or more pharmaceutically acceptable additives.

135. The method according to any one of claims 96-134, further comprising tableting or encapsulating the therapeutic polypeptide-coated filler in a tablet or capsule, respectively.
136. The method according to claim 135, wherein the therapeutic polypeptide-coated filler
5 is encapsulated in a capsule.
137. The method according to claim 136, wherein the capsule is a gelatin capsule.
138. The method according to either of claims 136 or 137, wherein each capsule contains
10 25 µg to 1 g therapeutic polypeptide.
139. The method according to claim 138, wherein each capsule contains 100 µg to 500 mg therapeutic polypeptide.
- 15 140. The method according to claim 139, wherein each capsule contains 200 µg to 100 mg therapeutic polypeptide.
141. A pharmaceutical composition comprising therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the chromatographic purity of the therapeutic
20 polypeptide is greater than or equal to 90% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.
- 25 142. The pharmaceutical composition according to claim 141, wherein the chromatographic purity of the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95% or 96% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed
30 container containing a desiccant.
143. A unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the chromatographic purity of the therapeutic polypeptide is greater than or equal to 90% after (a) 18 months of storage

of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

5 144. The unit dosage form according to claim 143, wherein the chromatographic purity of the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95% or 96% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

10 145. A sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide and a pharmaceutically acceptable excipient wherein the chromatographic purity of the therapeutic polypeptide is greater than or equal to 90% after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 15 60% relative humidity or (b) 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

146. The sealed container according to claim 145, wherein the chromatographic purity of the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95% or 96% 20 after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

147. A pharmaceutical composition comprising therapeutic polypeptide and a 25 pharmaceutically acceptable excipient, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis is greater than or equal to 90% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

30 148. The pharmaceutical composition according to claim 147, wherein the assay value for the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% after (a) 18 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of

storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

149. A unit dosage form of a pharmaceutical composition comprising therapeutic polypeptide and a pharmaceutically acceptable excipient, wherein the assay value for therapeutic polypeptide determined on a weight/weight basis is greater than or equal to 90% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

150. The unit dosage form according to claim 149, wherein the assay value for the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% after (a) 18 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

151. A sealed container comprising a plurality of unit dosage forms of a pharmaceutical composition comprising therapeutic polypeptide and a pharmaceutically acceptable excipient wherein the assay value for therapeutic polypeptide in the unit dosage forms determined on a weight/weight basis is greater than or equal to 90% after (a) 18 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage the sealed container containing a desiccant at 40°C at 75% relative humidity.

152. The sealed container according to claim 151, wherein the assay value for the therapeutic polypeptide is greater than or equal to 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% after (a) 18 months of storage the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) 6 months of storage the sealed container containing a desiccant at 40°C at 75% relative humidity.

152. The pharmaceutical composition according to either of claims 141 or 142, wherein the chromatographic purity of the therapeutic polypeptide is greater than 90% after (a) 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

153. The unit dosage form according to either of claims 143 or 144, wherein the chromatographic purity of the therapeutic polypeptide is greater than 90% after (a) 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

154. The sealed container according to either of claims 145 or 146, wherein the chromatographic purity of the therapeutic polypeptide is greater than 90% after (a) a first 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) a first 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

155. The pharmaceutical composition according to either of claims 147 or 148, wherein the assay value of the therapeutic polypeptide is greater than 90% after (a) a first 24 months of storage of the pharmaceutical composition at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) a first 6 months of storage of the pharmaceutical composition at 40°C at 75% relative humidity in a sealed container containing a desiccant.

156. The unit dosage form according to either of claims 149 or 150, wherein the assay value of the therapeutic polypeptide is greater than 90% after (a) a first 24 months of storage of the unit dosage form at 25°C at 60% relative humidity in a sealed container containing a desiccant or (b) a first 6 months of storage of the unit dosage form at 40°C at 75% relative humidity in a sealed container containing a desiccant.

157. The sealed container according to either of claims 151 or 152, wherein the assay value of the therapeutic polypeptide is greater than 90% after (a) a first 24 months of storage of the sealed container containing a desiccant at 25°C at 60% relative humidity or (b) a first 6 months of storage of the sealed container containing a desiccant at 40°C at 75% relative humidity.

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(54) Title: GUANYLATE CYCLASE RECEPTOR AGONISTS FOR THE TREATMENT OF TISSUE INFLAMMATION AND CARCINOGENESIS

(57) Abstract: A method of treatment of inflamed, pre-cancerous or cancerous tissue or polyps in a mammalian subject is disclosed. The treatment involves administration of a composition of at least one peptide agonist of a guanylate cyclase receptor and/or other small molecules that enhance intracellular production of cGMP. The at least one peptide agonist of a guanylate cyclase receptor may be administered either alone or in combination with an inhibitor of cGMP-dependent phosphodiesterase. The inhibitor may be a small molecule, peptide, protein or other compound that inhibits the degradation of cGMP. Without requiring a particular mechanism of action, this treatment may restore a healthy balance between proliferation and apoptosis in the subject's population of epithelial cells, and also suppress carcinogenesis. Thus, the method may be used to treat, <i>inter alia</i>, inflammation, including gastrointestinal inflammatory disorders, general organ inflammation and asthma, and carcinogenesis of the lung, gastrointestinal tract, bladder, testis, prostate and pancreas, or polyps.

Guanylate Cyclase Receptor Agonists for the Treatment of Tissue Inflammation and Carcinogenesis

Cross Reference to Related Applications

5 The present application claims the benefit of U.S. provisional application nos. 60/279,438, filed on March 29, 2001; 60/279,437, filed on March 29, 2001; 60/300,850, filed on June 27, 2001; 60/303,806, filed on July 10, 2001; 60/307,358, filed on July 25, 2001; and 60/348,646, filed on January 17, 2002.

10 Field of the Invention

 The present invention relates to the therapeutic use of guanylate cyclase receptor agonists as a means for enhancing the intracellular production of cGMP. The agonists may be used either alone or in combination with inhibitors of cGMP-specific phosphodiesterase to prevent or treat cancerous, pre-cancerous and metastatic growths, particularly in the
15 gastrointestinal tract and lungs. In addition, the agonists may be used in the treatment of inflammatory disorders such as ulcerative colitis and asthma.

Background of the Invention

 Uroguanylin, guanylin and bacterial ST peptides are structurally related peptides that
20 bind to a guanylate cyclase receptor and stimulate intracellular production of cyclic guanosine monophosphate (cGMP) (1-6). This results in the activation of the cystic fibrosis transmembrane conductance regulator (CFTR), an apical membrane channel for efflux of chloride from enterocytes lining the intestinal tract (1-6). Activation of CFTR and the
25 subsequent enhancement of transepithelial secretion of chloride leads to stimulation of sodium and water secretion into the intestinal lumen. Therefore, by serving as paracrine regulators of CFTR activity, cGMP receptor agonists regulate fluid and electrolyte transport in the GI tract (1-6; US patent 5,489,670).

 The process of epithelial renewal involves the proliferation, migration, differentiation,
30 senescence, and eventual loss of GI cells in the lumen (7,8). The GI mucosa can be divided into three distinct zones based on the proliferation index of epithelial cells. One of these zones, the proliferative zone, consists of undifferentiated stem cells responsible for providing a constant source of new cells. The stem cells migrate upward toward the lumen to which they

are extruded. As they migrate, the cells lose their capacity to divide and become differentiated for carrying out specialized functions of the GI mucosa (9). Renewal of GI mucosa is very rapid with complete turnover occurring within a 24-48 hour period (9). During this process mutated and unwanted cells are replenished with new cells. Hence, homeostasis of the GI mucosa is regulated by continual maintenance of the balance between proliferation and apoptotic rates (8).

The rates of cell proliferation and apoptosis in the gut epithelium can be increased or decreased in a wide variety of different circumstances, *e.g.*, in response to physiological stimuli such as aging, inflammatory signals, hormones, peptides, growth factors, chemicals and dietary habits. In addition, an enhanced proliferation rate is frequently associated with a reduction in turnover time and an expansion of the proliferative zone (10). The proliferation index has been observed to be much higher in pathological cases of ulcerative colitis and other GI disorders (11). Thus, intestinal hyperplasia is the major promoter of gastrointestinal inflammation and carcinogenesis.

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In addition to a role for uroguanylin and guanylin as modulators of intestinal fluid and ion secretion, these peptides may also be involved in the continual renewal of GI mucosa. Previously published data in WO 01/25266 suggests a peptide with the active domain of uroguanylin may function as an inhibitor of polyp development in the colon and may constitute a treatment of colon cancer. However, the mechanism by which this is claimed to occur is questionable in that WO 01/25266 teaches uroguanylin agonist peptides that bind specifically to a guanylate cyclase receptor, termed GC-C, that was first described as the receptor for *E. coli* heat-stable enterotoxin (ST) (4). Knockout mice lacking this guanylate cyclase receptor show resistance to ST in intestine, but effects of uroguanylin and ST are not disturbed in the kidney *in vivo* (3). These results were further supported by the fact that membrane depolarization induced by guanylin was blocked by genistein, a tyrosine kinase inhibitor, whereas hyperpolarization induced by uroguanylin was not effected (12,13). Taken together these data suggest that uroguanylin also binds to a currently unknown receptor, which is distinct from GC-C.

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Other papers have reported that production of uroguanylin and guanylin is dramatically decreased in pre-cancerous colon polyps and tumor tissues (14-17). In addition, genes for both uroguanylin and guanylin have been shown to be localized to regions of the genome frequently

associated with loss of heterozygosity in human colon carcinoma (18-20). Taken together, these findings indicate that uroguanylin, guanylin and other peptides with similar activity may be used in the prevention or treatment of abnormal colon growths. This proposal is bolstered by a recent study demonstrating oral administration of uroguanylin inhibits polyp formation in
5 mice (15,16).

Uroguanylin and guanylin peptides also appear to promote apoptosis by controlling cellular ion flux. Alterations in apoptosis have been associated with tumor progression to the metastatic phenotype. While a primary gastrointestinal (GI) cancer is limited to the small
10 intestine, colon, and rectum, it may metastasize and spread to such localities as bone, lymph nodes, liver, lung, peritoneum, ovaries, brain. By enhancing the efflux of K^+ and influx of Ca^{++} , uroguanylin and related peptides may promote the death of transformed cells and thereby inhibit metastasis.

One of the clinical manifestations of reduced CFTR activity is the inflammation of
15 airway passages (21). This effect may be due to CFTR regulating the expression of NF-kB, chemokines and cytokines (22-25). Recent reports have also suggested that the CFTR channel is involved in the transport and maintenance of reduced glutathione, an antioxidant that plays an important role in protecting against inflammation caused by oxidative stress (39).
20 Enhancement of intracellular levels of cGMP by way of guanylate cyclase activation or by way of inhibition of cGMP-specific phosphodiesterase would be expected to down-regulate these inflammatory stimuli. Thus, uroguanylin-type agonists should be useful in the prevention and treatment of inflammatory diseases of the lung (*e.g.*, asthma), bowel (*e.g.*, ulcerative colitis and Crohn's disease), pancreas and other organs.

25 Overall, it may be concluded that agonists of guanylate cyclase receptor such as uroguanylin have potential therapeutic value in the treatment of a wide variety of inflammatory conditions, cancer (particularly colon cancer) and as anti-metastatic agents. The development of new agonists is therefore of substantial clinical importance.

30

Summary of the Invention

The present invention is based upon the development of new agonists of guanylate cyclase receptor, and new uses of naturally occurring agonists. The agonists are analogs of

uroguanylin, many of which have superior properties either in terms of improved receptor activation, stability, activity at low pH or reduced adverse effects. The peptides may be used to treat any condition that responds to enhanced intracellular levels of cGMP. Intracellular levels of cGMP can be increased by enhancing intracellular production of cGMP and/or by inhibition of its degradation by cGMP-specific phosphodiesterases. Among the specific conditions that can be treated or prevented are inflammatory conditions, cancer, polyps, and metastasis.

In its first aspect, the present invention is directed to a peptide consisting essentially of the amino acid sequence of any one of SEQ ID NOs:2-21 and to therapeutic compositions which contain these peptides. The term “consisting essentially of” includes peptides that are identical to a recited sequence identification number and other sequences that do not differ substantially in terms of either structure or function. For the purpose of the present application, a peptide differs substantially if its structure varies by more than three amino acids from a peptide of SEQ ID NOs:2-21 or if its activation of cellular cGMP production is reduced or enhanced by more than 50%. Preferably, substantially similar peptides should differ by no more than two amino acids and not differ by more than about 25% with respect to activating cGMP production. The most preferred peptide is a bicycle having the sequence of SEQ ID NO:20.

The peptides may be in a pharmaceutical composition in unit dose form, together with one or more pharmaceutically acceptable excipients. The term “unit dose form” refers to a single drug delivery entity, *e.g.*, a tablet, capsule, solution or inhalation formulation. The amount of peptide present should be sufficient to have a positive therapeutic effect when administered to a patient (typically, between 100 µg and 3 g). What constitutes a “positive therapeutic effect” will depend upon the particular condition being treated and will include any significant improvement in a condition readily recognized by one of skill in the art. For example, it may constitute a reduction in inflammation, a shrinkage of polyps or tumors, a reduction in metastatic lesions, etc.

The invention also encompasses combination therapy utilizing a guanylate cyclase receptor agonist administered either alone or together with an inhibitor of cGMP-dependent phosphodiesterase, an anti-inflammatory agent or an anticancer agent. These agents should be present in amounts known in the art to be therapeutically effective when administered to a

patient. Anti-neoplastic agents may include alkylating agents, epipodophyllotoxins, nitrosoureas, antimetabolites, vinca alkaloids, anthracycline antibiotics, nitrogen mustard agents, and the like. Particular anti-neoplastic agents may include tamoxifen, taxol, etoposide and 5-fluorouracil. Antiviral and monoclonal antibody therapies may be combined with
5 chemotherapeutic compositions comprising at least one guanylate cyclase receptor agonist in devising a treatment regimen tailored to a patient's specific needs.

In another aspect, the invention is directed to a method for preventing, treating or retarding the onset of cancer, particularly cancer of epithelial cells, or polyps in a subject by
10 administering a composition comprising an effective amount of a guanylate cyclase receptor agonist, preferably a synthetic guanylate cyclase receptor agonist. The term "effective amount" refers to sufficient agonist to measurably increase intracellular levels of cGMP. The term "synthetic" refers to a peptide created to bind a guanylate cyclase receptor, but containing certain amino acid sequence substitutions not present in known endogenous guanylate cyclase
15 agonists, such as uroguanylin. The agonist should be a peptide selected from those defined by SEQ ID NOs:2-21 and which are listed in Tables 2 and 3. Also included in the invention are methods of treating primary cancers, other than primary colon cancer, by administering an effective dosage of a peptide selected from the group consisting of: uroguanylin; guanylin; and *E. coli* ST peptide. Any known form of uroguanylin or guanylin can be used for this purpose,
20 although the human peptides are preferred.

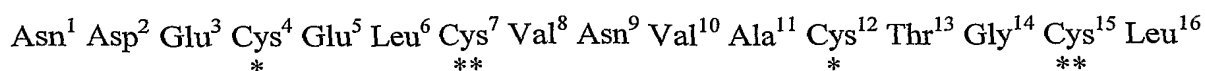
The invention also includes methods of preventing or treating tumor metastasis from a primary tumor mass. Metastatic tumor cells having guanylate cyclase receptors may be targeted by peptides generated according to the invention. In a preferred embodiment, the
25 targeted receptor is found on cells of gastrointestinal (GI) cancers and on metastasized cells derived from those cancers. Such receptors are typically transmembrane proteins with an extracellular ligand-binding domain, a membrane-spanning domain, and an intracellular domain with guanylate cyclase activity. Although the invention is not bound by any particular mechanism of action, it is believed that the peptides will act by binding to these cellular
30 receptors and inducing apoptosis. Metastatic tumors may also be treated by administering any known form of uroguanylin or guanylin (preferably human) or by administering *E. coli* ST peptide.

Peptides may be administered either alone or together with one or more inhibitors of cGMP dependent phosphodiesterase. Examples of cGMP dependent phosphodiesterase inhibitors include suldinac sulfone, zaprinast, and motapizone. Treatable forms of cancer include breast cancer, colorectal cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, and testicular cancer. Colon carcinogenesis may be prevented by inhibiting pre-cancerous colorectal polyp development via administration of a composition according to the invention. It is believed that the peptides should be especially effective with respect to the treatment of colon cancer and in preventing the metastasis of colon tumors.

In another aspect, the invention is directed to a method for treating, preventing, or retarding the onset of organ inflammation (*e.g.*, inflammation associated with the GI tract, asthma, nephritis, hepatitis, pancreatitis, bronchitis, or cystic fibrosis) of a subject by administering a composition comprising an agonist of a guanylate cyclase receptor that enhances intracellular production of cGMP. Preferred peptide agonists are selected from the group defined by SEQ ID NOs:2-21 shown in Tables 2 and 3, or uroguanylin, or guanylin, or *E. coli* ST peptide. These peptides may optionally be administered with one or more inhibitors of cGMP dependent phosphodiesterase, *e.g.*, suldinac sulfone, zaprinast, or motapizone. In a preferred embodiment, the invention is directed to a method of treating an inflammatory disorder in a mammalian gastrointestinal tract. The inflammatory disorder may be classified as an inflammatory bowel disease, and more particularly may be Crohn's disease or ulcerative colitis. Administration may be enteric, and employ formulations tailored to target enterocytes.

In a broader sense, the invention includes methods of inducing apoptosis in a patient by administering an effective amount of a peptide having the sequence of any one of SEQ ID NO:2 - SEQ ID NO:21, or uroguanylin, or guanylin or *E. coli* ST peptide. An "effective amount" of peptide, in this sense, refers to an amount sufficient to increase apoptosis in a target tissue. For example, sufficient peptide may be given to induce an increased rate of cell death in a neoplastic growth.

The most preferred peptide for use in the methods described above is the peptide defined by SEQ ID NO:20. The sequence is as follows (see also Table 3):



and wherein there is one disulfide linkage between the cysteine at position 4 and the cysteine at position 12; and a second disulfide linkage between the cysteine at position 7 and the cysteine at position 15 (SEQ ID NO:20). This peptide has been found to have enhanced biological activity as an agonist of cGMP production due to its enhanced binding constant for the guanylate cyclase receptor, and is superior to uroguanylin with regard to temperature and protease stability and with regard to its biological activity at the physiologically favorable pH range (pH 6 to 7) in the large intestine.

The guanylate cyclase receptor agonists used in the methods described above may be administered either orally, systemically or locally. Dosage forms include preparations for inhalation or injection, solutions, suspensions, emulsions, tablets, capsules, topical salves and lotions, transdermal compositions, other known peptide formulations and pegylated peptide analogs. An effective dosage of the composition will typically be between about 1 μ g and about 10 mg per kilogram body weight, preferably between about 10 μ g to 5 mg of the compound per kilogram body weight. Adjustments in dosage will be made using methods that are routine in the art and will be based upon the particular composition being used and clinical considerations. Agonists may be administered as either the sole active agent or in combination with other drugs, *e.g.*, an inhibitor of cGMP-dependent phosphodiesterase. In all cases, additional drugs should be administered at a dosage that is therapeutically effective using the existing art as a guide. Drugs may be administered in a single composition or sequentially.

Detailed Description of the Invention

The present invention is based upon several concepts. The first is that there is a cGMP-dependent mechanism which regulates the balance between cellular proliferation and apoptosis and that a reduction in cGMP levels, due to a deficiency of uroguanylin/guanylin and/or due to the activation of cGMP-specific phosphodiesterases, is an early and critical step in neoplastic transformation. A second concept is that the release of arachidonic acid from membrane phospholipids, which leads to the activation of cPLA₂, COX-2 and possibly 5-lipoxygenase during the process of inflammation, is down-regulated by a cGMP-dependent mechanism, leading to reduced levels of prostaglandins and leukotrienes, and that increasing intracellular levels of cGMP may therefore produce an anti-inflammatory response. In addition, a cGMP-dependent mechanism, is thought to be involved in the control of proinflammatory processes. Therefore, elevating intracellular levels of cGMP may be used as a means of treating and

controlling inflammatory bowel diseases such as ulcerative colitis and Crohn's disease and other organ inflammation (e.g., associated with asthma, nephritis, hepatitis, pancreatitis, bronchitis, cystic fibrosis).

5 Without intending to be bound by any theory, it is envisioned that ion transport across the plasma membrane may prove to be an important regulator of the balance between cell proliferation and apoptosis that will be affected by compositions altering cGMP concentrations. Uroguanylin has been shown to stimulate K^+ efflux, Ca^{++} influx and water transport in the gastrointestinal tract (3). Moreover, atrial natriuretic peptide (ANP), a peptide
10 that also binds to a specific guanylate cyclase receptor, has also been shown to induce apoptosis in rat mesangial cells, and to induce apoptosis in cardiac myocytes by a cGMP mechanism (26-29). It is believed that binding of the present agonists to a guanylate cyclase receptor stimulates production of cGMP. This ligand-receptor interaction, via activation of a cascade of cGMP-dependent protein kinases and CFTR, is then expected to induce apoptosis in
15 target cells. Therefore, administration of the novel peptides defined by SEQ ID NOs:2-21, as shown in Tables 2 and 3, or uroguanylin, or guanylin or *E. coli* ST peptide is expected to eliminate or, at least retard, the onset of inflammatory diseases of the GI tract and general organ inflammation (e.g., asthma, nephritis, hepatitis, pancreatitis, bronchitis, cystic fibrosis).

20 In another aspect, the invention is directed to a method for preventing, treating or retarding the onset of cancer, particularly cancer of epithelial cells, in a subject by administering a composition comprising an effective amount of a guanylate cyclase receptor agonist, preferably a synthetic a guanylate cyclase receptor agonist. The term "effective amount" refers to sufficient agonist to measurably increase intracellular levels of cGMP. The
25 term "synthetic" refers to a peptide created to bind a guanylate cyclase receptor, but containing certain amino acid sequence substitutions not present in known endogenous guanylate cyclase agonists, such as uroguanylin. The agonist should be a peptide selected from those defined by SEQ ID NOs:2-21 and which are listed in Tables 2 and 3. Also included in the invention are methods of treating primary and metastatic cancers, other than primary colon cancer, by
30 administering an effective dosage of a peptide selected from the group consisting of: uroguanylin; guanylin; and *E. coli* ST peptide. Any known form of uroguanylin or guanylin can be used for this purpose, although the human peptides are preferred.

The cGMP-dependent mechanism that regulates the balance between cellular proliferation and apoptosis in metastatic tumor cells may serve as a mechanism for targeting and treating metastatic tumors. The liver is the most common site of metastasis from a primary colorectal cancer. Toward later stages of disease, colorectal metastatic cells may also invade other parts of the body. It is important to note that metastatic cells originating from the primary site in the gastrointestinal tract typically continue to express guanylate cyclase receptors and therefore, these cells should be sensitive to apoptosis therapy mediated by intestinal guanylate cyclase receptors. Peptides having uroguanylin activity, when used either alone or in combination with specific inhibitors of cGMP-phosphodiesterase, also retard the onset of carcinogenesis in gut epithelium by restoring a healthy balance between cell proliferation and apoptosis via a cGMP-mediated mechanism.

As used herein, the term “guanylate cyclase receptor” refers to the class of guanylate cyclase receptors on any cell type to which the inventive agonist peptides or natural agonists described herein bind.

As used herein, the term “guanylate cyclase receptor-agonist” refers to peptides and/or other compounds that bind to a guanylate cyclase receptor and stimulate cGMP production. The term also includes all peptides that have amino acid sequences substantially equivalent to at least a portion of the binding domain comprising amino acid residues 3-15 of SEQ ID NO:1. This term also covers fragments and pro-peptides that bind to guanylate cyclase receptor and stimulate cGMP production. The term “substantially equivalent” refers to a peptide that has an amino acid sequence equivalent to that of the binding domain where certain residues may be deleted or replaced with other amino acids without impairing the peptide’s ability to bind to a guanylate cyclase receptor and stimulate cGMP production.

Strategy and design of novel guanylate cyclase receptor agonists

Uroguanylin is a peptide secreted by the goblet and other epithelial cells lining the gastrointestinal mucosa as pro-uroguanylin, a functionally inactive form. The human pro-peptide is subsequently converted to the functionally active 16 amino acid peptide set forth in SEQ ID NO:1 (human uroguanylin sequence, see Table 2) in the lumen of the intestine by endogenous proteases. Since uroguanylin is a heat-resistant, acid-resistant, and proteolysis-resistant peptide, oral or systemic administration of this peptide and/or other peptides similar to

the functionally active 16 amino acid peptide sequence of SEQ ID NO:1 may be effectively employed in treatment methods.

Peptides similar to, but distinct from, uroguanylin are described below, including some which produce superior cGMP enhancing properties and/or other beneficial characteristics (e.g., improved temperature stability, enhanced protease stability, or superior activity at preferred pH's) compared to previously known uroguanylin peptides. The peptides may be used to inhibit GI inflammation and for treating or preventing the onset of polyp formation associated with gut inflammation. Epithelial tissues susceptible to cancer cell formation may also be treated. The guanylate cyclase receptor agonists described have the amino acid sequences shown in Tables 2 and 3. The "binding domain" for agonist-receptor interaction includes the amino acid residues from 3-15 of SEQ ID NO:1.

Molecular modeling was applied to the design of novel guanylate cyclase receptor agonists using methods detailed in (30). It consisted of energy calculations for three compounds known to interact with guanylate cyclase receptors, namely for human uroguanylin, bicyclo [4,12; 7,15]Asn¹-Asp²-Asp³-Cys⁴-Glu⁵-Leu⁶-Cys⁷-Val⁸-Asn⁹-Val¹⁰-Ala¹¹-Cys¹²-Thr¹³-Gly¹⁴-Cys¹⁵-Leu¹⁶ (UG, SEQ ID NO:1); human guanylin, bicyclo [4,12; 7,15]Pro¹-Gly²-Thr³-Cys⁴-Glu⁵-Ile⁶-Cys⁷-Ala⁸-Tyr⁹-Ala¹⁰-Ala¹¹-Cys¹²-Thr¹³-Gly¹⁴-Cys¹⁵ (GU, SEQ ID NO:22); and *E. coli* small heat-stable enterotoxin, tricyclo [6,10; 7,15; 11-18] Asn¹-Ser²-Ser³-Asn⁴-Tyr⁵-Cys⁶-Cys⁷-Glu⁸-Leu⁹-Cys¹⁰-Cys¹¹-Asn¹²-Pro¹³-Ala¹⁴-Cys¹⁵-Thr¹⁶-Gly¹⁷-Cys¹⁸-Tyr¹⁹ (ST, SEQ ID NO:23). Geometrical comparisons of all possible low-energy conformations for these three compounds were used to reveal the common 3D structures that served as the "templates" for the bioactive conformation, i.e., for the conformation presumably adopted by GU, UG and ST during interaction with receptor. It allowed designing novel analogs with significantly increased conformational population of the bioactive conformation at the expense of other low-energy conformations by selecting individual substitutions for various amino acid residues.

Energy calculations were performed by use of build-up procedures (30). The ECEPP/2 potential field (31,32) was used assuming rigid valence geometry with planar *trans*-peptide bonds, including that for Pro¹³ in ST. The ω angle in Pro¹³ was allowed to vary. Aliphatic and

aromatic hydrogens were generally included in united atomic centers of CH_n type; H^α -atoms and amide hydrogens were described explicitly.

The main calculation scheme involved several successive steps. First, the sequences of the two monocyclic model fragments (three fragments for ST), *Ac-cyclo* ($\text{Cys}^i \dots \text{Cys}^j$)-NMe, were considered, where all residues except Cys, Gly and Pro were replaced by alanines; the *i* and *j* values corresponded to the sequences of GU, UG and ST. At this step, all possible combinations of local minima for the peptide backbone for each amino acid residue were considered, *i.e.*, the minima in the Ramachandran map of *E*, *F*, *C*, *D*, *A* and *A*^{*} types (according to the notation in (33)) for the Ala residue; of *E*^{*}, *F*^{*}, *C*^{*}, *D*^{*}, *A*, *E*, *F*, *C* *D* and *A*^{*} types for the Gly residue; and of *F*, *C* and *A* types for Pro. For each backbone conformation, one optimal possibility to close a cycle employing the parabolic potential functions, intrinsic to the ECEPP force field, was found by checking an energy profile of rotation around the dihedral angle χ_1 for the D-Cys residue.

Totally, as many as *ca.* 180,000 conformations for each of the cyclic moieties were considered. Then, the conformers satisfying the $E - E_{\min} < \Delta E = 15$ kcal/mol criterion and differing by more than 40° in at least one value of any backbone dihedral angle were selected (from *ca.* 3,000 to 8,000 conformations for different model fragments). At the next step, the selected conformations of the matching monocyclic fragments were overlapped to create possible conformations of the bicyclic model fragments (the tricyclic fragments in the case of ST). Typically, this procedure yielded *ca.* 20,000–30,000 conformations. All these conformations were submitted for a new cycle of energy calculations, which resulted in 191 conformations satisfying the $E - E_{\min} < \Delta E = 20$ kcal/mol criterion for the ST model fragment and in 6,965 conformations satisfying the same criterion for the GU/UG model fragment. After that, the missing side chains in the model fragments were restored, and energy calculations were performed again, the dihedral angle values of side chain groups (except the χ_1 angle for the Cys residues) and of the terminal groups of the backbone being optimized before energy minimization to achieve their most favorable spatial arrangements, employing an algorithm previously described (34). For the UG 4-15 fragment, 632 conformations satisfied the criterion of $\Delta E = 20$ kcal/mol; 164 of them satisfied the more stringent criterion of $\Delta E = 12$ kcal/mol, which corresponds to the accepted criterion of 1 kcal/mol/residue (30). Subsequent elongation

of the UG 4-15 fragment to 3-16, and then to the entire UG molecule was performed by the same build-up procedure. Finally, 31 backbone conformations of UG were found as satisfying the criterion of $\Delta E = 16$ kcal/mol.

5 Geometrical comparison of conformers was performed in the following manner. The best fit in the superposition for the atomic centers in a pair of conformers was assessed to check the level of geometrical similarity between the two conformers, according to (35). The criterion for geometrical similarity was the rms value, which was calculated for a pair of conformations A and B as follows:

$$10 \quad \text{rms} = (1/N) \sum_{i=1}^N [(x^A_i - x^B_i)^2 + (y^A_i - y^B_i)^2 + (z^A_i - z^B_i)^2]^{1/2},$$

where N is the number of the C $^\alpha$ -atom pairs chosen for superposition, and x, y and z are the Cartesian coordinates. By the criterion of geometrical similarity of $\text{rms} < 2.0$ Å, low-energy conformations of the rigid conformational fragment UG 4-15 fell into seven conformational families. One of them consists of the same six conformers that are similar both to 1UYA and 15 1ETN; this family contains also the lowest-energy conformer of UG. (1UYA and 1ETN are the experimentally defined 3D structures of UG and ST, respectively, which are known to possess high biological activity (36,37); the 3D structures were available in the Protein Data Bank.)

20 **Table 1.** The values of dihedral angles (in degrees) for peptide backbone in the “template” conformation of UG

Residue	Angle	Conformer's #					
		1	3	9	22	25	27
Cys ⁴	ψ	-37	-41	-40	-55	-38	-54
	ϕ	-71	-67	-72	-69	-68	-70
Glu ⁵	ψ	-50	-47	-48	-33	-43	-22
	ϕ	-86	-86	-85	-81	-88	-91
Leu ⁶	ψ	163	165	160	153	160	156
	ϕ	-79	-82	-79	-83	-79	-81
Cys ⁷	ψ	74	68	78	67	75	72
	ϕ	-120	-114	-126	-124	-125	-128
Val ⁸	ψ	-65	-57	-62	-55	-60	-64
	ϕ	-83	-95	-82	-88	-89	-82
Asn ⁹	ψ	119	113	134	118	111	116

Val ¹⁰	ϕ	-84	-82	-97	-90	-82	-82
	ψ	-21	-13	-16	-4	-15	-16
Ala ¹¹	ϕ	-79	-86	-87	-89	-85	-80
	ψ	-32	-21	-35	-35	-18	-27
Cys ¹²	ϕ	-86	-92	-78	-79	-95	-90
	ψ	-52	-53	-55	-57	-53	-54
Thr ¹³	ϕ	-129	-121	-127	-119	-118	-130
	ψ	111	153	141	155	141	119
Gly ¹⁴	ϕ	-64	-78	-78	-80	-78	-68
	ψ	83	64	68	62	67	78
Cys ¹⁵	ϕ	-139	-160	-150	-156	-78	-131

The dihedral angles ϕ and ψ , values that determine the overall 3D shape of this UG fragment, are similar (Table 1). It allowed performing preliminary design of new analogs aimed at stabilizing this particular family of conformations employing the known local conformational limitations imposed by various types of amino acids.

For instance, it is known that Gly is more conformationally flexible compared to any other L-amino acid residue, since Gly may adopt conformations with any of the four combinations of signs for ϕ and ψ , *i.e.*, $-,+$; $-,-$; $+,+$; and $+,-$. The last combination is sterically forbidden for the L-amino acids, as Ala. Therefore, substitution of Gly¹⁴ for Ala¹⁴ should limit conformational flexibility in position 14 preserving the conformations described in Table 1. Also, substitution for Aib (α -Me-Ala, di- α -methyl-alanine) should limit the local conformational flexibility by two regions only, namely for $-,-$ and $+,+$, the first one being compatible with conformers of Ala¹¹ in Table 1. Therefore, one more desirable substitution is Aib¹¹. In Pro, the ϕ value is fixed at -75° ; this residue is also similar to valine by its hydrophobic properties. Therefore, Val¹⁰ may be replaced by Pro¹⁰, which adds more local conformational constraints to the UG conformers in Table 1. Replacement by Pro also requires that the preceding residue possesses only positive ψ values; Asn⁹ in Table 1 fulfills this requirement. The Pro residue already exists in the corresponding position of ST. All suggested substitutions within SEQ ID NO:1 shown below (*e.g.*, Pro¹⁰, Aib¹¹ or Ala¹⁴) do not change the chemical nature of the non-aliphatic amino acids (such as Asn, Asp or Thr), which may be

important for the actual interaction with receptor. The former substitutions should lead only to conformational limitations shifting conformational equilibrium in UG towards the suggested “template” 3-D shape.

5 Based on the 3D structures defined in Table 1, a three-dimensional pharmacophore for uroguanylin was defined, enabling the determination of distances between functional groups of uroguanylin thought to directly interact with the receptor. Those groups thought to directly interact with the receptor are side groups of residues in positions 3, 5, 9 and 13 of the backbone sequence. Preferably, the residues are Glu3, Glu5, Asn9, and Thr13, as shown in SEQ ID NO:2
10 and SEQ ID NO:20. Thus, a three dimensional pharmacophore of uroguanylin is described in which the spatial arrangement of the four side chains of the residues at positions 3, 5, 9 and 13 may be created such that the distances between these side chains enable optional biological activity. Those distances (measured as distances between C β atoms of corresponding residues) are as follows: from 5.7 to 7.6 Å for the 3-5 distance, from 4.0 to 6.0 Å for 3-9; from 7.7 to 8.3
15 Å for 3-13, from 9.4 to 9.5 Å for 5-9, from 9.4 to 9.5 Å for 5-13, and from 5.8 to 6.3 Å for 9-13.

The distances above depend only on conformations of the peptide backbone. In some cases, however, conformations of side chains themselves are also important. For instance,
20 calculations showed that there is no conformational difference between the backbones of UG (SP301), [Glu²]-UG (SP303), [Glu³]-UG (SP304) and [Glu², Glu3]-UG (SP302) in terms of their low-energy conformations. However, there is a distinct difference in the spatial positions of the β -carboxyls of Asp and γ -carboxyls of Glu in position 3. Namely, γ -carboxyls of the Glu residues in position 3 are clearly stretched “outwards” of the bulk of the molecules farther than
25 the corresponding β -carboxyls of the Asp residues. The above observation strongly suggests that the negatively charged carboxyl group of the side chain in position 3 specifically interacts with a positively charged binding site on the receptor; therefore, analogs containing Glu3 instead of Asp3 should be more active. At the same time, to ensure efficiency of this particular interaction, an entire system of the long-range electrostatic interactions between ligand and
30 receptor should be well balanced. Since the Glu² side chain presents more conformational possibilities compared to the Asp² side chain, this balance may be slightly changed in SP302 (double substitution of Asp’s for Glu’s) compared to SP304 (single substitution of Asp³ for Glu³).

Compounds capable of adopting low-energy conformations described in Table 1 are listed in Table 2. All compounds are [4,12; 7,15] bicycles.

Table 2

5 **1. Parent compound: uroguanylin**

SEQ ID NO:1

Asn¹-Asp²-Asp³-Cys⁴-Glu⁵-Leu⁶-Cys⁷-Val⁸-Asn⁹-Val¹⁰-Ala¹¹-Cys¹²-Thr¹³-Gly¹⁴-Cys¹⁵-Leu¹⁶

2. Compounds without modifications of cysteines:

10 Common sequence (SEQ ID NO:2):

Asn¹-Aaa²-Bbb³-Cys⁴-Glu⁵-Leu⁶-Cys⁷-Val⁸-Asn⁹-Xxx¹⁰-Yyy¹¹-Cys¹²-Thr¹³-Zzz¹⁴-Cys¹⁵-Leu¹⁶

where Aaa = Asp, Glu; Bbb = Asp, Glu

with the exception that Aaa and Bbb are not both Asp in same molecule

And where Xxx = Val, Pro; Yyy = Ala, Aib; Zzz = Gly, Ala

15

3. Compounds with mercaptoproline (Mpt) substituted for cysteine in position 7:

Common sequence (SEQ ID NO:3):

20 Asn¹-Aaa²-Bbb³-Cys⁴-Glu⁵-Leu⁶-Mpt⁷-Val⁸-Asn⁹-Xxx¹⁰-Yyy¹¹-Cys¹²-Thr¹³-Zzz¹⁴-Cys¹⁵-
Leu¹⁶

where Aaa = Asp, Glu; Bbb = Asp, Glu

where Xxx = Val, Pro; Yyy = Ala, Aib; Zzz = Gly, Ala

25

4. Compounds with penicillamines (β,β-dimethylcysteines, Pen) substituted for cysteines:

Common sequence (SEQ ID NO:4):

30 Asn¹-Aaa²-Bbb³-Kkk⁴-Glu⁵-Leu⁶-Lll⁷-Val⁸-Asn⁹-Xxx¹⁰-Yyy¹¹-Mmm¹²-Thr¹³-Zzz¹⁴-Nnn¹⁵-
Leu¹⁶

where Aaa = Asp, Glu; Bbb = Asp, Glu

where Xxx = Val, Pro; Yyy = Ala, Aib; Zzz = Gly, Ala

35 and Kkk, Lll, Mmm and Nnn are either Cys or Pen (except not all are Cys in the same conformer)

5. Compounds with lactam bridges substituted for disulfide bridges:

Common sequence (SEQ ID NO:5):

5 Asn¹-Aaa²-Bbb³-Kkk⁴-Glu⁵-Leu⁶-Lll⁷-Val⁸-Asn⁹-Xxx¹⁰-Yyy¹¹-Mmm¹²-Thr¹³-Zzz¹⁴-Nnn¹⁵-
Leu¹⁶

where Aaa = Asp, Glu; Bbb = Asp, Glu

where Xxx = Val, Pro; Yyy = Ala, Aib; Zzz = Gly, Ala;

10 and all combinations of the following (Dpr is diaminopropionic acid):

Kkk is Dpr and Mmm is either Asp or Glu;

Kkk is either Asp or Glu, and Mmm is Dpr;

Lll is either Cys or Pen;

Nnn is either Cys or Pen;

15 or:

Lll is Dpr and Nnn is either Asp or Glu;

Lll is either Asp or Glu, and Nnn is Dpr;

Kkk is either Cys or Pen;

Mmm is either Cys or Pen.

20

Some of the peptides shown in Table 2 contain 16 amino acid residues in which cysteine residues form disulfide bridges between Cys⁴ and Cys¹², and Cys⁷ and Cys¹⁵, respectively. These peptides differ from the peptide sequences described in WO 01/25266, and are designed on the basis of peptide conformation and energy calculations.

25

In addition, peptides, varying in length from 13 to 16 amino acids, shown in Table 3, are designed, based on energy calculations and three-dimensional structures, to promote stabilization of the biologically active conformer and minimize or eliminate interconversion to biologically inactive conformers. These peptides are also designed to promote stability against proteolysis and higher temperatures. The design of these peptides involves modifications of amino acid residues that contain ionic charges at lower pH values, such as glutamic and aspartic acids.

30

35

Table 3

	SEQ ID NO:6	X1 Glu Glu Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys X9
5	SEQ ID NO:7	X1 Glu Asp Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:8	X1 Asp Glu Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys X9
10	SEQ ID NO:9	X1 Asp Asp Cys X2 X3 Cys X4 Tyr X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:10	X1 Glu Glu Cys X2 X3 Cys X4 Tyr X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:11	X1 Asp Glu Cys X2 X3 Cys X4 Tyr X5 X6 Cys X7 X8 Cys X9
15	SEQ ID NO:12	X1 Glu Asp Cys X2 X3 Cys X4 Tyr X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:13	X1 Asp Asp Cys X2 X3 Cys X4 Gln X5 X6 Cys X7 X8 Cys X9
20	SEQ ID NO:14	X1 Glu Glu Cys X2 X3 Cys X4 Gln X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:15	X1 Asp Glu Cys X2 X3 Cys X4 Gln X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO:16	X1 Glu Asp Cys X2 X3 Cys X4 Gln X5 X6 Cys X7 X8 Cys X9
25	SEQ ID NO: 17	Glu Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys X9
	SEQ ID NO: 18	Glu Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys
30	SEQ ID NO: 19	X1 Glu Cys X2 X3 Cys X4 Asn X5 X6 Cys X7 X8 Cys X9 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
	SEQ ID NO:20	Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu
35	SEQ ID NO:21	Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

40 X1 to X9 can be any amino acid. The disulfide bridges are formed between Cys residues at 4 and 12 and between 7 and 15, respectively. SEQ ID NO:18 represents the minimum length requirement for these peptides to bind a guanylate cyclase receptor.

Pharmaceutical Compositions and Formulations

45 The guanylate cyclase receptor agonists of the present invention (Table 2; SEQ ID NOs:2-5 and Table 3; SEQ ID NOs:6-21), as well as uroguanylin, guanylin and/or bacterial enterotoxin ST, may be combined or formulated with various excipients, vehicles or adjuvants for oral, local or systemic administration. Peptide compositions may be administered in solutions, powders, suspensions, emulsions, tablets, capsules, transdermal patches, ointments, or other formulations. Formulations and dosage forms may be made using methods well known

in the art (see, *e.g.*, Remington's Pharmaceutical Sciences, 16th ed., A. Oslo ed., Easton, PA (1980)).

5 Inhibitors of cGMP-dependent phosphodiesterase may be small molecules, peptides, proteins or other compounds that specifically prevent the degradation of cGMP. Inhibitory compounds include suldinac sulfone, zaprinast, motapizone and other compounds that block the enzymatic activity of cGMP-specific phosphodiesterases. One or more of these compounds may be combined with a guanylate cyclase receptor agonist exemplified in SEQ ID NOs:2-21, uroganylin, guanylin and *E. coli* ST peptide.

10 The selection of carriers (*e.g.*, phosphate-buffered saline or PBS) and other components suitable for use in compositions is well within the level of skill in this art. In addition to containing one or more guanylate cyclase receptor agonists, such compositions may incorporate pharmaceutically acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake. Other formulations, such as microspheres, nanoparticles, liposomes, pegylated protein or peptide, and immunologically-based systems may also be used. Examples include formulations employing polymers (*e.g.*, 20% w/v polyethylene glycol) or cellulose, or enteric formulations and pegylated peptide analogs for increasing systemic half-life and stability.

20 **Treatment Methods**

The term "treatment" refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, or preventing disease development. For a given subject, improvement in a symptom, its worsening, regression, or progression may be determined by any objective or subjective measure typically employed by one of skill in the art. Efficacy of the treatment in the case of cancer may be measured as an improvement in morbidity or mortality (*e.g.*, lengthening of the survival curve for a selected population). Thus, effective treatment would include therapy of existing disease, control of disease by slowing or stopping its progression, prevention of disease occurrence, reduction in the number or severity of symptoms, or a combination thereof. The effect may be shown in a controlled study using one or more statistically significant criteria.

Combination therapy with one or more medical/surgical procedures and/or at least one other chemotherapeutic agent may be practiced with the invention. Other suitable agents useful in combination therapy include anti-inflammatory drugs such as, for example, steroids or non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin and the like. Prophylactic methods for preventing or reducing the incidence of relapse are also considered treatment.

Cancers expected to be responsive to compositions include breast, colorectal, lung, ovarian, pancreatic, prostatic, renal, stomach, bladder, liver, esophageal and testicular carcinoma. Further examples of diseases involving cancerous or precancerous tissues that should be responsive to a therapeutic comprising at least one guanylate cyclase receptor agonist include: carcinoma (*e.g.*, basal cell, basosquamous, Brown-Pearce, ductal, Ehrlich tumor, in situ, Krebs, Merkel cell, small or non-small cell lung, oat cell, papillary, bronchiolar, squamous cell, transitional cell, Walker), leukemia (*e.g.*, B-cell, T-cell, HTLV, acute or chronic lymphocytic, mast cell, myeloid), histiocytoma, histiocytosis, Hodgkin disease, non-Hodgkin lymphoma, plasmacytoma, reticuloendotheliosis, adenoma, adeno-carcinoma, adenofibroma, adenolymphoma, ameloblastoma, angiokeratoma, angiolymphoid hyperplasia with eosinophilia, sclerosing angioma, angiomatosis, apudoma, branchioma, malignant carcinoid syndrome, carcinoid heart disease, carcinosarcoma, cementoma, cholangioma, cholesteatoma, chondrosarcoma, chondroblastoma, chondrosarcoma, chordoma, choristoma, craniopharyngioma, chondroma, cylindroma, cystadenocarcinoma, cystadenoma, cystosarcoma phyllodes, dysgerminoma, ependymoma, Ewing sarcoma, fibroma, fibrosarcoma, giant cell tumor, ganglioneuroma, glioblastoma, glomangioma, granulosa cell tumor, gynandroblastoma, hamartoma, hemangioendothelioma, hemangioma, hemangio-pericytoma, hemangiosarcoma, hepatoma, islet cell tumor, Kaposi sarcoma, leiomyoma, leiomyosarcoma, leukosarcoma, Leydig cell tumor, lipoma, liposarcoma, lymphangioma, lymphangiomyoma, lymphangiosarcoma, medulloblastoma, meningioma, mesenchymoma, mesonephroma, mesothelioma, myoblastoma, myoma, myosarcoma, myxoma, myxosarcoma, neurilemmoma, neuroma, neuroblastoma, neuroepithelioma, neurofibroma, neurofibromatosis, odontoma, osteoma, osteosarcoma, papilloma, paraganglioma, paraganglioma nonchromaffin, pinealoma, rhabdomyoma, rhabdomyosarcoma, Sertoli cell tumor, teratoma, theca cell tumor, and other diseases in which cells have become dysplastic, immortalized, or transformed.

A bolus of the inventive composition may be administered over a short time. Once a day is a convenient dosing schedule to treat, *inter alia*, one of the above-mentioned disease states. Alternatively, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to twelve doses per day. The dose level selected for use will depend on the bioavailability, activity, and stability of the compound, the route of administration, the severity of the disease being treated, and the condition of the subject in need of treatment. It is contemplated that a daily dosage will typically be between about 10 μg and about 2 mg (*e.g.*, about 100 μg to 1 mg) of the compound per kilogram body weight. The amount of compound administered is dependent upon factors known to a person skilled in this art such as, for example, chemical properties of the compound, route of administration, location and type of cancer, and the like. The subject mammal may be any animal or human patient. Thus, both veterinary and medical treatments are envisioned according to the invention.

The invention will be further described by the following non-limiting example.

EXAMPLE

Materials and Methods

Cell Culture: Human T84 colon carcinoma cells were obtained from the American Type Culture Collection at passage 52. Cells were grown in a 1:1 mixture of Ham's F-12 medium and Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U penicillin/ml, and 100 $\mu\text{g}/\text{ml}$ streptomycin. The cells were fed fresh medium every third day and split at a confluence of approximately 80%.

T84 cell-based assay for determining the intracellular levels of cGMP: Peptide analogs were custom synthesized by Multiple Peptide Systems, San Diego, CA., and by Princeton Biomolecules, Langhorne, PA. Biological activity of the synthetic peptides was assayed as previously reported (15). Briefly, the confluent monolayers of T-84 cells in 24-well plates were washed twice with 250 μl of DMEM containing 50 mM HEPES (pH 7.4), pre-incubated at 37°C for 10 min with 250 μl of DMEM containing 50 mM HEPES (pH 7.4) and 1 mM isobutylmethylxanthine (IBMX), followed by incubation with peptide analogs (0.1 nM to 10 μM) for 30 min. The medium was aspirated, and the reaction was terminated by the addition of

3% perchloric acid. Following centrifugation, and neutralization with 0.1 N NaOH, the supernatant was used directly for measurements of cGMP using an ELISA kit (Caymen Chemical, Ann Arbor, MI.).

5 Results

Peptides shown in Table 4 were custom synthesized and purified (>95% purity) using a published procedure (38). Peptide analogs were evaluated in the T84 cell-based assay for their ability to enhance intracellular levels of cGMP. As shown in Table 4, SP304 (SEQ ID NO:20) gave the greatest enhancement of intracellular cGMP of all the analogs tested. SP316 (SEQ ID
10 NO:21) was second in effectiveness, whereas the biological activities of SP301, SP302 and SP303 were all somewhat weaker. The peptide analogs SP306 and SP310 were not active in this assay. These results indicate that SP304 is the most potent peptide for enhancing cGMP. These results also suggest that the cysteine residue at position 7 cannot be substituted with penicillamine as a component of the [7,15] disulfide linkage, and that the Asn residue at
15 position 9 cannot be changed to a Gln.

Table 4: Peptide agonists evaluated for biological activity in the T84 cell bioassay.

20	SEQ ID NO.*	Compound Code	cGMP Level** (pmol/well)
	1	SP 301	205
	6	SP 302	225
25	7	SP 303	195
	20	SP 304	315
30	14	SP 306	0
	4	SP 310	0
35	21	SP 316	275

* SEQ ID's for SP301, SP304 and SP316 are the precise amino acid sequences for these analogs as given in the text.

40 ** Intracellular cGMP level observed in T84 cells following treatment with 1 micromolar solution of the respective peptide agonist for 30 minutes. The value observed for SP304 was statistically significant with a $p > 0.5$.

To examine heat stability, 10 micromolar solutions of peptide analogs were heated at 95°C for up to 90 minutes. At specific times during the treatment, samples were tested for their biological activity in the T84 cell-based assay. Biological activity of SP301, SP302, SP303 and SP304 did not change significantly after 60 minutes of heating. After 90 minutes, the activities of SP301, SP302 and SP303 were reduced to about 80% of their original values, whereas the biological activity of SP304 remained unaltered. This indicates that SP304 is more stable to heat denaturation compared to the other peptides tested. Based on energy calculations and 3D structure, we expected that the negatively charged carboxyl group of the side chain in position 3 of SEQ ID NO:1 specifically interacts with a positively charged binding site on the receptor. In the case where this interaction can be enhanced, analogs containing Glu3 instead of Asp3 should be more active, as was found to be the case with SP304. At the same time, to ensure efficiency of this particular interaction, an entire system of the long-range electrostatic interactions between ligand and receptor should be well balanced. Since the Glu² side chain presents more conformational possibilities compared to the Asp² side chain, this balance may be slightly changed in SP302 (double substitution of Asp's for Glu's) compared to SP304 (single substitution of Asp³ for Glu³). Indeed, biological activity of SP 304 is the best amongst the analogs evaluated.

Synthetic peptides SP301, SP302, SP303 and SP304 were also tested for their activities at different pH values of the T84 cell-based assay. Whereas all of these peptides showed enhanced intracellular production of cGMP at pH's ranging from 5 to 7, SP304 showed the greatest enhancement in the range between 6.5 and 7. It is important to note that the physiological pH of the large intestine is in a similar range, and, therefore, SP304 would be expected to be especially efficacious for colon cancer treatment.

We also evaluated peptides used either alone or in combination with inhibitors of cGMP dependent phosphodiesterase (*e.g.*, zaprinast or sulindac sulfone) in T84 cell-based assays for enhancement of intracellular levels of cGMP. Combinations of an inhibitor of cGMP dependent phosphodiesterase with SP304 displayed a dramatic effect in enhancing cGMP levels in these experiments. Synthetic peptide SP304 substantially increased the cGMP level over the level reached in the presence of either zaprinast or sulindac sulfone alone. Treatment of wells with SP304 in combination with either Zaprinast or sulindac sulfone resulted in synergistic increases in intracellular cGMP levels. These increases were statistically

significant, with p values of <0.5. These data indicate that treatments combining a peptide agonist of a guanylate cyclase receptor with one or more inhibitors of cGMP dependent phosphodiesterase result in a greater than additive increase in cGMP concentrations.

5 While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to those of ordinary skill in the art that various changes and modifications can be made without departing from the spirit and scope of the invention.

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What is Claimed is:

1. A peptide consisting essentially of the amino acid sequence of any one of SEQ ID NO:2 - SEQ ID NO:21.
5
2. The peptide of claim 1, wherein said peptide is a (4,12; 7,15) bicycle having the sequence of SEQ ID NO:20.
3. The peptide of either claim 1 or claim 2, wherein said peptide consists of the amino acid sequence of any one of SEQ ID NO:2-SEQ ID NO:21.
10
4. A method for preventing or treating primary or metastatic cancer or polyps in a patient comprising administering to said patient an effective dosage of a guanylate cyclase receptor agonist having the sequence of any one of SEQ ID NO:2 - SEQ ID NO:21.
15
5. A method for treating metastatic cancer in a patient comprising administering to said patient an effective dosage of a guanylate cyclase receptor agonist selected from the group consisting of: uroguanylin; guanylin; and *E. coli* ST peptide.
- 20 6. A method for treating primary cancers other than colon cancer in a patient, comprising administering to said patient an effective dosage of a guanylate cyclase receptor agonist selected from the group consisting of: uroguanylin; guanylin; and *E. coli* ST peptide.
7. The method of claim 4, wherein said peptide is a (4,12; 7,15) bicyclic peptide having the sequence of SEQ ID NO:20.
25
8. The method of claim 4, wherein said primary cancer is a member selected from the group consisting of the breast, colon, rectum, lung, ovary, pancreas, bladder, prostate, kidney or testis.
30
9. The method of any one of claims 4-8, further comprising administering to said patient an effective dose of an inhibitor of cGMP-dependent phosphodiesterase either concurrently or sequentially with said guanylate cyclase receptor agonist.

10. A method of treating a patient for colon cancer or polyps comprising administering to said patient an effective dose of an inhibitor of cGMP-dependent phosphodiesterase either concurrently or sequentially with uroguanylin, guanylin or *E. coli* ST peptide.
- 5 11. The method of claim 9 and 10, wherein said cGMP-dependent phosphodiesterase inhibitor is selected from the group consisting of suldinac sulfone, zaprinast, and motapizone.
12. A method for preventing or treating inflammation in a patient comprising administering
10 to said patient an effective dosage of a guanylate cyclase receptor agonist having the sequence of any one of: SEQ ID NO:2 - SEQ ID NO:21; uroguanylin; guanylin; or *E. coli* ST peptide.
13. The method of claim 12, wherein said peptide is a (4,12; 7,15) bicyclic peptide having
15 the sequence of SEQ ID NO:20.
14. The method of claim 12, wherein said inflammation is an inflammatory disease selected from the group consisting of: asthma; nephritis, hepatitis, pancreatitis, bronchitis and cystic fibrosis.
20
15. The method of claim 12, wherein said patient is treated for an inflammatory disorder of the gastrointestinal tract.
16. The method of claim 15, wherein said inflammatory disorder of the gastrointestinal
25 tract is an inflammatory bowel disease selected from the group consisting of: ulcerative colitis and Crohn's disease.
17. The method of claim 12, further comprising administering to said patient an effective
30 dose of an inhibitor of cGMP-dependent phosphodiesterase either concurrently or sequentially with said guanylate cyclase receptor agonist.
18. The method of claim 17, wherein said cGMP-dependent phosphodiesterase is selected from the group consisting of suldinac sulfone, zaprinast, and motapizone.

19. A method of treating a patient for primary or metastatic cancer, polyps or inflammation comprising administering to said patient:

5 a) a guanylate cyclase receptor agonist peptide having the sequence of any one of: SEQ ID NOs:2-21; uroguanylin; guanylin; or *E. coli* ST peptide; and

b) at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor; an anti-inflammatory agent; an antiviral agent; and an anticancer agent;

10 wherein said guanylate cyclase receptor agonist and said compound are each administered in a therapeutically effective amount.

20. A pharmaceutical composition in unit dose form comprising a guanylate cyclase receptor agonist peptide having the sequence of any one of SEQ ID NOs:2-21 present in a therapeutically effective amount.

15 21. A pharmaceutical composition in unit dose form comprising:

a) a guanylate cyclase receptor agonist peptide having the sequence of any one of: SEQ ID NOs:2-21; uroguanylin; guanylin; or *E. coli* ST peptide; and

20 b) at least one compound selected from the group consisting of: a cGMP-dependent phosphodiesterase inhibitor, an anti-inflammatory agent, an antiviral agent and an anticancer agent;

wherein said guanylate cyclase receptor agonist and said compound are each present in a therapeutically effective amount.

25 22. The pharmaceutical composition of either claim 20 or 21, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution or inhalation formulation.

30 23. The pharmaceutical composition of either claim 20 nor 21, further comprising one or more excipients.

24. A method of inducing apoptosis in the cells of a subject, comprising administering to said subject an effective amount of agonist peptide having the sequence of any one of SEQ ID NO:2 - SEQ ID NO:21.
- 5 25. A method of inducing apoptosis in the cells of a subject, comprising administering to said subject an effective amount of uroguanylin, guanylin or *E. coli* ST peptide for cancers other than colon cancer.
- 10 26. A peptide conjugate comprising polyethylene glycol (PEG) attached to a peptide having the sequence of any of: SEQ ID NO:2 - SEQ ID NO:21; uroguanylin; guanylin; or *E. coli* ST peptide.
- 15 27. A method of treating cancer, inflammation or polyps in a patient comprising administering to said patient a therapeutically effective amount of the peptide conjugate of claim 26.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/09551

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 31/00, 38/00; A01N 61/00; C12Q 1/00; C07K 2/00, 4/00, 5/00, 7/00, 14/00, 16/00, 17/00; G01N 33/53, 33/48, 33/567, 574
 US CL : 435/4, 7.1, 7.21, 7.23; 436/64; 514/1, 2, 10, 14; 530/300, 317

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 435/4, 7.1, 7.21, 7.23; 436/64; 514/1, 2, 10, 14; 530/300, 317

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 GenCore, EAST, WEST, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HILL, O. et al. A new human guanylate cyclase-activating peptide (GCAP-II, uroguanylin): precursor cDNA and colonic expression. <i>Biochimica et Biophysica Acta</i> , 1995, Vol 1253, pages 146-149.	1-27
Y,P	US 6,235,782 B1 (PAMUKCU et al) 22 May 2001 (22.05.2001)	9-11, 19 and 21-23
X	US 5,879,656 (WALDMAN) 9 March 1999 (9.3.1999)	5 and 6
---		-----
Y		9-11, 19, 21-23 and 25-27
Y	US 5,578,709 (WOISZWILLO) 26 November 1996 (26.11.1996)	26 and 27

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 08 August 2002 (08.08.2002)	Date of mailing of the international search report 18 SEP 2002
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Alana M. Harris, Ph.D. Telephone No. (703)908-0196

(FILE 'HOME' ENTERED AT 09:00:26 ON 13 AUG 2014)

FILE 'CAPLUS' ENTERED AT 09:00:56 ON 13 AUG 2014

E COMISKEY ST?/AU
L1 14 S E4-E7
L2 14 S L1
L3 6 S L2 AND (GUANYLATE CYCLASE)
L4 8363 S (GUANYLATE CYCLASE)
L5 691 S L4 AND AGONIST
L6 21 S L5 AND EXCIPIENT
L7 8 S L6 AND (AMINO ACID)
E FENG RO?/AU
L8 115 S E18-E36
L9 5 S L8 AND (GUANYLATE CYCLASE)
E FOSS JO?/AU
L10 62 S E52-E57
L11 4 S L10 AND (GUANYLATE CYCLASE)
E SHAILUBHAI KU?/AU
L12 34 S E64
L13 34 S L12
L14 17 S L13 AND (GUANYLATE CYCLASE)
L15 4 S L14 AND (LEUCINE OR ARGININE OR HISTIDINE)

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	1	peptide same (liquid formulation) same (blister pack)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:29
L2	102	(liquid formulation) same (blister pack)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:29
L3	78	L2 and peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:30
L4	34	L3 and @py<"2010"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:31
L6	6968	guanylate cyclase	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:37
L8	1	L2 and L6	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:38
L9	200	L6 and (liquid formulation)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:39
L10	59	L9 and blister	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:39
L11	28	L10 and @py<"2010"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 18:40
L12	35	9 AND ((A61K2300/00 OR A61K38/10 OR A61K31/215 OR A61K8/731 OR C07D213/81 OR C07D213/56).CPC.)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/12 19:23
S1	39681	(guanylate near cyclase near C) or GOC	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 20:24
S2	201	S1 with agonist	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 20:24


S3	72	S2 and @py<"2011"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 20:25
S4	22	GOC agonist peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 20:29
S5	22	GOC agonist peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 21:54
S6	7	S5 and tablet and process	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/05/26 21:54
S7	611	(blister pack) with liquid	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:46
S8	94	S7 with capsule	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:47
S9	0	S8 same peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:47
S10	48	S8 and peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:48
S11	46	S10 and oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:48
S12	2	S11 and cyclase	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 16:58
S13	17	(oral dosage) same (inorganic acid) same (carboxylic acid)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:12
S14	19367	excipient same (leucine or histidine or arginine or amine)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:18
S15	12456	S14 and oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:19
S16	1829	S14 same oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:19
S17	763	S16 and peptide	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT;	ADJ	ON	2014/08/11 17:19

			IBM_TDB			
S18	493	S17 and lubricant	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:20
S19	487	S18 and pharmaceutical	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:21
S20	299	S19 and blister	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:21
S21	299	S20 and capsule	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:22
S22	138	S21 and @py<"2010"	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:22
S23	134	S22 and liquid	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 17:22
S24	96	(Stephen near3 Comiskey).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:35
S25	2	S24 and (Oral dosage)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:36
S26	197	(Rong near3 Feng).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:37
S27	6	S26 and (oral)	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:38
S28	118	(John near3 Foss).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:39
S29	2	S28 and oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:39
S30	133	(Kunwar near3 Shailubhai).in.	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:40
S31	41	S30 and oral	US-PGPUB; USPAT; USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB	ADJ	ON	2014/08/11 18:40
S32	28	S31 and arginine	US-PGPUB; USPAT;	ADJ	ON	2014/08/11

			USOCR; FPRS; EPO; JPO; DERWENT; IBM_TDB		18:40
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8/ 12/ 2014 7:26:19 PM

C:\Users\jlee24\Documents\EAST\Workspaces\13 421769.wsp

Search Notes 	Application/Control No. 13421769	Applicant(s)/Patent Under Reexamination COMISKEY ET AL.
	Examiner JIA-HAI LEE	Art Unit 1676

CPC- SEARCHED		
Symbol	Date	Examiner
(A61K2300/00 OR A61K38/10 OR A61K31/215 OR A61K8/731 OR C07D213/81 OR C07D213/56).CPC.	8/13/2014	JL

CPC COMBINATION SETS - SEARCHED		
Symbol	Date	Examiner

US CLASSIFICATION SEARCHED			
Class	Subclass	Date	Examiner

SEARCH NOTES		
Search Notes	Date	Examiner
see search printout	8/13/2014	JL
inventor (Comiskey; Feng; Foss; Shailubhai) in EAST,STN,PALM	8/13/2014	JL

INTERFERENCE SEARCH			
US Class/ CPC Symbol	US Subclass / CPC Group	Date	Examiner

/J.L./ Examiner.Art Unit 1676	
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POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(b).

I hereby appoint:

Practitioners associated with the Customer Number:
OR

58249

Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number	Name	Registration Number

as attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(b).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(b) to:

The address associated with Customer Number:
OR

58249

<input type="checkbox"/> Firm or Individual Name			
Address			
City	State	Zip	
Country			
Telephone	Email		

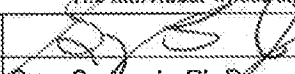
Assignee Name and Address:

Synergy Pharmaceuticals Inc.
420 Lexington Avenue, Suite 2012
New York, NY 10170

A copy of this form, together with a statement under 37 CFR 3.73(b) (Form PTO/SB/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(b) may be completed by one of the practitioners appointed in this form if the appointed practitioner is authorized to act on behalf of the assignee, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date	Oct. 6, 2014
Name	Gary S. Jacob, Ph.D.	Telephone	
Title	President and Chief Executive Officer		

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Stephen Comiskey et al.

Application No./Patent No.: 13/421,769

Filed/Issue Date: 03/15/2012

Titled: FORMULATIONS OF GUANYLATE CYCLASE C AGONISTS AND METHODS OF USE

Synergy Pharmaceuticals Inc., a corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

- 1. the assignee of the entire right, title, and interest in;
- 2. an assignee of less than the entire right, title, and interest in
(The extent (by percentage) of its ownership interest is _____ %); or
- 3. the assignee of an undivided interest in the entirety of (a complete assignment from one of the joint inventors was made)

the patent application/patent identified above, by virtue of either:

A. An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 028079, Frame 0873, or for which a copy therefore is attached.

OR

B. A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

Additional documents in the chain of title are listed on a supplemental sheet(s).

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

[Signature]
Signature

Oct 6, 2014
Date

Gary S. Jacob, Ph.D.

President and Chief Executive Officer

Printed or Typed Name

Title

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt

EFS ID:	20467658
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	30623
Filer:	Cynthia A. Kozakiewicz/Donna Doyle
Filer Authorized By:	Cynthia A. Kozakiewicz
Attorney Docket Number:	40737-509001US
Receipt Date:	24-OCT-2014
Filing Date:	15-MAR-2012
Time Stamp:	18:17:16
Application Type:	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	Power of Attorney	SYPA_SB80_GeneralPOA.pdf	110734 e5cda96054c9fa05e6d856e71accc68030e266e6	no	1

Warnings:

Information:

2	Assignee showing of ownership per 37 CFR 3.73.	SYPA_009X01US_Statement.pdf	88906 a1cfaee9dea888b968b61f97a2fca8d5a4dc7e54	no	1
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Warnings:

Information:

Total Files Size (in bytes):	199640
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

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

National Stage of an International Application under 35 U.S.C. 371

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

New International Application Filed with the USPTO as a Receiving Office

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



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/421,769	03/15/2012	Stephen Comiskey	40737-509001US

CONFIRMATION NO. 3135

POA ACCEPTANCE LETTER



58249
COOLEY LLP
ATTN: Patent Group
1299 Pennsylvania Avenue, NW
Suite 700
Washington, DC 20004

Date Mailed: 10/29/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/24/2014.

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

/dtdinh/

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



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
13/421,769	03/15/2012	Stephen Comiskey	40737-509001US

CONFIRMATION NO. 3135

POWER OF ATTORNEY NOTICE

30623
Mintz Levin/Boston Office
One Financial Center
Boston, MA 02111



Date Mailed: 10/29/2014

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 10/24/2014.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/dtdinh/

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

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Stephen Comiskey *et al* Confirmation No.: 3135

Application No.: 13/421,769 Group Art Unit: 1676

Filed: March 15, 2012 Examiner: LEE, Jia-Hai

FOR: **FORMULATIONS OF GUANYLATE CYCLASE C AGONISTS AND METHODS OF USE**

EFS

Mail Stop Amendment

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

RESPONSE TO NON-FINAL OFFICE ACTION

In response to the Non-Final Office Action mailed August 19, 2014 please enter the following amendments and remarks. Applicants submit concurrently herewith a request for a three-month extension of time making this response timely filed by February 19, 2014.

Amendments to the Claims begin on page 2.

Remarks begin on page 7.

Amendments to the Claims:

This listing of claims will replace all prior listings in the application. Please amend the claims as follows.

1. (Withdrawn –Currently Amended) An oral dosage formulation comprising at least one Guanylate Cyclase C (GCC) [GCC] agonist peptide and one or more pharmaceutically acceptable excipients, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, and the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 9 and 8.
2. (Currently Amended) An oral dosage formulation comprising at least one Guanylate Cyclase C (GCC) [GCC] agonist peptide and one or more pharmaceutically acceptable excipients, wherein the amount of GCC agonist peptide per unit dose is from 0.01 mg to 10 mg, the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1-54 and 56-249, [[and]] the GCC agonist peptide has a chromatographic purity of no less than 91%, and the formulation comprises an inert low moisture carrier.
3. (Currently Amended) The oral dosage formulation of claim 2, wherein the GCC agonist peptide has a chromatographic purity of no less than 92% ~~or no less than~~ to 95%.
4. (Currently Amended) The oral dosage formulation of claim 2, wherein the GCC agonist peptide has a total impurity content of no greater than ~~9%, 7%, 6%, or 5%,~~ .
5. (Original) The oral dosage formulation of claim 2, wherein the formulation is substantially free of inorganic acids and carboxylic acids.
6. (Original) The oral dosage formulation of claim 2, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56.
7. (Currently Amended) The oral dosage formulation of claim 2, wherein the amount of GCC agonist peptide per unit dose is selected from the group consisting of 0.1 mg, 0.3 mg, 1.0 mg, 3.0 mg, 6.0 mg, 9.0 mg or 9.5 mg.
8. (Original) The oral dosage formulation of claim 2, wherein the formulation is a solid formulation and the unit dose is a powder, granule, sachet, troche, tablet, or capsule.

9. (Original) The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise an inert carrier.
10. (Currently Amended) The oral dosage formulation of claim 9, wherein the inert carrier is ~~a selected from mannitol, lactose, a microcrystalline cellulose, or starch.~~
11. (Original) The oral dosage formulation of claim 10, wherein the inert carrier has a particle size of from 50 to 900 microns.
12. (Withdrawn) The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise a divalent cation salt.
13. (Withdrawn) The oral dosage formulation of claim 12, wherein the salt is calcium chloride or calcium ascorbate.
14. (Original) The oral dosage formulation of claim 2, wherein the one or more pharmaceutically acceptable excipients comprise an amino acid or amine, and the molar ratio between the amino acid and GCC agonist peptide is 2:1 to 30:1.
15. (Original) The oral dosage formulation of claim 14, wherein the amino acid is leucine, histidine, or arginine.
16. (Currently Amended) The oral dosage formulation of claim 2, wherein the formulation consists of the GCC agonist peptide,[[an]] the inert low moisture carrier, and a lubricant.
17. (Withdrawn) The oral dosage formulation of claim 2, wherein the formulation consists of the GCC agonist peptide, an inert carrier, a divalent cation salt, an amino acid, a coating agent and optionally a lubricant.
18. (Withdrawn) The oral dosage of formulation of claim 17, wherein the inert carrier is microcrystalline cellulose and the lubricant is magnesium stearate.
19. (Withdrawn) The oral dosage of formulation of claim 18, wherein the divalent cation salt is calcium chloride or calcium ascorbate, the amino acid is leucine, histidine, or arginine, and the coating agent is hypromellose.
20. (Original) The oral dosage formulation of claim 2, wherein the GCC agonist peptide is stabilized against degradation for a period of at least 18 months at 30 °C and 65% relative

- humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.
21. (Original) The oral dosage formulation of claim 2, wherein the formulation is in the form of a capsule or tablet.
 22. (Original) The oral dosage formulation of claim 21, wherein the capsule or tablet is in a blister pack or strip.
 23. (Original) The oral dosage formulation of claim 22, wherein the GCC agonist peptide is in solution or suspension in a lipophilic liquid.
 24. (Original) The oral dosage formulation of claim 23, wherein the unit dosage form is a liquid-filled capsule.
 25. (Currently Amended) The oral dosage formulation of claim ~~[[2]]~~23, wherein the liquid is a refined specialty oil or a medium chain triglyceride or related ester.
 26. (Withdrawn) A process for making an oral dosage formulation comprising at least one GCC agonist peptide, the method comprising:
 - a) providing an aqueous solution comprising: a GCC agonist peptide selected from the group consisting of SEQ ID NOs: 1-54 and 56-249, and one or more pharmaceutically acceptable excipients, wherein the concentration of the GCC agonist peptide ranges from 10 to 60 mg/mL; and
 - b) applying the aqueous solution to a pharmaceutically acceptable carrier to generate a GCC agonist peptide-coated carrier.
 27. (Withdrawn) The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise a divalent cation salt wherein the divalent cation is selected from Ca^{2+} , Mg^{2+} , Zn^{2+} , and Mn^{2+}
 28. (Withdrawn) The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise an amino acid selected from leucine, histidine, and arginine.

29. (Withdrawn) The process of claim 26, wherein the one or more pharmaceutically acceptable excipients comprise a coating agent.
30. (Withdrawn) The process of claim 29, wherein the coating agent is hypromellose.
31. (Withdrawn) The process of claim 26, wherein the aqueous solution has a pH greater than 4 or 5.
32. (Withdrawn) The process of claim 26, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, and 56.
33. (Withdrawn) The process of claim 26, wherein the aqueous solution is substantially free of inorganic acids and carboxylic acids.
34. (Withdrawn) The process of claim 26, further comprising drying the GCC agonist peptide-coated carrier.
35. (Withdrawn) An oral dosage formulation made by the process of claim 26, wherein the GCC agonist peptide is stabilized against degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.
36. (Withdrawn) A method for treating or preventing a disease or disorder in a subject in need thereof, comprising administering to the subject an oral dosage formulation of claim 2.
37. (Withdrawn) The method of claim 36, wherein the disease or disorder is a gastrointestinal disease or disorder selected from the group consisting of irritable bowel syndrome, chronic idiopathic constipation, non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastro esophageal reflux disease, constipation, gastroparesis, heartburn, gastric cancer, and H. pylori infection.
38. (Withdrawn) The method of claim 36, wherein the GCC agonist peptide is selected from the group consisting of SEQ ID NOs: 1, 8, 9, or 56.

39. (Withdrawn) The method of claim 36, further comprising administering to the subject an effective amount of an inhibitor of a cGMP-specific phosphodiesterase.
40. (Withdrawn) The method of claim 36, further comprising administering to the subject an effective amount of at least one laxative.
41. (Withdrawn) The method of claim 36, further comprising administering to the subject an effective amount of at least one anti-inflammatory agent.
42. (Withdrawn) A pharmaceutical composition comprising the oral dosage formulation of claim 2.
43. (New) The oral dosage formulation of claim 2, wherein the GCC agonist peptide is SEQ ID NO: 1 and the per unit dose is 3.0 mg or 6.0 mg.
44. (New) The oral dosage formulation of claim 43, wherein the GCC agonist peptide is stabilized against degradation for a period of at least 18 months at 30 °C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C.

REMARKS

Status of the Claims

Claims 1-44 are pending. Claims 1, 12, 13, 17-19, and 26-41 have been withdrawn from consideration. Applicants reserve the right to request rejoinder of claims to non-elected subject matter, upon the allowance of a claim directed to the elected invention. Applicants further reserve the right to file one or more divisional applications directed to the non-elected subject matter in this application. Claims 1-4, 7, 10, 16, and 25 are amended. New claims 43 and 44 are added.

Claim 2 and withdrawn claim 1 are amended to recite Guanylate Cyclase C (GCC). Support for this amendment can be found throughout the specification as filed, and specifically for example, at paragraph [02]. Claims 2 and 16 have been amended to recite the formulation comprises an inert low moisture carrier. Support for this amendment can be found throughout the specification as filed, and specifically for example, at paragraphs [044] and [184]. Claim 3 is amended to recite the GCC agonist peptide has a chromatographic purity of no less than 92% to 95%. Support for this amendment can be found throughout the specification as filed, and specifically for example, in claim 3 as originally filed. Claim 4 has been amended to recite the GCC agonist peptide has a total impurity content of no greater than 9%. Support for this amendment can be found throughout the specification as filed, and specifically for example, in claim 4 as originally filed. Claim 7 has been amended to recite the amount of GCC agonist peptide per unit dose is selected from the group of recited doses. Support for this amendment can be found throughout the specification as filed, and specifically for example, in claim 7 as originally filed. Claim 10 is amended to recite the inert carrier is a microcrystalline cellulose. Support for this amendment can be found throughout the specification as filed, and specifically for example, in claim 10 as originally filed. Claim 25 is amended to depend from claim 24. Support for this amendment can be found throughout the specification as filed, and specifically for example in paragraph [46].

New claim 43 recites the GCC agonist peptide is SEQ ID NO: 1 and the per unit dose is 3.0 mg or 6.0 mg. Support for this claim can be found throughout the specification as filed, and specifically for example in paragraph [11]. New claim 44 recites the GCC agonist peptide is

stabilized against degradation for a period of at least 18 months at 30°C and 65% relative humidity, or at least 18 months at 25 °C and 60% relative humidity, or at least 18 months at 2-8 °C. Support for this claim can be found throughout the specification as filed, and specifically for example in paragraph [15].

No new matter is added.

Claim Objections

The Examiner has objected to claims 2-4, 6-7, 14, 16-17, and 20 for informally reciting the abbreviation GCC. Applicants have herein amended claims 1 and 2 to recite the full name for this abbreviation, Guanylate Cyclase C, thereby obviating this objection.

Rejection of claims 3-4, 7, and 25 under 35 U.S.C. § 112, second paragraph

The Examiner has rejected claims 3-4, 7, and 25 under 35 U.S.C. § 112, second paragraph as allegedly being indefinite. Specifically, the Examiner contends the recitation of a broad range together with a narrow range that falls within the broader range is considered indefinite since the claim does not recite the metes and bounds of the protection desired.

The Examiner contends that claims 3, 4, and 7 are indefinite for reciting both a broad and a narrower range limitation. Solely in the interests of furthering prosecutions, Applicants have herein amended claim 3 to recite a chromatographic purity of no less than 92% to 95%, claim 4 to recite the GCC agonist peptide has a total impurity content of no greater than 9%, and claim 7 to recite the dose is selected from a group, thereby obviating the rejection as it pertains to these claims.

The Examiner contends that claim 25 lacks antecedent basis for the term “the liquid”. Solely in the interests of furthering prosecution, claim 25 has been amended to depend from claim 23 which recites a liquid-filled capsule, thereby obviating the rejection as it pertains to this claim.

The Examiner contends claim 20 is indefinite for reciting the term “temperature, relative humidity, and a period of 18 months” in claim 2 while claim 2 does not refer to any of the recited terminology. MPEP § 2173.02 states, in part, that definiteness of claims is not to be analyzed in a vacuum, but rather in light of:

- (A) The content of the particular application disclosure;
- (B) The teachings of the prior art; and
- (C) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

“The test for definiteness under the second paragraph of 35 U.S.C. § 112 is whether ‘those skilled in the art would understand what is claimed when the claim is read in light of the specification.’ *Orthokinetics, Inc. v. Safety Travel Chairs, Inc.*, 806 F.2d 1565, 1576, 1 USPQ2d 1081, 1088 (Fed. Cir. 1986)” (*Id.*). Applicants submit the skilled artisan reading claim 20 in light of the instant application would understand that claim 20 is drawn to those formulations of claim 2 that exhibit the recited stability characteristics, and consequently claim 20 is not indefinite.

Accordingly, Applicants request withdrawal of the rejection of claims 3-4, 7, and 25 under 35 U.S.C. § 112, second paragraph.

Rejection of claim 20 under 35 U.S.C. § 112, fourth paragraph

The Examiner has rejected claim 20 under U.S.C. § 112, fourth paragraph as allegedly being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends. Specifically, the Examiner contends that claim 20 is drawn to an oral dosage formulation comprising at least one GCC agonist peptide and excipient, and has the same scope of claim 2. Applicants respectfully disagree.

Claim 2 is drawn to an oral dosage form comprising a GCC peptide and one or more pharmaceutically acceptable excipients, while claim 20 is drawn to formulations of claim 2 that are stabilized against degradation under specific conditions. Applicants submit, that contrary to

the Examiner's assertion, the claims are not the same in scope. Accordingly, Applicants request withdrawal of the rejection of claim 20 under U.S.C. § 112, fourth paragraph.

Rejection of claims 2-11, 14-16, 20-22, 25, and 42 under 35 U.S.C. § 102

The Examiner has rejected claims 2-11, 14-16, 20-22, 25, and 42 under 35 U.S.C. § 102(b) as allegedly being anticipated by Shailubhai et al. (WO 2002/078683). Specifically, the Examiner contends Shailubhai *et al.* teaches a pharmaceutical composition comprising a guanylate cyclase C (GCC) agonist peptide having the sequence of SEQ ID NO:1, formulated with pharmaceutically acceptable excipients for oral administration, in a dosage between 100 µg to 3 g, with a purity of greater than 95%.

Applicants respectfully disagree. However, without acquiescing to the propriety of the rejection, and solely in the interests of furthering prosecution, Applicants have herein amended claim 2 to recite the formulation comprises an inert low moisture carrier. This element is not taught in Shailubhai *et al.* Accordingly, Applicants submit amended claim 2 is not anticipated by Shailubhai *et al.* Claims 3-11, 14-16, 20-22, 25, and 42 depend from claim 2 are not anticipated for the same reasons. Applicants request withdrawal of claims 2-11, 14-16, 20-22, 25, and 42 under 35 U.S.C. § 102(b).

Rejection of claims 2-11, 14-16, 20-25, and 42 under 35 U.S.C. § 103

The Examiner has rejected claims 2-11, 14-16, 20-22, 25, and 42 under 35 U.S.C. § 103(a) as allegedly being obvious over Shailubhai *et al.* (WO 2002/078683) in view of Fretzen *et al.* (WO 2010/027404). Specifically, the Examiner contends Shailubhai *et al.* teach an oral dosage formulation comprising a GCC agonist peptide of SEQ ID NO:1 and excipients, but does not specify the intrinsic stability of the GCC agonist peptide formulation. The Examiner argues Fretzen teaches a method and composition comprising a stable solid formulation of therapeutic polypeptides suitable for oral administration. The Examiner states it would have been obvious to the skilled artisan at the time of the invention to combine Shailubhai's oral dosage formulation

with Fretzen's method and composition for manufacturing a stable peptide formulation with predictable results.

The Examiner has also rejected claims 23-24 under 35 U.S.C. § 103(a) as allegedly being obvious over Shailubhai *et al.* (WO 2002/078683) and Fretzen *et al.* (WO 2010/027404), and further in view of Currie (US 2009/0253634). Specifically, the Examiner argues Shailubhai in view of Fretzen teach an oral dosage formulation comprising a GCC agonist peptide and one or more excipients with a purity greater than or equal to 95%, and that Currie teaches various forms of a GCC agonist peptide, including a solution, suspension in an aqueous or a non-aqueous liquid, capsule, or powder.

Applicants respectfully disagree. Amended claim 2 requires that the formulation includes an inert low moisture carrier.

There is no objective reason provided by the teachings of Shailubhai in view of Fretzen, and Currie that would lead the skilled artisan to combine these references, nor is there any evidence that the resultant combination of these references would lead the skilled artisan to arrive at the claimed invention with predictable results. These references, when considered in their entirety, fail to provide the skilled artisan with a reasonable expectation that an oral dosage formulation of the specifically recited Guanylate Cyclase C (GCC) agonist peptide and an inert low moisture carrier, as recited in claim 2, would have increased stability compared to any other inert carrier. This is especially true given the teaching of the instant specification and the accompanying §1.132 declaration of Steve Comiskey ("Comiskey Decl.") as discussed in detail below.

First, the improved stability of the GCC agonist formulation comprising a low-moisture inert carrier is shown superior results compared with formulations taught in the art. GCC agonist peptide formulated with a low moisture carrier are more stable than expected compared to formulations comprising a regular-grade carrier. (Comiskey Decl. at ¶5) As described in the Comiskey Decl. showing that formulations with a low moisture carrier decreased the amount of impurities dramatically, and more than had been expected. (Comiskey Decl. at ¶6 and 7). These data demonstrate that the claimed formulation provides an unexpectedly superior result relative to the formulations taught in the cited prior art.

Second, the Examiner has failed to make a *prima facie* case of obviousness. A *prima facie* case of “obviousness requires a suggestion of all limitations in a claim.” *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). *Shailubhai* does not teach or suggest a formulation comprising an inert low moisture carrier. Nothing in *Shailubhai* teaches or suggests a formulation comprising an inert low moisture carrier.

Nor do *Fretzen* or *Currie* cure the deficiencies of *Shailubhai*. Neither *Fretzen* nor *Currie* teach or suggest the use of an inert low moisture carrier in a formulation comprising a therapeutic peptide. The cited art therefore does not provide a suggestion of all elements of claim 2. Nor do they provide any reason to arrive at the subject matter of claim 2. The Examiner has therefore not made a *prima facie* case of obviousness with respect to claim 2. Claims 3-11, 14-16, 20-25, and 42 depend from claim 2 and are therefore not obvious for the same reasons.

In view of the foregoing, Applicants therefore respectfully request that the rejection be withdrawn.

Non-statutory double patenting rejection

Claims 2 and 8 are provisionally rejected on the ground of nonstatutory double patenting over claims 2-3 of co-pending U.S. Application Number 14/228,843. It is respectfully requested that this provisional rejection be held in abeyance until the finding of allowable subject matter in either the present application, or in U.S. Application Number 14/228,843.

CONCLUSION

In view of the foregoing, Applicant respectfully submits that no further impediments exist to the allowance of this application. However, the Examiner is requested to call the undersigned if any questions or comments arise.

The Director is hereby authorized to charge any appropriate fees, including those under 37 C.F.R. §§1.16, 1.17, and 1.21, that may be required by this paper, and to credit any overpayment, to Deposit Account No. 50-1283.

Dated: February 19, 2015

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of: Stephen Comiskey *et al* Confirmation No.: 3135
Application No.: 13/421,769 Group Art Unit: 1676
Filed: March 15, 2012 Examiner: LEE, Jia-Hai
FOR: FORMULATIONS OF GUANYLATE CYCLASE C AGONISTS AND METHODS OF USE

U.S. Patent and Trademark Office
Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

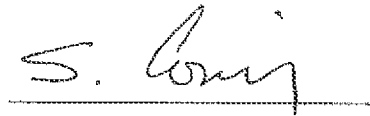
I, the undersigned Stephen Comiskey, declare and as follows:

1. I am the Vice President of Product Development of Synergy Pharmaceutical's the assignee of the above reference patent application I received a B.S. in Biochemistry, M.S. in Food Chemistry, and Ph.D. in Pharmaceutics from the University of Wisconsin-Madison.
2. I understand that the present claims are directed to an oral dosage formulation comprising at least one GCC agonist peptide and an inert low moisture carrier.
3. I have reviewed the Non-Final Office Action dated September 19, 2014. I understand the rejected pending claims as allegedly obvious over Shailubhai *et al.* (WO2002/078683) in view of Fretzen *et al.* (WO 2010/027404), and Currie *et al.* (US 2009/0253634).
4. I make this declaration to rebut the Examiner's rejection, with which I do not agree.

5. It is my opinion that the claimed methods are not obvious over the above cited references, for at least the following reasons. I have discovered that a low-moisture carrier improved the stability of a GCC agonist peptide compared to a regular grade carrier.
6. Appendix A shows total impurities and individual impurities with relative retention times (RRT) at both 25°C and 40°C in formulations of GCC agonist peptides comprising the low-moisture carrier (Avicel PH112) compared with the regular grade carrier (Avicel PH102). Formulations of plecanatide (a GCC agonist peptide) tablet with low moisture Avicel PH112 shows improved stability compared to regular grade Avicel.
7. This reduction in total impurities and impurities with relative retention times (RRT) with the low-moisture carrier (Avicel PH112) is surprising. Other than the low-moisture element of Avicel PH112, the two carriers are the same, but the reduced moisture content of the low-moisture carrier (~1.5%)¹ had a greater effect on peptide stability than expected. As shown in Appendix A, reducing the Avicel water content to not more than 1.5% resulted in approximately a 33% decrease in total impurities at 9 months at 25°C (2.733±0.289 for Avicel PH102; 1.82±0.217 and approximately a 34% decrease in total impurities at 12 months at 25°C (3.1±0.361 for Avicel PH102; 2.06±0.305 for Avicel PH112) and approximately a 30% decrease in total impurities at 6 months at 40°C (4.767±0.322 for Avicel PH102; 3.36±0.207 for Avicel PH112). This dramatic reduction in impurities was unexpected.

¹ <http://www.fmcbiopolymer.com/Portals/bio/content/Docs/PS-Section%2011.pdf>, at page 9.

8. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements 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 above-referenced application or any patent issuing thereon.



Stephen Comiskey

Signed, February 8, 2015

APPENDIX A

Improved Plicanamide Tablet Stability (reduced impurity levels) using low moisture Avicel PH112 compared to regular Avicel PH102
Created February 18, 2015

Batch (dosage form)	Months→ Avicel↓	Sum of Total Impurities (Area%)																	
		25C									40C								
		0	1	2	3	6	9	12	18	24	0	1	2	3	6				
2011F100A (3mg capsule)	PH102	2.4	2.6	2.6	2.9	2.9	2.9	3.2	3.3	3.6	2.4	3.2	3.6	4.2	4.9				
11H140 (3mg capsule)	PH102	2.1	2.5	2.7	2.6	2.8	2.9	3.4	3.6	3.7	2.1	3.5	3.6	4.0	5.0				
12G080 (3mg tablet)	PH102	1.9	2.7	2.2	2.3	2.4	2.4	2.7	3.1	3.2	1.9	3.0	2.8	3.2	4.4				
13C049 (3mg tablet)	PH112	1.2	1.2	1.4	1.4	1.7	1.7	2.0	2.2		1.2	1.7	2.1	3.1	3.2				
13C050 (3mg tablet)	PH112	1.2	1.2	1.3	1.3	1.4	1.4	1.7	1.9	2.4	1.2	1.7	2.1	2.3	3.4				
13C051 (6mg tablet)	PH112	1.1	1.2	1.3	1.4	1.5	1.7	1.9	2.3		1.1	1.7	2.5	2.2	3.2				
13E090 (3mg tablet)	PH112	1.3	1.2	1.2	1.4	1.6	1.8	1.9			1.3	1.8	2.5	2.6	3.3				
13F106 (6mg tablet)	PH112	1.7	1.6	1.5	1.9	2.1	2.2	2.6			1.7	1.9	2.6	2.8	3.7				

Batch (dosage form)	Months→ Avicel↓	Impurity RRT 0.97 (Area%)																	
		25C									40C								
		0	1	2	3	6	9	12	18	24	0	1	2	3	6				
2011F100A (3mg capsule)	PH102	0.48	0.26	0.34	0.00	0.43	0.33	0.38	0.20	0.37	0.48	0.29	0.31	0.31	0.33				
11H140 (3mg capsule)	PH102	0.11	0.20	0.26	0.00	0.31	0.36	0.63	0.15	0.24	0.11	0.22	0.25	0.24	0.25				
12G080 (3mg tablet)	PH102	0.00	0.30	0.00	0.30	0.00	0.00	0.00	0.11	0.00	0.00	0.34	0.00	0.00	0.00				
13C049 (3mg tablet)	PH112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00				
13C050 (3mg tablet)	PH112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00				
13C051 (6mg tablet)	PH112	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00				
13E090 (3mg tablet)	PH112	0.00	0.00	0.00	0.00	0.00	0.00	0.10			0.00	0.00	0.00	0.00	0.00				

13F106 (6mg tablet)	PH112	0.00	0.12	0.00	0.15	0.19	0.19			0.00	0.00	0.18	0.00	0.12
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Batch (dosage form)	Months→ Avicel↓	25C												40C					
		0	1	2	3	6	9	12	18	24	0	1	2	3	6				
2011F100A (3mg capsule)	PH102	0.29	0.49	0.54		0.69	0.92	1.10	1.32	1.44	0.29	1.00	1.36	1.73	2.49				
11H140 (3mg capsule)	PH102	0.31	0.45	0.54	0.60	0.79	0.95	1.04	1.48	1.69	0.31	1.10	1.48	1.84	2.77				
12G080 (3mg tablet)	PH102	0.12	0.21	0.29	0.34	0.50	0.57	0.61	0.76	0.92	0.12	0.54	0.83	0.97	1.53				
13C049 (3mg tablet)	PH112	0.22	0.29	0.36	0.38	0.56	0.58	0.70	0.84		0.22	0.65	0.82	1.54	1.45				
13C050 (3mg tablet)	PH112	0.21	0.32	0.33	0.39	0.54	0.58	0.67	0.85		0.21	0.62	0.84	0.95	1.42				
13C051 (6mg tablet)	PH112	0.22	0.26	0.35	0.38	0.50	0.58	0.66	0.82		0.22	0.66	1.15	0.90	1.39				
13E090 (3mg tablet)	PH112	0.25	0.31	0.37	0.43	0.51	0.66	0.69			0.25	0.65	0.94	1.11	1.43				
13F106 (6mg tablet)	PH112	0.20	0.26	0.29	0.43	0.47	0.57	0.67			0.20	0.59	0.76	1.03	1.38				

Impurity RRT 1.33 (Area%)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Stephen Comiskey *et al.* Confirmation No.: 3135

Application No.: 13/421,769 Group Art Unit: 1676

Filed: March 15, 2012 Examiner: Jia-Hai Lee

For: **Formulations of Guanylate Cyclase C Agonists and Methods of Use**

Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window, **Mail Stop Amendment**
Randolph Building
401 Dulany Street
Alexandria, VA 22314

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97(c)

In accordance with the duty of disclosure set forth in 37 C.F.R. §1.56, Applicant(s) hereby submits the following information in conformance with 37 C.F.R. §§1.97 and 1.98.

- Pursuant to 37 C.F.R. §1.98, copies of documents 60-249 cited in the attached Form PTO/SB/08a are enclosed.

- No copies of any U.S. patents or U.S. patent application publications listed on the attached Form PTO/SB/08a are being provided pursuant to 37 C.F.R. §1.98.

This Information Disclosure Statement is filed after the period specified in 37 C.F.R. § 1.97(b), but before the mailing of:

- a final action under 37 C.F.R. §1.113;
- a notice of allowance under 37 C.F.R. §1.311; or
- an action that otherwise closes prosecution in this application.

In accordance with 37 C.F.R. §1.97(c) also enclosed is:

- Fee under 37 C.F.R. §1.17(p) in the amount of \$90.00; or
- Statement as specified in 37 C.F.R. §1.97(e):

- Each item of information contained in the Information Disclosure Statement cited herein was first cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing date of the Information Disclosure Statement; or
- No item of information contained in the Information Disclosure Statement submitted herewith was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the undersigned, having made a reasonable inquiry, no item of information contained in the Information Disclosure Statement was known to any individual designated in 37 C.F.R. §1.56(c) more than three months prior to the filing date of the Information Disclosure Statement.

It is respectfully requested that the Examiner consider the above-noted information and return an initialed copy of the attached Forms PTO/SB/08a to the undersigned. The U.S. Patent and Trademark Office is hereby authorized to charge any fee deficiency, or credit any overpayment, to our Deposit Account No. 50-1283.

Dated: February 19, 2015

Respectfully submitted,
COOLEY LLP

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SHEET 1 OF 19

INFORMATION DISCLOSURE STATEMENT LIST (Use as many sheets as necessary)	Complete if Known	
	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Art Unit	1676
	Examiner Name	Jia-Hai Lee
	Attorney Docket Number	SYPA-009/X001US 321994-2142

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No. ¹	Document Number Number-Kind Code ² (if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
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	20.	2010/0069306 A1	03-18-2010	Shailubhai et al.	

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	39.	5,601,990	02-11-1997	Waldman et al.	
	40.	5,731,159	03-24-1998	Waldman et al.	

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FOREIGN PATENT DOCUMENTS

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	60.	DE 19744027	04-08-1999	Hoechst Marion Rouseel Deutschland GmbH		
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	109	DeSavage et al. "Precursor structure, expression and tissue distribution of human guanylin" Proc. Natl. Acad. Sci USA 89:9089-9093 (1992).	

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INFORMATION DISCLOSURE STATEMENT LIST (Use as many sheets as necessary)	Complete if Known	
	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Art Unit	1676
	Examiner Name	Jia-Hai Lee
	Attorney Docket Number	SYPA-009/X001US 321994-2142

NON PATENT LITERATURE DOCUMENTS

Examiner's Initials	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ⁶
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	119	European Patent 1,379,224: CombiMab, Inc. Annex to Notice of Opposition dated April 22, 2010 (41 pages)	
	120	European Patent 1,379,224:: Summons to attend oral hearing dated June 6, 2011 (23 pages)	
	121	European Patent 1,379,224: Response to Communication from Opposition division dated October 8, 2010 (44 pages)	
	122	European Patent 1,379,224: Written submission dated October 7, 2011 in response to the June 24, 2011 preliminary opinion of the Opposition Division (7 pages)	

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	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Art Unit	1676
	Examiner Name	Jia-Hai Lee
	Attorney Docket Number	SYPA-009/X001US 321994-2142

NON PATENT LITERATURE DOCUMENTS

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	186	Mahato et al. "Emerging trends in oral delivery of peptide and protein drugs" Crit. Rev. Ther. Drug Carrier Systems 20(2-3):153-214 (2003).	
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	Art Unit	1676
	Examiner Name	Jia-Hai Lee
	Attorney Docket Number	SYPA-009/X001US 321994-2142

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	198	Ohbayashi et al., "Effects of uroguanylin and guanylin against antigen-induced bronchoconstriction and airway microvascular leakage in sensitized guinea-pigs" Life Sci., 62(20):1883-1844 (1998)	
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	Application Number	13/421,769
	Filing Date	March 15, 2012
	First Named Inventor	Stephen Comiskey
	Art Unit	1676
	Examiner Name	Jia-Hai Lee
	Attorney Docket Number	SYPA-009/X001US 321994-2142

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	211	Schulz et al., "Guanylyl Cyclase Is a Heat-Stable Enterotoxin Receptor," Cell 63:941-948 (1990)	
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	246	Written Opinion of the International Searching Authority, PCT Appl. No. PCT/US2013/030551, 6 pages (June 18, 2013)	
	247	Wu et al. "Atrial natriuretic peptide induces apoptosis in neonatal rat cardia myocytes" J. Biol. Chem. 272(23):14860-14866 (1997)	
	248	Zhang et al. "Gene expression profiles in normal and cancer cells" Science 276:1268-1272 (1997)	
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Electronic Patent Application Fee Transmittal

Application Number:	13421769			
Filing Date:	15-Mar-2012			
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use			
First Named Inventor/Applicant Name:	Stephen Comiskey			
Filer:	Anne Elizabeth Fleckenstein			
Attorney Docket Number:	40737-509001US			
Filed as Small Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Claims in excess of 20	2202	2	40	80
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Extension - 3 months with \$0 paid	2253	1	700	700
Miscellaneous:				
Submission- Information Disclosure Stmt	2806	1	90	90
Total in USD (\$)				870

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EFS ID:	21547745
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	58249
Filer:	Anne Elizabeth Fleckenstein
Filer Authorized By:	
Attorney Docket Number:	40737-509001US
Receipt Date:	19-FEB-2015
Filing Date:	15-MAR-2012
Time Stamp:	19:02:26
Application Type:	Utility under 35 USC 111(a)

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Payment Type	Deposit Account
Payment was successfully received in RAM	\$870
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Req. Reconsideration-After Non-Final Reject	SYPA_009_X01US_Response.pdf	165753	no	13
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Information:					
2	Miscellaneous Incoming Letter	SYPA_009_X01US_Declaration_under_1_132.pdf	604923	no	5
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Warnings:					
Information:					
4	Information Disclosure Statement (IDS) Form (SB08)	SYPA_009_X01US_SB08A.pdf	361187	no	19
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5	Fee Worksheet (SB06)	fee-info.pdf	33723	no	2
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Electronic Acknowledgement Receipt

EFS ID:	21547745
Application Number:	13421769
International Application Number:	
Confirmation Number:	3135
Title of Invention:	Formulations of Guanylate Cyclase C Agonists and Methods of Use
First Named Inventor/Applicant Name:	Stephen Comiskey
Customer Number:	58249
Filer:	Anne Elizabeth Fleckenstein
Filer Authorized By:	
Attorney Docket Number:	40737-509001US
Receipt Date:	19-FEB-2015
Filing Date:	15-MAR-2012
Time Stamp:	19:02:26
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$870
RAM confirmation Number	5721
Deposit Account	501283
Authorized User	

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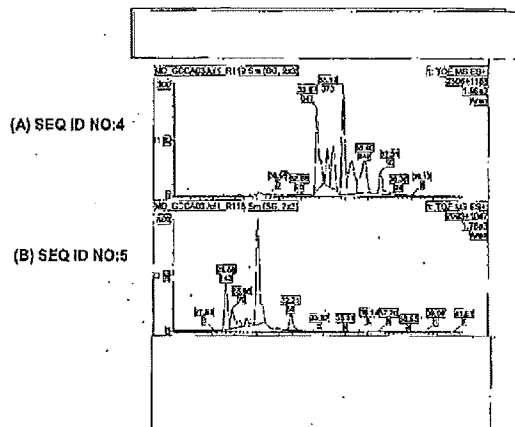
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(54) Title: METHODS AND COMPOSITIONS FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS

LCMS analysis of recombinant peptide variants



(57) Abstract: The present invention features compositions and related methods for treating IBS and other gastrointestinal disorders and conditions (e.g., gastrointestinal motility disorders, functional gastrointestinal disorders, gastroesophageal reflux disease (GERD), duodenogastric reflux, Crohn's disease, ulcerative colitis, Inflammatory bowel disease, functional heartburn, dyspepsia (including functional dyspepsia or nonulcer dyspepsia), gastroparesis, chronic intestinal pseudo-obstruction (or colonic pseudo-obstruction), and disorders and conditions associated with constipation, e.g., constipation associated with use of opiate pain killers, post-surgical constipation (post-operative ileus), and constipation associated with neuropathic disorders as well as other conditions and disorders using peptides and other agents that activate the guanylate cyclase C (GC-C) receptor.

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FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
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Methods and Compositions for the Treatment of Gastrointestinal Disorders

TECHNICAL FIELD

This invention relates to methods and compositions for treating various disorders,
5 including gastrointestinal disorders, obesity, congestive heart failure and benign prostatic
hyperplasia.

BACKGROUND

Irritable bowel syndrome (IBS) is a common chronic disorder of the intestine that affects
10 20 to 60 million individuals in the US alone (Lehman Brothers, Global Healthcare-
Irritable bowel syndrome industry update, September 1999). IBS is the most common
disorder diagnosed by gastroenterologists (28% of patients examined) and accounts for
12% of visits to primary care physicians (Camilleri 2001, Gastroenterology 120:652-
668). In the US, the economic impact of IBS is estimated at \$25 billion annually,
15 through direct costs of health care use and indirect costs of absenteeism from work
(Talley 1995, Gastroenterology 109:1736-1741). Patients with IBS have three times
more absenteeism from work and report a reduced quality of life. Sufferers may be
unable or unwilling to attend social events, maintain employment, or travel even short
distances (Drossman 1993, Dig Dis Sci 38:1569-1580). There is a tremendous unmet
20 medical need in this population since few prescription options exist to treat IBS.

Patients with IBS suffer from abdominal pain and a disturbed bowel pattern. Three
subgroups of IBS patients have been defined based on the predominant bowel habit:
constipation-predominant (c-IBS), diarrhea-predominant (d-IBS) or alternating between
25 the two (a-IBS). Estimates of individuals who suffer from c-IBS range from 20-50% of
the IBS patients with 30% frequently cited. In contrast to the other two subgroups that

have a similar gender ratio, c-IBS is more common in women (ratio of 3:1) (Talley et al. 1995, *Am J Epidemiol* 142:76-83).

The definition and diagnostic criteria for IBS have been formalized in the "Rome
5 Criteria" (Drossman et al. 1999, *Gut* 45:Suppl II: 1-81), which are well accepted in
clinical practice. Briefly, the criteria specify that for at least 12 weeks (consecutive or
non-consecutive in the preceding 12 months of abdominal discomfort or pain at least two
of the following three features must occur: (1) relieved with defecation, (2) onset
associated with a change in frequency of stool, and (3) onset associated with a change in
10 form (appearance) of stool. The Rome II criteria also state that the symptoms that
cumulatively support the diagnosis of irritable bowel syndrome include: abnormal stool
frequency ("abnormal" may be defined as greater than 3 bowel movements per day and
less than 3 bowel movements per week), abnormal stool form (lumpy/hard or
loose/watery stool), abnormal stool passage (straining, urgency, or feeling of incomplete
15 evacuation), passage of mucus, and bloating or feeling of abdominal distension.
However, the complexity of symptoms has not been explained by anatomical
abnormalities or metabolic changes. This has led to the classification of IBS as a
functional GI disorder, which is diagnosed on the basis of the Rome criteria and limited
evaluation to exclude organic disease (Ringel et al. 2001, *Annu Rev Med* 52: 319-338).
20 IBS is considered to be a "biopsychosocial" disorder resulting from a combination of
three interacting mechanisms: altered bowel motility, an increased sensitivity of the
intestine or colon to pain stimuli (visceral sensitivity) and psychosocial factors (Camilleri
2001, *Gastroenterology* 120:652-668). Recently, there has been increasing evidence for a
role of inflammation in etiology of IBS. Reports indicate that subsets of IBS patients
25 have small but significant increases in colonic inflammatory and mast cells, increased
inducible nitric oxide (NO) and synthase (iNOS) and altered expression of inflammatory
cytokines (reviewed by Talley 2000, *Medscape Coverage of DDW week*).

The present invention features peptides that activate and/or bind the guanylate cyclase-C (GC-C) receptor (reviewed by Lucas et al. 2000 Pharmacol. Rev 52:375-414 and Vaandrager et al. 2002 Molecular and Cellular Biochemistry 230:73-83) and any of its variants, including but not limited to insertion, deletion, mutation, and splice variants.

5 GC-C is a key regulator in mammals of intestinal function (although low levels of GC-C have been detected in other tissues). GC-C responds to the endogenous hormones, guanylin and uroguanylin, and to enteric bacterial peptides from the heat stable enterotoxin family (ST peptides). When agonists bind to GC-C, there is an elevation of the second messenger, cyclic GMP, and an increase in chloride and bicarbonate secretion,

10 resulting in an increase in intestinal fluid secretion. The Genbank GI accession number for guanylyl cyclase C homologs from multiple organisms are:

Genbank GI number	organism
27806993	cattle
16555439	eel
16555437	eel
4521169	fish
1850774	frog
1495352	Guinea pig
2494861	Guinea pig
4826752	human
4505441	human
1184046	human
1230617	mouse
2708786	mouse
71894985	mouse
47523018	pig

5930067	rabbit
6981000	rat
40445437	worm

SUMMARY

The present invention features compositions and related methods for treating IBS and
5 other gastrointestinal disorders and conditions (e.g., gastrointestinal motility disorders,
chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease,
duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional
gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD),
gastroparesis, irritable bowel syndrome (IBS, e.g., constipation predominant-IBS,
10 diarrhea predominant-IBS, and/or alternating-IBS)), post-operative ileus, ulcerative colitis,
chronic constipation, and disorders and conditions associated with constipation (e.g.
constipation associated with use of opiate pain killers, post-surgical constipation, and
constipation associated with neuropathic disorders as well as other conditions and
disorders are described herein. The compositions feature peptides that activate the
15 guanylate cyclase C (GC-C) receptor.

Also described herein are compositions and related methods for treating obesity,
congestive heart failure (including congestive heart failure at any of stages I-IV according
to New York Heart Association (NYHA) Functional Classification) and benign prostatic
hyperplasia (BPH).

20 Without being bound by any particular theory, in the case of IBS and other
gastrointestinal disorders the peptides are useful because they may increase
gastrointestinal motility.

Without being bound by any particular theory, in the case of IBS and other gastrointestinal disorders the peptides are useful, in part, because they may decrease inflammation.

- 5 Without being bound by any particular theory, in the case of IBS and other gastrointestinal disorders the peptides are also useful because they may decrease gastrointestinal pain, visceral pain, chronic visceral hypersensitivity, or hypersensitivity to colorectal distension.
- 10 Without being bound by any particular theory, in the case of salt retention, fluid retention disorders and combinations thereof the peptides are also useful because they may elicit one or more of diuresis, naturesis and/or kaliuresis. Thus the peptides described herein may be diuretics.
- 15 The invention features pharmaceutical compositions comprising certain peptides that are capable of activating the guanylate-cyclase C (GC-C) receptor. Also within the invention are pharmaceutical compositions comprising a peptide or GC-C agonist described herein and one or more additional therapeutic agents including, without limitation, the agents described herein. The other agents can be administered with the peptides described
- 20 herein (simultaneously or sequentially). They can also be linked to a peptide described herein to create therapeutic conjugates.

The invention includes methods for treating various gastrointestinal disorders by administering a peptide that acts as a partial or complete agonist of the GC-C receptor.

- 25 The peptide includes at least six cysteines that can form three disulfide bonds. In certain embodiments the disulfide bonds are replaced by other covalent cross-links and in some cases the cysteines are substituted by other residues to provide for alternative covalent cross-links. The peptides may also include at least one trypsin or chymotrypsin cleavage site and/or an amino or carboxy-terminal analgesic peptide or small molecule, e.g.,

AspPhe or some other analgesic peptide. When present within the peptide, the analgesic peptide or small molecule may be preceded by a chymotrypsin or trypsin cleavage site that allows release of the analgesic peptide or small molecule. The peptides and methods described herein are also useful for treating pain and inflammation associated with
5 various disorders, including gastrointestinal disorders. Certain peptides include a functional chymotrypsin or trypsin cleavage site located so as to allow inactivation of the peptide upon cleavage. Certain peptides having a functional cleavage site undergo cleavage and gradual inactivation in the digestive tract, and this is desirable in some circumstances. In certain peptides, a functional chymotrypsin site is altered, increasing
10 the stability of the peptide *in vivo*.

The invention includes: a method for increasing intestinal motility comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof.
15

The invention includes a method treating a disorder associated with reduced gastrointestinal transit rates or reduced gastrointestinal motility comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof

20 The invention also includes a method treating a gastrointestinal hypomotility disorder comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof.

The disorders which can be treated by administering a GC-C receptor agonist include
25 constipation, constipation dominant irritable bowel syndrome and pelvic floor dyssynergia.

The invention features a method treating a non-inflammatory gastrointestinal disorder comprising administering a GC-C receptor agonist, e.g., a peptide described herein, to a patient in need thereof.

- 5 The invention includes a method treating a gastrointestinal disorder other than Crohn's disease and ulcerative colitis comprising administering a GC-C receptor agonist to a patient in need thereof.

The invention includes methods for treating other disorders such as congestive heart failure and benign prostatic hyperplasia by administering a peptide or small molecule (parenterally or orally) that acts as an agonist of the GC-C receptor. Such agents can be used in combination with natriuretic peptides (e.g., atrial natriuretic peptide, brain natriuretic peptide or C-type natriuretic peptide), a diuretic, or an inhibitor of angiotensin converting enzyme.

15

The invention features methods and compositions for increasing intestinal motility. Intestinal motility involves spontaneous coordinated dissections and contractions of the stomach, intestines, colon and rectum to move food through the gastrointestinal tract during the digestive process.

20

In certain embodiments the patient has been diagnosed as suffering from IBS according to the Rome criteria. In certain embodiments the patient is female.

In certain embodiments the peptides include either one or two or more contiguous negatively charged amino acids (e.g., Asp or Glu) or one or two or more contiguous positively charged residues (e.g., Lys or Arg) or one or two or more contiguous positively or negatively charged amino acids at the carboxy terminus. In these embodiments all of the flanking amino acids at the carboxy terminus are either positively or negatively charged. In other embodiments the carboxy terminal charged amino acids are preceded

by a Leu. For example, any of the following amino acid sequences can be added to the carboxy terminus of the peptide: Asp; Asp Lys; Lys Lys Lys Lys Lys Lys; Asp Lys Lys Lys Lys Lys Lys; Leu Lys Lys; and Leu Asp. It is also possible to simply add Leu at the carboxy terminus.

5

In a first aspect, the invention features a peptide comprising, consisting of, or consisting essentially of the amino acid sequence (I):

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃
 10 Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (SEQ ID NO: 1)

In some embodiments Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is Asn Ser Ser Asn Tyr or is missing or Xaa₁ Xaa₂ Xaa₃ Xaa₄ is missing.

15 In certain embodiments Xaa₈, Xaa₉, Xaa₁₂, Xaa₁₄, Xaa₁₆, Xaa₁₇, and Xaa₁₉ can be any amino acid. In certain embodiments Xaa₈, Xaa₉, Xaa₁₂, Xaa₁₄, Xaa₁₆, Xaa₁₇, and Xaa₁₉ can be any natural or non-natural amino acid or amino acid analog.

In certain embodiments, the peptide does not include the sequence of E. coli ST peptide.

20 In other embodiment, the peptide does not include the sequence of any of the peptides in Table I, below.

In certain embodiments Xaa₅ is Asn, Trp, Tyr, Asp, or Phe. In other embodiments, Xaa₅ can also be Thr or Ile. In other embodiments Xaa₅ is Tyr, Asp or Trp. In certain

25 embodiments Xaa₅ is Asn, Trp, Tyr, Asp, Ile, Thr or Phe. In certain embodiments Xaa₅ is Asn.

In some embodiments Xaa₈ is Glu, Asp, Gln, Gly or Pro. In other embodiments Xaa₈ is Glu. In other embodiments Xaa₈ is Glu or Asp. In others it is Asn, Glu, or Asp. In others

it is Glu, His, Lys, Gln, Asn, or Asp. In others it is Glu, His, Gln, Asn, or Asp. In others it is Glu, Asn, His, Gln, Lys, Asp or Ser. In still others it is Pro. In certain embodiments it is any natural or non-natural amino acid or amino acid analog.

5 In some embodiments Xaa₉ is Leu, Ile, Val, Ala, Lys, Arg, Trp, Tyr or Phe. In some embodiments Xaa₉ is Leu, Ile, Val, Lys, Arg, Trp, Tyr or Phe. In others it is Leu, Ile, Val, Trp, Tyr or Phe. In others it is Leu, Ile or Val. In others it is Trp, Tyr or Phe. In others it is Leu, Ile, Lys, Arg, Trp, Tyr, or Phe. In others it is Leu, Val, Ile, or Met. In others it is Leu or Phe. In others it is Leu, Phe, or Tyr. In others it is Tyr, Phe or His. In others it is
10 Phe, His, Trp, or Tyr. In certain embodiments, Xaa₉ is not Leu. In others it is Tyr. In other embodiments it is any natural or non-natural aromatic amino acid or amino acid analog. In certain embodiments it is any natural or non-natural amino acid or amino acid analog.

15 In certain embodiments, Xaa₁₂ is Asn, Tyr, Asp or Ala. In others it is Asn. In others it is Asn, Met, Arg, Lys, His, or Gln. In others it is Asn, Lys, His, or Gln. In others it is Asn, Asp, Glu or Gln. In others it is Asn, Thr, Ser, Arg, Lys, Gln, or His. In others it is Asn, Ser, or His. In certain embodiments it is any natural or non-natural amino acid or amino acid analog.

20 In certain embodiments, Xaa₁₃ is Ala, Pro or Gly. In others it is Pro or Gly. In others it is Pro and in still others it is Gly.

In certain embodiments, Xaa₁₄ is Ala, Leu, Ser, Gly, Val, Glu, Gln, Ile, Leu, Thr, Lys,
25 Arg, or Asp. In others it is Ala or Gly. In others it is Val or Ala. In others it is Ala or Thr. In others it is Ala. In others it is Val, Gln, Asn, Glu, Asp, Thr, or Ala. In others it is Gly, Cys or Ser. In still others it is Thr. In certain embodiments it is any natural or non-natural amino acid or amino acid analog.

In certain embodiments Xaa₁₆ is Thr, Ala, Asn, Lys, Arg, Trp, Gly or Val. In others it is Thr, Ala, Asn, Lys, Arg or Trp. In others it is Thr, Ala, Lys, Arg or Trp. In certain embodiments it is Thr, Ala or Trp. In others it is Thr. In certain embodiments it is Trp, Tyr or Phe. In certain embodiments it is Thr or Ala. In certain embodiments it is Val. In certain embodiments it is Gly. In others it is Thr, Ser, Met or Val. In others it is Val, Ala, or Thr. In others it is Ile, Val, Lys, Asn, Glu, Asp, or Thr. In certain embodiments it is any natural or non-natural amino acid or amino acid analog. In certain embodiments it is any natural or non-natural non-aromatic amino acid or amino acid analog.

10 In certain embodiments Xaa₁₇ is Gly, Pro or Ala. In certain embodiments it is Gly. In certain embodiments it is Ala. In others it is Gly or Ala. In others it is Gly, Asn, Ser or Ala. In others it is Asn, Glu, Asp, Thr, Ala, Ser, or Gly. In others it is Asp, Ala, Ser, or Gly. In certain embodiments it is any natural or non-natural amino acid or amino acid analog.

15 In certain embodiments Xaa₁₉ is Trp, Tyr, Phe, Asn, Ile, Val, His, Leu, or Arg. In certain embodiments it is Trp, Tyr, Asn or Leu. In certain embodiments it is Trp, Tyr or Phe. In others it is Tyr, Phe or His. In others it is Tyr or Trp. In others it is Tyr. In certain embodiments it is Leu, Ile or Val. In certain embodiments it is His. In certain
20 embodiments it is Trp, Tyr, Phe, Asn, Ile, Val, His or Leu. In certain embodiments it is Trp, Tyr, Phe or Leu. In certain embodiments it is Tyr or Leu. In certain embodiments it is Lys or Arg. In certain embodiments it is any amino acid other than Pro, Arg, Lys, Asp or Glu. In certain embodiments it is any amino acid other than Pro. In certain
25 embodiments it is any natural or non-natural amino acid or amino acid analog. In certain
embodiments it is missing.

In certain embodiments Xaa₂₀ is Asp or Asn. In certain embodiments Xaa₂₀ Xaa₂₁ is AspPhe or is missing or Xaa₂₀ is Asn or Glu and Xaa₂₁ is missing or Xaa₁₉ Xaa₂₀ Xaa₂₁ is missing.

In certain embodiments, the invention features, a purified polypeptide comprising the amino acid sequence (II):

Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃
 5 Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁

wherein Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is Asn Ser Ser Asn Tyr or is missing or Xaa₁ Xaa₂ Xaa₃ Xaa₄ is missing and Xaa₅ is Asn;

Xaa₈ is Glu or Asp;

Xaa₉ is Leu, Ile, Val, Trp, Tyr or Phe;

10 Xaa₁₆ is Thr, Ala, Trp;

Xaa₁₉ is Trp, Tyr, Phe or Leu or is missing; and Xaa₂₀ Xaa₂₁ is AspPhe.

In various embodiments the invention features a purified polypeptide comprising the amino acid sequence (II): Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁
 15 Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ wherein, Xaa₉ is Leu, Ile or Val and Xaa₁₆ is Trp, Tyr or Phe; Xaa₉ is Trp, Tyr or Phe, and Xaa₁₆ is Thr or Ala; Xaa₁₉ is Trp, Tyr, Phe and Xaa₂₀ Xaa₂₁ is AspPhe; and Xaa₁ Xaa₂ Xaa₃ Xaa₄ is missing and Xaa₅ is Asn; the peptide comprises fewer than 50, 40, 30 or 25 amino acids; or fewer than five amino acids precede Cys₆.

20

In certain embodiments the peptide includes a peptide comprising, consisting essentially, or consisting of the amino acid sequence Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys Cys Glu Xaa₉ Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr Xaa₂₀ Xaa₂₁ (II) (SEQ ID NO:2) wherein Xaa₉ is any amino acid; wherein Xaa₉ is any amino acid other than Leu; wherein Xaa₉ is selected from Phe, Trp and Tyr; wherein Xaa₉ is selected from any other natural or non-natural aromatic amino acid; wherein Xaa₉ is Tyr; wherein Xaa₉ is Phe; wherein Xaa₉ is Trp; wherein Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is Asn Ser Ser Asn Tyr; wherein Xaa₁, Xaa₂, Xaa₃, Xaa₄, and Xaa₅ are missing; wherein Xaa₁, Xaa₂, Xaa₃ and Xaa₄ are missing; wherein Xaa₁, Xaa₂ and Xaa₃ are missing; wherein Xaa₁ and Xaa₂ are missing; wherein Xaa₁ is
 25

missing; wherein Xaa₂₀ Xaa₂₁ is AspPhe or is missing or Xaa₂₀ is Asn or Glu and Xaa₂₁ is missing or Xaa₁₉ Xaa₂₀ Xaa₂₁ is missing; wherein Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ and Tyr Xaa₂₀ Xaa₂₁ are missing. In the case of a peptide comprising the sequence (I): Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ wherein: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is missing and/or the
5 sequence Xaa₁₉ Xaa₂₀ Xaa₂₁ is missing, the peptide can still contain additional carboxyterminal or amino terminal amino acids or both. In the case of peptides missing one or more terminal amino acids such as Xaa₁ or Xaa₂₁, the peptide can still contain additional carboxyterminal or amino terminal amino acids or both.

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In certain embodiments, the peptide includes disulfide bonds between Cys₆ and Cys₁₁, between Cys₇ and Cys₁₅ and between Cys₁₀ and Cys₁₈. In other embodiments, the peptide is a reduced peptide having no disulfide bonds. In still other embodiments the peptide has one or two disulfide bonds chosen from: a disulfide bond between Cys₆ and
15 Cys₁₁, a disulfide bond between Cys₇ and Cys₁₅ and a disulfide bond between Cys₁₀ and Cys₁₈.

In certain embodiments the peptide includes a peptide comprising, consisting essentially, or consisting of the amino acid sequence Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys
20 Thr Gly Cys Xaa₁₄ (SEQ ID NO:XXX) wherein Xaa₄ is any amino acid: wherein Xaa₄ is any amino acid other than Leu; wherein Xaa₄ is selected from Phe, Trp and Tyr; wherein Xaa₄ is selected from any other natural aromatic amino acid or non-natural aromatic amino acid; wherein Xaa₄ is Tyr; wherein Xaa₄ is Phe; wherein Xaa₄ is Trp; wherein Xaa₁₄ is Tyr, wherein Xaa₁₄ is missing, and wherein Xaa₁₄ is selected from any other
25 natural or non-natural amino acid. In certain embodiments, the peptide may contain additional carboxyterminal or amino terminal amino acids or both. In some embodiments the peptide is 13, 14, 15, or 16 amino acids long.

In certain embodiments, the peptide includes disulfide bonds between Cys₁ and Cys₆, between Cys₂ and Cys₁₀ and between Cys₅ and Cys₁₃. In other embodiments, the peptide is a reduced peptide having no disulfide bonds. In still other embodiments the peptide has one or two disulfide bonds chosen from: a disulfide bond between Cys₁ and Cys₆, a
5 disulfide bond between Cys₂ and Cys₁₀ and a disulfide bond between Cys₅ and Cys₁₃.

In certain embodiments, one or more amino acids can be replaced by a non-naturally occurring amino acid or a naturally or non-naturally occurring amino acid analog. In certain embodiments, one or more L-amino acids can be substituted with a D-
10 amino acid. There are many amino acids beyond the standard 20 amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val). Some are naturally-occurring others are not (see, for example, Hunt, *The Non-Protein Amino Acids: In Chemistry and Biochemistry of the Amino Acids*, Barrett, Chapman and Hall, 1985). For example, an aromatic amino acid can be replaced by 3,4-
15 dihydroxy-L-phenylalanine, 3-iodo-L-tyrosine, triiodothyronine, L-thyroxine, phenylglycine (Phg) or nor-tyrosine (norTyr). Phg and norTyr and other amino acids including Phe and Tyr can be substituted by, e.g., a halogen, -CH₃, -OH, -CH₂NH₃, -C(O)H, -CH₂CH₃, -CN, -CH₂CH₂CH₃, -SH, or another group. Any amino acid can be substituted by the D-form of the amino acid.

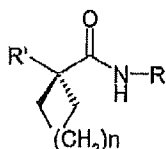
20 With regard to non-naturally occurring amino acids or naturally and non-naturally occurring amino acid analogs, a number of substitutions in the peptide of formula I or the peptide of formula II are possible alone or in combination.

25 Xaa₈ can be replaced by *gamma*-Hydroxy-Glu or *gamma*-Carboxy-Glu.

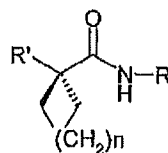
Xaa₉ can be replaced by an alpha substituted amino acid such as L-*alpha*-methylphenylalanine or by analogues such as: 3-Amino-Tyr; Tyr(CH₃); Tyr(PO₃(CH₃)₂); Tyr(SO₃H); *beta*-Cyclohexyl-Ala; *beta*-(1-Cyclopentenyl)-Ala; *beta*-Cyclopentyl-Ala; *beta*-

Cyclopropyl-Ala; *beta*-Quinolyl-Ala; *beta*-(2-Thiazolyl)-Ala; *beta*-(Triazole-1-yl)-Ala; *beta*-(2-Pyridyl)-Ala; *beta*-(3-Pyridyl)-Ala; Amino-Phe; Fluoro-Phe; Cyclohexyl-Gly; *t*Bu-Gly; *beta*-(3-benzothieryl)-Ala; *beta*-(2-thienyl)-Ala; 5-Methyl-Trp; and 4-Methyl-Trp.

- 5 Xaa₁₃ can be an N(*alpha*)-C(*alpha*) cyclized amino acid analogues with the structure:



$n = 0, 1, 2, 3$. Xaa₁₃ can also be homopro (L-pipecolic acid); hydroxy-Pro; 3,4-Dehydro-Pro; 4-fluoro-Pro; or *alpha*-methyl-Pro.



- 10 When Xaa₁₃ is Gly, Ala, Leu or Val, Xaa₁₄ can be: $n = 0, 1, 2, 3$

Xaa₁₄ can also be an *alpha*-substitued or N-methylated amino acid such as *alpha*-amino isobutyric acid (*aib*), L/D-*alpha*-ethylalanine (L/D-isovaline), L/D-methylvaline, or L/D-*alpha*-methylleucine or a non-natural amino acid such as *beta*-fluoro-Ala.

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Xaa₁₇ can be *alpha*-amino isobutyric acid (*aib*) or L/D-*alpha*-ethylalanine (L/D-isovaline).

Further examples of unnatural amino acids include: an unnatural analogue of tyrosine; an unnatural analogue of glutamine; an unnatural analogue of phenylalanine; an
 20 unnatural analogue of serine; an unnatural analogue of threonine; an alkyl, aryl, acyl, azido, cyano, halo, hydrazine, hydrazide, hydroxyl, alkenyl, alkynyl, ether, thiol, sulfonyl, seleno, ester, thioacid, borate, boronate, phospho, phosphono, phosphine, heterocyclic, enone, imine, aldehyde, hydroxylamine, keto, or amino substituted amino acid, or any

combination thereof; an amino acid with a photoactivatable cross-linker; a spin-labeled amino acid; a fluorescent amino acid; an amino acid with a novel functional group; an amino acid that covalently or noncovalently interacts with another molecule; a metal binding amino acid; an amino acid that is amidated at a site that is not naturally amidated, 5 a metal-containing amino acid; a radioactive amino acid; a photocaged and/or photoisomerizable amino acid; a biotin or biotin-analogue containing amino acid; a glycosylated or carbohydrate modified amino acid; a keto containing amino acid; amino acids comprising polyethylene glycol or polyether; a heavy atom substituted amino acid (e.g., an amino acid containing deuterium, tritium, ^{13}C , ^{15}N , or ^{18}O); a chemically 10 cleavable or photocleavable amino acid; an amino acid with an elongated side chain; an amino acid containing a toxic group; a sugar substituted amino acid, e.g., a sugar substituted serine or the like; a carbon-linked sugar-containing amino acid; a redox-active amino acid; an α -hydroxy containing acid; an amino thio acid containing amino acid; an α , α disubstituted amino acid; a β -amino acid; a cyclic amino acid other than proline; an 15 O-methyl-L-tyrosine; an L-3-(2-naphthyl)alanine; a 3-methyl-phenylalanine; a *p*-acetyl-L-phenylalanine; an O-4-allyl-L-tyrosine; a 4-propyl-L-tyrosine; a tri-O-acetyl-GlcNAc β -serine; an L-Dopa; a fluorinated phenylalanine; an isopropyl-L-phenylalanine; a *p*-azido-L-phenylalanine; a *p*-acyl-L-phenylalanine; a *p*-benzoyl-L-phenylalanine; an L-phosphoserine; a phosphoserine; a phosphotyrosine; a *p*-iodo-phenylalanine; a 4- 20 fluorophenylglycine; a *p*-bromophenylalanine; a *p*-amino-L-phenylalanine; an isopropyl-L-phenylalanine; L-3-(2-naphthyl)alanine; an amino-, isopropyl-, or O-allyl-containing phenylalanine analogue; a dopa, O-methyl-L-tyrosine; a glycosylated amino acid; a *p*-(propargyloxy)phenylalanine; dimethyl-Lysine; hydroxy-proline; mercaptopropionic acid; methyl-lysine; 3-nitro-tyrosine; norleucine; pyro-glutamic acid; Z (Carbobenzoxyl); 25 ϵ -Acetyl-Lysine; β -alanine; aminobenzoyl derivative; aminobutyric acid (Abu); citrulline; aminohexanoic acid; aminoisobutyric acid; cyclohexylalanine; d-cyclohexylalanine; hydroxyproline; nitro-arginine; nitro-phenylalanine; nitro-tyrosine; norvaline; octahydroindole carboxylate; ornithine; penicillamine; tetrahydroisoquinoline; acetamidomethyl protected amino acids and pegylated amino acids. Further examples of

unnatural amino acids and amino acid analogs can be found in U.S. 20030108885, U.S. 20030082575, US20060019347 (paragraphs 410-418) and the references cited therein. The polypeptides described herein can include further modifications including those described in US20060019347, paragraph 589.

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In some embodiments, an amino acid can be replaced by a naturally-occurring, non-essential amino acid, e.g., taurine.

Methods to manufacture peptides containing unnatural amino acids can be found in, for example, U.S. 20030108885, U.S. 20030082575, US20060019347, Deiters et al., J Am Chem Soc. (2003) 125:11782-3, Chin et al., Science (2003) 301:964-7, and the references cited therein.

Peptides that include non-natural amino acids can also be prepared using the methods described in WO02086075

The peptides described herein can have one or more conventional peptide bonds replaced by an alternative bond. Such replacements can increase the stability of the peptide. For example, replacement of the peptide bond between Cys₁₈ and Xaa₁₉ with an alternative bond can reduce cleavage by carboxy peptidases and may increase half-life in the digestive tract. Bonds that can replace peptide bonds include: a retro-inverso bonds (C(O)-NH instead of NH-C(O)); a reduced amide bond (NH-CH₂); a thiomethylene bond (S-CH₂ or CH₂-S); an oxomethylene bond (O-CH₂ or CH₂-O); an ethylene bond (CH₂-CH₂); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); an fluoro substituted trans-olefine bond (CF=CH); a ketomethylene bond (C(O)-CHR or CHR-C(O) wherein R is H or CH₃); and a fluoro-ketomethylene bond (C(O)-CFR or CFR-C(O) wherein R is H or F or CH₃).

The peptides described herein can be modified using standard modifications. Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a

combination of any of the preceding. In one aspect described herein, there may be more than one type of modification of the peptide. Modifications include but are not limited to: acetylation, amidation, biotinylation, cinnamoylation, farnesylation, formylation, myristoylation, palmitoylation, phosphorylation (Ser, Tyr or Thr), stearoylation, succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cy3 or Cy5. The peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methyl-coumarin (AMC), fluorescein, NBD (7-Nitrobenz-2-Oxa-1,3-Diazole), p-nitro-anilide, rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid), dabcyf, dabsyl, dansyl, texas red, Fmoc, and Tamra (Tetramethylrhodamine). The peptides described herein may also be conjugated to, for example, polyethylene glycol (PEG); alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; combinations of PEG, alkyl groups and fatty acid radicals (see U.S. Patent 6,309,633; Soltero et al., 2001 Innovations in Pharmaceutical Technology 106-110); BSA and KLH (Keyhole Limpet Hemocyanin). The addition of PEG and other polymers which can be used to modify polypeptides described herein is described in US2006019347 section IX.

The peptides and agonists described herein can be chemically modified to increase therapeutic activity by synthetically adding sugar moieties (WO 88/02756; WO 89/09786; DE 3910667 A1, EP 0 374 089 A2; and U.S. 4,861,755), adding cationic anchors (EP0363589), lipid moieties (WO91/09837; U.S. 4,837,303) or the substituents described as compounds I, II, and III in US5552520.

When Xaa₉ is Trp, Tyr or Phe or when Xaa₁₆ is Trp the peptide has a potentially functional chymotrypsin cleavage site that is located at a position where cleavage may alter GC-C receptor binding by the peptide. When Xaa₉ is Lys or Arg or when Xaa₁₆ is Lys or Arg, the peptide has a potentially functional trypsin cleavage site that is located at a position where cleavage may alter GC-C receptor binding by the peptide.

When Xaa₁₉ is Trp, Tyr or Phe, the peptide has a chymotrypsin cleavage site that is located at a position where cleavage will liberate the portion of the peptide carboxy-terminal to Xaa₁₉. When Xaa₁₉ is Leu, Ile or Val, the peptide can have a chymotrypsin cleavage site that is located at a position where cleavage will liberate the portion of the peptide amino-terminal to Xaa₁₉. At relatively high pH the same effect is seen when Xaa₁₉ is His. When Xaa₁₉ is Lys or Arg, the peptide has a trypsin cleavage site that is located at a position where cleavage will liberate portion of the peptide carboxy-terminal to Xaa₁₉. Thus, if the peptide includes an analgesic peptide carboxy-terminal to Xaa₁₉, the peptide will be liberated in the digestive tract upon exposure to the appropriate protease. Among the analgesic peptides which can be included in the peptide and/or coadministered with the peptide are: AspPhe (as Xaa₂₀Xaa₂₁), endomorphin-1, endomorphin-2, nocistatin, dalargin, lupron, ziconotide, and substance P and other analgesic peptides described herein. These peptides can, for example, be used to replace Xaa₂₀Xaa₂₁.

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When Xaa₁ or the amino-terminal amino acid of the peptide described herein (e.g., Xaa₂ or Xaa₃) is Trp, Tyr or Phe, the peptide has a chymotrypsin cleavage site that is located at a position where cleavage will liberate the portion of the peptide amino-terminal to Xaa₁ (or Xaa₂ or Xaa₃) along with Xaa₁, Xaa₂ or Xaa₃. When Xaa₁ or the amino-terminal amino acid of the peptide described herein (e.g., Xaa₂ or Xaa₃) is Lys or Arg, the peptide has a trypsin cleavage site that is located at a position where cleavage will liberate portion of the peptide amino-terminal to Xaa₁ along with Xaa₁, Xaa₂ or Xaa₃). When Xaa₁ or the amino-terminal amino acid of the peptide described herein is Leu, Ile or Val, the peptide can have a chymotrypsin cleavage site that is located at a position where cleavage will liberate the portion of the peptide amino-terminal to Xaa₁. At relatively high pH the same effect is seen when Xaa₁ is His. Thus, for example, if the peptide includes an analgesic peptide amino-terminal to Xaa₁, the peptide will be liberated in the digestive tract upon exposure to the appropriate protease. Among the analgesic peptides which can be included in the peptide are: AspPhe, endomorphin-1, endomorphin-2,

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nocistatin, dalargin, luproin, and substance p and other analgesic peptides described herein.

In certain embodiments, when fully folded, disulfide bonds may be present between: Cys₆ and Cys₁₁; Cys₇ and Cys₁₅; and Cys₁₀ and Cys₁₈. In other embodiments, when fully
5 folded, disulfide bonds may be present between: Cys₁ and Cys₆; Cys₂ and Cys₁₀; and Cys₅ and Cys₁₃. The peptides described herein bear some sequence similarity to ST peptides. However, they include amino acid changes and/or additions that improve functionality. These changes can, for example, increase or decrease activity (e.g., increase or decrease
10 the ability of the peptide to stimulate intestinal motility), alter the ability of the peptide to fold correctly, alter the stability of the peptide, alter the ability of the peptide to bind the GC-C receptor and/or decrease toxicity. In some cases the peptides may function more desirably than wild-type ST peptide. For example, they may limit undesirable side effects such as diarrhea and dehydration.

15 In some embodiments one or both members of one or more pairs of Cys residues which normally form a disulfide bond can be replaced by homocysteine, penicillamine, 3-mercaptoproline (Kolodziej et al. 1996 Int J Pept Protein Res 48:274); β , β dimethylcysteine (Hunt et al. 1993 Int J Pept Protein Res 42:249) or diaminopropionic acid (Smith et al. 1978 J Med Chem 21:117) to form alternative internal cross-links at the
20 positions of the normal disulfide bonds.

In addition, one or more disulfide bonds can be replaced by alternative covalent cross-
links, e.g., an amide linkage (-CH₂CH(O)NHCH₂- or -CH₂NHCH(O)CH₂-), an ester
25 linkage, a thioester linkage, a lactam bridge, a carbamoyl linkage, a urea linkage, a thiourea linkage, a phosphonate ester linkage, an alkyl linkage (-CH₂CH₂CH₂CH₂-), an alkenyl linkage(-CH₂CH=CHCH₂-), an ether linkage (-CH₂CH₂OCH₂- or -CH₂OCH₂CH₂-), a thioether linkage (-CH₂CH₂SCH₂- or -CH₂SCH₂CH₂-), an amine linkage (-CH₂CH₂NHCH₂- or -CH₂NHCH₂CH₂-) or a thioamide linkage (-

CH₂CH(S)HNHCH₂- or -CH₂NHCH(S)CH₂-). For example, Ledu et al. (Proc Nat'l Acad. Sci. 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links. Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000) describes stable, hydrocarbon cross-links. Hydrocarbon cross links can be produced via metathesis (or methathesis followed by hydrogenation in the case of saturated hydrocarbons cross-links) using one or another of the Grubbs catalysts (available from Materia, Inc. and Sigma-Aldrich and described, for example, in U.S. Patent No. 5,831,108 and 6,111,121). In some cases, the generation of such alternative cross-links requires replacing the Cys residues with other residues such as Lys or Glu or non-naturally occurring amino acids. In addition the lactam, amide and hydrocarbon cross-links can be used to stabilize the peptide even if they link amino acids at positions other than those occupied by Cys. Such cross-links can occur between two amino acids that are separated by two amino acids or between two amino acids that are separated by six amino acids (see, e.g., Schafmeister et al. (J. Am. Chem. Soc. 122:5891, 2000)).

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In the case of a peptide comprising the sequence (I): Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys Cys Glu Xaa₉ Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr Xaa₂₀ Xaa₂₁ (II) wherein: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is missing and/or the sequence Xaa₁₉ Xaa₂₀ Xaa₂₁ is missing, the peptide can still contain additional carboxyterminal or amino terminal amino acids or both. For example, the peptide can include an amino terminal sequence that facilitates recombinant production of the peptide and is cleaved prior to administration of the peptide to a patient. The peptide can also include other amino terminal or carboxyterminal amino acids. In some cases the additional amino acids protect the peptide, stabilize the peptide or alter the activity of the peptide. In some cases some or all of these additional amino acids are removed prior to administration of the peptide to a patient. The peptide can include 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70 80, 90, 100 or more amino acids at its amino terminus or carboxy terminus or both. The number of flanking amino acids need not be the same. For example, there can

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be 10 additional amino acids at the amino terminus of the peptide and none at the carboxy terminus.

In one embodiment the peptide comprises the amino acid sequence (I): Xaa₁ Xaa₂ Xaa₃
 5 Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈
 Xaa₁₉ Xaa₂₀ Xaa₂₁ wherein: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ is missing; Xaa₈ is Glu; Xaa₉ is
 Leu, Ile, Lys, Arg, Trp, Tyr or Phe; Xaa₁₂ is Asn; Xaa₁₃ is Pro; Xaa₁₄ is Ala; Xaa₁₆ is Thr,
 Ala, Lys, Arg, Trp; Xaa₁₇ is Gly; Xaa₁₉ is Tyr or Leu; and Xaa₂₀ Xaa₂₁ is AspPhe or is
 missing. Where Xaa₂₀ Xaa₂₁ and/or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ are missing, there may be
 10 additional flanking amino acids in some embodiments. In certain embodiments of a
 composition comprising a peptide having the sequence (I): Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅
 Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀
 Xaa₂₁, the peptide does not comprise or consist of any of the peptides of Table I.

15 In a second aspect, the invention also features a therapeutic or prophylactic method
 comprising administering to a patient a pharmaceutical composition comprising or
 consisting essentially of a purified peptide comprising, consisting of or consisting
 essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉
 Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁
 20 Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆
 Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr
 Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described herein.

The peptides can be co-administered with or linked, e.g., covalently linked to any of a
 25 variety of other peptides or compounds including analgesic peptides or analgesic
 compounds including, without limitation, the agents described herein.

Amino acid, non-amino acid, peptide and non-peptide spacers can be interposed between
 a peptide that is a GC-C receptor agonist and a peptide that has some other biological

function, e.g., an analgesic peptide or a peptide used to treat obesity. The linker can be one that is cleaved from the flanking peptides *in vivo* or one that remains linked to the flanking peptides *in vivo*. For example, glycine, beta-alanine, glycyL-glycine, glycyL-beta-alanine, gamma-aminobutyric acid, 6-aminocaproic acid, L-phenylalanine, L-tryptophan and glycyL-L-valil-L-phenylalanine can be used as spacers (Chaltin et al. 2003 Helvetica Chimica Acta 86:533-547; Caliceti et al. 1993 FARMCO 48:919-32) as can polyethylene glycols (Butterworth et al. 1987 J. Med. Chem 30:1295-302) and maleimide derivatives (King et al. 2002 Tetrahedron Lett. 43:1987-1990). Various other linkers are described in the literature (Nestler 1996 Molecular Diversity 2:35-42; Finn et al. 1984 Biochemistry 23:2554-8; Cook et al. 1994 Tetrahedron Lett. 35:6777-80; Brokx et al. 2002 Journal of Controlled Release 78:115-123; Griffin et al. 2003 J. Am. Chem. Soc. 125:6517-6531; Robinson et al. 1998 Proc. Natl. Acad. Sci. USA 95:5929-5934). Linkers are also described in US20050171014, for example, amino acid linkers such as FALA, VLALA, ALAL, ALALA, 2-cyclohexyl-L-alanine-LALA, 2-cyclohexyl-L-alanine-2-cyclohexyl-L-alanine-LAL, 1-naphtyl-alanine-ChaLAL and 1-naphtyl-alanine-LALA. Peptides and agonists described herein can also be conjugated to: an affinity tag (such as (histidine 6) H6), a HIV tat peptide residues 49-57, HIV tat peptide residues 49-56, the tat sequence YGRKKRRQRRR, a polyarginine peptide having from 6 to 20 residues (such as R6) and the following peptide sequences: YARKARRQARR, YARAAARQARA, YARAARRAARR, YARAARRAARA, ARRRRRRRRR, and YAAARRRRRRR, which are disclosed in WO 99/29721 and in US patent No. 6,221,355 (seq. id. nos. 3-8).

The peptides described herein can be attached to one, two or more different moieties each providing the same or different functions. For example, the peptide can be linked to a molecule that is an analgesic and to a peptide that is used to treat obesity. The peptide and various moieties can be ordered in various ways. For example, a peptide described herein can have an analgesic peptide linked to its amino terminus and an anti-obesity peptide linked to its carboxy terminus. The additional moieties can be directly covalently bonded to the peptide or can be bonded via linkers.

The peptides described herein can be a cyclic peptide or a linear peptide. In addition, multiple copies of the same peptide can be incorporated into a single cyclic or linear peptide.

5 The peptides can include the amino acid sequence of a peptide that occurs naturally in a vertebrate (e.g., mammalian) species or in a bacterial species. In addition, the peptides can be partially or completely non-naturally occurring peptides. Also within the invention are peptidomimetics corresponding to the peptides described herein.

In various embodiments, the patient is suffering from a gastrointestinal disorder; the
10 patient is suffering from a disorder selected from the group consisting of: gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus,
15 ulcerative colitis, chronic constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders are described herein); the patient is suffering from a gastrointestinal motility disorder, chronic intestinal pseudo-obstruction, colonic pseudo-
20 obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, inflammatory bowel disease, irritable bowel syndrome (e.g. d-IBS, c-IBS, and/or a-IBS), post-operative ileus, ulcerative colitis, chronic constipation, and disorders and conditions associated with
25 constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other conditions and disorders are described herein); the patient has been diagnosed with a functional gastrointestinal disorder according to the Rome Criteria (e.g. Rome II), the

patient has been diagnosed with irritable bowel syndrome (e.g. (e.g. diarrhea predominant-IBS, constipation predominant-IBS, and/or alternating-IBS), according to the Rome Criteria (e.g. Rome II); the composition is administered orally; the peptide comprises 30 or fewer amino acids, the peptide comprises 20 or fewer amino acids, the peptide comprises no more than 5 amino acids prior to Cys₆; the peptide comprises 14 amino acids, the peptide comprises 13 amino acids; the peptide comprises 150, 140, 130, 120, 110, 100, 90, 80, 70, 60, 50, 40, or 30 or fewer amino acids. In other embodiments, the peptide comprises 20 or fewer amino acids. In other embodiments the peptide comprises no more than 20, 15, 10, or 5 peptides subsequent to Cys₁₈. In certain 10 embodiments Xaa₁₉ is a chymotrypsin or trypsin cleavage site and an analgesic peptide is present immediately following Xaa₁₉.

In a third aspect, the invention features a method for treating a patient suffering from constipation. Clinically accepted criteria that define constipation include the frequency of 15 bowel movements, the consistency of feces and the ease of bowel movement. One common definition of constipation is less than three bowel movements per week. Other definitions include abnormally hard stools or defecation that requires excessive straining (Schiller 2001, *Aliment Pharmacol Ther* 15:749-763). Constipation may be idiopathic (functional constipation or slow transit constipation) or secondary to other causes 20 including neurologic, metabolic or endocrine disorders. These disorders include diabetes mellitus, hypothyroidism, hyperthyroidism, hypocalcaemia, Multiple Sclerosis, Parkinson's disease, spinal cord lesions, Neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung's disease and Cystic fibrosis. Constipation may also be the result of surgery (postoperative ileus) or due to the use of drugs such as analgesics (like 25 opioids), antihypertensives, anticonvulsants, antidepressants, antispasmodics and antipsychotics. The method of treating constipation comprises administering a pharmaceutical composition comprising or consisting essentially of a peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇

Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀
 Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys Glu
 Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described
 herein.

5

In various embodiments, the constipation is associated with use of a therapeutic agent;
 the constipation is associated with a neuropathic disorder; the constipation is post-
 surgical constipation (postoperative ileus); and the constipation associated with a
 gastrointestinal disorder; the constipation is idiopathic (functional constipation or slow
 10 transit constipation); the constipation is spinal chord injury induced; the constipation is
 thyroid disease related; the constipation is associated with neuropathic, metabolic or
 endocrine disorder (e.g., diabetes mellitus, hypothyroidism, hyperthyroidism,
 hypocalcaemia, Multiple Sclerosis, Parkinson's disease, spinal cord lesions,
 neurofibromatosis, autonomic neuropathy, Chagas disease, Hirschsprung's disease or
 15 cystic fibrosis). Constipation may also be the result of surgery (postoperative ileus) or
 due the use of drugs such as analgesics (e.g., opioids), antihypertensives, anticonvulsants,
 antidepressants, antispasmodics and antipsychotics.

In a fourth aspect, the invention features a method for treating a patient suffering a
 20 gastrointestinal disorder, the method comprising administering to the patient a
 pharmaceutical composition comprising or consisting essentially of a purified peptide
 comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂
 Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇
 Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀
 25 Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys Glu
 Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described
 herein.

In various embodiments, the patient is suffering from a gastrointestinal disorder; the patient is suffering from a disorder selected from the group consisting of: gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer
 5 dyspepsia, a functional gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-operative ileus, ulcerative colitis, chronic constipation, and disorders and conditions associated with constipation (e.g. constipation associated with use of opiate pain killers, post-surgical constipation, and constipation associated with neuropathic disorders as well as other
 10 conditions and disorders are described herein), obesity, congestive heart failure, or benign prostatic hyperplasia.

In a fifth aspect, the invention features a method for increasing gastrointestinal motility in a patient, the method comprising administering to a patient a pharmaceutical composition
 15 comprising a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID
 20 NO:XXX) as described herein.

In a sixth aspect, the invention features a method for increasing the activity of (activating) an intestinal guanylate cyclase (GC-C) receptor in a patient, the method comprising administering to a patient a pharmaceutical composition comprising a
 25 purified peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II)

or Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described herein.

In a seventh aspect, the invention features an isolated nucleic acid molecule comprising a
5 nucleotide sequence encoding a polypeptide comprising the amino acid sequence: Xaa₁
Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆
Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉
Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys
10 Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described
herein.

In an eighth aspect the invention features a method for treating constipation, the method
comprising administering an agonist of the intestinal guanylate cyclase (GC-C) receptor.
In various embodiments: the agonist is a peptide, the peptide includes two Cys that form
15 one disulfide bond, the peptide includes four Cys that form two disulfide bonds, and the
peptide includes six Cys that form three disulfide bonds.

In a ninth aspect, the invention features a method for treating a gastrointestinal disorder,
gastrointestinal motility disorders, chronic intestinal pseudo-obstruction, colonic pseudo-
20 obstruction, Crohn's disease, duodenogastric reflux, dyspepsia, functional dyspepsia,
nonulcer dyspepsia, a functional gastrointestinal disorder, functional heartburn,
gastroesophageal reflux disease (GERD), gastroparesis, irritable bowel syndrome, post-
operative ileus, ulcerative colitis, chronic constipation, and disorders and conditions
associated with constipation (e.g. constipation associated with use of opiate pain killers,
25 post-surgical constipation, and constipation associated with neuropathic disorders as well
as other conditions and disorders are described herein), obesity, congestive heart failure,
or benign prostatic hyperplasia, the method comprising administering an agonist of the
intestinal guanylate cyclase (GC-C) receptor either orally, by rectal suppository, or
parenterally. In various embodiments: the agonist is a peptide, the peptide includes two

Cys that form one disulfide bond, the peptide includes four Cys that form two disulfide bonds, and the peptide includes six Cys that form three disulfide bonds.

In a tenth aspect, the invention features a method for treating a gastrointestinal disorder
5 selected from the group consisting of: gastrointestinal motility disorders, chronic
intestinal pseudo-obstruction, colonic pseudo-obstruction, Crohn's disease,
duodenogastric reflux, dyspepsia, functional dyspepsia, nonulcer dyspepsia, a functional
gastrointestinal disorder, functional heartburn, gastroesophageal reflux disease (GERD),
gastroparesis, irritable bowel syndrome, post-operative ileus, ulcerative colitis, chronic
10 constipation, and disorders and conditions associated with constipation (e.g. constipation
associated with use of opiate pain killers, post-surgical constipation, and constipation
associated with neuropathic disorders as well as other conditions and disorders are
described herein), the method comprising administering an agonist of the intestinal
guanylate cyclase (GC-C) receptor. In various embodiments the composition is
15 administered orally; the peptide comprises 30 or fewer amino acids, the peptide
comprises 20 or fewer amino acids, the peptide comprises, consists essentially, consists
of 14 amino acids, the peptide comprises, consists essentially, consists of 13 amino acids,
and the peptide comprises no more than 5 amino acids prior to Cys₆.

20 In various embodiments: the agonist is a peptide, the peptide includes two Cys that form
one disulfide bond, the peptide includes four Cys that form two disulfide bonds, and the
peptide includes six Cys that form three disulfide bonds.

In an eleventh aspect, the invention features a method for treating obesity, the method
25 comprising administering a complete or partial agonist of the intestinal guanylate cyclase
(GC-C) receptor. In various embodiments: the agonist is a peptide, the peptide includes
two Cys that form one disulfide bond, the peptide includes four Cys that form two
disulfide bonds, and the peptide includes six Cys that form three disulfide bonds. The
agonist can be administered alone or in combination with one or more agents for

treatment of obesity, including but not limited to the anti-obesity agents described herein. Thus, for example, PYY₃₋₃₆ can be fused to the carboxy or amino terminus of a peptide described herein. Such a fusion protein can include a chymotrypsin or trypsin cleavage site that can permit cleavage to separate the two peptides.

5

In a twelfth aspect, the invention features a method for treating obesity, the method comprising administering to a patient a pharmaceutical composition comprising or consisting essentially of a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉
 10 Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁
 Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆
 Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (II) or Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr
 Gly Cys Xaa₁₄ (SEQ ID NO:XXX) as described herein.

15 In a thirteenth aspect, the invention features a composition comprising or consisting essentially of a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂
 Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I) or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅
 Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅ Xaa₁₆ Gly₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀
 20 Xaa₂₁ (II) or Cys Cys Glu Xaa₄ Cys Cys Asn Pro Ala Cys Thr Gly Cys Xaa₁₄ (SEQ ID
 NO:XXX) as described herein. In one embodiment, the composition is a pharmaceutical composition.

In a fourteenth aspect, the invention features a method for treating congestive heart
 25 failure, the method comprising administering to a patient a pharmaceutical composition comprising or consisting essentially of a purified peptide comprising, consisting of or consisting essentially of the amino acid sequence: Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇
 Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Xaa₁₂ Xaa₁₃ Xaa₁₄ Cys₁₅ Xaa₁₆ Xaa₁₇ Cys₁₈ Xaa₁₉ Xaa₂₀ Xaa₂₁ (I)
 or Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Cys₆ Cys₇ Xaa₈ Xaa₉ Cys₁₀ Cys₁₁ Asn₁₂ Pro₁₃ Ala₁₄ Cys₁₅