determined as the activity of a positive ST control, Cys-Cys-Glu-Leu-Cys-Cys-Asn-Pro-Ala-Cys-Thr-Gly-Cys-Tyr and set to 100%.

Example 3: Anion Secretion in T84 cells

The ability of agents to increase chloride anion secretion can be examined using the T84 human colon carcinoma cell line (American Type Culture Collection (Bethesda, Md)). Briefly, cells are grown to confluency in 24-well culture plates with a 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), supplemented with 5% fetal calf serum and were used at between passages 54 and 60. Chloride ion secretion is measured in the presence of vehicle only or test article similar the methods described in Dharmasthaphorn et al. (1985) J Clin Invest 75:462-471 and Barrett and Bigby (1993) Am J Physiol 264:C446-52. Briefly, an Ussing chamber is modified to allow maintenance of the integrity of the cell monolayers during the study. The modified chamber is designed to minimize turbulence created by the air lift system and to avoid edge damage to the monolayers. 10^6 T84 cells are plated on a permeable support (1.98 cm^2 surface area) and maintained for 5-6 day before use. The supports are suspended over the bottom of a 100-mm culture dish to permit "bottom feeding" by laying them on top of a layer of glass beads as described in Barret and Bigby supra. After cell growth, the entire ring assembly is inserted into the Ussing chamber. No pressure is exerted directly on the monolayers and hence edge damage is avoided. Mucosal and serosal reservoirs contain identical volumes of oxygenated Ringer's solution (pH 7.4, at 37°C) that contained (in millimolar): Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO_3, 25; H_2PO_4, 2.4; HPO_4, 0.4; and glucose, 10. Potential difference (PD) across the cell monolayer is measured by calomel electrodes in 3 M KCl and monitored with a potentiometer. Throughout the experiment, except for 5-10 seconds every 5 min while the PD is being recorded, spontaneous tissue PD is short circuited and nullified by an automatic voltage clamp (WPI, New Haven, CT) with $Ag:AgCl_2$ electrodes. Tissue conductance (G) is calculated from the PD and the imposed current according to Ohm's law. The magnitude of changes in the short circuit current (Isc) is used as an index of chloride secretion.

Example 4: Effect on fluid secretion and sodium excretion in ligated loops rodent models

The effect of agents described herein on fluid and sodium secretion can be assessed by injecting vehicle or a test agent (e.g., one or more agents described herein) directly into an isolated loop. This is done by surgically ligating a loop in the small intestine of a mouse. The methodology for ligated loop formation is described in London et al. 1997 Am J Physiol p.G93-105. The loop is roughly centered and is approximately 1-3 cm. The loops are injected with a test agent or vehicle. Following a recovery time of 90 minutes the loops are excised. Weights are recorded for each loop before and after removal of the fluid contained therein. The length of each loop is also recorded. A weight to length ratio (W/L) for each loop is calculated to determine the effects of test agent as compared to vehicle. To determine the effect of a test agent on sodium excretion, fluid from the loops is collected and profiled for electrolyte levels. Similar assays can be performed using rats instead of mice.

Example 5: Animal Models of Hypertension

Various animal models of hypertension can be used to screen the agents described herein for anti-hypertensive activity. In general, hypertension can be induced in rats in at least four ways, including: genetically-induced, environmentally-induced, pharmacologically-induced, and renal-induced. A variety of rodent hypertension models are described in Pinto et al. (1998 Cardiovascular Research 39:77-88), Badyal et al. (2003 Indian Journal of Pharmacology 35: 349-362) and the references cited therein. One of the most widely used rodent models of hypertension is the Spontaneously Hypertensive Rat (SHR). Other models include: (1) the two-kidney one-clip, (2) transgenic rats overexpressing the murine Ren2 gene, (3) DOCA (deoxycorticosterone acetate)-salt model and (4) the Dahl salt sensitive rat. Thus, for example, agents described herein, can be administered to Dahl salt sensitive rats (Rapp and Dene 1985 Hypertension 7:340-9) to determine effects on blood pressure, urine volume and urinary sodium excretion and left ventricular wall thickness (for example as described in examples 4 and 5 herein).

Example 6: Measurement of the Effects of Lubiprostone and ST peptide on Urinary Sodium Excretion and Urine Volume.

All experimental subjects were female Sprague-Dawley rats which weighed between 200-230 g at the time of experimentation. Following arrival at the animal facility, rats were housed in solid bottom cages in groups of three, where they had unlimited access to food and water.

Temperature was maintained at 21 ± 2°C, and lights were on a 12:12 hr cycle (with lights on at 6:00AM).

Following at least 3 days of acclimation to the facility prior to experimentation, rats were dosed orally (PO) with either vehicle (phosphate buffered saline) or test article, and transferred to individual metabolism cages where they had access to food and water. The volume of urine excreted was recorded from 0-3 hours, and 4-6 hours post dose. In addition, 0.5-1.0mL urine samples were taken at each of the above time points and frozen for later analysis. Urine samples were analyzed for sodium concentration using ISE crown-ether membrane methodology on an Olympus AU5400 chemistry immuno analyzer (Olympus America Inc).

Figures 1, 2, and 3 demonstrate the effects of Lubiprostone and ST peptide (SEQ ID NO: 1: CCELCCNPACTGCY) on urine sodium and urine volume in this assay.

Example 7. Effects of a test agent of left ventricular wall thickness in salt-sensitive and salt-resistant rats.

Salt-sensitive and salt-resistant, 4-5 week-old male Dahl rats (Brookhaven National Laboratory, Upton, New York, USA) are fed with Purina rat chow with 0.4% NaCl for the first 3-4 weeks. Thereafter salt-sensitive and salt-resistant rats are randomized into two populations receiving either a high-salt (8% NaCl) or a low-salt (0.4% NaCl) diet for a further 3 weeks. Following this, each population is separated into two groups, one receiving test agent in tap water and the other vehicle only. Test agent is given in incremental doses until systolic blood pressure (tail-cuff measurement) is < 140 mmHg.

At the end of the study rats are anaesthetized with intraperitoneal sodium pentobarbital (45 mg/kg), and systolic and diastolic blood pressures are measured directly through catheterization of the right femoral artery, using a Beckman R611 recorder. Blood (8-10 ml) for determination of plasma rennin activity (New England Nuclear Corporation, Boston, Massachusetts, USA) and aldosterone concentration (Diagnostic Products Corporation, Los Angeles, USA) is obtained by decapitation. Hearts are removed and placed in a Petri dish, and blood and blood clots are flushed out with cold saline. Superficial water is removed by blotting. The whole heart is weighed, thereafter the atria and the right ventricular free wall are dissected from the

interventricular septum. The remaining interventricular septum and the left ventricle represented left ventricular weight, and the left ventricular weight: body weight ratio is taken as a measure for left ventricular mass or left ventricular hypertrophy.

Methods of Treatment

A number of disorders associated with fluid or salt retention may be prevented or treated with agents that reduce sodium absorption in the intestine and/or increase anion secretion (e.g., in the instestine). Useful agents include: guanylate cyclase receptor C agonists, soluble guanylate cyclase modulators, prostanoids including prostaglandin E and derivatives thereof, chloride channel activators (e.g. Amitiza® (lubiprostone)), 5HT4 agonists, cyclic nucleotides, laxatives, CFTR (cystic fibrosis transmembrane conductance regulator) modulators, agents which affect cAMP levels, sodium transport inhibitors (e.g. sodium channel inhibitors such as amiloride), phosphodiesterase inhibitors, renin inhibitors and aldosterone antagonists, potassium, polymer resins, and combinations thereof described herein. The agents that reduce sodium absorption in the intestine and/or increase anion secretion can be used alone or in combination with one or more agents useful in the treatment of congestive heart failure, and/or one or more lipid lowering agent and/or one or more anti-hypertensive agents.

Agents useful in the treatment of congestive heart failure

The agents described herein can be administered together with one or more agents useful in the treatment of congestive heart failure including, for example, nesiritide, dobutamine (beta receptor antagonist), milrinone (phosphodiesterase inhibitor), Levosimendan (Simdax®), adenosine, an adenosine analog (e.g. N^6 -[(1,2-dihydro-1-acenaphthylenyl)methyl]adenosine, dipyridamole or iodotubercidin), an agent which increases the cellular availability of adenosine, an adenosine A_2 receptor agonist, an adenosine transport inhibitor, or an adenosine deaminase inhibitor.

Lipid Lowering Agents

Lipid lowering agents or dilipidemia agents are those agents that act directly or indirectly to reduce serum cholesterol. Such agents include, but are not limited to, bile acid sequestrants such as cholestyramine (a styrene-divinylbenzene copolymer containing quaternary ammonium

cationic groups capable of binding bile acids, such as QUESTRAN® or QUESTRAN LIGHT® cholestyramine which are available from Bristol-Myers Squibb), colesevelam hydrochloride (such as WELCHOL® Tablets (polyallylamine hydrochloride) cross-linked with epichlorohydrin and alkylated with 1-bromodecane and (6-bromohexyl)-trimethylammonium bromide) which are available from Sankyo), colestipol (a copolymer of diethylenetriamine and 1-chloro-2,3-epoxypropane, such as COLESTID® tablets which are available from Pharmacia), dialkylaminoalkyl derivatives of a cross-linked dextran, LOCHOLEST®, DEAE-Sephadex (SECHOLEX®, POLICEXIDE®), water soluble derivatives such as 3,3-ioene, N-(cycloalkyl)alkylamines and poliglusam, insoluble quaternized polystyrenes, saponins and mixtures thereof and those bile acid sequestrants disclosed in WO97/11345, WO98/57652, US3692895, and US5703188. Suitable inorganic cholesterol sequestrants include bismuth salicylate plus montmorillonite clay, aluminum hydroxide and calcium carbonate antacids.

HMG-CoA reductase inhibitors are dyslipidemic agents that can be used in therapeutic combination with GC-C receptor agonist. s with compounds described herein. Suitable HMG-CoA reductase inhibitors for use in therapeutic combination with a compounds described herein include: atorvastatin (LIPITOR®; disclosed in US4681893, US5385929 and US5686104), atorvastatin calcium (disclosed in US5273995), dihydrocompactin, (disclosed in US4450171), bervastatin (disclosed in US5082859), carvastatin, cerivastatin (BAYCOL®; disclosed in US5006530, US5502199, and US5177080), crilvastatin, dalvastatin (disclosed in EP738510A2), fluvastatin (LESCOL®; disclosed in US4739073 and US534772), glenvastatin, fluindostatin (disclosed in EP363934A1), velostatin (visinolin; disclosed in US4448784 and US4450171), lovastatin (mevinolin; MEVACOR® (Merck and Co.) and related compounds disclosed in US4231938), mevastatin (and related compound disclosed in US3983140), compactin (and related compounds disclosed in US4804770), pitavastatin (also known as NK-104, itavastatin, nisvastatin, nisbastatin disclosed in US5102888), pravastatin (PRAVACHOL® (Bristol Myers Squibb) and related compounds disclosed in US4346227), rivastatin (sodium 7-(4-fluorophenyl)-2,6-diisopropyl-5-methoxymethylpyridin-3-yl)-3,5-dihydroxy-6-heptanoate), rosuvastatin (CRESTOR®; also known as ZD-4522 disclosed in US5260440), atavastatin, visastatin, simvastatin (ZOCOR® (Merck and Co.) and related compounds as disclosed in US4448784 and US4450171), sirrivastatin, CI-981, compounds disclosed in WO03/033481, US4231938,

US4444784, US4647576, US4686237, US4499289, US4346227, US5753675, US4613610, EP0221025, and EP491226, and optical or geometric isomers thereof; and nontoxic pharmaceutically acceptable salts, N-oxides, esters, quaternary ammonium salts, and prodrugs thereof. In HMG-CoA reductase inhibitors where an open-acid form can exist, salt and ester forms may preferably be formed from the open-acid, and all such forms are included within the meaning of the term "HMG-CoA reductase inhibitor" as used herein. Pharmaceutically acceptable salts with respect to the HMG-CoA reductase inhibitor includes non-toxic salts of the compounds which are generally prepared by reacting the free acid with a suitable organic or inorganic base, particularly those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc and tetramethylammonium, as well as those salts formed from amines such as ammonia, ethylenediamine, N-methylglucamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, 1-p-chlorobenzyl-2-pyrrolidine-1'-yl-methylbenzim- idazole, diethylamine, piperazine, and tris(hydroxymethyl) aminomethane. Further examples of salt forms of HMG-CoA reductase inhibitors may include, but are not limited to, acetate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, calcium edetate, camsylate, carbonate, chloride, clavulanate, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, gluceptate, gluconate, glutamate, glycollylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynapthoate, iodide, isothionate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, oleate, oxalate, pamaote, palmitate, panthothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, and valerate.

Other dyslipidemic agents which can be used in therapeutic combination with a GC-C receptor agonist described herein include:

HMG-CoA synthase inhibitors such as L-659,699 ((E,E)-11-[3'R-(hydroxy-methyl)-4'-oxo-2'R-oxetanyl]-3,5,7R-trimethyl-2,4-undecadienoic acid) and those disclosed in US5120729, US5064856, and US4847271;

cholesterol absorption inhibitors such as plant sterols, plant stanols and/or fatty acid esters of plant stanols such as sitostanol ester used in BENECOL® margarine, stanol esters, beta-sitosterol, and sterol glycosides such as tiqueside. Other cholesterol absorption inhibitors include 1,4-Diphenylazetidin-2-ones; 4-biarylyl-1-phenylazetidin-2-ones; 4-(hydroxyphenyl)azetidin-2-ones; 1,4-diphenyl-3-hydroxyalkyl-2-azetidinones; 4-biphenyl-1-phenylazetidin-2-ones; 4-biarylyl-1-phenylazetidin-2-ones; and 4-biphenylylazetidinones.

acyl coenzyme A -cholesterol acyl transferase (ACAT) inhibitors such as avasimibe (Current Opinion in Investigational Drugs. 3(9):291-297 (2003)), eflucimibe, HL-004, lecimibe, DuP-128, KY505, SMP 797, CL-277,082 (Clin Pharmacol Ther. 48(2):189-94 (1990)) and the like; and those disclosed in US5510379, WO96/26948 and WO96/10559;

CETP inhibitors such as JTT 705 identified as in Nature 406, (6792):203-7 (2000), torcetrapib (CP-529,414 described in US20030186952 and WO00/017164), CP 532,632, BAY63-2149, SC 591, SC 795, and the like including those described in Current Opinion in Investigational Drugs. 4(3):291-297 (2003) and those disclosed in J. Antibiot., 49(8): 815-816 (1996), and Bioorg. Med. Chem. Lett., 6:1951-1954 (1996) and patent publications US5512548, US6147090, WO99/20302, WO99/14204, WO99/41237, WO95/04755, WO96/15141, WO96/05227, WO038721, EP796846, EP818197, EP818448, DE19704244, DE19741051, DE19741399, DE197042437, DE19709125, DE19627430, DE19832159, DE19741400, JP 11049743, and JP 09059155;

squalene synthetase inhibitors such as squalestatin-1, TAK-475, and those disclosed in US4871721, US4924024, US5712396 (α-phosphono-sulfonates), Biller et al (1988) J. Med. Chem., 31:1869 (e.g. isoprenoid (phosphinyl-methyl)phosphonates), Biller et al (1996) Current Pharmaceutical Design, 2:1, P. Ortiz de Montellano et al (1977) J. Med. Chem. 20:243 (terpenoid pyrophosphates), Corey and Volante (1976) J. Am. Chem. Soc., 98:1291 (farnesyl diphosphate analog A and presqualene pyrophosphate (PSQ-PP) analogs), McClard et al (1987) J.A.C.S., 109:5544 (phosphinylphosphonates), Capson, T. L., PhD dissertation, June, 1987, Dept. Med. Chem. U of Utah, Abstract, Table of Contents, pp 16, 17, 40-43, 48-51, Summary,

(cyclopropanes), Curr. Op. Ther. Patents (1993) 861, and patent publications EP0567026A1, EP0645378A1, EP0645377A1, EP0611749A1, EP0705607A2, EP0701725A1, and WO96/09827;

antioxidants such as probucol (and related compounds disclosed in US3674836), probucol derivatives such as AGI-1067 (and other derivatives disclosed in US6121319 and US6147250), tocopherol, ascorbic acid, β-carotene, selenium and vitamins such as vitamin B6 or vitamin B12 and pharmaceutically acceptable salts and esters thereof;

PPARα agonists such as those disclosed in US6028109 (fluorophenyl compounds), WO00/75103 (substituted phenylpropionic compounds), WO98/43081 and fibric acid derivatives (fibrates) such as beclofibrate, benzafibrate, bezafibrate (C.A.S. Registry No. 41859-67-0, see US3781328), binifibrate (C.A.S. Registry No. 69047-39-8, see BE884722), ciprofibrate (C.A.S. Registry No. 52214-84-3, see US3948973), clinofibrate (C.A.S. Registry No. 30299-08-2, see US3716583), clofibrate (such as ethyl 2-(p-chlorophenoxy)-2-methyl-propionate, e.g. Atromid-S® capsules (Wyeth-Ayerst), etofibrate, fenofibrate (such as Tricor® micronized fenofibrate ((2-[4-(4-chlorobenzoyl)phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester; Abbott Laboratories) or Lipanthyl® micronized fenofibrate (Labortoire Fournier, France)), gemcabene, gemfibrozil (such as 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid, e.g. Lopid® tablets (Parke Davis)), lifibrol, GW 7647, BM 170744, LY518674 and those fibrate and fibrate acid derivatives disclosed in WO03/033456, WO03/033481, WO03/043997, WO03/048116, WO03/053974, WO03/059864, and WO03/05875;

FXR receptor modulators such as GW 4064, SR 103912, and the like;

LXR receptor modulators such as GW 3965, T9013137, and XTC0179628, and those disclosed in US20030125357, WO03/045382, WO03/053352, WO03/059874, and the like;

HM74 and HM74A (human HM74A is Genbank Accession No. AY148884 and rat HM74A is EMM_patAR098624) receptor agonists such as nicotinic acid (niacin) and derivatives thereof (e.g. compounds comprising a pyridine-3-carboxylate structure or a pyrazine-2-carboxylate structure, including acid forms, salts, esters, zwitterions and tautomers, where available) including but not limited to those disclosed in Wise et al (2003) J. Biol. Chem. 278: 9869 (e.g. 5-

methylpyrazole-3-carboxylic acid and acifran (4,5-dihydro-5-methyl-4-oxo-5-phenyl-2-furan carboxylic acid pyradine-3-acetic acid)), as well as 5-methyl nicotinic acid, nicotinuric acid, niceritrol, nicofuranose, acipimox (5-methylpyrazine-2-carboxylic acid 4-oxide), Niaspan® (niacin extended-release tablets; Kos) and those which can be easily identified by one skilled in the art which bind to and agonize the HM74A or HM74 receptor (for example using the assays disclosed in Wise et al (2003) J. Biol. Chem 278:9869 (nicotine binding and [35S]-GTPγS binding assays), Soga et al (2003) Biochem. Biophys. Res. Comm. 303:364 (radiolabel binding assay using the HM74 receptor which could be adapted to the HM74A receptor), Tunaru et al (2003) Nature Medicine 9:352 (calcium mobilization assay using the HM74 receptor which could be adapted to the HM74A receptor) and US6420183 (FLIPR assays are described generally in and may be adapted to the HM74A or HM74 receptor);

renin angiotensin system inhibitors;

bile acid reabsorption inhibitors (bile acid reuptake inhibitors), such as BARI 1453, SC435, PHA384640, S8921, AZD7706, and the like;

PPARδ agonists (including partial agonists) such as GW 501516, and GW 590735, and those disclosed in US5859051 (acetophenols), WO03/024395, W097/28149, WO01/79197, WO02/14291, WO02/46154, WO02/46176, WO02/076957, WO03/016291, WO03/033493, WO99/20275 (quinoline phenyl compounds), WO99/38845 (aryl compounds), WO00/63161 (1,4-disubstituted phenyl compounds), WO01/00579 (aryl compounds), WO01/12612 & WO01/12187 (benzoic acid compounds), and WO97/31907 (substituted 4-hydroxy-phenylalconic acid compound);

sterol biosynthesis inhibitors such as DMP-565;

triglyceride synthesis inhibitors;

microsomal triglyceride transport (MTTP) inhibitors, such as inplitapide, LAB687, and CP346086, AEGR 733, implitapide and the like;

HMG-CoA reductase gene expression inhibitors (e.g. compounds that decrease HMG-CoA reductase expression by affecting (e.g. blocking) transcription or translation of HMG-CoA reductase into protein or compounds that may be biotransformed into compounds that have the aforementioned attributes by one or more enzymes in the cholesterol biosynthetic cascade or may lead to the accumulation of an isoprene metabolite that has the aforementioned activities (such regulation is readily determined by those skilled in the art according to standard assays (Methods of Enzymology, 110:9-19 1985))) such as those disclosed in US5041432 (certain 15-substituted lanosterol derivatives) and E. I. Mercer (1993) Prog. Lip. Res. 32:357 (oxygenated sterols that suppress the biosynthesis of HMG-CoA reductase);

squalene epoxidase inhibitors such as NB-598 ((E)-N-ethyl-N-(6,6-dimethyl-2-hepten-4-y- nyl)-3-[(3,3'-bithiophen-5-yl)methoxy]benzene-methanamine hydrochloride);

low density lipoprotein (LDL) receptor inducers such as HOE-402 (an imidazolidinyl-pyrimidine derivative that directly stimulates LDL receptor activity, see Huettinger et al (1993) Arterioscler. Thromb. 13:1005);

platelet aggregation inhibitors;

5-LO or FLAP inhibitors;

PPAR modulators (including compounds that may have multiple functionality for activating various combinations of PPARα, PPARγ, and PPARδ) such as those disclosed in US6008237, US6248781, US6166049, WO00/12491, WO00/218355, WO00/23415, WO00/23416, WO00/23425, WO00/23442, WO00/23445, WO00/23451, WO00/236331, WO00/236332, WO00/238553, WO00/50392, WO00/53563, WO00/63153, WO00/63190, WO00/63196, WO00/63209, WO00/78312, WO00/78313, WO01/04351, WO01/14349, WO01/14350, WO01/16120, WO01/17994, WO01/21181, WO01/21578, WO01/25181, WO01/25225, WO01/25226, WO01/40192, WO01/79150, WO02/081428, WO02/100403, WO02/102780, WO02/79162, WO03/016265, WO03/033453, WO03/042194, WO03/043997, WO03/066581, WO97/25042, WO99/07357, WO99/11255, WO99/12534, WO99/15520, WO99/46232, and

WO98/05331 (including GW2331 or (2-(4-[difluorophenyl]-1 heptylureido)ethyl]phenoxy)-2-methylbutyric));

niacin-bound chromium, as disclosed in WO03/039535;

substituted acid derivatives disclosed in WO03/040114;

apolipoprotein B inhibitors such as those disclosed in WO02/090347, WO02/28835, WO03/045921, WO03/047575;

Factor Xa modulators such as those disclosed in WO03/047517, WO03/047520, WO03/048081;

ileal bile acid transport ("IBAT") inhibitors (or apical sodium co-dependent bile acid transport ("ASBT") inhibitors) such as benzothiepines (including 1,2-benzothiazepines; 1,4-benzothiazepines; 1,5-benzothiazepines; 1,2, 5-benzothiadiazepines);

PPARδ activators such as disclosed in WO01/00603 (thiazole and oxazole derivates (e.g. C.A.S. Registry No. 317318-32-4), WO97/28149 (fluoro, chloro and thio phenoxy phenylacetic), US5093365 (non-1-oxidizable fatty acid analogues), and WO99/04815.

Anti-hypertensive agents

The agents described herein can be used in therapeutic combination with one or more anti-hypertensive agents, including but 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 396-01-0)), and aldosterone antagonists (e.g., spironolactone (CAS Number 52-01-7 and active metabolites thereof including canrenone), epirenone, and the like);

β-adrenergic blockers such as Amiodarone (Cordarone, Pacerone), bunolol hydrochloride (CAS RN 31969-05-8, Parke-Davis), acebutolol (±N-[3-Acetyl-4-[2-hydroxy-3-[(1 methylethyl)amino]propoxy]phenyl]-butanamide, or (±)-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, Corgard, 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(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-, hemihydrate, (S)-, CAS RN 91524-16-2), timolol maleate (S)-1-[(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]phenoxyl]-3-[(1-meth- ylethyl)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), nebivalol (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-methylethy)-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), flestolol sulfate (Benzoic acid, 2-fluro-,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-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[1-methylethyl)amino]-; CAS RN 37350-58-6), metoprolol tartrate (such as 2-Propanol, 1-[4-(2-methoxyethyl)phenoxy]-3-[(1-methylethyl)amino]-, e.g., Lopressor®, Novartis), pamatolol sulfate (Carbamic acid, [2-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxyl]phenyl]-ethyl]-, methyl ester, (±) sulfate (salt) (2:1), CAS RN 59954-01-7), penbutolol sulfate (2-Propanol, 1-(2-cyclopentylphenoxy)-3-[1,1-dimethylethyl)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 (Propanol, 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 (e.g., Visken), propanolol (e.g., Inderal, Inderal-LA), tertatolol, Coreg (carvedilol), 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 is 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 benzeneacetronitrile, (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-phenoxyethyl)-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)thiep- in-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-(1S)-propyl]amino]-2,3,4,5-tetrahydro-2-oxo-1H -1-(3S)-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), enalopril, enaloprilat, fosinopril, ((such as *trans*-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-[[(R)-[(1S)-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 (Hoechsst) 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-2-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 C.A. 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)-[[(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 (phosphonamidates);

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), nicotinyl 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® Titradose®, Wyeth-Ayerst), sosorbide mononitrate (such as 1,4:3,6-dianhydro-D-glucito- 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]-Nisoldipine3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester CAS RN 63675-72-9), nifedipine3,5-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, pratosartan, Fl6828K, RNH6270, candesartan (l 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-l-[[2'-(lH-tetrazol-5-yl)biphenyl-4-yl]-lH-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'-(lh-tetrazol-5-yl)biphenyl-4-yl]methyl]1,3-diazazspiro[4,4]non-l-en-4-one, US5270317 and US5352788), losartan (2-N-butyl-4-chloro-5-hydroxymethyl-l-[(2'-(lH-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole, potassium salt, US5138069, US5153197 and US5128355), tasosartan (5,8-dihydro-2,4-dimethyl-8-[(2'-(lH-tetrazol-5-yl)[1,1'-biphenyl]4-yl)methyl]-pyrido[2,3-d]pyrimidin-7(6H)-one, US5149699), telmisartan (4'-[(1,4-dimethyl-2'-propyl-(2,6'-bi-lH-benzimidazol)-l'-yl)]-[1,1'-biphenyl]-2-carboxylic acid, CAS RN 144701-48-4, US5591762), milfasartan, abitesartan, valsartan (Diovan® (Novartis), (S)-N-valeryl-N-[[2'-(lH-tetrazol-5-yl)biphenyl-4-yl)methyl]valine, US5399578), EXP-3137 (2-N-butyl-4-chloro-l-[(2'-(lH-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid, US5138069, US5153197 and US5128355), 3-(2'-(tetrazol-5-yl)-1,1'-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]-1,1'-biphenyl]-2- carboxylic acid, 2-butyl-6-(1-methoxy-1-methylethyl)-2-[2'-)lH-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-(ethoxycarbonyl-oxy)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-tetrazoly)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[4,5-6]pyridine, 2-[5-[(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridine-3-yl)methyl]-2-quinolinyl]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-tetrazacyclopentanaphthalene-9-one, 4-[1-[2'-(1,2,3,4-tetrazol-5-yl)biphen-4-yl)methylamino]-5,6,7,8-tetrahydro-2-trifylquinazoline, 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-imidzole-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, WO9210179, 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 (±)-1-(Carbazol-4-yloxy)-3-[[2-(o-methoxyphenoxy)ethyl]amino]-2-propanol, e.g., Coreg®, SmithKline Beecham), labetalol (such as 5-[1-hydroxy-2-[(1-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, XEN010, fenspiride hydrochloride (which may be prepared as disclosed in US3399192), proroxan (CAS RN 33743-96-3), and labetalol hydrochloride and combinations thereof;

α2 agonists such as methyldopa, methyldopa HCL, lofexidine, tiamenidine, moxonidine, rilmenidine, guanobenz, and the like;

aldosterone inhibitors, and the like;

angiopoietin-2-binding agents such as those disclosed in WO03/030833;

anti-angina agents such as ranolazine (hydrochloride1-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), butoprozine hydrochloride (Methanone, [4-[3(dibutylamino)propoxy]phenyl](2-ethyl-3-indolizinyl)-, monohydrochloride CAS RN 62134-34-3), cinepazet maleate1-Piperazineacetic acid, 4-[1-oxo-3-(3,4,5-trimethoxyphenyl)-2-propenyl]-, ethyl ester, (2Z)-2-butenedioate (1:1) CAS RN 50679-07-7), tosifen (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-meth-oxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); tosifen (Benzenesulfonamide, 4-methyl-N-[[[(1S)-1-methyl-2-phenylethyl]amino]carbonyl]- CAS RN 32295-184); and

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), methyldopa-chlorothiazide (such as 6-chloro-2H-1, 2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide and methyldopa 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); and those agents disclosed in US20030069221.

Agents useful in the treatment of obesity

The agents described herein can be administered together with one or more agents useful in the treatment of obesity. Suitable anti-obesity agents include, but are not limited to:

11β HSD-1 (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-(l-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-l,2,4-triazolo[4,3-a][11]annulene, and those compounds disclosed in WO01/90091, WO01/90090, WO01/90092 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), SR146131 (Sanofi Synthelabo), butabindide, PD170,292, 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 inhibtors, 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 WR1339, 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, USUS5512565, 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, WO01/991752, WO01/25192, WO01/52880, WO01/74844, WO01/70708, 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 WO01/21169, 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;

serotoninergic 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/ReductilTM) 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, WO01/27068, and WO01/62341;

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, WO01/44201, 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 US6734188, 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;

Neuropeptide Y2 (NPY2) agonists include but are not limited to: peptide 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 (ProzacTM), fluvoxamine, sertraline, citalopram, and imipramine, and those disclosed in US6162805, US6365633, WO03/00663, WO01/27060, and WO01/162341;

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-1 (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-napthalenyl)-1-propenyl]benzoic acid (TTNPB), retinoic acid, and those disclosed in WO99/00123;

β3 (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-dihydro-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-Ayerst), 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, sercloremine, 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), peptide hormones and variants thereof which affect the islet cell secretion, such as the hormones of the secretin/gastric inhibitory peptide (GIP)/vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase activating peptide (PACAP)/glucagon-like peptide II (GLP-II)/glicentin/glucagon gene family and/or those of the adrenomedullin/amylin/calcitonin gene related peptide (CGRP) gene family includingGLP-1 (glucagon-like peptide 1) agonists (e.g. (1) exendin-4, (2) those GLP-1 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-1 peptides 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 peptide-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-1 (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-1), β-hydroxy-β-methylbutyrate, p57 (Pfizer), Zonisamide (ZonegranTM, indicated as an anti-epileptic which has been shown to lead to weight loss), and the agents disclosed in US20030119428 paragraphs 20-26.

Anti-diabetic Agents

The agents described herein can be administered together with one or more agents useful in the treatment of diabetes. Suitable anti-diabetic agents include, but are not limited to:

PPARγ 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 (Mitsibishi disclosed in US5594016), pioglitazone (such as such as Actos™ pioglitazone; Takeda), rosiglitazone (Avandia™;Smith Kline Beecham), rosiglitazone maleate, troglitazone (Rezulin, disclosed in US4572912), rivoglitazone (CS-011, 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, US6303640, US6329404, US5994554, W097/10813, 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 Glucovance™, Bristol-Myers Squibb); buformin (Imidodicarbonimidic diamide, N-butyl-); etoformine (1-Butyl-2-ethylbiguanide, Schering A. G.); other metformin salt forms (including where the salt is chosen from the group of, acetate, benzoate, citrate, fumarate, embonate, chlorophenoxyacetate, glycolate, palmoate, aspartate, methanesulphonate, maleate, parachlorophenoxyisobutyrate, formate, lactate, succinate, sulphate, tartrate, 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-1B (PTP-1B) inhibitors, such as A-401,674, KR 61639, OC-060062, OC-83839, OC-297962, 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 acetohexamide (e.g. Dymelor, Eli Lilly), carbutamide, chlorpropamide (e.g. Diabinese, Pfizer), gliamilide (Pfizer), gliclazide (e.g. Diamcron, Servier 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. Pranidin□, Novo 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]-alpha-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 WO01/47528 (polyamines);

α-amylase inhibitors such as tendamistat, trestatin, and A1-3688, and the compounds disclosed in US4451455, US4623714, and US4273765;

SGLT2 inhibitors including those disclosed in US6414126 and US6515117;

an aP2 inhibitor such as disclosed in US6548529;

insulin secreatagogues such as linogliride, A-4166, forskilin, dibutyrl 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, deriglidole, 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-1 (1-36) amide, GLP-1 (73-7) (insulintropin, disclosed in US5614492), LY-315902 (Lilly), GLP-1 (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;

PPARα/γ dual agonists such as AR-HO39242 (Aztrazeneca), 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), tesaglitzar (Astrazeneca), reglitazar (JTT-501) and those disclosed in WO99/16758, WO99/19313, WO99/20614, WO99/38850, 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, such as those disclosed in WO03/015774;

retinoid modulators such as those disclosed in WO03/000249;

GSK 3β/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 compounds disclosed in WO01/94300, WO02/20530, WO03/037864, and pharmaceutically acceptable salts or esters thereof;

ATP consumption promotors 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

agents such as those disclosed in WO99/51225, US20030134890, WO01/24786, and WO03/059870;

insulin-responsive DNA binding protein-1 (IRDBP-1) as disclosed in WO03/057827, and the like;

adenosine A2 antagonists such as those disclosed in WO03/035639, WO03/035640, and the like;

PPARδ agonists such as GW 501516, GW 590735, and compounds disclosed in JP10237049 and WO02/14291;

dipeptidyl peptidase IV (DP-IV) inhibitors, such as 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, 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, EP1258476, WO02/083128, WO02/062764, WO03/000250, WO03/002530, WO03/002531, WO03/002553, WO03/002593, WO03/000180, and WO03/000181;

GLP-1 agonists such as exendin-3 and exendin-4 (including the 39 aa peptide 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 (pramlintide acetate); and

glycokinase activators such as those disclosed in US2002103199 (fused heteroaromatic compounds) and WO02/48106 (isoindolin-1-one-substituted propionamide compounds).

Administration of agents

For therapeutic and preventive treatment of disorders described herein, the agents described herein can be administered orally, e.g., as a tablet or cachet containing a predetermined amount of the active ingredient, pellet, gel, paste, syrup, bolus, electuary, slurry, sachet; capsule; powder; lyophilized powder; granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, via a liposomal formulation (see, e.g., EP 736299) or in some other form. Orally administered compositions can include binders, lubricants, inert diluents, lubricating, surface active or dispersing agents, flavoring agents, 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 active ingredient therein. The agents can be co-administered with other agents used to treat gastrointestinal disorders including but not limited to the agents described herein. The agents can also be administered by rectal suppository. For the treatment

of disorders outside the gastrointestinal tract such as congestive heart failure and benign prostatic hypertrophy, agents are preferably administered parenterally or orally.

The agent described herein can be administered alone or in combination with other agents. For example, the agents can be administered together with an analgesic agent. The analgesic agent can be covalently attached to an agent described herein or it can be a separate agent that is administered together with or sequentially with an agent described herein in a combination therapy.

Combination therapy can be achieved by administering two or more agents, e.g., an agent described herein and an analgesic agent or 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.

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.

Combination therapy can also include the administration of two or more agents via different routes or locations. For example, (a) one agent is administered orally and another agent is administered intravenously or (b) one agent is administered orally and another is administered locally. In each case, the agents can either simultaneously or sequentially. Approximated

dosages for some of the combination therapy agents described herein are found in the "BNF Recommended Dose" column of tables on pages 11-17 of WO01/76632 (the data in the tables being attributed to the March 2000 British National Formulary) and can also be found in other standard formularies and other drug prescribing directories. For some drugs, the customary prescribed dose for an indication will vary somewhat from country to country.

The agents, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose (e.g. celphere, Celphere beads®), diluents, lubricants, binders, disintegrating agents, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

Compositions of the present disclosure may also optionally include other therapeutic ingredients, anti-caking agents, preservatives, sweetening agents, colorants, flavors, desiccants, plasticizers, dyes, glidants, anti-adherents, anti-static agents, surfactants (wetting agents), anti-oxidants, film-coating agents, and the like. Any such optional ingredient must be compatible with the compound described herein to insure the stability of the formulation.

The composition 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 peptides and proteins, for example albumen.

Examples of excipients for use as the pharmaceutically acceptable carriers and the pharmaceutically acceptable inert carriers and the aforementioned additional ingredients include, but are not limited to binders, fillers, disintegrants, lubricants, anti-microbial agents, 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 (*e.g.* AVICEL™, such as, AVICEL-PH-101™, -103™ and -105™, sold by FMC Corporation, Marcus Hook, PA, USA), 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, dextrates, 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, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums (like gellan), low-substituted hydroxypropyl cellulose, or mixtures thereof,

LUBRICANTS: calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, sodium stearyl fumarate, 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., Plano, 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, 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.

The formulation can also include other excipients and categories thereof including but not limited to L-histidine, Pluronic®, Poloxamers (such as Lutrol® and Poloxamer 188), ascorbic acid, glutathione, permeability enhancers (e.g. lipids, sodium cholate, acylcarnitine, salicylates, mixed bile salts, fatty acid micelles, chelators, fatty acid, surfactants, medium chain glycerides), protease inhibitors (e.g. soybean trypsin inhibitor, organic acids), pH lowering agents and absorption enhancers effective to promote bioavailability (including but not limited to those described in US6086918 and US5912014), 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); spheronization 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.

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-1-8106).

The agents either in their free form or as a salt can be combined with a polymer such as polylactic-glycoloic acid (PLGA), poly-(l)-lactic-glycolic-tartaric acid (P(l)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. Such formulations can be used to implants that release a peptide or another agent over a period of a few days, a few weeks or several months depending on the polymer, the particle size of the polymer, and the size of the implant (see, e.g., U.S. 6,620,422). Other sustained release formulations and polymers for use in 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 9726015, WO 97/04744, and. US20020019446. In such sustained release formulations microparticles (Delie and Blanco-Prieto 2005 Molecule 10:65-80) of peptide are combined with microparticles of polymer. One or more sustained release implants can be placed in the large intestine, the small intestine or both. 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 releaseof the agent within the GI tract. Additional controlled release formulations are described in U.S. 6,734,188, WO 02/38129, EP 326 151, 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.

The agents can be administered, e.g., by intravenous injection, intramuscular injection, subcutaneous injection, intraperitoneal injection, topical, sublingual, intraarticular (in the joints), intradermal, buccal, ophthalmic (including intraocular), intranasaly (including using a cannula), intraspinally, intrathecally, or by other routes. The agents can be administered orally, e.g., as a tablet or cachet containing a predetermined amount of the active ingredient, gel, pellet, paste, syrup, bolus, electuary, slurry, capsule, powder, lyophilized powder, granules, sachet, as a solution or a suspension in an aqueous liquid or a non-aqueous liquid, as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion, via a micellar formulation (see, e.g. WO 97/11682) via a liposomal formulation (see, e.g., EP 736299,WO 99/59550 and WO 97/13500), via formulations described in WO 03/094886, via bilosome (bile-salt based vesicular system), via a dendrimer, or in some other form. Orally administered compositions can include binders, lubricants, inert diluents, lubricating, surface active or dispersing agents, flavoring agents, 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 active ingredient therein. The agents can also be administered transdermally (i.e. via reservoir-type or matrix-type patches, microneedles, thermal poration, hypodermic needles, iontophoresis, electroporation, ultrasound or other forms of sonophoresis, jet injection, or a combination of any of the preceding methods (Prausnitz et al. 2004, Nature Reviews Drug Discovery 3:115-124)). The agents can be administered using high-velocity transdermal particle injection techniques

using the hydrogel particle formulation described in U.S. 20020061336. Additional particle formulations are described in WO 00/45792, WO 00/53160, and WO 02/19989. An example of a transdermal formulation containing plaster and the absorption promoter dimethylisosorbide can be found in WO 89/04179. WO 96/11705 provides formulations suitable for transdermal administration. The agents can be administered in the form a suppository or by other vaginal or rectal means. The agents can be administered in a transmembrane formulation as described in WO 90/07923. The agents can be administed non-invasively via the dehydrated particicles described in U.S. 6,485,706. The agent can be administered in an enteric-coated drug formulation as described in WO 02/49621. The agents can be administered intranassaly using the formulation described in U.S. 5,179,079. Formulations suitable for parenteral injection are described in WO 00/62759. The agents can be administered using the casein formulation described in U. S. 20030206939 and WO 00/06108. The agents can be administered using the particulate formulations described in U.S. 20020034536.

The agents, alone or in combination with other suitable components, can be administered by pulmonary route utilizing several techniques including but not limited to intratracheal instillation (delivery of solution into the lungs by syringe), intratracheal delivery of liposomes, insufflation (administration of powder formulation by syringe or any other similar device into the lungs) and aerosol inhalation. Aerosols (e.g., jet or ultrasonic nebulizers, metered-dose inhalers (MDIs), and dry-powder inhalers (DPIs)) can also be used in intranasal applications. Aerosol formulations are stable dispersions or suspensions of solid material and liquid droplets in a gaseous medium and can be placed into pressurized acceptable propellants, such as hydrofluroalkanes (HFAs, i.e. HFA-134a and HFA-227, or a mixture thereof), dichlorodifluoromethane (or other chlorofluocarbon propellants such as a mixture of Propellants 11, 12, and/or 114), propane, nitrogen, and the like. Pulmonary formulations may include permeation enhancers such as fatty acids, saccharides, chelating agents, enzyme inhibitors (e.g., protease inhibitors), adjuvants (e.g., glycocholate, surfactin, span 85, and nafamostat), preservatives (e.g., benzalkonium chloride or chlorobutanol), and ethanol (normally up to 5% but possibly up to 20%, by weight). Ethanol is commonly included in aerosol compositions as it can improve the function of the metering valve and in some cases also improve the stability of the dispersion. Pulmonary formulations may also include surfactants which include but are not

limited to bile salts and those described in U.S. 6,524,557 and references therein. The surfactants described in U.S. 6,524,557, e.g., a C8-C16 fatty acid salt, a bile salt, a phospholipid, or alkyl saccaride are advantageous in that some of them also reportedly enhance absorption of the peptide in the formulation. Also suitable in the disclosure are dry powder formulations comprising a therapeutically effective amount of active compound blended with an appropriate carrier and adapted for use in connection with a dry-powder inhaler. Absorption enhancers which can be added to dry powder formulations of the present disclosure include those described in U.S. 6,632,456. WO 02/080884 describes new methods for the surface modification of powders. Aerosol formulations may include U.S. 5,230,884, U.S. 5,292,499, WO 017/8694, WO 01/78696, U.S. 2003019437, U. S. 20030165436, and WO 96/40089 (which includes vegetable oil). Sustained release formulations suitable for inhalation are described in U.S. 20010036481A1, 20030232019A1, and U.S. 20040018243A1 as well as in WO 01/13891, WO 02/067902, WO 03/072080, and WO 03/079885. Pulmonary formulations containing microparticles are described in WO 03/015750, U.S. 20030008013, and WO 00/00176. Pulmonary formulations containing stable glassy state powder are described in U.S. 20020141945 and U.S. 6,309,671. Other aerosol formulations are desribed in EP 1338272A1 WO 90/09781, U. S. 5,348,730, U.S. 6,436,367, WO 91/04011, and U.S. 6,294,153 and U.S. 6,290,987 describes a liposomal based formulation that can be administered via aerosol or other means. Powder formulations for inhalation are described in U.S. 20030053960 and WO 01/60341. The agents can be administered intranasally as described in U.S. 20010038824. The agents can be incorporated into microemulsions, which generally are thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules (Encyclopedia of Pharmaceutical Technology (New York: Marcel Dekker, 1992), volume 9). For the preparation of microemulsions, surfactant (emulsifier), co-surfactant (co-emulsifier), an oil phase and a water phase are necessary. Suitable surfactants include any surfactants that are useful in the preparation of emulsions, e.g., emulsifiers that are typically used in the preparation of creams. The co-surfactant (or "co-emulsifer") is generally selected from the group of polyglycerol derivatives, glycerol derivatives and fatty alcohols. Preferred emulsifier/co-emulsifier combinations are generally although not necessarily selected from the group consisting of: glyceryl monostearate and polyoxyethylene stearate; polyethylene glycol and ethylene glycol palmitostearate; and caprilic and capric

triglycerides and oleoyl macrogolglycerides. The water phase includes not only water but also, typically, buffers, glucose, propylene glycol, polyethylene glycols, preferably lower molecular weight polyethylene glycols (e.g., PEG 300 and PEG 400), and/or glycerol, and the like, while the oil phase will generally comprise, for example, fatty acid esters, modified vegetable oils, silicone oils, mixtures of mono- di- and triglycerides, mono- and di-esters of PEG (e.g., oleoyl macrogol glycerides), etc.

The agents described herein can be incorporated into pharmaceutically-acceptable nanoparticle, nanosphere, and nanocapsule formulations (Delie and Blanco-Prieto 2005 Molecule 10:65-80). Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland et al., 1987; Quintanar-Guerrero et al., 1998; Douglas et al., 1987). To avoid side effects due to intracellular polymeric overloading, ultrafine particles (sized around 0.1 μm) can be designed using polymers able to be degraded in vivo (e.g. biodegradable polyalkyl-cyanoacrylate nanoparticles). Such particles are described in the prior art (Couvreur et al, 1980; 1988; zur Muhlen et al., 1998; Zambaux et al. 1998; Pinto-Alphandry et al., 1995 and U.S. Pat. No. 5,145,684).

The agents described herein can be formulated with pH sensitive 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 US4910021 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. The agents 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 hyrdratable polymers); WO0243767 (enzyme cleavable membrane translocators); WO03007913 and WO03086297 (mucoadhesive systems); WO02072075 (bilayer laminated formulation comprising pH lowering agent and absorption enhancer); WO04064769 (amidated peptides); 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); WO04112711 (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); US5108758 (glassy amylose matrix delivery); US 5840860 (modified starch based delivery). JP10324642 (delivery system comprising chitosan and gastric resistant material such as wheat gliadin or zein); US5866619 and US6368629 (saccharide containing polymer); US 6531152 (describes a drug delivery system containing a water soluble core (Ca pectinate or other water-insoluble polymers) and outer coat which bursts (eg hydrophobic polymer-Eudragrit)); US 6234464; US 6403130 (coating with polymer containing casein and high methoxy pectin; WO0174175 (Maillard reaction product); WO05063206 (solubility increasing formulation); WO04019872 (transferring fusion proteins). The agents 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. 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.

The agents described herein can be formulated in an osmotic device including the ones disclosed in US4503030, US5609590 and US5358502. US4503030 discloses an osmotic device for dispensing a drug to certain pH regions of the gastrointestinal tract. More particularly, the disclosure 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.

The agents described herein may be formulated based on the disclosure described in U. S. Patent No. 5,316, 774 which discloses a composition for the controlled release of an active substance comprising a polymeric particle matrix, where each particle defines a network of internal pores. The active substance is entrapped within the pore network together with a blocking agent having physical and chemical characteristics selected to modify the release rate of the active substance from the internal pore network. In one embodiment, drugs may be selectively delivered to the intestines using an enteric material as the blocking agent. The enteric material remains intact in the stomach but degrades under the pH conditions of the intestines. In another embodiment, the sustained release formulation employs a blocking agent, which remains stable under the expected conditions of the environment to which the active substance is to be released. The use of pH-sensitive materials alone to achieve site-specific delivery is difficult because of leaking of the beneficial agent prior to the release site or desired delivery time and it is difficult to achieve long time lags before release of the active ingredient after exposure to high pH (because of rapid dissolution or degradation of the pH-sensitive materials).

The agents may also be formulated in a hybrid system which combines pH-sensitive materials and osmotic delivery systems. These hybrid devices provide delayed initiation of sustained-release of the beneficial agent. In one device a pH-sensitive matrix or coating dissolves releasing

osmotic devices that provide sustained release of the beneficial agent see U. S. Patent Nos. 4,578, 075, 4,681, 583, and 4,851, 231. A second device consists of a semipermeable coating made of a polymer blend of an insoluble and a pH-sensitive material. As the pH increases, the permeability of the coating increases, increasing the rate of release of beneficial agent see U. S. Patent Nos. 4,096, 238,4, 503,030, 4, 522, 625, and 4,587, 117.

The agents described herein may be formulated in terpolumers according to U. S. Patent No. 5,484, 610 which discloses terpolymers which are sensitive to pH and temperature which are useful carriers for conducting bioactive agents through the gastric juices of the stomach in a protected form. The terpolymers swell at the higher physiologic pH of the intestinal tract causing release of the bioactive agents into the intestine. The terpolymers are linear and are made up of 35 to 99 wt % of a temperature sensitive component, which imparts to the terpolymer LCST (lower critical solution temperature) properties below body temperatures, 1 to 30 wt % of a pH sensitive component having a pKa in the range of from 2 to 8 which functions through ionization or deionization of carboxylic acid groups to prevent the bioactive agent from being lost at low pH but allows bioactive agent release at physiological pH of about 7.4 and a hydrophobic component which stabilizes the LCST below body temperatures and compensates for bioactive agent effects on the terpolymers. The terpolymers provide for safe bioactive agent loading, a simple procedure for dosage form fabrication and the terpolymer functions as a protective carrier in the acidic environment of the stomach and also protects the bioactive agents from digestive enzymes until the bioactive agent is released in the intestinal tract.

The agents described herein may be formulated in pH sensitive polymers according to those described in U. S. Patent No. 6,103, 865. U. S. Patent No. 6,103, 865 discloses pH-sensitive polymers containing sulfonamide groups, which can be changed in physical properties, such as swellability and solubility, depending on pH and which can be applied for a drug-delivery system, bio-material, sensor, and the like, and a preparation method therefore. The pH-sensitive polymers are prepared by introduction of sulfonamide groups, various in pKa, to hydrophilic groups of polymers either through coupling to the hydrophilic groups of polymers, such as acrylamide, N, N- dimethylacrylamide, acrylic acid, N-isopropylacrylamide and the like or

copolymerization with other polymerizable monomers. These pH-sensitive polymers may have a structure of linear polymer, grafted copolymer, hydrogel or interpenetrating network polymer.

The agents described herein may be formulated according U. S. Patent No. 5, 656, 292 which discloses a composition for pH dependent or pH regulated controlled release of active ingredients especially drugs. The composition consists of a compactable mixture of the active ingredient and starch molecules substituted with acetate and dicarboxylate residues. The preferred dicarboxylate acid is succinate. The average substitution degree of the acetate residue is at least 1 and 0. 2-1.2 for the dicarboxylate residue. The starch molecules can have the acetate and dicarboxylate residues attached to the same starch molecule backbone or attached to separate starch molecule backbones. The present disclosure also discloses methods for preparing said starch acetate dicarboxylates by transesterification or mixing of starch acetates and starch dicarboxylates respectively.

The agents described herein may be formulated according to the methods described in U. S. Patent Nos. 5,554, 147,5, 788, 687, and 6,306, 422 which disclose a method for the controlled release of a biologically active agent wherein the agent is released from a hydrophobic, pH-sensitive polymer matrix. The polymer matrix swells when the environment reaches pH 8.5, releasing the active agent. A polymer of hydrophobic and weakly acidic comonomers is disclosed for use in the controlled release system. Also disclosed is a specific embodiment in which the controlled release system may be used. The pH-sensitive polymer is coated onto a latex catheter used in ureteral catheterization. A ureteral catheter coated with a pH-sensitive polymer having an antibiotic or urease inhibitor trapped within its matrix will release the active agent when exposed to high pH urine.

The agents described herein may be formulated in/with bioadhesive polymers according to US Patent No. 6,365, 187. Bioadhesive polymers in the form of, or as a coating on, microcapsules containing drugs or bioactive substances which may serve for therapeutic, or diagnostic purposes in diseases of the gastrointestinal tract, are described in US6365187. The polymeric microspheres all have a bioadhesive force of at least 11 mN/cm^2 (110 N/m2) Techniques for the fabrication of bioadhesive microspheres, as well as a method for measuring bioadhesive forces

between microspheres and selected segments of the gastrointestinal tract in vitro are also described. This quantitative method provides a means to establish a correlation between the chemical nature, the surface morphology and the dimensions of drug-loaded microspheres on one hand and bioadhesive forces on the other, allowing the screening of the most promising materials from a relatively large group of natural and synthetic polymers which, from theoretical consideration, should be used for making bioadhesive microspheres. Solutions of medicament in buffered saline and similar vehicles are commonly employed to generate an aerosol in a nebulizer. Simple nebulizers operate on Bernoulli's principle and employ a stream of air or oxygen to generate the spray particles. More complex nebulizers employ ultrasound to create the spray particles. Both types are well known in the art and are described in standard textbooks of pharmacy such as Sprowls' American Pharmacy and Remington's The Science and Practice of Pharmacy. Other devices for generating aerosols employ compressed gases, usually hydrofluorocarbons and chlorofluorocarbons, which are mixed with the medicament and any necessary excipients in a pressurized container, these devices are likewise described in standard textbooks such as Sprowls and Remington.

The agents can be a free acid or base, or a pharmacologically acceptable salt thereof. Solids can be dissolved or dispersed immediately prior to administration or earlier. In some circumstances the preparations include a preservative to prevent the growth of microorganisms. The pharmaceutical forms suitable for injection can include sterile aqueous or organic solutions or dispersions which include, e.g., water, an alcohol, an organic solvent, an oil or other solvent or dispersant (e.g., glycerol, propylene glycol, polyethylene glycol, and vegetable oils). The formulations may contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. Pharmaceutical agents can be sterilized by filter sterilization or by other suitable means. The agent can be fused to immunoglobulins or albumin, albumin variants or fragments thereof, or incorporated into a liposome to improve half-life. Thus the agents described herein may be fused directly or via a peptide linker, water soluble polymer, or prodrug linker to albumin or an analog, fragment, or derivative thereof. Generally, the albumin proteins that are part of the fusion proteins of the present disclosure may be derived from albumin cloned

from any species, including human. Human serum albumin (HSA) consists of a single non-glycosylated peptide chain of 585 amino acids with a formula molecular weight of 66,500. The amino acid sequence of human HSA is known [See Meloun, et al. (1975) FEBS Letters 58:136; Behrens, et al. (1975) Fed. Proc. 34:591; Lawn, et al. (1981) Nucleic Acids Research 9:6102-6114; Minghetti, et al. (1986) J. Biol. Chem. 261:6747, each of which are incorporated by reference herein]. A variety of polymorphic variants as well as analogs and fragments of albumin have been described. [See Weitkamp, et al., (1973) Ann. Hum. Genet. 37:219]. For example, in EP 322,094, various shorter forms of HSA. Some of these fragments of HSA are disclosed, including HSA(1-373), HSA(1-388), HSA(1-389), HSA(1-369), and HSA(1-419) and fragments between 1-369 and 1-419. EP 399,666 discloses albumin fragments that include HSA(1-177) and HSA(1-200) and fragments between HSA(1-177) and HSA(1-200). Methods related to albumin fusion proteins can be found in US 7,056,701, US 6,994,857, US 6,946,134, US6,926,898, and US 6,905,688 and the related priority documents and references cited therein. The agent can also be conjugated to polyethylene glycol (PEG) chains. Methods for pegylation and additional formulations containing PEG-conjugates (i.e. PEG-based hydrogels, PEG modified liposomes) can be found in Harris and Chess, Nature Reviews Drug Discovery 2: 214-221 and the references therein. Agents can also be modified with alkyl groups (e.g., C1-C20 straight or branched alkyl groups); fatty acid radicals; and 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). The agent can be administered via a nanocochleate or cochleate delivery vehicle (BioDelivery Sciences International). The agents can be delivered transmucosally (i.e. across a mucosal surface such as the vagina, eye or nose) using formulations such as that described in U.S. 5,204,108. The agents can be formulated in microcapsules as described in WO 88/01165. The agent can be administered intra-orally using the formulations described in U.S. 20020055496, WO 00/47203, and U.S. 6,495,120. The agent can be delivered using nanoemulsion formulations described in WO 01/91728A2.

Controlled release formulations

In general, one can provide for controlled release of the agents described herein through the use of a wide variety of polymeric carriers and controlled release systems including erodible and

non-erodible matrices, osmotic control devices, various reservoir devices, enteric coatings and multiparticulate control devices.

Matrix devices are a common device for controlling the release of various agents. In such devices, the agents described herein are generally present as a dispersion within the polymer matrix, and are typically formed by the compression of a polymer/drug mixture or by dissolution or melting. The dosage release properties of these devices may be dependent upon the solubility of the agent in the polymer matrix or, in the case of porous matrices, the solubility in the sink solution within the pore network, and the tortuosity of the network. In one instance, when utilizing an erodible polymeric matrix, the matrix imbibes water and forms an aqueous-swollen gel that entraps the agent. The matrix then gradually erodes, swells, disintegrates or dissolves in the GI tract, thereby controlling release of one or more of the agents described herein. In non-erodible devices, the agent is released by diffusion through an inert matrix.

Agents described herein can be incorporated into an erodible or non-erodible polymeric matrix controlled release device. By an erodible matrix is meant aqueous-erodible or water-swellable or aqueous-soluble in the sense of being either erodible or swellable or dissolvable in pure water or requiring the presence of an acid or base to ionize the polymeric matrix sufficiently to cause erosion or dissolution. When contacted with the aqueous environment of use, the erodible polymeric matrix imbibes water and forms an aqueous-swollen gel or matrix that entraps the agent described herein. The aqueous-swollen matrix gradually erodes, swells, disintegrates or dissolves in the environment of use, thereby controlling the release of a compound described herein to the environment of use.

The erodible polymeric matrix into which an agent described herein can be incorporated may generally be described as a set of excipients that are mixed with the agent following its formation that, when contacted with the aqueous environment of use imbibes water and forms a water-swollen gel or matrix that entraps the drug form. Drug release may occur by a variety of mechanisms, for example, the matrix may disintegrate or dissolve from around particles or granules of the agent or the agent may dissolve in the imbibed aqueous solution and diffuse from the tablet, beads or granules of the device. One ingredient of this water-swollen matrix is the

water-swellable, erodible, or soluble polymer, which may generally be described as an osmopolymer, hydrogel or water-swellable polymer. Such polymers may be linear, branched, or crosslinked. The polymers may be homopolymers or copolymers. In certain embodiments, they may be synthetic polymers derived from vinyl, acrylate, methacrylate, urethane, ester and oxide monomers. In other embodiments, they can be derivatives of naturally occurring polymers such as polysaccharides (e.g. chitin, chitosan, dextran and pullulan; gum agar, gum arabic, gum karaya, locust bean gum, gum tragacanth, carrageenans, gum ghatti, guar gum, xanthan gum and scleroglucan), starches (e.g. dextrin and maltodextrin), hydrophilic colloids (e.g. pectin), phosphatides (e.g. lecithin), alginates (e.g. ammonium alginate, sodium, potassium or calcium alginate, propylene glycol alginate), gelatin, collagen, and cellulosics. Cellulosics are cellulose polymer that has been modified by reaction of at least a portion of the hydroxyl groups on the saccharide repeat units with a compound to form an ester-linked or an ether-linked substituent. For example, the cellulosic ethyl cellulose has an ether linked ethyl substituent attached to the saccharide repeat unit, while the cellulosic cellulose acetate has an ester linked acetate substituent. In certain embodiments, the cellulosics for the erodible matrix comprises aqueous-soluble and aqueous-erodible cellulosics can include, for example, ethyl cellulose (EC), methylethyl cellulose (MEC), carboxymethyl cellulose (CMC), CMEC, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), cellulose acetate (CA), cellulose propionate (CP), cellulose butyrate (CB), cellulose acetate butyrate (CAB), CAP, CAT, hydroxypropyl methyl cellulose (HPMC), HPMCP, HPMCAS, hydroxypropyl methyl cellulose acetate trimellitate (HPMCAT), and ethylhydroxy ethylcellulose (EHEC). In certain embodiments, the cellulosics comprises various grades of low viscosity (MW less than or equal to 50,000 daltons, for example, the Dow Methocel™ series E5, E15LV, E50LV and K100LY) and high viscosity (MW greater than 50,000 daltons, for example, E4MCR, E10MCR, K4M, K15M and K100M and the Methocel™ K series) HPMC. Other commercially available types of HPMC include the Shin Etsu Metolose 90SH series.

The choice of matrix material can have a large effect on the maximum drug concentration attained by the device as well as the maintenance of a high drug concentration. The matrix material can be a concentration-enhancing polymer, for example, as described in WO05/011634.

Other materials useful as the erodible matrix material include, but are not limited to, pullulan, polyvinyl pyrrolidone, polyvinyl alcohol, polyvinyl acetate, glycerol fatty acid esters, polyacrylamide, polyacrylic acid, copolymers of ethacrylic acid or methacrylic acid (EUDRAGITO, Rohm America, Inc., Piscataway, New Jersey) and other acrylic acid derivatives such as homopolymers and copolymers of butylmethacrylate, methylmethacrylate, ethylmethacrylate, ethylacrylate, (2-dimethylaminoethyl) methacrylate, and (trimethylaminoethyl) methacrylate chloride.

The erodible matrix polymer may contain a wide variety of the same types of additives and excipients known in the pharmaceutical arts, including osmopolymers, osmagens, solubility-enhancing or-retarding agents and excipients that promote stability or processing of the device.

Alternatively, the agents of the present disclosure may be administered by or incorporated into a non-erodible matrix device. In such devices, an agent described herein is distributed in an inert matrix. The agent is released by diffusion through the inert matrix. Examples of materials suitable for the inert matrix include insoluble plastics (e.g methyl acrylate-methyl methacrylate copolymers, polyvinyl chloride, polyethylene), hydrophilic polymers (e.g. ethyl cellulose, cellulose acetate, crosslinked polyvinylpyrrolidone (also known as crospovidone)), and fatty compounds (e.g. carnauba wax, microcrystalline wax, and triglycerides). Such devices are described further in Remington: The Science and Practice of Pharmacy, 20th edition (2000). Matrix controlled release devices may be prepared by blending an agent described herein and other excipients together, and then forming the blend into a tablet, caplet, pill, or other device formed by compressive forces. Such compressed devices may be formed using any of a wide variety of presses used in the fabrication of pharmaceutical devices. Examples include single-punch presses, rotary tablet presses, and multilayer rotary tablet presses, all well known in the art. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000. The compressed device may be of any shape, including round, oval, oblong, cylindrical, or triangular. The upper and lower surfaces of the compressed device may be flat, round, concave, or convex.

In certain embodiments, when formed by compression, the device has a strength of at least 5 Kiloponds (Kp)/cm^2 (for example, at least 7 Kp/cm^2). Strength is the fracture force, also known

as the tablet hardness required to fracture a tablet formed from the materials, divided by the maximum cross-sectional area of the tablet normal to that force. The fracture force may be measured using a Schleuniger Tablet Hardness Tester, Model 6D. The compression force required to achieve this strength will depend on the size of the tablet, but generally will be greater than about 5 kP/cm^2. Friability is a well-know measure of a device's resistance to surface abrasion that measures weight loss in percentage after subjecting the device to a standardized agitation procedure. Friability values of from 0.8 to 1.0% are regarded as constituting the upper limit of acceptability. Devices having a strength of greater than 5 kP/cm^2 generally are very robust, having a friability of less than 0. 5%. Other methods for forming matrix controlled-release devices are well known in the pharmaceutical arts. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000.

As noted above, the agents described herein may also be incorporated into an osmotic control device. Such devices generally include a core containing one or more agents as described herein and a water permeable, non-dissolving and non-eroding coating surrounding the core which controls the influx of water into the core from an aqueous environment of use so as to cause drug release by extrusion of some or all of the core to the environment of use. In certain embodiments, the coating is polymeric, aqueous-permeable, and has at least one delivery port. The core of the osmotic device optionally includes an osmotic agent which acts to imbibe water from the surrounding environment via such a semi-permeable membrane. The osmotic agent contained in the core of this device may be an aqueous-swellable hydrophilic polymer or it may be an osmogen, also known as an osmagent. Pressure is generated within the device which forces the agent(s) out of the device via an orifice (of a size designed to minimize solute diffusion while preventing the build-up of a hydrostatic pressure head).

Osmotic agents create a driving force for transport of water from the environment of use into the core of the device. Osmotic agents include but are not limited to water- swellable hydrophilic polymers, and osmogens (or osmagens). Thus, the core may include water-swellable hydrophilic polymers, both ionic and nonionic, often referred to as osmopolymers and hydrogels. The amount of water-swellable hydrophilic polymers present in the core may range from about 5 to about 80 wt% (including for example, 10 to 50 wt%). Nonlimiting examples of core materials include hydrophilic vinyl and acrylic polymers, polysaccharides such as calcium alginate,

polyethylene oxide (PEO), polyethylene glycol (PEG), polypropylene glycol (PPG), poly (2-hydroxyethyl methacrylate), poly (acrylic) acid, poly (methacrylic) acid, polyvinylpyrrolidone (PVP) and crosslinked PVP, polyvinyl alcohol (PVA), PVA/PVP copolymers and PVA/PVP copolymers with hydrophobic monomers such as methyl methacrylate, vinyl acetate, and the like, hydrophilic polyurethanes containing large PEO blocks, sodium croscarmellose, carrageenan, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), carboxymethyl cellulose (CMC) and carboxyethyl cellulose (CEC), sodium alginate, polycarbophil, gelatin, xanthan gum, and sodium starch glycolat. Other materials include hydrogels comprising interpenetrating networks of polymers that may be formed by addition or by condensation polymerization, the components of which may comprise hydrophilic and hydrophobic monomers such as those just mentioned. Water-swellable hydrophilic polymers include but are not limited to PEO, PEG, PVP, sodium croscarmellose, HPMC, sodium starch glycolate, polyacrylic acid and crosslinked versions or mixtures thereof.

The core may also include an osmogen (or osmagent). The amount of osmogen present in the core may range from about 2 to about 70 wt% (including, for example, from 10 to 50 wt%). Typical classes of suitable osmogens are water-soluble organic acids, salts and sugars that are capable of imbibing water to thereby effect an osmotic pressure gradient across the barrier of the surrounding coating. Typical useful osmogens include but are not limited to magnesium sulfate, magnesium chloride, calcium chloride, sodium chloride, lithium chloride, potassium sulfate, sodium carbonate, sodium sulfite, lithium sulfate, potassium chloride, sodium sulfate, mannitol, xylitol, urea, sorbitol, inositol, raffinose, sucrose, glucose, fructose, lactose, citric acid, succinic acid, tartaric acid, and mixtures thereof. In certain embodiments, the osmogen is glucose, lactose, sucrose, mannitol, xylitol, sodium chloride, including combinations thereof.

The core may include a wide variety of additives and excipients that enhance the performance of the dosage form or that promote stability, tableting or processing. Such additives and excipients include tableting aids, surfactants, water- soluble polymers, pH modifiers, fillers, binders, pigments, disintegrants, antioxidants, lubricants and flavorants. Nonlimiting examples of additives and excipients include but are not limited to those described elsewhere herein as well as microcrystalline cellulose, metallic salts of acids (e.g. aluminum stearate, calcium stearate, magnesium stearate, sodium stearate, zinc stearate), pH control agents (e.g. buffers, organic

acids, organic acid salts, organic and inorganic bases), fatty acids, hydrocarbons and fatty alcohols (e.g. stearic acid, palmitic acid, liquid paraffin, stearyl alcohol, and palmitol), fatty acid esters (e.g. glyceryl (mono-and di-) stearates, triglycerides, glyceryl (palmiticstearic) ester, sorbitan esters (e.g. sorbitan monostearate, saccharose monostearate, saccharose monopalmitate, sodium stearyl fumarate), polyoxyethylene sorbitan esters), surfactants (e.g. alkyl sulfates (e.g. sodium lauryl sulfate, magnesium lauryl sulfate), polymers (e.g. polyethylene glycols, polyoxyethylene glycols, polyoxyethylene, polyoxypropylene ethers, including copolymers thereof), polytetrafluoroethylene), and inorganic materials (e.g. talc, calcium phosphate), cyclodextrins, sugars (e.g. lactose, xylitol), sodium starch glycolate). Nonlimiting examples of disintegrants are sodium starch glycolate (e. g., Explotab™ CLV, (microcrystalline cellulose (e. g., Avicel™), microcrystalline silicified cellulose (e.g., ProSolv™), croscarmellose sodium (e. g., Ac-Di-Sol™). When the agent described herein is a solid amorphous dispersion formed by a solvent process, such additives may be added directly to the spray-drying solution when forming an agent described herein/concentration-enhancing polymer dispersion such that the additive is dissolved or suspended in the solution as a slurry, Alternatively, such additives may be added following the spray-drying process to aid in forming the final controlled release device.

A nonlimiting example of an osmotic device consists of one or more drug layers containing an agent described herein, such as a solid amorphous drug/polymer dispersion, and a sweller layer that comprises a water-swellable polymer, with a coating surrounding the drug layer and sweller layer. Each layer may contain other excipients such as tableting aids, osmagents, surfactants, water-soluble polymers and water-swellable polymers.

Such osmotic delivery devices may be fabricated in various geometries including bilayer (wherein the core comprises a drug layer and a sweller layer adjacent to each other), trilayer (wherein the core comprises a sweller layer sandwiched between two drug layers) and concentric (wherein the core comprises a central sweller agent surrounded by the drug layer). The coating of such a tablet comprises a membrane permeable to water but substantially impermeable to drug and excipients contained within. The coating contains one or more exit passageways or ports in communication with the drug-containing layer(s) for delivering the drug agent. The drug-containing layer(s) of the core contains the drug agent (including optional osmagents and

hydrophilic water-soluble polymers), while the sweller layer consists of an expandable hydrogel, with or without additional osmotic agents.

When placed in an aqueous medium, the tablet imbibes water through the membrane, causing the agent to form a dispensable aqueous agent, and causing the hydrogel layer to expand and push against the drug-containing agent, forcing the agent out of the exit passageway. The agent can swell, aiding in forcing the drug out of the passageway. Drug can be delivered from this type of delivery system either dissolved or dispersed in the agent that is expelled from the exit passageway.

The rate of drug delivery is controlled by such factors as the permeability and thickness of the coating, the osmotic pressure of the drug-containing layer, the degree of hydrophilicity of the hydrogel layer, and the surface area of the device. Those skilled in the art will appreciate that increasing the thickness of the coating will reduce the release rate, while any of the following will increase the release rate: increasing the permeability of the coating; increasing the hydrophilicity of the hydrogel layer; increasing the osmotic pressure of the drug-containing layer; or increasing the device's surface area.

Other materials useful in forming the drug-containing agent, in addition to the agent described herein itself, include HPMC, PEO and PVP and other pharmaceutically acceptable carriers. In addition, osmagents such as sugars or salts, including but not limited to sucrose, lactose, xylitol, mannitol, or sodium chloride, may be added. Materials which are useful for forming the hydrogel layer include sodium CMC, PEO (e.g. polymers having an average molecular weight from about 5,000,000 to about 7,500,000 daltons), poly (acrylic acid), sodium (polyacrylate), sodium croscarmellose, sodium starch glycolat, PVP, crosslinked PVP, and other high molecular weight hydrophilic materials.

In the case of a bilayer geometry, the delivery port(s) or exit passageway(s) may be located on the side of the tablet containing the drug agent or may be on both sides of the tablet or even on the edge of the tablet so as to connect both the drug layer and the sweller layer with the exterior of the device. The exit passageway(s) may be produced by mechanical means or by laser

drilling, or by creating a difficult-to-coat region on the tablet by use of special tooling during tablet compression or by other means.

The osmotic device can also be made with a homogeneous core surrounded by a semipermeable membrane coating, as in US3845770. The agent described herein can be incorporated into a tablet core and a semipermeable membrane coating can be applied via conventional tablet-coating techniques such as using a pan coater. A drug delivery passageway can then be formed in this coating by drilling a hole in the coating, either by use of a laser or mechanical means. Alternatively, the passageway may be formed by rupturing a portion of the coating or by creating a region on the tablet that is difficult to coat, as described above. In one embodiment, an osmotic device comprises: (a) a single-layer compressed core comprising: (i) an agent described herein, (ii) a hydroxyethylcellulose, and (iii) an osmagent, wherein the hydroxyethylcellulose is present in the core from about 2.0% to about 35% by weight and the osmagent is present from about 15% to about 70% by weight; (b) a water-permeable layer surrounding the core; and (c) at least one passageway within the water-permeable layer (b) for delivering the drug to a fluid environment surrounding the tablet. In certain embodiments, the device is shaped such that the surface area to volume ratio (of a water-swollen tablet) is greater than 0.6 mm^{-1} (including, for example, greater than 1.0 mm^{-1}). The passageway connecting the core with the fluid environment can be situated along the tablet band area. In certain embodiments, the shape is an oblong shape where the ratio of the tablet tooling axes, i.e., the major and minor axes which define the shape of the tablet, are between 1.3 and 3 (including, for example, between 1.5 and 2.5). In one embodiment, the combination of the agent described herein and the osmagent have an average ductility from about 100 to about 200 Mpa, an average tensile strength from about 0.8 to about 2.0 Mpa, and an average brittle fracture index less than about 0.2. The single-layer core may optionally include a disintegrant, a bioavailability enhancing additive, and/or a pharmaceutically acceptable excipient, carrier or diluent.

In certain embodiments, entrainment of particles of agents described herein in the extruding fluid during operation of such osmotic device is desirable. For the particles to be well entrained, the agent drug form is dispersed in the fluid before the particles have an opportunity to settle in the tablet core. One means of accomplishing this is by adding a disintegrant that serves to break up

the compressed core into its particulate components. Nonlimiting examples of standard disintegrants include materials such as sodium starch glycolate (e.g., Explotab™ CLV), microcrystalline cellulose (e. g., Avicel™), microcrystalline silicified cellulose (e. g., ProSolv™) and croscarmellose sodium (e. g., Ac-Di-Sol™), and other disintegrants known to those skilled in the art. Depending upon the particular formulation, some disintegrants work better than others. Several disintegrants tend to form gels as they swell with water, thus hindering drug delivery from the device. Non-gelling, non-swelling disintegrants provide a more rapid dispersion of the drug particles within the core as water enters the core. In certain embodiments, non-gelling, non-swelling disintegrants are resins, for example, ion-exchange resins. In one embodiment, the resin is Amberlite™ IRP 88 (available from Rohm and Haas, Philadelphia, PA). When used, the disintegrant is present in amounts ranging from about 50-74% of the core agent.

Water-soluble polymers are added to keep particles of the agent suspended inside the device before they can be delivered through the passageway(s) (e.g., an orifice). High viscosity polymers are useful in preventing settling. However, the polymer in combination with the agent is extruded through the passageway(s) under relatively low pressures. At a given extrusion pressure, the extrusion rate typically slows with increased viscosity. Certain polymers in combination with particles of the agent described herein form high viscosity solutions with water but are still capable of being extruded from the tablets with a relatively low force. In contrast, polymers having a low weight-average, molecular weight (< about 300,000) do not form sufficiently viscous solutions inside the tablet core to allow complete delivery due to particle settling. Settling of the particles is a problem when such devices are prepared with no polymer added, which leads to poor drug delivery unless the tablet is constantly agitated to keep the particles from settling inside the core. Settling is also problematic when the particles are large and/or of high density such that the rate of settling increases.

In certain embodiments, the water-soluble polymers for such osmotic devices do not interact with the drug. In certain embodiments the water-soluble polymer is a non-ionic polymer. A nonlimiting example of a non-ionic polymer forming solutions having a high viscosity yet still extrudable at low pressures is Natrosol™ 250H (high molecular weight hydroxyethylcellulose, available from Hercules Incorporated, Aqualon Division, Wilmington, DE; MW equal to about 1

million daltons and a degree of polymerization equal to about 3,700). Natrosol 250H™ provides effective drug delivery at concentrations as low as about 3% by weight of the core when combined with an osmagent. Natrosol 250H™ NF is a high-viscosity grade nonionic cellulose ether that is soluble in hot or cold water. The viscosity of a 1% solution of Natrosol 250H using a Brookfield LVT (30 rpm) at 25°C is between about 1, 500 and about 2,500 cps.
In certain embodiments, hydroxyethylcellulose polymers for use in these monolayer osmotic tablets have a weight-average, molecular weight from about 300,000 to about 1.5 million. The hydroxyethylcellulose polymer is typically present in the core in an amount from about 2.0% to about 35% by weight.

Another example of an osmotic device is an osmotic capsule. The capsule shell or portion of the capsule shell can be semipermeable. The capsule can be filled either by a powder or liquid consisting of an agent described herein, excipients that imbibe water to provide osmotic potential, and/or a water-swellable polymer, or optionally solubilizing excipients. The capsule core can also be made such that it has a bilayer or multilayer agent analogous to the bilayer, trilayer or concentric geometries described above.

Another class of osmotic device useful in this disclosure comprises coated swellable tablets, for example, as described in EP378404. Coated swellable tablets comprise a tablet core comprising an agent described herein and a swelling material, preferably a hydrophilic polymer, coated with a membrane, which contains holes, or pores through which, in the aqueous use environment, the hydrophilic polymer can extrude and carry out the agent. Alternatively, the membrane may contain polymeric or low molecular weight water-soluble porosigens. Porosigens dissolve in the aqueous use environment, providing pores through which the hydrophilic polymer and agent may extrude. Examples of porosigens are water-soluble polymers such as HPMC, PEG, and low molecular weight compounds such as glycerol, sucrose, glucose, and sodium chloride. In addition, pores may be formed in the coating by drilling holes in the coating using a laser or other mechanical means. In this class of osmotic devices, the membrane material may comprise any film-forming polymer, including polymers which are water permeable or impermeable, providing that the membrane deposited on the tablet core is porous or contains water-soluble porosigens or possesses a macroscopic hole for water ingress and drug release. Embodiments of

this class of sustained release devices may also be multilayered, as described, for example, in EP378404.

When an agent described herein is a liquid or oil, such as a lipid vehicle formulation, for example as described in WO05/011634, the osmotic controlled-release device may comprise a soft-gel or gelatin capsule formed with a composite wall and comprising the liquid formulation where the wall comprises a barrier layer formed over the external surface of the capsule, an expandable layer formed over the barrier layer, and a semipermeable layer formed over the expandable layer. A delivery port connects the liquid formulation with the aqueous use environment. Such devices are described, for example, in US6419952, US6342249, US5324280, US4672850, US4627850, US4203440, and US3995631.

The osmotic controlled release devices of the present disclosure can also comprise a coating. In certain embodiments, the osmotic controlled release device coating exhibits one or more of the following features: is water-permeable, has at least one port for the delivery of drug, and is non-dissolving and non-eroding during release of the drug formulation, such that drug is substantially entirely delivered through the delivery port(s) or pores as opposed to delivery primarily via permeation through the coating material itself. Delivery ports include any passageway, opening or pore whether made mechanically, by laser drilling, by pore formation either during the coating process or *in situ* during use or by rupture during use. In certain embodiments, the coating is present in an amount ranging from about 5 to 30 wt% (including, for example, 10 to 20 wt%) relative to the core weight.

One form of coating is a semipermeable polymeric membrane that has the port(s) formed therein either prior to or during use. Thickness of such a polymeric membrane may vary between about 20 and 800 μm (including, for example, between about 100 to 500 μm). The diameter of the delivery port (s) may generally range in size from 0.1 to 3000 μm or greater (including, for example, from about 50 to 3000 μm in diameter). Such port(s) may be formed post-coating by mechanical or laser drilling or may be formed *in situ* by rupture of the coatings; such rupture may be controlled by intentionally incorporating a relatively small weak portion into the coating. Delivery ports may also be formed *in situ* by erosion of a plug of water-soluble material or by rupture of a thinner portion of the coating over an indentation in the core. In addition, delivery

ports may be formed during coating, as in the case of asymmetric membrane coatings of the type disclosed in US5612059 and US5698220. The delivery port may be formed *in situ* by rupture of the coating, for example, when a collection of beads that may be of essentially identical or of a variable agent are used. Drug is primarily released from such beads following rupture of the coating and, following rupture, such release may be gradual or relatively sudden. When the collection of beads has a variable agent, the agent may be chosen such that the beads rupture at various times following administration, resulting in the overall release of drug being sustained for a desired duration.

Coatings may be dense, microporous or asymmetric, having a denser region supported by a thick porous region such as those disclosed in US5612059 and US5698220. When the coating is dense the coating can be composed of a water-permeable material. When the coating is porous, it may be composed of either a water-permeable or a water-impermeable material. When the coating is composed of a porous water-impermeable material, water permeates through the pores of the coating as either a liquid or a vapor. Nonlimiting examples of osmotic devices that utilize dense coatings include US3995631 and US3845770. Such dense coatings are permeable to the external fluid such as water and may be composed of any of the materials mentioned in these patents as well as other water-permeable polymers known in the art.

The membranes may also be porous as disclosed, for example, in US5654005 and US5458887 or even be formed from water-resistant polymers. US5120548 describes another suitable process for forming coatings from a mixture of a water-insoluble polymer and a leachable water-soluble additive. The porous membranes may also be formed by the addition of pore-formers as disclosed in US4612008. In addition, vapor-permeable coatings may even be formed from extremely hydrophobic materials such as polyethylene or polyvinylidene difluorid that, when dense, are essentially water-impermeable, as long as such coatings are porous. Materials useful in forming the coating include but are not limited to various grades of acrylic, vinyls, ethers, polyamides, polyesters and cellulosic derivatives that are water-permeable and water-insoluble at physiologically relevant pHs, or are susceptible to being rendered water-insoluble by chemical alteration such as by crosslinking. Nonlimiting examples of suitable polymers (or crosslinked versions) useful in forming the coating include plasticized, unplasticized and reinforced cellulose acetate (CA), cellulose diacetate, cellulose triacetate, CA propionate, cellulose nitrate, cellulose

acetate butyrate (CAB), CA ethyl carbamate, CAP, CA methyl carbamate, CA succinate, cellulose acetate trimellitate (CAT), CA dimethylaminoacetate, CA ethyl carbonate, CA chloroacetate, CA ethyl oxalate, CA methyl sulfonate, CA butyl sulfonate, CA p-toluene sulfonate, agar acetate, amylose triacetate, beta glucan acetate, beta glucan triacetate, acetaldehyde dimethyl acetate, triacetate of locust bean gum, hydroxiated ethylene-vinylacetate, EC, PEG, PPG, PEG/PPG copolymers, PVP, HEC, HPC, CMC, CMEC, HPMC, HPMCP, HPMCAS, HPMCAT, poly (acrylic) acids and esters and poly- (methacrylic) acids and esters and copolymers thereof, starch, dextran, dextrin, chitosan, collagen, gelatin, polyalkenes, polyethers, polysulfones, polyethersulfones, polystyrenes, polyvinyl halides, polyvinyl esters and ethers, natural waxes and synthetic waxes. In various embodiments, the coating agent comprises a cellulosic polymer, in particular cellulose ethers, cellulose esters and cellulose ester-ethers, i.e., cellulosic derivatives having a mixture of ester and ether substituents, the coating materials are made or derived from poly (acrylic) acids and esters, poly (methacrylic) acids and esters, and copolymers thereof, the coating agent comprises cellulose acetate, the coating comprises a cellulosic polymer and PEG, the coating comprises cellulose acetate and PEG.

Coating is conducted in conventional fashion, typically by dissolving or suspending the coating material in a solvent and then coating by dipping, spray coating or by pan-coating. In certain embodiments, the coating solution contains 5 to 15 wt% polymer. Typical solvents useful with the cellulosic polymers mentioned above include but are not limited to acetone, methyl acetate, ethyl acetate, isopropyl acetate, n-butyl acetate, methyl isobutyl ketone, methyl propyl ketone, ethylene glycol monoethyl ether, ethylene glycol monoethyl acetate, methylene dichloride, ethylene dichloride, propylene dichloride, nitroethane, nitropropane, tetrachloroethane, 1,4-dioxane, tetrahydrofuran, diglyme, water, and mixtures thereof. Pore-formers and non- solvents (such as water, glycerol and ethanol) or plasticizers (such as diethyl phthalate) may also be added in any amount as long as the polymer remains soluble at the spray temperature. Pore-formers and their use in fabricating coatings are described, for example, in US5612059. Coatings may also be hydrophobic microporous layers wherein the pores are substantially filled with a gas and are not wetted by the aqueous medium but are permeable to water vapor, as disclosed, for example, in US5798119. Such hydrophobic but water-vapor permeable coatings are typically composed of hydrophobic polymers such as polyalkenes, polyacrylic acid derivatives, polyethers,

polysulfones, polyethersulfones, polystyrenes, polyvinyl halides, polyvinyl esters and ethers, natural waxes and synthetic waxes. Hydrophobic microporous coating materials include but are not limited to polystyrene, polysulfones, polyethersulfones, polyethylene, polypropylene, polyvinyl chloride, polyvinylidene fluoride and polytetrafluoroethylene. Such hydrophobic coatings can be made by known phase inversion methods using any of vapor-quench, liquid quench, thermal processes, leaching soluble material from the coating or by sintering coating particles. In thermal processes, a solution of polymer in a latent solvent is brought to liquid-liquid phase separation in a cooling step. When evaporation of the solvent is not prevented, the resulting membrane will typically be porous. Such coating processes may be conducted by the processes disclosed, for example, in US4247498, US4490431 and US4744906. Osmotic controlled-release devices may be prepared using procedures known in the pharmaceutical arts. See for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000.

As further noted above, the agents described herein may be provided in the form of microparticulates, generally ranging in size from about 10μm to about 2mm (including, for example, from about 100μm to 1mm in diameter). Such multiparticulates may be packaged, for example, in a capsule such as a gelatin capsule or a capsule formed from an aqueous-soluble polymer such as HPMCAS, HPMC or starch; dosed as a suspension or slurry in a liquid ; or they may be formed into a tablet, caplet, or pill by compression or other processes known in the art. Such multiparticulates may be made by any known process, such as wet- and dry-granulation processes, extrusion/spheronization, roller-compaction, melt-congealing, or by spray-coating seed cores. For example, in wet-and dry- granulation processes, the agent described herein and optional excipients may be granulated to form multiparticulates of the desired size. Other excipients, such as a binder (e. g., microcrystalline cellulose), may be blended with the agent to aid in processing and forming the multiparticulates. In the case of wet granulation, a binder such as microcrystalline cellulose may be included in the granulation fluid to aid in forming a suitable multiparticulate. See, for example, Remington : The Science and Practice of Pharmacy, 20 Edition, 2000. In any case, the resulting particles may themselves constitute the therapeutic composition or they may be coated by various film-forming materials such as enteric polymers or water-swellable or water-soluble polymers, or they may be combined with other excipients or vehicles to aid in dosing to patients.

Suitable pharmaceutical compositions in accordance with the disclosure will generally include an amount of the active compound(s) with an acceptable pharmaceutical diluent or excipient, such as a sterile aqueous solution, to give a range of final concentrations, depending on the intended use. The techniques of preparation are generally well known in the art, as exemplified by Remington's Pharmaceutical Sciences (18th Edition, Mack Publishing Company, 1995).

Kits

The agents 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 formulated in combination. Thus, one or more agents 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. A kit can include additional components such as syringes or other means for administering the agents as well as diluents or other means for formulation.

Thus, the kits can comprise: a) a pharmaceutical composition comprising an agent described herein and a pharmaceutically acceptable carrier, vehicle or diluent; and b) a container or packaging. The kits may optionally comprise instructions describing a method of using the pharmaceutical compositions in one or more of the methods described herein (e.g. disorders associated with fluid and sodium retention (such as diseases of the electrolyte-water/electrolyte transport system within the kidney, gut and urogenital system, heart failure (e.g. congestive heart failure including heart failure at any of stages I-IV according to New York Heart Association (NYHA) Functional Classification), hypertension, salt dependent forms of high blood pressure, hepatic edema, liver cirrhosis, kidney disease, polycystic kidney disease) and gastrointestinal disorders (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, 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 described herein)). The kit may optionally comprise a second pharmaceutical composition comprising one or more additional agents including but not limited to those including analgesic agents, an agent used to treat heart failure (Diuretics (e.g. furesomide (Lasix), bumetanide (Bumex), ethacrynic acid (Edecrin), torsemide (Demadex), amiloride (Midamor), spironolactone (Aldactone), canrenone, chorthiazide (Diuril), metolazone (Zaroxylyn)), Angiotension-Converting Enzyme (ACE) inhibitors (e.g. captopril (Capoten), enalopril (Vasotec), lisinopril (Prinivil, Zestril), ramipril (Altace)), Beta blockers (e.g. carvedilol (Coreg) and Inotropes (e.g. digoxin, dobutaimine, dopamine Milrinone)), a phosphodiesterase inhibitor, an agent used to treat gastrointestinal and other disorders (including those described herein), an agent used to treat constipation, an antidiarrheal agent, an insulin or related compound (including those described herein), an anti-hypertensive agent, an agent useful in the treatment of respiratory and other disorders, an anti-obesity agent, an anti-diabetic agents, an agent that activates soluble guanylate cyclase and a pharmaceutically acceptable carrier, vehicle or diluent. The pharmaceutical composition comprising the compound described herein and the second pharmaceutical composition contained in the kit may be optionally combined in the same pharmaceutical composition.

A kit includes a container or packaging for containing the pharmaceutical compositions and may also include divided containers such as a divided bottle or a divided foil packet. The container can be, for example 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 market a single dosage form. For example, tablets may be contained in a bottle which is in turn contained within a box.

An example of a kit is a so-called blister pack. Blister packs are well known in the packaging industry and are being widely used for the packaging of pharmaceutical unit dosage forms (tablets, capsules, and the like). Blister packs generally consist of a sheet of relatively stiff

material covered with a foil of a preferably transparent plastic material. During the packaging process, recesses are formed in the plastic foil. The recesses have the size and shape of individual tablets or capsules to be packed or may have the size and shape to accommodate multiple tablets and/or capsules to be packed. Next, the tablets or capsules are placed in the recesses accordingly and the sheet of relatively stiff material is sealed against the plastic foil at the face of the foil which is opposite from the direction in which the recesses were formed. As a result, the tablets or capsules are individually sealed or collectively sealed, as desired, in the recesses between the plastic foil and the sheet. Preferably the strength of the sheet is such that the tablets or capsules can be removed from the blister pack by manually applying pressure on the recesses whereby an opening is formed in the sheet at the place of the recess. The tablet or capsule can then be removed via said opening.

It maybe desirable to provide a written memory aid containing information and/or instructions for the physician, pharmacist or subject regarding when the medication is to be taken. A "daily dose" can be a single tablet or capsule or several tablets or capsules to be taken on a given day. When the kit contains separate compositions, a daily dose of one or more compositions of the kit can consist of one tablet or capsule while a daily dose of another one or more compositions of the kit can consist of several tablets or capsules. A kit can take the form of a dispenser designed to dispense the daily doses one at a time in the order of their intended use. The dispenser can be equipped with a memory-aid, so as to further facilitate compliance with the regimen. An example of such a memory-aid is a mechanical counter which indicates the number of daily doses that have been dispensed. Another example of such a memory-aid is a battery-powered micro-chip memory coupled with a liquid crystal readout, or audible reminder signal which, for example, reads out the date that the last daily dose has been taken and/or reminds one when the next dose is to be taken.

Methods to increase chemical and/or physical stability of the agents the described herein are found in U.S. 6,541,606, U.S. 6,068,850, U.S. 6,124,261, U.S. 5,904,935, and WO 00/15224, U.S. 20030069182 (via the additon of nicotinamide), U.S. 20030175230A1, U.S. 20030175230A1, U.S. 20030175239A1, U.S. 20020045582, U.S. 20010031726, WO 02/26248,

WO 03/014304, WO 98/00152A1, WO 98/00157A1, WO 90/12029, WO 00/04880, and WO 91/04743, WO 97/04796 and the references cited therein.

Methods to increase bioavailability of the agents described herein are found in U.S. 6,008,187, U.S. 5,424,289, U.S. 20030198619, WO 90/01329, WO 01/49268, WO 00/32172, and WO 02/064166. Glycyrrhizinate can also be used as an absorption enhancer (see, e.g., EP397447). WO 03/004062 discusses Ulex europaeus I (UEAI) and UEAI mimetics which may be used to target the agents described herein to the GI tract. The bioavailability of the agents described herein can also be increased by addition of oral bioavailability-enhancing agents such as those described in U.S. 6,818,615 including but not limited to: cyclosporins (including cyclosporins A through Z as defined in Table 1 of U.S. 6,818,615), for example, cyclosporin A (cyclosporin), cyclosporin F, cyclosporin D, dihydro cyclosporin A, dihydro cyclosporin C, acetyl cyclosporin A, PSC-833, (Me-Ile-4)-cyclosporin (SDZ-NIM 811) (both from Sandoz Pharmaceutical Corp.), and related oligopeptides produced by species in the genus Topycladium); antifungals including but not limited to ketoconazole; cardiovascular drug including but not limited to MS-209 (BASF), amiodarone, nifedipine, reserpine, quinidine, nicardipine, ethacrynic acid, propafenone, reserpine, amiloride; anti-migraine natural products including but not limited to ergot alkaloids; antibiotics including but not limited to cefoperazone, tetracycline, chloroquine, fosfomycin; antiparasitics including but not limited to ivermectin; multi-drug resistance reversers including but not limited to VX-710 and VX-853 (Vertex Pharmaceutical Incorporated); tyrosine kinase inhibitors including but not limited to genistein and related isoflavonoids, quercetin; protein kinase C inhibitors including but not limited to calphostin; apoptosis inducers including but not limited to ceramides; and agents active against endorphin receptors including but not limited to morphine, morphine congeners, other opioids and opioid antagonists including (but not limited to) naloxone, naltrexone and nalmefene).

The agents described herein can be fused to a modified version of the blood serum protein transferrin. U.S. 20030221201, U.S. 20040023334, U.S. 20030226155, WO 04/020454, and WO 04/019872 discuss the manufacture and use of transferrin fusion proteins. Transferrin fusion proteins may improve circulatory half life and efficacy, decrease undesirable side effects and allow reduced dosage.

The GCC agonist peptides described herein can be recombinantly expressed in bacteria. Bacteria expressing the peptide or agonists can be administered orally, rectally, mucosally or in via some other mode of administration including but not limited to those described herein. Bacterial hosts suitable for such administration include but are not limited to certain *Lactobacteria (e.g. Lactococcus lactis, Lactobacillus plantarum, Lact. rhamnosus and Lact. paracasei ssp. Paracasie* and other species found in normal human flora (Ahrne et al. Journal of Applied Microbiology 1998 85:88)), certain *Streptococcus sp. (e.g. S. gordonii)*, and certain *B. subtilis* strains (including pSM539 described in Porzio et al. BMC Biotechnology 2004 4:27). The peptides and agonists described herein can be administered using the Heliobacter based preparation methods described in WO06/015445.

Dosage

The dose range for adult humans for various drugs is generally from 0.005 mg to 10 g/day orally. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of compound described herein which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg. 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 dosage unit (e.g. an oral dosage unit) can include from, for example, 1 to 30 μg, 1 to 40 μg, 1 to 50 μg, 1 to 100 μg, 1 to 200 μg, 1 to 300 μg, 1 to 400 μg, 1 to 500 μg, 1 to 600 μg, 1 to 700 μg, 1 to 800 μg, 1 to 900 μg, 1 to 1000 μg, 10 to 30 μg, 10 to 40 μg, 10 to 50 μg, 10 to 100 μg, 10 to 200 μg, 10 to 300 μg, 10 to 400 μg, 10 to 500 μg, 10 to 600 μg, 10 to 700 μg, 10 to 800 μg, 10 to 900 μg, 10 to 1000 μg, 100 to 200 μg, 100 to 300 μg, 100 to 400 μg, 100 to 500 μg, 100 to 600 μg, 100 to 700 μg, 100 to 800 μg, 100 to 900 μg, 100 to 1000 μg, 100 to 1250 μg, 100 to 1500 μg, 100 to 1750 μg, 100 to 2000 μg, 100 to 2250 μg, 100 to 2500 μg, 100 to 2750 μg, 100 to 3000 μg, 200 to 300 μg, 200 to 400 μg, 200 to 500 μg, 200 to 600 μg, 200 to 700 μg, 200 to 800 μg, 200 to 900 μg, 200 to 1000 μg, 200 to 1250 μg, 200 to 1500 μg, 200 to 1750 μg, 200 to 2000 μg, 200 to 2250 μg, 200 to 2500 μg, 200 to 2750 μg, 200 to 3000 μg, 300 to 400 μg, 300 to 500

μg, 300 to 600 μg, 300 to 700 μg, 300 to 800 μg, 300 to 900 μg, 300 to 1000 μg, 300 to 1250 μg, 300 to 1500 μg, 300 to 1750 μg, 300 to 2000 μg, 300 to 2250 μg, 300 to 2500 μg, 300 to 2750 μg, 300 to 3000 μg, 400 to 500 μg, 400 to 600 μg, 400 to 700 μg, 400 to 800 μg, 400 to 900 μg, 400 to 1000 μg, 400 to 1250 μg, 400 to 1500 μg, 400 to 1750 μg, 400 to 2000 μg, 400 to 2250 μg, 400 to 2500 μg, 400 to 2750 μg, 400 to 3000 μg, 500 to 600 μg, 500 to 700 μg, 500 to 800 μg, 500 to 900 μg, 500 to 1000 μg, 500 to 1250 μg, 500 to 1500 μg, 500 to 1750 μg, 500 to 2000 μg, 500 to 2250 μg, 500 to 2500 μg, 500 to 2750 μg, 500 to 3000 μg, 600 to 700 μg, 600 to 800 μg, 600 to 900 μg, 600 to 1000 μg, 600 to 1250 μg, 600 to 1500 μg, 600 to 1750 μg, 600 to 2000 μg, 600 to 2250 μg, 600 to 2500 μg, 600 to 2750 μg, 600 to 3000 μg, 700 to 800 μg, 700 to 900 μg, 700 to 1000 μg, 700 to 1250 μg, 700 to 1500 μg, 700 to 1750 μg, 700 to 2000 μg, 700 to 2250 μg, 700 to 2500 μg, 700 to 2750 μg, 700 to 3000 μg, 800 to 900 μg, 800 to 1000 μg, 800 to 1250 μg, 800 to 1500 μg, 800 to 1750 μg, 800 to 2000 μg, 800 to 2250 μg, 800 to 2500 μg, 800 to 2750 μg, 800 to 3000 μg, 900 to 1000 μg, 900 to 1250 μg, 900 to 1500 μg, 900 to 1750 μg, 900 to 2000 μg, 900 to 2250 μg, 900 to 2500 μg, 900 to 2750 μg, 900 to 3000 μg, 1000 to 1250 μg, 1000 to 1500 μg, 1000 to 1750 μg, 1000 to 2000 μg, 1000 to 2250 μg, 1000 to 2500 μg, 1000 to 2750 μg, 1000 to 3000 μg, 2 to 500 μg, 50 to 500 μg, 3 to 100 μg, 5 to 20 μg, 5 to 100 μg, 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 75 μg, 80 μg, 90 μg, 100 μg, 150 μg, 200 μg, 250 μg, 300 μg, 350 μg, 400 μg, 450 μg, 500 μg, 550 μg, 600 μg, 650 μg, 700 μg, 750 μg, 800 μg, 850 μg, 900 μg, 950 μg, 1000 μg, 1050 μg, 1100 μg, 1150 μg, 1200 μg, 1250 μg, 1300 μg, 1350 μg, 1400 μg, 1450 μg, 1500 μg, 1550 μg, 1600 μg, 1650 μg, 1700 μg, 1750 μg, 1800 μg, 1850 μg, 1900 μg, 1950 μg, 2000 μg, 2050 μg, 2100 μg, 2150 μg, 2200 μg, 2250 μg, 2300 μg, 2350 μg, 2400 μg, 2450 μg, 2500 μg, 2550 μg, 2600 μg, 2650 μg, 2700 μg, 2750 μg, 2800 μg, 2850 μg, 2900 μg, 2950 μg, 3000 μg, 3250 μg, 3500 μg, 3750 μg, 4000 μg, 4250 μg, 4500 μg, 4750 μg, 5000 μg of a GCC peptide or agonist described herein. In various embodiments, the dosage unit is administered with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast), at bedtime after a low fat snack. In various embodiments, the dosage unit is administered once a day, twice a day, three times a day, four times a day, five times a day, six timesa day. The dosage unit can optionally comprise other agents.

A dosage unit (e.g. an oral dosage unit) can include, for example, from 1 to 30 μg, 1 to 40 μg, 1 to 50 μg, 1 to 100 μg, 1 to 200 μg, 1 to 300 μg, 1 to 400 μg, 1 to 500 μg, 1 to 600 μg, 1 to 700 μg, 1 to 800 μg, 1 to 900 μg, 1 to 1000 μg, 10 to 30 μg, 10 to 40 μg, 10 to 50 μg, 10 to 100 μg, 10 to 200 μg, 10 to 300 μg, 10 to 400 μg, 10 to 500 μg, 10 to 600 μg, 10 to 700 μg, 10 to 800 μg, 10 to 900 μg, 10 to 1000 μg, 100 to 200 μg, 100 to 300 μg, 100 to 400 μg, 100 to 500 μg, 100 to 600 μg, 100 to 700 μg, 100 to 800 μg, 100 to 900 μg, 100 to 1000 μg, 100 to 1250 μg, 100 to 1500 μg, 100 to 1750 μg, 100 to 2000 μg, 100 to 2250 μg, 100 to 2500 μg, 100 to 2750 μg, 100 to 3000 μg, 200 to 300 μg, 200 to 400 μg, 200 to 500 μg, 200 to 600 μg, 200 to 700 μg, 200 to 800 μg, 200 to 900 μg, 200 to 1000 μg, 200 to 1250 μg, 200 to 1500 μg, 200 to 1750 μg, 200 to 2000 μg, 200 to 2250 μg, 200 to 2500 μg, 200 to 2750 μg, 200 to 3000 μg, 300 to 400 μg, 300 to 500 μg, 300 to 600 μg, 300 to 700 μg, 300 to 800 μg, 300 to 900 μg, 300 to 1000 μg, 300 to 1250 μg, 300 to 1500 μg, 300 to 1750 μg, 300 to 2000 μg, 300 to 2250 μg, 300 to 2500 μg, 300 to 2750 μg, 300 to 3000 μg, 400 to 500 μg, 400 to 600 μg, 400 to 700 μg, 400 to 800 μg, 400 to 900 μg, 400 to 1000 μg, 400 to 1250 μg, 400 to 1500 μg, 400 to 1750 μg, 400 to 2000 μg, 400 to 2250 μg, 400 to 2500 μg, 400 to 2750 μg, 400 to 3000 μg, 500 to 600 μg, 500 to 700 μg, 500 to 800 μg, 500 to 900 μg, 500 to 1000 μg, 500 to 1250 μg, 500 to 1500 μg, 500 to 1750 μg, 500 to 2000 μg, 500 to 2250 μg, 500 to 2500 μg, 500 to 2750 μg, 500 to 3000 μg, 600 to 700 μg, 600 to 800 μg, 600 to 900 μg, 600 to 1000 μg, 600 to 1250 μg, 600 to 1500 μg, 600 to 1750 μg, 600 to 2000 μg, 600 to 2250 μg, 600 to 2500 μg, 600 to 2750 μg, 600 to 3000 μg, 700 to 800 μg, 700 to 900 μg, 700 to 1000 μg, 700 to 1250 μg, 700 to 1500 μg, 700 to 1750 μg, 700 to 2000 μg, 700 to 2250 μg, 700 to 2500 μg, 700 to 2750 μg, 700 to 3000 μg, 800 to 900 μg, 800 to 1000 μg, 800 to 1250 μg, 800 to 1500 μg, 800 to 1750 μg, 800 to 2000 μg, 800 to 2250 μg, 800 to 2500 μg, 800 to 2750 μg, 800 to 3000 μg, 900 to 1000 μg, 900 to 1250 μg, 900 to 1500 μg, 900 to 1750 μg, 900 to 2000 μg, 900 to 2250 μg, 900 to 2500 μg, 900 to 2750 μg, 900 to 3000 μg, 1000 to 1250 μg, 1000 to 1500 μg, 1000 to 1750 μg, 1000 to 2000 μg, 1000 to 2250 μg, 1000 to 2500 μg, 1000 to 2750 μg, 1000 to 3000 μg, 2 to 500 μg, 50 to 500 μg, 3 to 100 μg, 5 to 20 μg, 5 to 100 μg, 10 μg, 20 μg, 30 μg, 40 μg, 50 μg, 60 μg, 70 μg, 75 μg, 80 μg, 90 μg, 100 μg, 150 μg, 200 μg, 250 μg, 300 μg, 350 μg, 400 μg, 450 μg, 500 μg, 550 μg, 600 μg, 650 μg, 700 μg, 750 μg, 800 μg, 850 μg, 900 μg, 950 μg, 1000 μg, 1050 μg, 1100 μg, 1150 μg, 1200 μg, 1250 μg, 1300 μg, 1350 μg, 1400 μg, 1450 μg, 1500 μg, 1550 μg, 1600 μg, 1650 μg, 1700 μg, 1750 μg, 1800 μg, 1850 μg, 1900 μg, 1950 μg, 2000 μg, 2050 μg, 2100 μg, 2150 μg, 2200 μg, 2250 μg, 2300 μg, 2350

μg, 2400 μg, 2450 μg, 2500 μg, 2550 μg, 2600 μg, 2650 μg, 2700 μg, 2750 μg, 2800 μg, 2850 μg, 2900 μg, 2950 μg, 3000 μg, 3250 μg, 3500 μg, 3750 μg, 4000 μg, 4250 μg, 4500 μg, 4750 μg, 5000 μg of an agent that reduces sodium absorption in the intestine or increases anion secretion..

The precise amount of each of the two or more active ingredients in a dosage unit will depend on the desired dosage of each component. Thus, it can be useful to create a dosage unit that will, when administered according to a particular dosage schedule (e.g., a dosage schedule specifying a certain number of units and a particular timing for administration), deliver the same dosage of each component as would be administered if the patient was being treated with only a single component. In other circumstances, it might be desirable to create a dosage unit that will deliver a dosage of one or more components that is less than that which would be administered if the patient was being treated only with a single component. Finally, it might be desirable to create a dosage unit that will deliver a dosage of one or more components that is greater than that which would be administered if the patient was being treated only with a single component. The pharmaceutical composition can include additional ingredients including but not limited to the excipients described herein. In certain embodiments, one or more therapeutic agents of the dosage unit may exist in an extended or control release formulation and additional therapeutic agents may not exist in extended release formulation. For example, an agent described herein may exist in a controlled release formulation or extended release formulation in the same dosage unit with another agent that may or may not be in either a controlled release or extended release formulation. Thus, in certain embodiments, it may be desirable to provide for the immediate release of one or more of the agents described herein, and the controlled release of one or more other agents.

In certain embodiments the dosage unit and daily dose are equivalent. In certain embodiments the dosage unit and the daily dose are not equivalent. In various embodiments, the dosage unit is administered twenty minutes prior to food consumption, twenty minutes after food consumption, with food at anytime of the day, without food at anytime of the day, with food after an overnight fast (e.g. with breakfast), at bedtime after a low fat snack. In various embodiments, the dosage

unit is administered once a day, twice a day, three times a day, four times a day, five times a day, six times a day.

When two or more active ingredients are combined in single dosage form, chemical interactions between the active ingredients may occur. For example, acidic and basic active ingredients can react with each other and acidic active ingredients can facilitate the degradation of acid labile substances. Thus, in certain dosage forms, acidic and basic substances can be physically separated as two distinct or isolated layers in a compressed tablet, or in the core and shell of a press-coated tablet. Additional agents that are compatible with acidic as well as basic substances, have the flexibility of being placed in either layer. In certain multiple layer compositions at least one active ingredient can be enteric-coated. In certain embodiments thereof at least one active ingredient can be presented in a controlled release form. In certain embodiments where a combination of three or more active substances are used, they can be presented as physically isolated segments of a compressed multilayer tablet, which can be optionally film coated.

The therapeutic combinations described herein can be formulated as a tablet or capsule comprising a plurality of beads, granules, or pellets. All active ingredients including the vitamins of the combination are formulated into granules or beads or pellets that are further coated with a protective coat, an enteric coat, or a film coat to avoid the possible chemical interactions. Granulation and coating of granules or beads is done using techniques well known to a person skilled in the art. At least one active ingredient can present in a controlled release form. Finally these coated granules or beads are filled into hard gelatin capsules or compressed to form tablets.

The therapeutic combinations described herein can be formulated as a capsule comprising microtablets or minitablets of all active ingredients. Microtablets of the individual agents can be prepared using well known pharmaceutical procedures of tablet making like direct compression, dry granulation or wet granulation. Individual microtablets can be filled into hard gelatin capsules. A final dosage form may comprise one or more microtablets of each individual component. The microtablets may be film coated or enteric coated.

The therapeutic combinations described herein can be formulated as a capsule comprising one or more microtablets and powder, or one or more microtablets and granules or beads. In order to avoid interactions between drugs, some active ingredients of a said combination can be formulated as microtablets and the others filled into capsules as a powder, granules, or beads. The microtablets may be film coated or enteric coated. At least one active ingredient can be presented in controlled release form.

The therapeutic combinations described herein can be formulated wherein the active ingredients are distributed in the inner and outer phase of tablets. In an attempt to divide chemically incompatible components of proposed combination, few interacting components are converted in granules or beads using well known pharmaceutical procedures in prior art. The prepared granules or beads (inner phase) are then mixed with outer phase comprising the remaining active ingredients and at least one pharmaceutically acceptable excipient. The mixture thus comprising inner and outer phase is compressed into tablets or molded into tablets. The granules or beads can be controlled release or immediate release beads or granules, and can further be coated using an enteric polymer in an aqueous or non-aqueous system, using methods and materials that are known in the art.

The therapeutic combinations described herein can be formulated as single dosage unit comprising suitable buffering agent. All powdered ingredients of said combination are mixed and a suitable quantity of one or more buffering agents is added to the blend to minimize possible interactions.

The agents described herein, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose, diluents, lubricants, binders, disintegrating agents, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

When provided as a single dosage form, the potential exists for a chemical interaction between the combined active ingredients (for example, an lipid lowering agents and a GC-C agonist). For this reason, the preferred dosage forms of the combination products of this disclosure are formulated such that although the active ingredients are combined in a single dosage form, the physical contact between the active ingredients is minimized (that is, reduced).

In order to minimize contact, one embodiment of this disclosure where the product is orally administered provides for a combination product wherein one active ingredient is enteric coated. By enteric coating one or more of the active ingredients, it is possible not only to minimize the contact between the combined active ingredients, but also, it is possible to control the release of one of these components in the gastrointestinal tract such that one of these components is not released in the stomach but rather is released in the intestines. Another embodiment of this disclosure where oral administration is desired provides for a combination product wherein one of the active ingredients is coated with a sustained-release material which effects a sustained-release throughout the gastrointestinal tract and also serves to minimize physical contact between the combined active ingredients. Furthermore, the sustained-released component can be additionally enteric coated such that the release of this component occurs only in the intestine. Still another approach would involve the formulation of a combination product in which the one component is coated with a sustained and/or enteric release polymer, and the other component is also coated with a polymer such as a low-viscosity grade of hydroxypropyl methylcellulose (HPMC) or other appropriate materials as known in the art, in order to further separate the active components. The polymer coating serves to form an additional barrier to interaction with the other component.

Dosage forms of the combination products include those wherein one active ingredient is enteric coated can be in the form of tablets such that the enteric coated component and the other active ingredient are blended together and then compressed into a tablet or such that the enteric coated component is compressed into one tablet layer and the other active ingredient is compressed into an additional layer. Optionally, in order to further separate the two layers, one or more placebo layers may be present such that the placebo layer is between the layers of active ingredients. In

addition, dosage forms of the present disclosure can be in the form of capsules wherein one active ingredient is compressed into a tablet or in the form of a plurality of microtablets, particles, granules or non-perils, which are then enteric coated. These enteric coated microtablets, particles, granules or non-perils are then placed into a capsule or compressed into a capsule along with a granulation of the other active ingredient.

These as well as other ways of minimizing contact between the components of combination products of the present disclosure, whether administered in a single dosage form or administered in separate forms but at the same time by the same manner, will be readily apparent to those skilled in the art in light of the present disclosure.

Claims

1. A method of reducing the risk of or treating a disorder associated with fluid and/or salt retention in a patient, the method comprising administering to the patient an agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

2. The method of claim 1 wherein the agent reduces sodium absorption in the intestine.

3. The method of claim 1 wherein the agent increases anion secretion in the intestine.

4. The method of claim 1 wherein the agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

5. The method of claim 1 wherein the agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

6. The method of claim 5 wherein the agent is a guanylate cyclase receptor C agonist.

7. The method of claim 5 wherein the agent is a soluble guanylate cyclase modulator.

8. The method of claim 5 wherein the agent is a prostanoid.

9. The method of claim 5 wherein the agent is a chloride channel activator.

10. The method of claim 9 wherein the chloride channel activator is lubiprostone.

11. The method of claim 5 wherein the agent is a 5HT4 agonist.

12. The method of claim 5 wherein the agent is a cyclic nucleotide.

13 The method of claim 5 wherein the agent is a sodium transport inhibitor.

14. The method of claim 13 wherein the sodium transport inhibitor is amiloride.

15. The method of claim 13 wherein the sodium transport inhibitor is an NHE3 inhibitor.

16. The method of claim 5 wherein the agent is a laxative.

17. The method of claim 5 wherein the agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

18. The method of claim 5 wherein the agent is an agent that affects cAMP level.

19. The method of claim 5 wherein the agent is a phosphodiesterase inhibitor.

20. The method of claim 5 wherein the agent is a renin inhibitor.

21. The method of claim 5 wherein the agent is an aldosterone antagonist.

22. The method of claim 5 wherein the agent is potassium.

23. The method of claim 5 wherein the agent is a polymer resin.

24. The method of claim 11 wherein the 5HT4 agonist is Zelnorm.

25. The method of claim 8 wherein the prostanoid is selected from: the compound represented by CAS Registry No. 333963-40-9, the compound represented by CAS Registry No. 136790-76-6, (–)-7-[(2R,4aR,5R,7aR)-2-(1,1-difluoropentyl)-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl]heptanoic acid; and the 13, 14-dihydro-15-keto prostaglandins E disclosed in US5284858 including 13,14-dihydro-15-keto-PGE_2 alkyl ester, 13,14-dihydro-15-keto-PGE_2 cycloalkyl ester; 13,14-dihydro-15-keto-PGE_2 hydroxy alkyl ester, 13,14-dihydro-15-keto-PGE_2 benzyl ester, 13,14-dihydro-15-keto-PGE_1 alkyl ester, 13,14-dihydro-6,15-diketo-PGE_1 alkyl ester, 13,14-dihydro-15-keto-18-methoxy-19, 20-dinor-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-18-methoxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-Δ^2-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-methoxy-Δ^2-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-20-methoxy-PGE_2 or an alkyl ester thereof, 13, 14-dihydro-15-keto-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-hydroxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-20-methoxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-17S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-19-methy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-isopropropylidene PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-n-propyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16,16-dimethyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-19-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-20-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-methyl-PGE_1 or an alkyl ester thereof, 13, 14-dihydro-6,15-diketo-11-dehydroxy-11R-hydroxymethyl PGE_1 alkyl ester, 13,14-dihydro-15-keto-20-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-15-keto-Δ^2-PGE_1 or an alkyl ester thereof, 13,14-dihydro-15-

keto-16R,S-fluoro-20-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-difluoro-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-5,6-dehydro-20-methoxy-PGE_2 or an alkyl ester thereof, and 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE_1 or an alkyl ester thereof.

26. The method of claim 8 wherein the prostanoid is misoprostol.

27. The method of claim 8 wherein the prostanoid is the free acid of the compound associated with CAS registry NO. 59122-49-5

28. The method of claim 26 wherein the prostanoid comprises a mixture of steroisomers of misoprostol.

29. The method of claim 8 or 26 wherein only a single isomer of a prostanoid is administered.

30. The method of claim 16 wherein the laxative is selected from: a CCK-1 antagonist, a stimulant, a bulk-producing agent and a stool softener.

31. The method of claim 30 wherein the laxative is selected from dexloxiglumide, psyllium husk, docusate sodium, bisacodyl, and phenolphthalein.

32. The method of claim 23 wherein the polymer resin is selected from psyllium, lipid lowering polymers, nonabsorbed polymer resins, and sodium binding polymers.

33. The method of claim 32 wherein the lipid lowering polymer is selected from: Colesevelam, Sevalmer, or Cholestyramine.

34. The method of claim 32 wherein the nonabsorbed polymer resin is selected from: hyaluronic acid, polycarbophil calcium, polyvinyl acetate, polyvinyl pyrrolidone, polystyrene sulfate.

35. The method of claim 32 wherein the sodium-binding polymer is selected fromthe group consisting of: crosslinked polyvinylsulfamate polymer, N-(bis-phosphonic-ethyl) polyvinylamine polymer, poly-α-acrylic acid polymer, poly-α-fluoroacrylic acid polymer, polyvinylphosphoramidic polymer, polyvinylsulfamate polymer, polyvinylsulfamate/vinylsulfate copolymer, polyvinylsulfate polymer, polyvinylsulfonate polymer, polyvinylsulfonate polymer, vinylphosphonate/α-fluoroacrylic acid copolymer, vinylphosphonate/α-fluoroacrylic acid copolymer, and vinylphosphonate/acrylic acid copolymer.

36. The method of claim 32 wherein the sodium-binding polymer is administered as a core-shell composition which further comprises a semi-permeable shell.

37. The method of claim 36 wherein the semi-permeable shell comprises at least one of a poly-11 trimethylammonioundecylmethacrylate polymer, a styrene-vinylpyridine polymer, 11-dimethyl-aminodecylmethacrylate/laurylmethacrylate copolymer, or a polyallylamine/polystyrene sulfonate polymer.

38. The method of any of claims 5-37 further comprising administering an anti-diabetic agent.

39. The method of any of claims 5-37 further comprising administering an anti-obesity agent.

40. The method of any of claims 5-37 further comprising administering potassium or a salt thereof.

41. The method of any of claims 5-37 further comprising administering a PDE inhibitor.

42. The method of claim 41 wherein the PDE inhibitor is a PDE5-specific PDE inhibitor.

43. The method of claim 41 wherein the PDE inhibitor is a cGMP-specific PDE inhibitor.

44. The method of claim 41 wherein the PDE inhibitor is a cAMP-specific PDE inhibitor.

45. The method of any of claims 5-37 further comprising administering polymer resin.

46. The method of 45 wherein the polymer resin is psyllium.

47. The method of claim 45 wherein the polymer resin is a nonabsorbed polymer resin.

48. The method of claim 47 wherein the nonabsorbed polymer resin is selected from hyaluronic acid, polycarbophil calcium, polyvinyl acetate, and polyvinyl pyrrolidine.

49 The method of claim 45 wherein the polymer resin is a lipid lowering polymer.

50. The method of claim 49 wherein the lipid lowering polymer is selected from: cholestyramine, colesevelam and sevalmer.

51. The method of any of claims 5-37 further comprising administering an anti-hypertensive agent.

52. The method of claim 51 wherein the antihypertensive agent is selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist.

53. The method of claim 51 wherein the method comprises administering two or more anti-hypertensive agents wherein the two or more antihypertensive agents are independently selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist.

54. The method of claim 51 wherein the anti-hypertensive agent is a diuretic.

55. The method of claim 54 wherein the diuretic is selected from the group consisting of: a loop diuretic, a thiazide, a potassium sparing agent, and an osmotic diuretic.

56. The method of claim 54 wherein the diuretic is a loop diuretic.

57. The method of claim 54 wherein the diuretic is furosemide, bumetanide, ethacrynic or torsemide.

58. The method of claim 54 wherein the diuretic is a thiazide.

59. The method of claim 58 wherein the thiazide is bendroflumethiazide, hydrochlorothiazide, indapamide, chlortalidone or metolazone.

60. The method of claim 54 wherein the diuretic is a potassium sparing agent.

61. The method of claim 60 wherein the potassium sparing agent is amiloride or triamterene.

62. The method of claim 54 wherein the diuretic is an osmotic diuretic.

63. The method of claim 62 wherein the osmotic diuretic is glucose or mannitol.

64. The method of claim 51 wherein the antihypertensive agent is an angiotensin converting enzyme inhibitor.

65. The method of claim 64 wherein the angiotensin converting enzyme inhibitor is selected from: Benazepril (Lotensin), Captopril (Capoten), Enalapril/Enalaprilat (Vasotec), Fosinopril (Monopril), Lisinopril (Zestril and Prinivil), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and Trandolapril (Mavik).

66. The method of claim 51 wherein the antihypertensive agent is an angiotensin II receptor antagonist.

67. The method of claim 66 wherein the angiotensin II receptor antagonist is selected from: Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, and Valsartan.

68. The method of claim 51 wherein the antihypertensive agent is a calcium channel blocker.

69. The method of claim 68 wherein the calcium channel blocker is selected from: Amlodipine (Norvasc), Felodipine (Plendil), Nicardipine (Cardene), Nifedipine (Procardia, Adalat), Nimodipine (Nimotop), Nisoldipine (Sular), Nitrendipine (Cardif, Nitrepin), and Lacidipine (Motens), Lercanidipine (Zanidip), Verapamil (Calan, Isoptin), Gallopamil (D600), Diltiazem (Cardizem), and Menthol (mint oil).

70. The method of claim 69 wherein the calcium channel blocker is Amlodipine.

71. The method of claim 51 wherein the antihypertensive agent is a beta-adrenergic antagonist.

72. The method of claim 71 wherein the beta-adrenergic antagonist is selected from: Dichloroisoprenaline, Practolol, Pronethaolol, Alprenolol, Carteolol, Levobunolol, Mepindolol, Metipranolol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Propranolol, Sotalol, Timolol, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Esmolol Metoprolol, Nebivolol, Carvedilol, Celiprolol, Labetalol, and Butoxamine.

73. The method of claim 51 wherein the antihypertensive agent is an alpha-adrenergic antagonist.

74. The method of claim 73 wherein the alpha-adrenergic antagonist is selected from: Doxazosin (Cardura), Prazosin (Minipress), Phenoxybenzamine, Phentolamine (Regitine), Tamsulosin (Flomaxtra/Flomax), Alfuzosin (Uroxatral), and Terazosin (Hytrin).

75. The method of claim 51 wherein the antihypertensive agent is a renin inhibitor.

76. The method of claim 75 wherein the renin inhibitor is selected from: Tekturna® (Rasilez and Aliskiren) and SPP635.

77. The method of claim 51 wherein the antihypertensive agent is an aldosterone antagonist.

78. The method of claim 77 wherein the aldosterone antagonist is Spironolactone, Canrenone, or Eplerenone.

79. The method of any of claims 5-37 further comprising administering a lipid altering agent.

80. The method of claim 79 wherein the lipid altering agent is a cholesterol lowering agent.

81. The method of claim 80 wherein the agent lowers low density cholesterol.

82. The method of claim 79 wherein the lipid altering agent is selected from the group consisting of: a statin; a fibrate; niacin; a CETP inhibitor; a MTP inhibitor; a cholesterol absorption inhibitor; a squalene synthesis inhibitor; and a bile acid sequestrant.

83. The method of claim 82 wherein the lipid altering agent is a statin.

84. The method of claim 83 wherein the statin is chosen from simvastatin, rosuvastatin and atorvastatin.

85. The method of claim 79 wherein the lipid altering agent is a cholesterol absorption inhibitor.

86. The method of claim 85 wherein the cholesterol absorption inhibitor is ezetimibe.

87. The method of claim 79 wherein the lipid altering agent is a bile acid sequestrant.

88. The method of claim 87 wherein the bile acid sequestrant is chosen from cholestyramine, colesevelam and colestipol.

89. The method of claim 79 wherein the lipid altering agent is a fibrate.

90. The method of claim 89 wherein the fibrate is fenofibrate.

91. The method of claim 1 comprising administering misoprostol and psyllium.

92. The method of claim 1 comprising administering methyl 7-[(1R,2R,3R)-3-hydroxy-2-[(E,4S)-4-hydroxy-4-methyloct-1-enyl]-5-oxocyclopentyl]heptanoate.

93 The method of claim 1 comprising administering psyllium and a peptide that activates the guanylate cyclase C receptor.

94. The method of claim 93 wherein the peptide is selected from:
Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;
d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;
d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;
d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;

d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;

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

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

Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro; Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;

Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro; Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys; Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Val Thr Val Gln Asp Gly Asn Phe Ser Phe Ser Leu Glu Ser Val Lys Lys Leu Lys Asp Leu Gln Glu Pro Gln Glu Pro;

Arg Val Gly Lys Leu Arg Asn Phe Ala Pro Ile Pro Gly Glu Pro Val Val Pro Ile Leu Cys Ser Asn Pro Asn Phe Pro; Glu Glu Leu Lys Pro Leu Cys Lys Glu Pro Asn Ala Gln Glu Ile Leu Gln Arg Leu Glu Glu Ile Ala Glu Asp Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Met Pro Ser Thr Gln Tyr Ile Arg Arg Pro Ala Ser Ser Tyr Ala Ser Cys Ile Trp Cys Thr Thr Ala Cys Ala Ser Cys His Gly Arg Thr Thr Lys Pro Ser Leu Ala Thr;

Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Val Gln Val Glu Glu Gly Gly Phe Ser Phe Pro Leu Asp Ala Val Lys Lys Leu Glu Glu Leu Met Gly Val Asp Met; Thr Val Lys Gln Ser Pro Arg Leu Ala Lys Thr Ser Thr Thr Ala Val Cys Thr Asn Pro Asp Leu Pro Ala Val Phe Leu; Pro Leu Cys Lys Ser Lys Gly Ala Ala Asn Ser Phe Phe Arg Leu Gly Phe Val Ala Ala Arg Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr;
Val Tyr Ile Gln Tyr Glu Gly Phe Gln Val Asn Leu Asp Ser Val Lys Lys Leu Asp Lys Leu Leu Glu Gln Leu Arg Gly;
Phe His His Gln Met Gly Asp Gln Arg Asp Pro Ser Ile Leu Cys Ser Asp Pro Ala Leu Pro Ser Asp Leu Gln Pro Val; and
Cys Glu Asn Ser Gln Ala Val Asn Ile Phe Arg Ala Leu Arg Tyr Ile Asn Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr

95. The method of claim 1 wherein the disorder is associated with fluid retention.

96. The method of claim 1 wherein the disorder is associated with salt retention.

97. The method of any of claims 1-94 wherein the disorder is a cardiovascular disorder.

98. The method of claim 97 wherein the cardiovascular disorder is cardiomyopathy.

99. The method of claim 98 wherein the cardiomyopathy is associated with Chagas disease.

100. The method of claim 97 wherein the cardiovascular disorder is hypertension.

101. The method of claim 100 wherein the hypertension is salt-sensitive hypertension.

102. The method of claim 97 wherein the cardiovascular disorder is congestive heart failure.

103. The method of claim 97 wherein the cardiovascular disorder is cardiac hypertrophy.

104. The method of claim 97 wherein the cardiovascular disorder is a heart attack.

105. The method of claim 97 wherein the cardiovascular disorder is stroke.

106. The method of any of claims 1-94 wherein the patient is suffering from salt-sensitive hypertension.

107. The method of any of claims 1-94 wherein the patient is suffering from congestive heart failure.

108. The method of any of claims 1-94 wherein the patient is suffering from cardiac hypertrophy.

109. The method of any of claims 1-94 wherein the patient has suffered a heart attack.

110. The method of any of claims 1-94 wherein the patient has suffered a stroke.

111. The method of any of claims 1-94 wherein the patient is salt sensitive.

112. The method of any of claims 1-94 wherein the patient is suffering from hypertension.

113. The method of any of claims 1-94 wherein the disorder is a renal disorder.

114. The method of claim 113 wherein the renal disorder is selected from chronic renal failure or acute renal failure.

115. The method of any of claims 1-94 wherein the disorder is associated with ascites.

116. The method of any of claims 1-94 wherein the disorder is cirrhosis.

117. The method of any of claims 1-94 wherein the patient is suffering from alcoholism.

118. The method of any of claims 1-94 wherein the disorder hepatitis.

119. The method of claim 118 wherein the hepatitis is chronic hepatitis.

120. The method of claim 118 wherein the hepatitis is severe alcoholic hepatitis without cirrhosis.

121. The method of claim any of claims 1-94 wherein the disorder is Budd-Chiari syndrome.

122. The method of claim any of claims 1-94 wherein the disorder is constrictive pericarditis.

123. A pharmaceutical composition comprising: a first agent that is an anti-hypertensive agent and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

124. The pharmaceutical composition of claim 123 wherein the second agent reduces sodium absorption in the intestine.

125. The pharmaceutical composition of claim 123 wherein the second agent increases anion secretion in the intestine.

126. The pharmaceutical composition of claim 123 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

127. The pharmaceutical composition of claim 123 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

128. The pharmaceutical composition of claim 123 wherein the second agent is a guanylate cyclase receptor C agonist.

129. The pharmaceutical composition of claim 123 wherein the second agent is a soluble guanylate cyclase modulator.

130. The pharmaceutical composition of claim 123 wherein the second agent is a prostanoid.

131. The pharmaceutical composition of claim 123 wherein the second agent is a chloride channel activator.

132. The pharmaceutical composition of claim 123 wherein the second agent is a 5HT4 agonist.

133. The pharmaceutical composition of claim 123 wherein the second agent is a cyclic nucleotide.

134. The pharmaceutical composition of claim 123 wherein the second agent is a sodium transport inhibitor.

135. The pharmaceutical composition of claim 123 wherein the second agent is a laxative.

136. The pharmaceutical composition of claim 123 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

137. The pharmaceutical composition of claim 123 wherein the second agent is an agent that alters cAMP level.

138. The pharmaceutical composition of claim 137 wherein the second agent increases cAMP level.

139. The pharmaceutical composition of claim 123 wherein the second agent is a phosphodiesterase inhibitor.

140. The pharmaceutical composition of claim 123 wherein the second agent is a renin inhibitor.

141. The pharmaceutical composition of claim 123 wherein the second agent is an aldosterone antagonist.

142. The pharmaceutical composition of claim 123 wherein the second agent is potassium.

143. The pharmaceutical composition of claim 123 wherein the second agent is a polymer resin.

144. A pharmaceutical composition comprising: a first agent that is an anti-diabetic agent and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

145. The pharmaceutical composition of claim 144 wherein the second agent reduces sodium absorption in the intestine.

146. The pharmaceutical composition of claim 144 wherein the second agent increases anion secretion in the intestine.

147. The pharmaceutical composition of claim 144 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

148. The pharmaceutical composition of claim 144 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator

(CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

149. The pharmaceutical composition of claim 148 wherein the second agent is a guanylate cyclase receptor C agonist.

150. The pharmaceutical composition of claim 148 wherein the second agent is a soluble guanylate cyclase modulator.

151. The pharmaceutical composition of claim 148 wherein the second agent is a prostanoid.

152. The pharmaceutical composition of claim 148 wherein the second agent is a chloride channel activator.

153. The pharmaceutical composition of claim 148 wherein the second agent is a 5HT4 agonist.

154. The pharmaceutical composition of claim 148 wherein the second agent is a cyclic nucleotide.

155. The pharmaceutical composition of claim 148 wherein the second agent is a sodium transport inhibitor.

156. The pharmaceutical composition of claim 148 wherein the second agent is a laxative.

157. The pharmaceutical composition of claim148 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

158. The pharmaceutical composition of claim 148 wherein the second agent is an agent that affects cAMP level.

159. The pharmaceutical composition of claim 158 wherein the second agent increases cAMP level.

160. The pharmaceutical composition of claim 148 wherein the second agent is a phosphodiesterase inhibitor.

161. The pharmaceutical composition of claim148 wherein the second agent is a renin inhibitor.

162. The pharmaceutical composition of claim 148 wherein the second agent is an aldosterone antagonist.

163. The pharmaceutical composition of claim 148 wherein the second agent is potassium.

164. The pharmaceutical composition of claim 148 wherein the second agent is a polymer resin.

165. A pharmaceutical composition comprising: a first agent that is an anti-obesity agent and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

166. The pharmaceutical composition of claim 165 wherein the second agent reduces sodium absorption in the intestine.

167. The pharmaceutical composition of claim 165 wherein the second agent increases anion secretion in the intestine.

168. The pharmaceutical composition of claim 165 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

169. The pharmaceutical composition of claim 165 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

170. The pharmaceutical composition of claim 169 wherein the second agent is a guanylate cyclase receptor C agonist.

171. The pharmaceutical composition of claim 169 wherein the second agent is a soluble guanylate cyclase modulator.

172. The pharmaceutical composition of claim 169 wherein the second agent is a prostanoid.

173. The pharmaceutical composition of claim 169 wherein the second agent is a chloride channel activator.

174. The pharmaceutical composition of claim 169 wherein the second agent is a 5HT4 agonist.

175. The pharmaceutical composition of claim 169 wherein the second agent is a cyclic nucleotide.

176. The pharmaceutical composition of claim 169 wherein the second agent is a sodium transport inhibitor.

177. The pharmaceutical composition of claim 169 wherein the second agent is a laxative.

178. The pharmaceutical composition of claim 169 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

179. The pharmaceutical composition of claim 169 wherein the second agent is an agent that affects cAMP level.

180. The pharmaceutical composition of claim 179 wherein the second agent increases cAMP level.

181. The pharmaceutical composition of claim 169 wherein the second agent is a phosphodiesterase inhibitor.

182. The pharmaceutical composition of claim 169 wherein the second agent is a renin inhibitor.

183. The pharmaceutical composition of claim 169 wherein the second agent is an aldosterone antagonist.

184. The pharmaceutical composition of claim 169 wherein the second agent is potassium.

185. The pharmaceutical composition of claim 169 wherein the second agent is a polymer resin.

186. A pharmaceutical composition comprising: potassium or a salt thereof and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

187. The pharmaceutical composition of claim 186 wherein the second agent reduces sodium absorption in the intestine.

188. The pharmaceutical composition of claim 186 wherein the second agent increases anion secretion in the intestine.

189. The pharmaceutical composition of claim 186 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

190. The pharmaceutical composition of claim 186 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

191. The pharmaceutical composition of claim 190 wherein the second agent is a guanylate cyclase receptor C agonist.

192 The pharmaceutical composition of claim 190 wherein the second agent is a soluble guanylate cyclase modulator.

193. The pharmaceutical composition of claim 190 wherein the second agent is a prostanoid.

194. The pharmaceutical composition of claim 190 wherein the second agent is a chloride channel activator.

195. The pharmaceutical composition of claim 190 wherein the second agent is a 5HT4 agonist.

196. The pharmaceutical composition of claim 190 wherein the second agent is a cyclic nucleotide.

197. The pharmaceutical composition of claim 190 wherein the second agent is a sodium transport inhibitor.

198. The pharmaceutical composition of claim 190 wherein the second agent is a laxative.

199. The pharmaceutical composition of claim 190 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

200. The pharmaceutical composition of claim 190 wherein the second agent is an agent that affects cAMP level.

201. The pharmaceutical composition of claim 200 wherein the second agent increases cAMP level.

202. The pharmaceutical composition of claim 190 wherein the second agent is a phosphodiesterase inhibitor.

203. The pharmaceutical composition of claim 190 wherein the second agent is a renin inhibitor.

204. The pharmaceutical composition of claim190 wherein the second agent is an aldosterone antagonist.

205. The pharmaceutical composition of claim 190 wherein the second agent is potassium.

206. The pharmaceutical composition of claim 190 wherein the second agent is a polymer resin.

207. A pharmaceutical composition comprising: a first agent that is a PDE inhibitor and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

208. The pharmaceutical composition of claim 207 wherein the second agent reduces sodium absorption in the intestine.

209. The pharmaceutical composition of claim 207 wherein the second agent increases anion secretion in the intestine.

210. The pharmaceutical composition of claim 207 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

211. The pharmaceutical composition of claim 207 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

212. The pharmaceutical composition of claim 211 wherein the second agent is a guanylate cyclase receptor C agonist.

213. The pharmaceutical composition of claim 211 wherein the second agent is a soluble guanylate cyclase modulator.

214. The pharmaceutical composition of claim 211 wherein the second agent is a prostanoid.

215. The pharmaceutical composition of claim 211 wherein the second agent is a chloride channel activator.

216. The pharmaceutical composition of claim 211 wherein the second agent is a 5HT4 agonist.

217. The pharmaceutical composition of claim 211 wherein the second agent is a cyclic nucleotide.

218. The pharmaceutical composition of claim 211 wherein the second agent is a sodium transport inhibitor.

219. The pharmaceutical composition of claim 211 wherein the second agent is a laxative.

220. The pharmaceutical composition of claim 211 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

221. The pharmaceutical composition of claim 211 wherein the second agent is an agent that affects cAMP level.

222. The pharmaceutical composition of claim 221 wherein the second agent increases cAMP level.

223. The pharmaceutical composition of claim 211 wherein the second agent is a phosphodiesterase inhibitor.

224. The pharmaceutical composition of claim 211 wherein the second agent is a renin inhibitor.

225. The pharmaceutical composition of claim 211 wherein the second agent is an aldosterone antagonist.

226. The pharmaceutical composition of claim 211 wherein the second agent is potassium.

227. The pharmaceutical composition of claim 211 wherein the second agent is a polymer resin.

228. The method of any of claims 207-227 wherein the PDE inhibitor is a PDE5-specific PDE inhibitor.

229. The method of any of claims 207-227 wherein the PDE inhibitor is a cGMP-specific PDE inhibitor.

230. The method of any of claims 207-227 wherein the PDE inhibitor is a cAMP-specific PDE inhibitor.

231. A pharmaceutical composition comprising: a first agent that is a polymer resin and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

232. The pharmaceutical composition of claim 231 wherein the second agent reduces sodium absorption in the intestine.

233. The pharmaceutical composition of claim 231 wherein the second agent increases anion secretion in the intestine.

234. The pharmaceutical composition of claim 231 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

235. The pharmaceutical composition of claim 231 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

236. The pharmaceutical composition of claim 235 wherein the second agent is a guanylate cyclase receptor C agonist.

237. The pharmaceutical composition of claim 235 wherein the second agent is a soluble guanylate cyclase modulator.

238. The pharmaceutical composition of claim 235 wherein the second agent is a prostanoid.

239. The pharmaceutical composition of claim 235 wherein the second agent is a chloride channel activator.

240. The pharmaceutical composition of claim 235 wherein the second agent is a 5HT4 agonist.

241. The pharmaceutical composition of claim 235 wherein the second agent is a cyclic nucleotide.

242. The pharmaceutical composition of claim 235 wherein the second agent is a sodium transport inhibitor.

243. The pharmaceutical composition of claim 235 wherein the second agent is a laxative.

244. The pharmaceutical composition of claim 235 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

245. The pharmaceutical composition of claim 235 wherein the second agent is an agent that affects cAMP level.

246. The pharmaceutical composition of claim 235 wherein the second agent is a sodium transport inhibitor.

247. The pharmaceutical composition of claim 235 wherein the second agent is a phosphodiesterase inhibitor.

248. The pharmaceutical composition of claim 235 wherein the second agent is a renin inhibitor.

249. The pharmaceutical composition of claim 235 wherein the second agent is an aldosterone antagonist.

250. The pharmaceutical composition of claim 235 wherein the second agent is potassium.

251. The pharmaceutical composition of any of claims 231-250 wherein the polymer resin is psyllium.

252. The pharmaceutical composition of any of claims 235-250 wherein the polymer resin is a nonabsorbed polymer resin.

253. The pharmaceutical composition of claim 252 wherein the nonabsorbed polymer resin is selected from hyaluronic acid, polycarbophil calcium, polyvinyl acetate, and polyvinyl pyrrolidine.

254. The pharmaceutical composition of any of claims 231-250 wherein the polymer resin is a lipid lowering polymer.

255. The pharmaceutical composition of claim 254 wherein the lipid lowering polymer is selected from: cholestyramine, colesevelam or sevalmer.

256. A pharmaceutical composition comprising: a first agent that is an anti-hypertensive agent. and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

257. The pharmaceutical composition of claim 256 wherein the second agent reduces sodium absorption in the intestine.

258. The pharmaceutical composition of claim 256 wherein the second agent increases anion secretion in the intestine.

259. The pharmaceutical composition of claim 256 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

260. The pharmaceutical composition of claim 256 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

261. The pharmaceutical composition of claim 260 wherein the second agent is a guanylate cyclase receptor C agonist.

262. The pharmaceutical composition of claim 260 wherein the second agent is a soluble guanylate cyclase modulator.

263. The pharmaceutical composition of claim 260 wherein the second agent is a prostanoid.

264. The pharmaceutical composition of claim 260 wherein the second agent is a chloride channel activator.

265. The pharmaceutical composition of claim 260 wherein the second agent is a 5HT4 agonist.

266. The pharmaceutical composition of claim 260 wherein the second agent is a cyclic nucleotide.

267. The pharmaceutical composition of claim 260 wherein the second agent is a sodium transport inhibitor.

268. The pharmaceutical composition of claim 260 wherein the second agent is a laxative.

269. The pharmaceutical composition of claim 260 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

270. The pharmaceutical composition of claim 260 wherein the second agent is an agent that affects cAMP level.

271. The pharmaceutical composition of claim 270 wherein the second agent increases cAMP level.

272. The pharmaceutical composition of claim 260 wherein the second agent is a phosphodiesterase inhibitor.

273. The pharmaceutical composition of claim 260 wherein the second agent is a renin inhibitor.

274. The pharmaceutical composition of claim 260 wherein the second agent is an aldosterone antagonist.

275. The pharmaceutical composition of claim 260 wherein the second agent is potassium.

276. The pharmaceutical composition of claim 260 wherein the second agent is a polymer resin.

277. The pharmaceutical composition of any of claims 256-276 wherein the antihypertensive agent is selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist.

278. The pharmaceutical composition of any of claims 256-276 wherein the composition comprises two or more anti-hypertensive agents wherein the two or more antihypertensive agents are independently selected from: a diuretic, an inhibitor of angiotensin converting enzyme, an angiotensin II receptor antagonist, a calcium channel blocker, a beta-adrenergic antagonist, alpha-adrenergic antagonist, a renin inhibitor, and an aldosterone antagonist.

279. The pharmaceutical composition of claim 277 wherein the anti-hypertensive agent is a diuretic.

280. The pharmaceutical composition of claim 279 wherein the diuretic is selected from the group consisting of: a loop diuretic, a thiazide, a potassium sparing agent, and an osmotic diuretic.

281. The pharmaceutical composition of claim 279 wherein the diuretic is a loop diuretic.

282. The pharmaceutical composition of claim 279 wherein the diuretic is furosemide, bumetanide, ethacrynic or torsemide.

283. The pharmaceutical composition of claim 279 wherein the diuretic is a thiazide.

284. The pharmaceutical composition of claim 283 wherein the thiazide is bendroflumethiazide, hydrochlorothiazide, indapamide, chlortalidone or metolazone.

285. The pharmaceutical composition of claim 279 wherein the diuretic is a potassium sparing agent.

286. The pharmaceutical composition of claim 285 wherein the potassium sparing agents is amiloride or triamterene.

287. The pharmaceutical composition of claim 279 wherein the diuretic is an osmotic diuretic.

288. The pharmaceutical composition of claim 287 wherein the osmotic diuretic is glucose or mannitol.

289. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is an angiotensin converting enzyme inhibitor.

290. The pharmaceutical composition of claim 289 wherein the angiotensin converting enzyme inhibitor is selected from: Benazepril (Lotensin), Captopril (Capoten), Enalapril/Enalaprilat (Vasotec), Fosinopril (Monopril), Lisinopril (Zestril and Prinivil), Moexipril (Univasc), Perindopril (Aceon), Quinapril (Accupril), Ramipril (Altace), and Trandolapril (Mavik).

291. The method of claim 277 wherein the antihypertensive agent is an angiotensin II receptor antagonist.

292. The pharmaceutical composition of claim 291 wherein the angiotensin II receptor antagonist is selected from: Candesartan, Eprosartan, Irbesartan, Losartan, Olmesartan, Telmisartan, Valsartan.

293. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is a calcium channel blocker.

294. The pharmaceutical composition of claim 293 wherein the calcium channel blocker is selected from: Amlodipine (Norvasc), Felodipine (Plendil), Nicardipine (Cardene), Nifedipine (Procardia, Adalat), Nimodipine (Nimotop), Nisoldipine (Sular), Nitrendipine (Cardif, Nitrepin),

and Lacidipine (Motens), Lercanidipine (Zanidip), Verapamil (Calan, Isoptin), Gallopamil (D600), Diltiazem (Cardizem), and Menthol (mint oil).

295. The pharmaceutical composition of claim 294 wherein the calcium channel blocker is Amlodipine.

296. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is a beta-adrenergic antagonist.

297. The pharmaceutical composition of claim 296 wherein the beta-adrenergic antagonist is selected from: Dichloroisoprenaline, Practolol, Pronethaolol, Alprenolol, Carteolol, Levobunolol, Mepindolol, Metipranolol, Nadolol, Oxprenolol, Penbutolol, Pindolol, Propranolol, Sotalol, Timolol, Acebutolol, Atenolol, Betaxolol, Bisoprolol, Esmolol Metoprolol, Nebivolol, Carvedilol, Celiprolol, Labetalol, and Butoxamine.

298. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is an alpha-adrenergic antagonist.

299. The pharmaceutical composition of claim 298 wherein the alpha-adrenergic antagonist is selected from: Doxazosin (Cardura), Prazosin (Minipress), Phenoxybenzamine, Phentolamine (Regitine), Tamsulosin (Flomaxtra/Flomax), Alfuzosin (Uroxatral), and Terazosin (Hytrin).

300. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is a renin inhibitor.

301. The pharmaceutical composition of claim 300 wherein the renin inhibitor is selected from: Tekturna® (Rasilez and Aliskiren) and SPP635.

302. The pharmaceutical composition of claim 277 wherein the antihypertensive agent is an aldosterone antagonist.

303. The pharmaceutical composition of claim 302 wherein the aldosterone antagonist is Spironolactone, Canrenone, or Eplerenone.

304. A pharmaceutical composition comprising: a first agent that is a lipid altering agent, and a second agent selected from: a) an agent that reduces sodium absorption in the intestine; b) an agent that increases anion secretion in the intestine; or c) an agent that both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

305. The pharmaceutical composition of claim 304 wherein the second agent reduces sodium absorption in the intestine.

306. The pharmaceutical composition of claim 304 wherein the second agent increases anion secretion in the intestine.

307. The pharmaceutical composition of claim 304 wherein the second agent both reduces sodium absorption in the intestine and increases anion secretion in the intestine.

308. The pharmaceutical composition of claim 304 wherein the second agent is selected from: a) a guanylate cyclase receptor C agonist, b) a soluble guanylate cyclase modulator, c) a prostanoid, d) a chloride channel activator, e) a 5HT4 agonist, f) a cyclic nucleotide, g) a sodium transport inhibitor, h) a laxative, i) a cystic fibrosis transmembrane conductance regulator (CTFR) modulator, j) an agent that affects cAMP levels, k) a phosphodiesterase inhibitor, l) a renin inhibitor, m) an aldosterone antagonist, n) potassium, and o) a polymer resin.

309. The pharmaceutical composition of claim 308 wherein the second agent is a guanylate cyclase receptor C agonist.

310. The pharmaceutical composition of claim 308 wherein the second agent is a soluble guanylate cyclase modulator.

311. The pharmaceutical composition of claim 308 wherein the second agent is a prostanoid.

312. The pharmaceutical composition of claim 308 wherein the second agent is a chloride channel activator.

313. The pharmaceutical composition of claim 308 wherein the second agent is a 5HT4 agonist.

314. The pharmaceutical composition of claim 308 wherein the second agent is a cyclic nucleotide.

315. The pharmaceutical composition of claim 308 wherein the second agent is a sodium transport inhibitor.

316. The pharmaceutical composition of claim 308 wherein the second agent is a laxative.

317. The pharmaceutical composition of claim 308 wherein the second agent is a cystic fibrosis transmembrane conductance regulator (CTFR) modulator.

318. The pharmaceutical composition of claim 308 wherein the second agent is an agent that affects cAMP level.

319. The pharmaceutical composition of claim 318 wherein the second agent increases cAMP level.

320. The pharmaceutical composition of claim 308 wherein the second agent is a phosphodiesterase inhibitor.

321. The pharmaceutical composition of claim 308 wherein the second agent is a renin inhibitor.

322. The pharmaceutical composition of claim 308 wherein the second agent is an aldosterone antagonist.

323. The pharmaceutical composition of claim 308 wherein the second agent is potassium.

324. The pharmaceutical composition of claim 308 wherein the second agent is a polymer resin.

325. The pharmaceutical composition of any one of claims 304-324 wherein the lipid altering agent is a cholesterol lowering agent.

326. The pharmaceutical composition of claim 325 wherein the cholesterol lowering agent lowers low density cholesterol.

327. The pharmaceutical composition of any one of claims 304-324 wherein the lipid altering agent is selected from the group consisting of: a statin; a fibrate; niacin; a CETP inhibitor; a MTP inhibitor; a cholesterol absorption inhibitor; a squalene synthesis inhibitor; and a bile acid sequestrant.

328. The pharmaceutical composition of claim 327 wherein the lipid altering agent is a statin.

329. The pharmaceutical composition of claim 328 wherein the statin is chosen from simvastatin, rosuvastatin and atorvastatin.

330. The pharmaceutical composition of claim 327 wherein the lipid altering agent is a cholesterol absorption inhibitor.

331. The method of claim 330 wherein the cholesterol absorption inhibitor is ezetimibe.

332. The pharmaceutical composition of claim 327 wherein the lipid altering agent is a bile acid sequestrant.

333. The pharmaceutical composition of claim 332 wherein the bile acid sequestrant is chosen from cholestyramine, colesevelam and colestipol.

334. The pharmaceutical composition of claim 327 wherein the lipid altering agent is a fibrate.

335. The pharmaceutical composition of claim 334 wherein the fibrate is fenofibrate.

336. The pharmaceutical composition of any of claims 132, 153, 174, 195, 216, 240, 265, and 313 wherein the 5HT4 agonist is Zelnorm.

337. The pharmaceutical composition of any of claims 130, 151, 172, 193, 214, 238, 263 and 311 wherein the prostanoid is selected from: the compound represented by CAS Registry No. 333963-40-9, the compound represented by CAS Registry No. 136790-76-6, (–)-7-[(2R,4aR,5R,7aR)-2-(1,1-difluoropentyl)-2-hydroxy-6-oxooctahydrocyclopenta[b]pyran-5-yl]heptanoic acid; and the 13, 14-dihydro-15-keto prostaglandins E disclosed in US5284858 including 13,14-dihydro-15-keto-PGE_2 alkyl ester, 13,14-dihydro-15-keto-PGE_2 cycloalkyl ester; 13,14-dihydro-15-keto-PGE_2 hydroxy alkyl ester, 13,14-dihydro-15-keto-PGE_2 benzyl ester, 13,14-dihydro-15-keto-PGE_1 alkyl ester, 13,14-dihydro-6,15-diketo-PGE_1 alkyl ester, 13,14-dihydro-15-keto-18-methoxy-19, 20-dinor-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-18-methoxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-Δ^2-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-methoxy-Δ^2-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-3R,S-methyl-20-methoxy-PGE_2 or an alkyl ester thereof, 13, 14-dihydro-15-keto-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-hydroxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-dimethyl-20-methoxy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-17S-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-19-methy-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-

isopropylidene PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-11-dehydroxy-11R-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-n-propyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-20-ethyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-fluoro-11-dehydroxy-11R-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16R,S-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-16,16-dimethyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-19-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-20-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-6,15-diketo-11-dehydroxy-11R-methyl-PGE_1 or an alkyl ester thereof, 13, 14-dihydro-6,15-diketo-11-dehydroxy-11R-hydroxymethyl PGE_1 alkyl ester, 13,14-dihydro-15-keto-20-methyl-PGE_1 or an alkyl ester thereof, 13,14-dihydro-15-keto-Δ^2-PGE_1 or an alkyl ester thereof, 13,14-dihydro-15-keto-16R,S-fluoro-20-methyl-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-16,16-difluoro-PGE_2 or an alkyl ester thereof, 13,14-dihydro-15-keto-5,6-dehydro-20-methoxy-PGE_2 or an alkyl ester thereof, and 13,14-dihydro-6,15-diketo-16R,S-fluoro-PGE_1 or an alkyl ester thereof.

338. The pharmaceutical composition of any of claims 130, 151, 172, 193, 214, 238, 263 and 311 wherein the prostanoid is the free acid of the compound associated with CAS registry NO. 59122-49-5

339. The pharmaceutical composition of any of claims 130, 151, 172, 193, 214, 238, 263 and 311 wherein the prostanoid comprises a mixture of steroisomers.

340. The pharmaceutical composition of any of claims 130, 151, 172, 193, 214, 238, 263 and 311 wherein only a single isomer of a prostanoid is present.

341. The pharmaceutical composition of any of claims 135, 156, 177, 198, 219, 243, 268, and 316 wherein the laxative is selected from: a stimulant, a bulk-producing agent and a stool softener.

342. The pharmaceutical composition of any of claims 135, 156, 177, 198, 219, 243, 268, and 316 wherein the laxative is selected from dexloxiglumide, psyllium husk, docusate sodium, bisacodyl, and phenolphthalein.

343. The pharmaceutical composition of any of claims 143, 164, 185, 206, 227, 276, and 324 wherein the polymer resin is selected from psyllium, lipid lowering polymers, nonabsorbed polymer resins, and sodium binding polymers.

344. The pharmaceutical composition of claim 343 wherein the polymer resin is psyllium.

345. The pharmaceutical composition of claim 343 wherein the polymer resin is a lipid lowering polymer.

346. The pharmaceutical composition of claim 343 wherein the polymer resin is a nonabsorbed polymer resin.

347. The pharmaceutical composition of claim 343 wherein the polymer resin is a sodium binding polymer.

348. The pharmaceutical composition of claim 345 wherein the lipid lowering polymer is selected from: Colesevelam, Sevalmer, Cholestyramine.

349. The pharmaceutical composition of claim 346 wherein nonabsorbed polymer resin is selected from: hyaluronic acid, polycarbophil calcium, polyvinyl acetate, polyvinyl pyrrolidone, polystyrene sulfate.

350. The pharmaceutical composition of claim 347 wherein the sodium-binding polymer is selected from: crosslinked polyvinylsulfamate polymer, N-(bis-phosphonic-ethyl) polyvinylamine polymer, poly-α-acrylic acid polymer, poly-α-fluoroacrylic acid polymer, polyvinylphosphoramidic polymer, polyvinylsulfamate polymer, polyvinylsulfamate/vinylsulfate copolymer, polyvinylsulfate polymer, polyvinylsulfonate polymer, polyvinylsulfonate polymer,

vinylphosphonate/α-fluoroacrylic acid copolymer, vinylphosphonate/α-fluoroacrylic acid copolymer, or vinylphosphonate/acrylic acid copolymer.

351. The pharmaceutical composition of claim 350 wherein the sodium-binding polymer is administered as a core-shell composition which further comprises a semi-permeable shell.

352. The pharmaceutical composition of claim 351 wherein the semi-permeable shell comprises at least one of a poly-11 trimethylammonioundecylmethacrylate polymer, a styrene-vinylpyridine polymer, 11-dimethyl-aminodecylmethacrylate/laurylmethacrylate copolymer, or a polyallylamine/polystyrene sulfonate polymer.

353. A pharmaceutical composition comprising misoprostol and psyllium.

354. A pharmaceutical composition comprising psyllium and a peptide that activates the guanylate cyclase C receptor.

355. The pharmaceutical composition of claim 354 wherein the peptide comprises an amino acid sequence selected from:
Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;
Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;
d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys Tyr;
d-Cys Cys Glu Tyr Cys Cys Asn Pro Ala Cys Thr Gly Cys;

d-Cys Cys Glu Leu Cys Cys Asn Pro Ala Cys Thr Gly Cys;

d-Cys Cys Glu Phe Cys Cys Asn Pro Ala Cys Thr Gly Cys;

d-Cys Cys Glu Trp Cys Cys Asn Pro Ala Cys Thr Gly Cys;

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

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

Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro; Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys;

Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Asp Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Val Tyr Ile Gln Tyr Gln Gly Phe Arg Val Gln Leu Glu Ser Met Lys Lys Leu Ser Asp Leu Glu Ala Gln Trp Ala Pro; Ser Pro Arg Leu Gln Ala Gln Ser Leu Leu Pro Ala Val Cys His His Pro Ala Leu Pro Gln Asp Leu Gln Pro Val Cys; Ala Ser Gln Glu Ala Ser Ser Ile Phe Lys Thr Leu Arg Thr Ile Ala Asn Asp Glu Cys Glu Leu Cys Val Asn Val Ala Cys Thr Gly Cys Leu;

Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Val Thr Val Gln Asp Gly Asn Phe Ser Phe Ser Leu Glu Ser Val Lys Lys Leu Lys Asp Leu Gln Glu Pro Gln Glu Pro;

Arg Val Gly Lys Leu Arg Asn Phe Ala Pro Ile Pro Gly Glu Pro Val Val Pro Ile Leu Cys Ser Asn Pro Asn Phe Pro; Glu Glu Leu Lys Pro Leu Cys Lys Glu Pro Asn Ala Gln Glu Ile Leu Gln Arg Leu Glu Glu Ile Ala Glu Asp Pro Gly Thr Cys Glu Ile Cys Ala Tyr Ala Ala Cys Thr Gly Cys;

Met Pro Ser Thr Gln Tyr Ile Arg Arg Pro Ala Ser Ser Tyr Ala Ser Cys Ile Trp Cys Thr Thr Ala Cys Ala Ser Cys His Gly Arg Thr Thr Lys Pro Ser Leu Ala Thr;

Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

Val Gln Val Glu Glu Gly Gly Phe Ser Phe Pro Leu Asp Ala Val Lys Lys Leu Glu Glu Leu Met Gly Val Asp Met; Thr Val Lys Gln Ser Pro Arg Leu Ala Lys Thr Ser Thr Thr Ala Val Cys Thr Asn Pro Asp Leu Pro Ala Val Phe Leu; Pro Leu Cys Lys Ser Lys Gly Ala Ala Asn Ser Phe Phe

Arg Leu Gly Phe Val Ala Ala Arg Ala Asp Leu Cys Glu Ile Cys Ala Phe Ala Ala Cys Thr Gly Cys Leu;

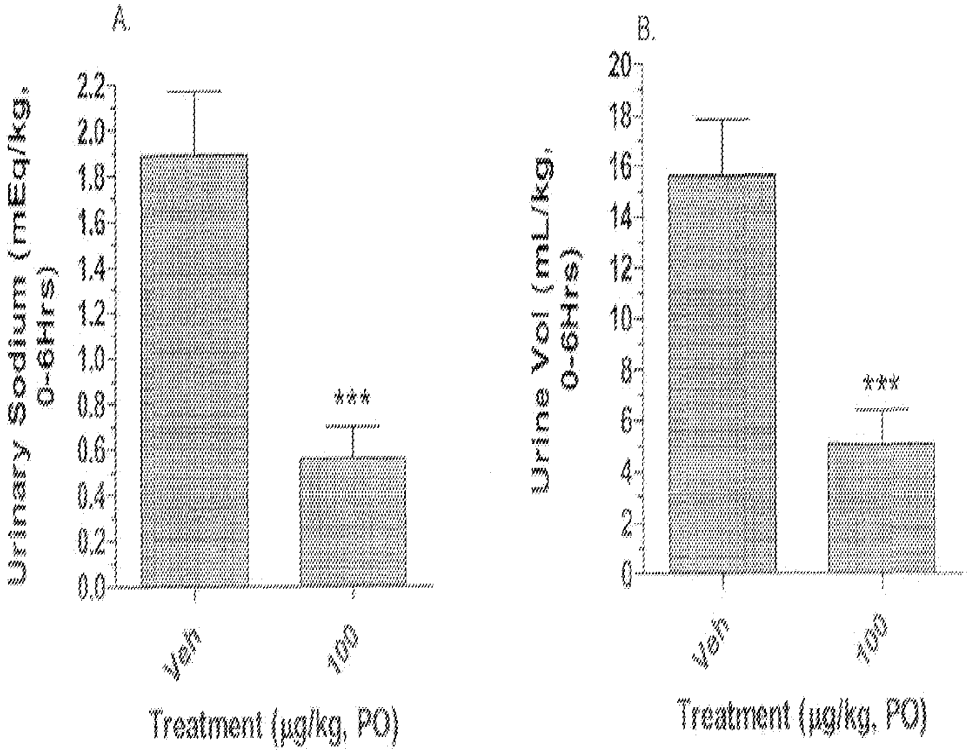
Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr;

Val Tyr Ile Gln Tyr Glu Gly Phe Gln Val Asn Leu Asp Ser Val Lys Lys Leu Asp Lys Leu Leu Glu Gln Leu Arg Gly;

Phe His His Gln Met Gly Asp Gln Arg Asp Pro Ser Ile Leu Cys Ser Asp Pro Ala Leu Pro Ser Asp Leu Gln Pro Val; and

Cys Glu Asn Ser Gln Ala Val Asn Ile Phe Arg Ala Leu Arg Tyr Ile Asn Gln Glu Glu Cys Glu Leu Cys Ile Asn Met Ala Cys Thr Gly Tyr.

Figure 1. The Effect of Lubiprostone (100µg/kg, PO) on (A) Urinary Sodium and (B) Urine Volume Measured Over 6 Hours in Female Sprague-Dawley Rats



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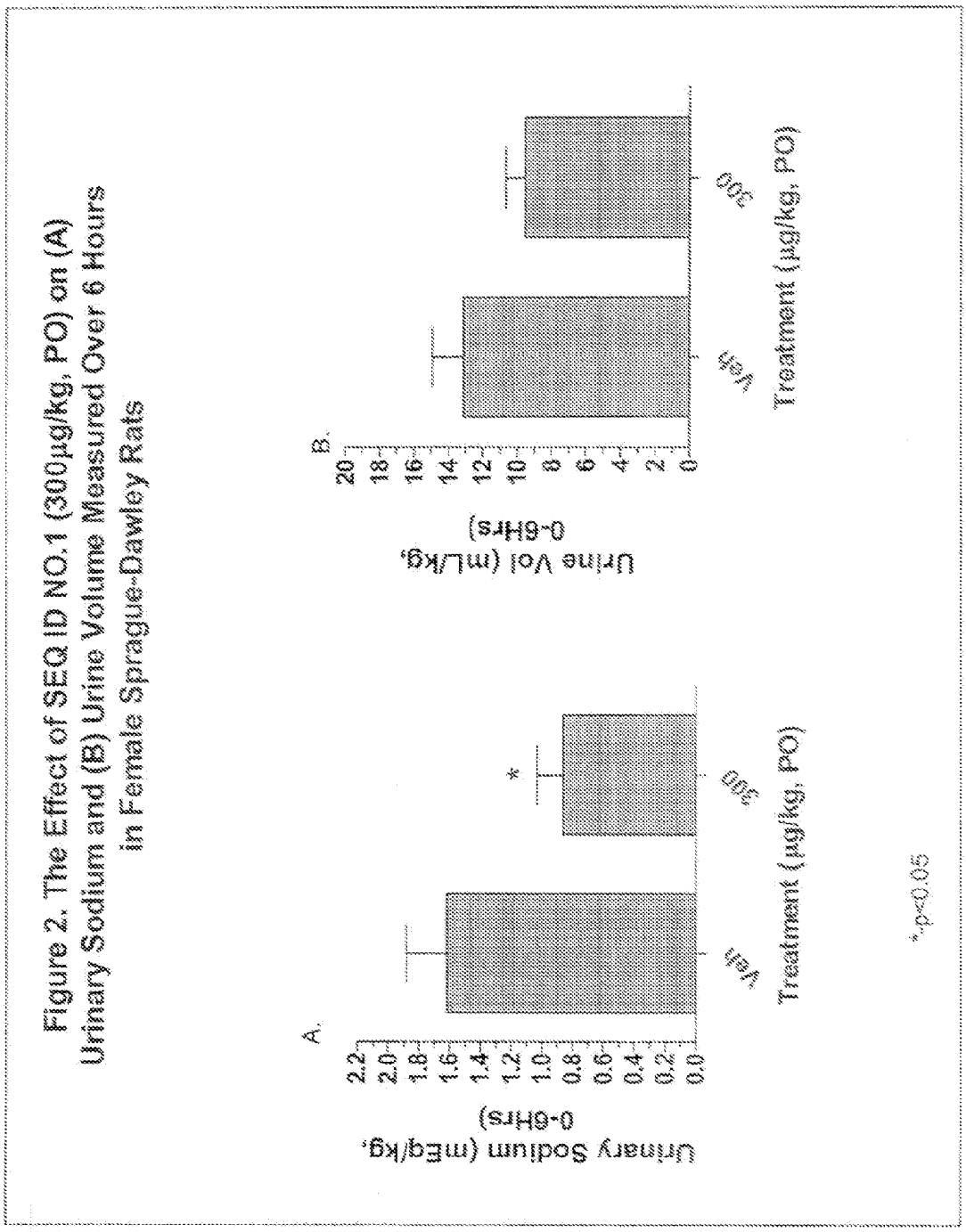

Figure 2. The Effect of SEQ ID NO.1 (300µg/kg, PO) on (A) Urinary Sodium and (B) Urine Volume Measured Over 6 Hours in Female Sprague-Dawley Rats

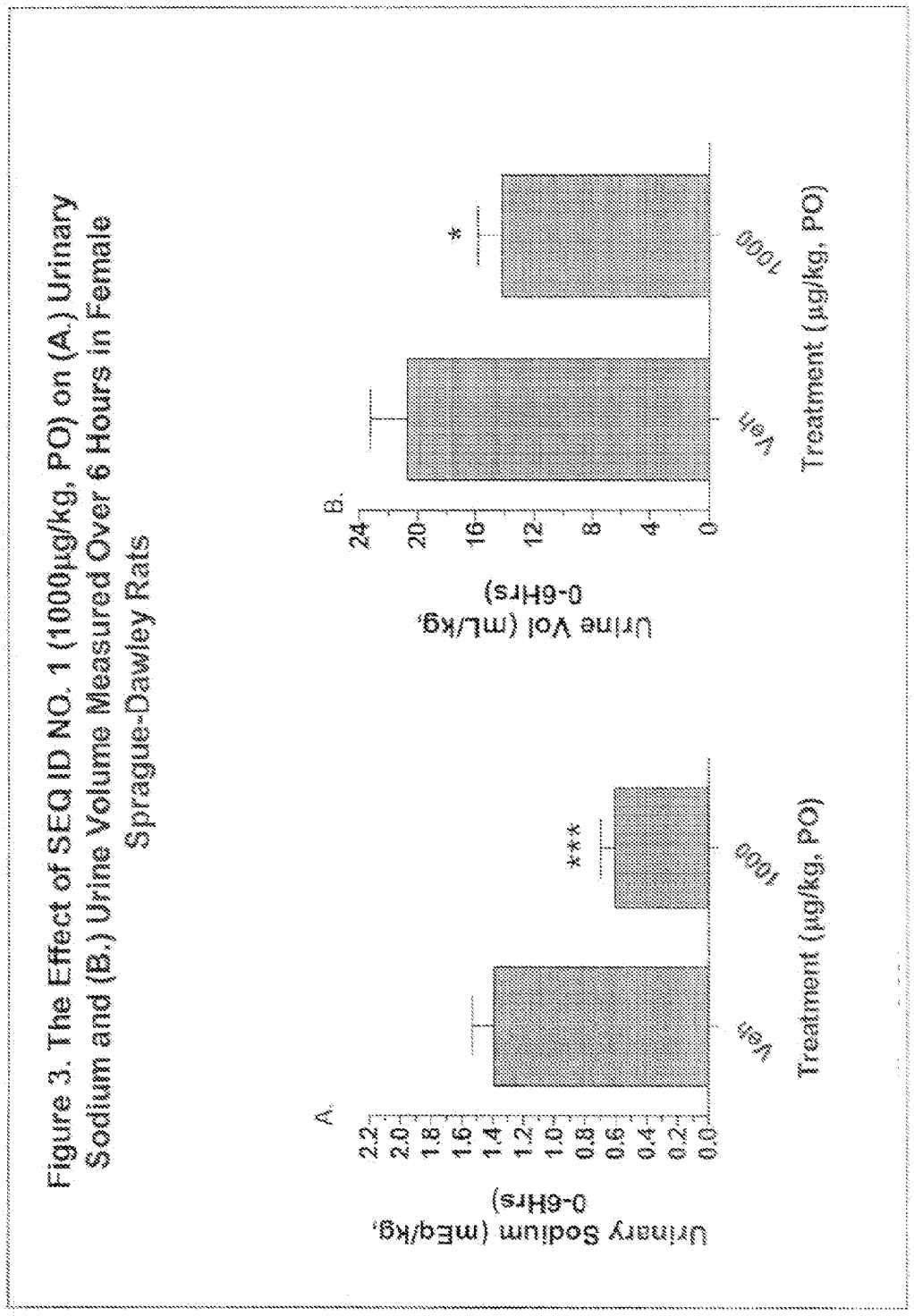
Figure 3. The Effect of SEQ ID NO. 1 (1000µg/kg, PO) on (A.) Urinary Sodium and (B.) Urine Volume Measured Over 6 Hours in Female Sprague-Dawley Rats
A.
Urinary Sodium (mEq/kg, 0-6Hrs)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
***
Veh
1000
Treatment (µg/kg, PO)
B.
Urine Vol (mL/kg, 0-6Hrs)
24
20
16
12
8
4
0
*
Veh
1000
Treatment (µg/kg, PO)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/061205

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/4706(2006.01)i, A61K 31/675(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 8: A61K

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)
eKIPASS(KIPO internal), Delphion, Pubmed (sodium absorption, guanylate cyclase, salt retention, hypertension, psyllium)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X/A	Hypertension 37(2 Part 2): 467-471 February 2001 See introduction & result	123-352/353-355
X/A	JP11240841A (NISSIN FOOD PROD CO LTD, UNIV KYOTO) 07 September 1999 See abstract	353-355/123-352
A	CA 2522895 A1 (WARATAH PHARMACEUTICALS, INC) 11 November 2004 See abstract	123-355
A	Vallon V et al., The salt paradox and its possible implications in managing hypertensive diabetic patients. Curr Hypertens Rep. 2005 Apr; 7(2):141-7. See abstract	123-355
A	Sica DA. Sodium and water retention in heart failure and diuretic therapy: basic mechanisms.Cleve Clin J Med. 2006 Jun; 73 Suppl 2:S2-7; discussion S30-33. See abstract	123-355
A	Sahay M et al., Sodium transporters in kidney role in health and disease. J Assoc Physicians India. 2007 Feb;55:135-139. See abstract	123-355

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 OCTOBER 2008 (08.10.2008)	**08 OCTOBER 2008 (08.10.2008)**

Name and mailing address of the ISA/KR
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Form PCT/ISA/210 (second sheet) (July 2008)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/061205

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Fordtran JS et al. The mechanisms of sodium absorption in the human small intestine. J Clin Invest. 1968 Apr;47(4):884-900. See abstract	123-355

Form PCT/ISA/210 (continuation of second sheet) (July 2008)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/061205

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

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

1. ☒ Claims Nos.: 1-122
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1 to 122 pertain to methods for treatment of the human or animal body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☒ Claims Nos.: 42-44, 46-50, 52-78, 80-90, 98-105, 114, 119, 120
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Said claims are unclear, since they refer to claim 38-41, 45, 51, 79, 97, 106-113, 115-118, 121, or 122, which does not follow the third sentences of Rule 6.4(a).

3. ☒ Claims Nos.: 38-41, 45, 51, 79, 97, 106-113, 115-118, 121, 122
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2008/061205

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
JP 11-240841 A	07.09.1999	JP 3345650 B2	18.11.2002
CA 2522895 A1	11.11.2004	AU 2004-233911 A1	11.11.2004
		EP 1620464 A1	01.02.2006
		JP 2007-523840 T2	23.08.2007
		US 2005-0217671 A1	06.10.2005
		WO 2004-096853 A1	11.11.2004

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A61K 38/10 (2006.01) *A61P 1/00* (2006.01)

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(54) Title: AGONISTS OF GUANYLATE CYCLASE USEFUL FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS, INFLAMMATION, CANCER AND OTHER DISORDERS

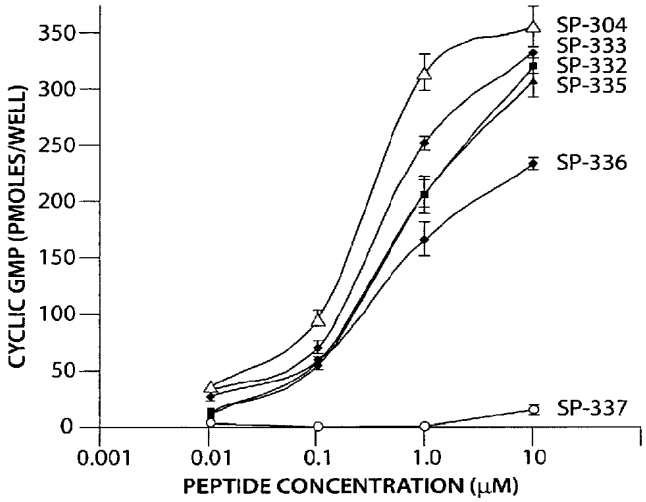

Fig. 5

(57) Abstract: The invention provides novel guanylate cyclase-C agonist peptides and their use in the treatment of human diseases including gastrointestinal disorders, inflammation or cancer (e.g., a gastrointestinal cancer). The peptides can be administered either alone or in combination with an inhibitor of cGMP-dependent phosphodiesterase. The gastrointestinal disorder may be classified as either irritable bowel syndrome, constipation, or excessive acidity etc. The gastrointestinal disease may be classified as either inflammatory bowel disease or other GI condition, including Crohn's disease and ulcerative colitis, and cancer.

WO 2008/151257 A2

AGONISTS OF GUANYLATE CYCLASE USEFUL FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS, INFLAMMATION, CANCER AND OTHER DISORDERS

RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Application No. 60/933,194 filed on June 4, 2007, the contents of which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the therapeutic use of guanylate cyclase C (GC-C) 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 inflammation, cancer and other disorders, particularly of the gastrointestinal tract and the lung.

BACKGROUND OF THE INVENTION

Uroguanylin, guanylin and bacterial ST peptides are structurally related peptides that 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 subsequent enhancement of transepithelial secretion of chloride lead 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). Thus, the cGMP-mediated activation of CFTR and the downstream signaling plays an important role in normal functioning of gut physiology. Therefore, any abnormality in this process could potentially lead to gastrointestinal disorders such as irritable bowel syndrome, inflammatory bowel disease, excessive acidity and cancer (25, 26).

The process of epithelial renewal involves the proliferation, migration, differentiation, 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.

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 by maintaining the balance between proliferation and apoptosis in cells lining GI mucosa. Therefore, any disruption in this renewal process, due to reduced production of uroguanylin and/or guanylin can lead to GI inflammation and cancer (25, 26). This is consistent with previously published data in WO 01/25266, which suggest 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, recent data also suggest that uroguanylin also binds to a currently unknown receptor, which is distinct from GC-C receptor (3,4). Knockout mice lacking this guanylate cyclase receptor show resistance to ST peptides in the intestine, but effects of uroguanylin and ST peptides 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). Thus, it is not clear if the anti-colon cancer and anti-inflammatory activities of uroguanylin and its analogs are mediated through binding to one or both of these receptors.

Inflammatory bowel disease is a general name given to a group of disorders that cause intestines to become inflamed, characterized by red and swollen tissue. Gastrointestinal (GI) inflammation can be a chronic condition and often leads to GI cancer (14). Examples of such inflammatory bowel diseases (IBD) include Crohn's disease and ulcerative colitis (UC). It is estimated that as many as 1,000,000 Americans are afflicted with IBD, 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.

Crohn's disease is a serious inflammatory disease that predominantly effects ileum and colon, but can also occur in other sections of the GI tract, whereas UC is exclusively an inflammatory disease of the colon, the large intestine (15). 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, UC affects only the innermost lining (mucosa) of the colon in a continuous manner (16). Depending on which portion of the GI tract is involved, Crohn's disease may be referred to as ileitis, regional enteritis, colitis, etc. Crohn's disease and UC differ from spastic colon or irritable bowel syndrome, which are motility disorders of the GI tract.

While the precise cause of IBD is not known, it is believed that the disruption of the process of continual renewal of GI mucosa may be involved in disease (17,18). The renewal process of the GI lining is an efficient and dynamic process involving the continual proliferation and replenishment of unwanted damaged cells. Proliferation rates of cells lining the GI mucosa are very high, second only to the hematopoietic system. Thus, the balance between proliferation and apoptosis is important to the maintenance of the homeostasis of the GI mucosa (19,20).

GI homeostasis depends on both proliferation and programmed cellular death (apoptosis) of epithelial cells lining the gut mucosa. Hence, 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. It has become increasingly apparent that the control of cell death is an equally, if not more, important regulator of cell number and proliferation index (19,20). Reduced rates of apoptosis are often associated with abnormal growth, inflammation, and neoplastic transformation. Thus, both decreased proliferation and/or increased cell death may reduce cell number, whereas increased proliferation and/or reduced cell death may increase the

proliferation index of intestinal tissue (20), which may lead to GI inflammatory diseases and cancer.

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 intestine, colon, and rectum, it may metastasize and spread to such localities as bone, lymph nodes, liver, lung, peritoneum, ovaries, and 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

Irritable bowel syndrome (IBS) and chronic idiopathic constipation are pathological conditions that can cause a great deal of intestinal discomfort and distress but unlike the IBD diseases such as ulcerative colitis and Crohn's disease, 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 (IBD), celiac disease and irritable bowel syndrome (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 (27). Serotonin (5-hydroxytryptamine [5-HT]) is a key modulator of gut function and is known to play a major role in pathophysiology of IBS. It has been shown that the activity of 5-HT is regulated by cGMP (28). Therefore, based on this observation as well as other effects of cGMP, we believe that GC-C agonists will be useful in the treatment of IBS.

Given the prevalence of inflammatory conditions in Western societies and the attendant risk of developing cancerous lesions from inflamed tissue, particularly intestinal tissue, a need exists to improve the treatment options for inflammatory conditions, particularly of the gastrointestinal tract.

SUMMARY OF THE INVENTION

The present invention is based upon the development of agonists of guanylate cyclase receptor. The agonists are analogs of uroguanylin and bacterial ST peptides and have superior properties such as for example high resistance to degradation at the N-terminus and C-terminus from carboxypeptidases and/or by other proteolytic enzymes present in the stimulated human intestinal juices and human gastric juices.

The peptides of the invention 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 gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity. Gastointestinal disorders include for example, irritable bowel syndrome (IBS), non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease (GERD), ileus inflammation (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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (e.g., pancreatis), lung inflammation (e.g., bronchitis or asthma) or skin inflammation (e.g., psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. 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.

In one aspect, the present invention is directed to a peptide consisting essentially of the amino acid sequence of, SEQ ID NOs: 2-54 and 57-98 and to therapeutic compositions which contain these peptides. Prefered peptides include SEQ ID NO: 8, 9, 10, 58 and 59. 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 2-54 and 57-98 or if its activation of cellular cGMP production is reduced by more than 50% compared to a control peptide such as SEQ ID NO:1, 55 or 56. 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 instant peptide sequences comprise at least 12 amino acid residues, preferably between 12 and 26 amino acids in length.

The peptides may be in a pharmaceutical composition in unit dose form, together with one or more pharmaceutically acceptable carrier, excipients or diluents. 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, shrinkage of polyps or tumors, a reduction in metastatic lesions, etc.

In yet another aspect, an invention provides administering to said patient an effective dose of an inhibitor of cGMP-specific phosphodiesterase (cGMP-PDE) either concurrently or sequentially with said guanylate cyclase receptor agonist. The cGMP-PDE inhibitor include for example suldinac sulfone, zaprinast, and motapizone, vardenifil, and sildenafil. In

addition, GC-C agonist peptides may be used in combination with inhibitors of cyclic nucleotide transporters.

Optionally, anti-inflammatory agents are also administered. Anti-inflammatory agents include for example steroids and non-steroidal anti-inflammatory drugs (NSAIDS).

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

Figure 1A is a bar chart showing the biological activity of SP-304 after incubation with simulated gastric fluid (SGF) for times as indicated. The biological activity of SP-304 was determined by measuring its ability to stimulate cGMP synthesis in T84 cells. Following the incubations, samples were used for their abilities to stimulate cGMP synthesis in T84 cells. The cGMP stimulation activity in sample at 0 min of incubation with SGF was taken as 100%. The activities in samples from other times of incubations with SGF were calculated as percentage of the activity in the sample at 0 min. The data is average of triplicates ± SD

Figure 1B is a schematic representation of the results of HPLC chromatographic analyses of SP-304 samples after incubation with SGF at indicated times. The major peak of SP-304 did not change following incubation with SGF, indicating that the peptide was resistant to SGF digestion. The arrows indicate the elution position of SP-304.

Figure 2A is a bar chart showing Cyclic GMP synthesis in T84 cells by SP304 samples after incubation with simulated intestinal fluid (SIF) for the indicated times. Following the incubations, samples were used for their abilities to stimulate cGMP synthesis in T84 cells. The cGMP stimulation activity in sample at 0 min of incubation with SIF was taken as 100%. The activities in samples from other times of incubations with SIF were calculated as percentage of the activity in the sample at 0 min. The data is average of triplicates ± SD

Figure 2B is a schematic representation of the results of HPLC chromatographic analyses of SP304 samples after incubation with (A) heat inactivated SIF for 300 min or with (B) SIF for 120 min. The incubation with SIF completely converted SP-304 into another peptide eluting at 9.4 min, as indicated by *. Arrows indicate the position of SP-304.

Figure 3 is a schematic representation of the possible degradation products of SP-304.

Figure 4 shows stimulation of cGMP synthesis in T84 cells by the truncated peptides of SP-304. Thus, SP-338 has the same peptide sequence as SP-304 except that it lacks Leu at the C-terminus. Similarly, SP-327, SP-329 and SP-331 have Leu at their C-termini deleted relative to their corresponding parents, SP-326, SP-328 and SP-330. Peptides were evaluated for their abilities to stimulate cGMP synthesis in T84 cells. The results are expressed as an average of duplicates.

Figure 5 shows stimulation of cGMP synthesis in T84 cells by SP-304 and similar peptides. Cells were exposed to peptide analogs for 30 min and cell lysates were used to determine intracellular cGMP levels. Results are expressed as an average of triplicates ± SD.

Figure 6 shows stimulation of cGMP synthesis in T84 cells by SP-339 and other peptides. T84 Cells were exposed to the indicated peptide for 30 min and cell lysates were used to determine intracellular cGMP levels. Results are expressed as an average of triplicates ± SD.

Figure 7A shows stability of SP-333 against digestion with simulated intestinal fluid (SIF) for indicated times. The control sample marked as C120 was produced by incubating peptides with heat inactivated SIF. Samples from the incubations were removed and heated at 95°C for 5 min to inactivate digestive enzymes and then used to stimulate cyclic GMP synthesis in T84 cells. The cGMP stimulation activity at 0 min was taken as 100% in each set. The data is average of triplicates ± SD.

Figure 7B shows stability of SP-332 against digestion with simulated intestinal fluid (SIF) for indicated times. The control sample marked as C120 was produced by incubating peptides with heat inactivated SIF. Samples from the digestions were removed and heated at 95°C for 5 min to inactivate digestive enzymes and then used to stimulate cyclic GMP synthesis in T84 cells. The cGMP stimulation activity at 0 min was taken as 100% in each set. The data is average of triplicates ± SD.

Figure 7C shows stability of SP-304 against digestion with simulated intestinal fluid (SIF) for indicated times. The control samples marked as C0 and C60 were produced by incubating peptides with heat inactivated SIF. Samples from the digestions were removed and heated at 95°C for 5 min to inactivate digestive enzymes and then used to stimulate cyclic GMP synthesis in T84 cells. The cGMP stimulation activity at 0 min was taken as 100% in each set. The data is average of 3 determinations ± SD.

Figure 7D shows HPLC analysis of samples of SP-304 at 0 and 60 minutes following incubation with SIF. Arrow indicates the elution position of SP-304 peptide. The data clearly shows that the SP-304 peak eluting at 14.3 min completely vanished and two new peaks emerged at 7.4 and 10.3 minutes. These new peptide peaks represent the possible degradation products of SP-304.

Figure 7E shows HPLC analysis of samples of SP-332 at 0 and 120 minutes following incubation with SIF. Arrow indicates the elution position of SP-332 peptide. The data shows that the peptide SP-332 eluting at 14.8 minutes was not changed following incubation with SIF, suggesting that SP-332 is not sensitive to proteolysis by proteases present in SIF.

Figure 7F shows HPLC analysis of samples of SP-333 at 0 and 120 minutes following incubation with SIF. Arrows indicate the elution position of SP-333. The data show that peptide SP-333, eluting at 14.8 minutes, was not changed following incubation with SIF, suggesting that SP-333 is not sensitive to proteolysis by proteases present in SIF during the 120 minute incubation period.

Figure 8 shows stimulation of cGMP synthesis in T84 cells by the peggylated analogs of SP-333. T84 cells were exposed to the indicated peptides for 30 min and cell lysates were used to determine intracellular cGMP levels. Results are expressed as an average of triplicates ± SD.

Figure 9 shows stimulation of cGMP synthesis in T84 cells by SP-304 (0.1 μM) either alone or in combination with the phosphodiesterase (PDE) inhibitors Sulindac Sulfone (100 μM) or Zaprinast (100 μM). T84 cells were exposed to various treatments, as indicated, for 30 min and the cell lysates were used to determine the intracellular cGMP levels. Results are expressed as an average of duplicates.

Figure 10 shows stimulation of cGMP synthesis in T84 cells by SP-304 (0.1 or 1.0 μM) either alone or in combination with incremental concentrations of phosphodiesterase (PDE) inhibitors, as indicated. T84 cells were exposed to various treatments, as indicated, for 30 min and the cell lysates were used to determine the intracellular cGMP levels. Results are expressed as an average of duplicates.

Figure 11 shows stimulation of cGMP synthesis in T84 by SP-333 (0.1 or 1.0 μM) either alone or in combination with incremental concentrations Zaprinast, as indicated. T84 cells

were exposed to various treatments, as indicated, for 30 min and the cell lysates were used to determine the intracellular cGMP levels. Results are expressed as an average of duplicates.

Figure 12 shows stimulation of cGMP synthesis in T84 by SP-333 (0.1 μM) either alone or in combination with incremental concentrations Sulindac Sulfone, as indicated. T84 cells were exposed to various treatments, as indicated, for 30 min and the cell lysates were used to determine the intracellular cGMP levels. Results are expressed as an average of duplicates.

Figure 13 shows a schematic of the mainatance of intracellular concentrations of cGMP levels . The intracellular levels of cGMP can be maintained by stimulating its synthesis via the activation of GC-C and by inhibiting its degradation by cGMP-PDE. Thus, a combination of a GC-C agonist with an inhibitor of PDE may produce a synergistic effect to enhance levels of cGMP in tissues and organs.

DETAILED DESCRIPTION

The present invention is based upon the development of agonists of guanylate cyclase-C (GC-C). The agonists are analogs of uroguanylin and bacterial ST peptides and have superior properties such as for example high resistance to degradation at the N-terminus and C-terminus from carboxypeptidases and/or by other proteolytic enzymes such as those present in the stimulated human intestinal juices and human gastric juices.

The GC-C is expressed on various cells including on gastrointestinal epithelial cells, and on extra-intestinal tissues including kidney, lung, pancreas, pituitary, adrenal, developing liver, heart and male and female reproductive tissues (reviewed in Vaandrager 2002 Mol Cell Biochem 230:73-83). The GC-C is a key regulator of fluid and electrolyte balance in the intestine and kidney. In the intestine, when stimulated, the GC-C causes an increase in intestinal epithelial cGMP. This increase in cGMP causes a decrease in water and sodium absorption and an increase in chloride and potassium ion secretion, leading to changes in intestinal fluid and electrolyte transport and increased intestinal motility.

The gualylate cyclase-C agonists according to the invention include SEQ ID NO:2-54, and SEQ ID NO: 57-98 and are summarized below in Table I and Table II. The gualylate cyclase-C agonists according to the invention are collectively refered to herein as "GCRA peptides".

Table I. GCRA peptides

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[16]	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,	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

	C7:C15		
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]-X3[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 (NEW)	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 (NEW)	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. GCRA Peptides

Name	Position of Disulfide bonds	Structure	SEQ ID NO:
SP-339	C1:C6, C2:C10, C5:13	Cys[1]-Cys[2]-Glu3-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,	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

	C7:15		
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,	Cys[1]-Cys[2]-Glu3-Ser[4]-Cys[5]-Cys[6]-Asn[7]-Pro[8]-Ala[9]-Cys[10]-Thr[11]-Gly[12]-Cys[13]-Tyr[14]	85

	C2:C10, C5:13		
N27	C1:C6, C2:C10, C5:13	Cys^{1}-Cys^{2}-Glu3-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}-Glu3-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}-Glu3-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}-Glu3-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}-Glu3-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,	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

	C7:15		
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}-Cys12- 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}-Glu3-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}-Glu3-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}-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

The GCRA peptides described herein bind the guanylate cyclase C (GC-C) and stimulate intracellular production of cyclic guanosine monophosphate (cGMP). Optionally, the GCRA peptides induce apoptosis. In some aspects, the GCRA peptides stimulate intracellular cGMP production at higher levels than naturally occurring GC-C agonists (*e.g.*, uroguanylin, guanylin, and ST peptides) and/or SP-304. For example, the GCRA peptides of the invention stimulate 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to naturally occurring GC-C angonists and/or SP-304. The terms induced and stimulated are used interchangeably throughout the specification. The GCRA peptides described herein are more stable than naturally occurring GC-C agonists and/or SP-304. By more stable it is meant that the peptide degrade less and/or more slowly in simulated gastrointestinal fluid and/or simulatd intestinal fluid compared to naturally occurring GC-C angonists and/or SP-304. For example, the GCRA peptide of the invention degrade 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GC-C angonists and/or SP-304.

The GCRA peptides described herein have therapeutic value in the treatment of a wide variety of disorders and conditions including for example gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity. Gastointestinal disorders include for example, 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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema). Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

As used herein, the term "guanylate cyclase C (GC-C)" refers to the class of guanylate cyclase C receptor on any cell type to which the inventive agonist peptides or natural agonists described herein bind. As used herein, "intestinal guanylate cyclase receptor" is found exclusively on epithelial cells lining the GI mucosa. Uroguanylin, guanylin, and ST peptides are expected to bind to these receptors and may induce apoptosis. The possibility that there may be different receptors for each agonist peptide is not excluded. Hence, the term refers to the class of guanylate cyclase receptors on epithelial cells lining the GI mucosa.

As used herein, the term "GCR agonist" is meant to refer to peptides and/or other compounds that bind to an intestinal guanylate cyclase C and stimulate fluid and electrolyte transport. This term also covers fragments and pro-peptides that bind to GC-C and stimulate fluid and water secretion.

As used herein, the term "substantially equivalent" is meant to refer 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.

Addition of carriers (*e.g.*, phosphate-buffered saline or PBS) and other components to the composition of the present invention is well within the level of skill in this art. In addition to the compound, such compositions may contain pharmaceutically acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake. Other formulations, such as microspheres, nanoparticles, liposomes, and immunologically-based systems may also be used

in accordance with the present invention. Other examples include formulations with polymers (*e.g.*, 20% w/v polyethylene glycol) or cellulose, or enteric formulations.

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 cytoplasmic phospholipase A2 (cPLA2), cyclooxygenase-2 (COX-2) and possibly 5-lipoxygenase (5-LO) 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 gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, 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, osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (e.g., psoriasis, eczema). Lung Disorders include for example COPD and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

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 agents 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 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 (21-24).

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, induces apoptosis in target cells. Therefore, administration of the novel peptides defined by SEQ ID NO:2-54, and SEQ ID NO: 57-98, as shown in Tables I and II, or peptides similar to uroguanylin, or guanylin or E. coli ST peptide are useful in eliminating or, at least retarding, the onset of gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, irritable bowel syndrome (IBS), non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease (GERD), ileus inflammation (*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, osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema). Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

Uroguanylin is a circulating peptide hormone with natriuretic activity and has been found to stimulate fluid and electrolyte transport in a manner similar to another 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.

GCRA PEPTIDES

In one aspect, the invention provides a GCRA peptide. The GCRA peptides are analogues uroguanylin and bacterial ST peptide. No particular length is implied by the term "peptide". In some embodiments, the GCRA peptide is less than 25 amino acids in length, *e.g.,* less than or equal to 20, 15, 14, 13, 12, 11, 10, or 5 amino acid in length.

The GCRA peptides 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 an D retro-inverso peptide by synthesizing a reverse of the sequence for the corresponding native L-amino acid sequence. For example a GCRA peptide includes the sequence of SEQ ID NO: SEQ ID NO:2-54, and SEQ ID NO: 57-98. In various embodiments, the GCRA peptide includes the amino acid sequence of SEQ ID NO:45-54 and SEQ ID NO:87-98 where the peptide induces cGMP production by a cell. In various embodiments the GCRA peptide of the invention includes the amino acid sequence according to Formulas I-IX (*e.g.* SEQ ID NO:45-54) with the proviso that the GCRA peptide is not SEQ ID NO:1. In further embodiments the GCRA peptide of the invention include the amino acid sequence according to Formulas X- XVII (*e.g.* SEQ ID NO:87-98) with the proviso that the GCRA peptide is not SEQ ID NO:55 or SEQ ID NO:56. By inducing cGMP production is meant that the GCRA peptide induces the production of intracellular cGMP. Intracellular cGMP is measured by methods known in the art. For example, the GCRA peptide of the invention stimulate 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to naturally occurring GC-C angonists. Optionally, the GCRA peptides of the invention of the invention stimulate 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared SP-304 (SEQ ID NO:1). In further embodiments, the GCRA peptide stimulates apoptosis, *e.g.*, programmed cell death or activate the cystic fibrosis transmembrane conductance regulator (CFTR). In some embodimenst the GCRA peptides described herein are more stable than naturally occurring GC-C agonists and/or SP-304 (SEQ ID NO:1), SP-339 (SEQ ID NO: 55) or SP-340 (SEQ ID NO: 56). By more stable it is meant that the peptide degrade less and/or more slowly in simulated gastric fluid and/or simulated ntestinal fluid compared to naturally occurring GC-C angonists and/or SP-304. For example, the GCRA peptide of the invention degrade 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GC-C angonists and/or SP-304, SP-339 or SP-340.

As used herein PEG3, 3 PEG, is meant to denote polyethylene glycol such as include aminocthyloxy-cthyloxy-acctic acid (AccA). As used hercin, (*e.g.*, in Formulas I- XVII, SEQ ID NO:45-54 and SEQ ID NO:87-98) X_{aa} is any any natural, unnatural amino acid or amino acid analogue; M_{aa} is a Cysteine (Cys), Penicillamine (Pen) homocysteine, or 3-mercaptoproline; Xaa_{n1} is meant to denote an amino acid sequence of any any natural, 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 of any any natural, unnatural amino acid or amino acid analogue that is zero or one residue in length; and Xaa_{n3} is meant to denote an amino acid sequence of any any natural, unnatural amino acid or amino acid analogue that is 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 GCRA peptide represented by Formulas I-VII may contain on or more polyethylene glycol residues at the the N- terminus, C-terminus or both. An exemplary polyethylene glycol include aminoethyloxy-ethyloxy-acetic acid and polymers thereof.

In some embodiments, GCRA peptides include peptides containing the amino acid sequence of Formula I, wherein at 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 position 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 acids or a methylated amino acid. Preferably, the amino acid at position Xaa^6 of Formula I is a leucine, serine or tyrosine.

In alternative embodiments, GCRA peptides include peptides containing containing 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 embodimenst the amino acid denoted by Xaa_{n2} of Formula II is a leucine, d-leucine, serine or d-serine. Preferably, the one or more of the 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 Xaa^6 of Formula II is a leucine, serine or tyrosine.

In some embodiments, GCRA peptides include peptides containing the amino acid sequence of Formula III, wherein 1) at at least one amino acid of Formula I is a D-amino acid or a methylated amino acid and/or 2) 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, d-leucine, serine or d-serine. Preferably, the one or more of the 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 Xaa^6 of Formula III is a leucine, serine or tyrosine.

In other embodiments, GCRA peptides include peptides containing containing 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 2) Maa is not a cysteine. Preferably, the Xaa_{n2} of Formula IV is a D-amino acid or a methylated amino acid. In some embodimenst the amino acid denoted by Xaa_{n2} of Formula IV is a leucine, d-leucine, serine or 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 Xaa^6 of Formula IV is a leucine , serine or tyrosine. In further embodiments, GCRA peptides include peptides containing containing the amino acid sequence of Formula V, wherein at 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., Xaa^{16}) 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 acids or a methylated amino acid. Preferably, the amino acid denoted at Xaa^6 of Formula V is a leucine, serine or tyrosine.

In additional embodiments, GCRA peptides include peptides containing containing the amino acid sequence of Formula VI, VII, VIII, IX. Preferably, the amino acid at position 6 of Formula VI, VII, VIII, IX. is a leucine, serine or tyrosine. In some aspects the amino acid at position 16 of Formula VI, VII, VIII, 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.

In prefered embodiments, the GCRA peptide is SP-332 (SEQ ID NO:8), SP-333 (SEQ ID NO:9) or SP-334 (SEQ ID NO:10).

In additional embodiments, GCRA peptides include peptides containing containing 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

Preferably, the amino acid denoted by Xaa^6 of Formula XIV is a tyrosine, phenyalanine or a serine. Most preferably the amino acid denoted by Xaa^6 of Formula XIV is a phenyalanine or a serine. Preferably, the amino acid denoted by Xaa^4 of Formula XV, XVI or XVII is a tyrosine, phenyalanine or a serine. Most preferably, the amino acid position Xaa^4 of Formula V, XVI or XVII is a phenyalanine or a serine.

In prefered embodiments, the GCRA peptide is SP-353 (SEQ ID NO:58) or SP-354 (SEQ ID NO:59).

In certain embodiments, one or more amino acids of the GCRA peptides can be replaced by a non-naturally occurring amino acid or a naturally or non-naturally occurring amino acid analog. There are many amino acids beyond the standard 20 (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-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.

With regard to non-naturally occurring amino acids or naturally and non-naturally occurring amino acid analogs, a number of substitutions in the polypeptide and agonists described herein are possible alone or in combination.

For example, glutamine residues can be substituted with gamma-Hydroxy-Glu or gamma- Carboxy-Glu. 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-(l-Cyclopentenyl)-Ala; beta-Cyclopentyl-Ala; beta-Cyclopropyl-Ala; beta-Quinolyl-Ala; beta-(2-Thiazolyl)-Ala; beta-(Triazole-l-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. 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 Alanine residues can be substituted with 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. 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).

Further examples of unnatural 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, alkynl, 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, ^{13}C, ^{15}N, or ^{18}O); 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 ρ-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 phosphonoserine; a phosphonotyrosine; 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 (Carbobenzoxyl); 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. Exempary GCRA peptides which include a n0n-naturally occurring amino acid include for example SP-368 and SP-369.

In some embodiments, an amino acid can be replaced by a naturally-occurring, non-essential amino acid, *e.g.*, taurine.

Alternatively, the GCRA peptides are cyclic peptides. GCRA cyclic peptide are 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 aspects the GCRA peptides are [4,12; 7,15] bicycles.

In some GCRA peptides one or both members of one or both 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 JPept Protein Res 42:249) or diaminopropionic acid (Smith et al. 1978 J Med Chem 2 1:117) to form alternative internal cross-links at the 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)NHCH 2- or -CH2NHCH(O)CH 2-), 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(-CH 2CH=CHCH 2-), an ether linkage (-CH2CH2OCH2- or -CH2OCH2CH2-), a thioether linkage (-CH2CH2SCH2- or - CH2SCH2CH2-), an amine linkage (-CH2CH2NHCH2- or -CH2NHCH 2CH2-) or a thioamide linkage (-CH2CH(S)HNHCH 2- or -CH2NHCH(S)CH 2-). For example, Ledu et al. (Proc Nat'l Acad. Sci. 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links. Exemplary GCRA peptides which include a lactam bridge include for example SP-370.

The GCRA peptides can 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 (0-CH 2 or CH2-O); an ethylene bond (CH2-CH2); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); a fluoro substituted trans-olefme 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 GCRA peptides can be modified using standard modifications. Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a combination of any of the preceeding. 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), stearoylation, succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cys3 or Cys5. The GCRA peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methyl- coumarin (AMC), flourescein, NBD (7-Nitrobenz-2-Oxa-l,3-Diazole), p-nitro-anilide, rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-l- sulfonic acid), dabcyl, dabsyl, dansyl, texas red, FMOC, and Tamra (Tetramethylrhodamine). The GCRA 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.

Also included in the invention are peptides that biologically or functional equivalent to the peptides described herein. The term "biologically equivalent" or functional equivalent" is intended to mean that the compositions of the present invention are capable of demonstrating some or all of the cGMP production modulatory effects.

GCRA peptides can also include derivatives of GCRA peptides which are intended to include hybrid and modified forms of GCRA peptides in which certain amino acids have been deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylation so long the modified form retains the biological activity of GCRA peptides. By retaining the biological activity, it is meant that cGMP and or apoptosis is induced by the GCRA peptide, although not necessarily at the same level of potency as that of a naturally-occurring GCRA peptide identified.

Preferred variants are those that have conservative amino acid substitutions made at one or more predicted non-essential amino acid residues. A "conservative amino acid 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). Thus, a predicted nonessential amino acid residue in a GCRA polypeptide is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a GCRA coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened to identify mutants that retain activity.

Also included within the meaning of substantially homologous is any GCRA peptide which may be isolated by virtue of cross-reactivity with antibodies to the GCRA peptide.

PREPARATION OF GCRA PEPTIDES

GCRA peptides are easily prepared using modern cloning techniques, or may be synthesized by solid state methods or by site-directed mutagenesis. A GCRA peptide may include dominant negative forms of a polypeptide.

Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, 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.

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.

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.

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.

Alternatively the GCRA peptides are produced by modern cloning techniques For example, the GCRA 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 GCRA 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.

The sequence encoding a GCRA 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.

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.

The protein coding sequence that includes a GCRA 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.

Genetic constructs and methods suitable for production of immature and mature forms of the GCRA 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.

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.

THERAPEUTIC METHODS

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated that is mediated by guanylate cyclase receptor agonists. Disorders mediated by the guanylate cyclase receptor agonists include gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, 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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (e.g., pancreatis), lung inflammation (e.g., bronchitis or asthma) or skin inflammation (e.g., psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

The term "treatment" refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, and/or preventing disease in a subject who is free therefrom. For a given subject, improvement in a symptom, its worsening, regression, or progression may be determined by any objective or subjective measure. Efficacy of the treatment may be measured as an improvement in morbidity or mortality (*e.g.*, lengthening of 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.

Intracellular cGMP induced by exposing, *e.g.*, contacting a tissue (*e.g.*, gastrointestinals tissue) or cell with GCRA agonists. GC-C receptors are expressed throughout the GI tract starting from esophagus, duodenum, jejunum, ilium, caecum and colon. Human colon cancer cell lines (T81, CaCo-2 and HT-29) also express GC-C receptors. By inducing is meant an increase in cGMP production compared to a tissue or cell that has not been in contact with GCRA peptide or variant. Tissues or cells are directly contacted with a GCRA peptide or variant. Alternatively, the GCRA peptide or variant is administered systemically. GCRA peptide or variant are administered in an amount sufficient to increase intracellular cGMP concentration. cGMP production is measured by a cell-based assay known in the art (25).

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 GCRA peptide. The GCRA 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 10 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.

The GCRA peptides can be administered alone or in combination with other agents. For example the GCRA peptides can be administered in combination with inhibitors of cGMP dependent phosphodiesterase, such as, for example, suldinac sulfone, zaprinast, motapizone, vardenafil or sildenifil; one or more other chemotherapeutic agents; or anti-inflammatory drugs

such as, for example, steroids or non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin.

Combination therapy can be achieved by administering two or more agents, *e.g.*, a GCRA 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.

The GCRA 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.

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.

Combination therapy can also include the administration of two or more agents via different routes or locations. For example, (a) one agent is administered orally and another agents is administered intravenously or (b) one agent is administered orally and another is administered locally. In each case, the agents can either simultaneously or sequentially. Approximated dosages for some of the combination therapy agents described herein are found in the "BNF Recommended Dose" column of tables on pages 11-17 of WO01/76632 (the data in the tables being attributed to the March 2000 British National Formulary) and can also be found

in other standard formularies and other drug prescribing directories. For some drugs, the customary prescribed dose for an indication will vary somewhat from country to country.

The GCRA peptides, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose (*e.g.* celphere, Celphere beads®), diluents, lubricants, binders, disintegrating agents, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium

containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a GCRA agonist) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. Such as mannitol, fructooligosaccharides, polyethylene glycol and other excepients. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent

such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811, incorporated fully herein by reference.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit 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.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Compositions of the present invention may also optionally include other therapeutic ingredients, anti-caking agents, preservatives, sweetening agents, colorants, flavors, desiccants, plasticizers, dyes, glidants, anti-adherents, anti-static agents, surfactants (wetting agents), anti-oxidants, film- coating agents, and the like. Any such optional ingredient must be compatible with the compound described herein to insure the stability of the formulation.

The composition may contain other additives as needed, including for exanple lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffnose, 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.

Examples of excipients for use as the pharmaceutically acceptable carriers and the pharmaceutically acceptable inert carriers and the aforementioned additional ingredients include, but are not limited to binders, fillers, disintegrants, lubricants, anti-microbial agents, 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), 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, dextrates, 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, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums (like gellan), low-substituted hydroxypropyl cellulose, or mixtures thereof, LUBRICANTS: calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, sodium stearyl fumarate, 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, 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.

The formulation can also include other excipients and categories thereof including but not limited to L-histidine, Pluronic®, Poloxamers (such as Lutrol® and Poloxamer 188), ascorbic acid, glutathione, permeability enhancers (e.g. lipids, sodium cholate, acylcarnitine, salicylates, mixed bile salts, fatty acid micelles, chelators, fatty acid, surfactants, medium chain glycerides), protease inhibitors *(e.g.* soybean trypsin inhibitor, organic acids), pH lowering agents and absorption enhancers effective to promote bioavailability (including but not limited to those described in US6086918 and US5912014), 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); spheronization 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.

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).

The agents either in their free form or as a salt can be combined with a polymer such as polylactic-glycoloic acid (PLGA), poly-(I)-lactic-glycolic-tartaric acid (P(I)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. Such formulations can be used to implants that release a polypeptide or another agent over a period of a few days, a few weeks or several months depending on the polymer, the

particle size of the polymer, and the size of the implant (*See, e.g.*, U.S. 6,620,422). Other sustained release formulations and polymers for use in 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 9726015, WO 97/04744, and US200200 19446. In such sustained release formulations microparticles (Delie and Blanco-Prieto 2005 Molecule 10:65-80) of polypeptide are combined with microparticles of polymer. One or more sustained release implants can be placed in the large intestine, the small intestine or both. U.S. 6,011,0 1 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 releaseof 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.

The GCRA peptideds 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 hyrdratable 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); US5,866,619 and US6,368,629 (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-Eudragrit)); 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).

The GCRA 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. 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.

The GCRA peptides described herein can be formulated in an osmotic device including the ones disclosed in US4,503,030, US5,609,590 and US5,358,502. US4,503,030 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.

EXEMPLARY AGENTS FOR COMBINATION THERAPY

Analgesic Agents

The GCRA peptides described herein can be used in combination therapy with an analgesic agent, e.g., an analgesic compound or an analgesic polypeptide. These polypeptides and compounds can be administered with the GCRA peptides described herein (simultaneously or sequentially). They can also be optionally covalently linked or attached to an agent described herein to create therapeutic conjugates. Among the useful analgesic agents are: Ca channel blockers, 5HT receptor antagonists (for example 5HT3, 5HT4 and 5HTl receptor antagonists), opioid receptor agonists (loperamide, fedotozine, and fentanyl), NKl receptor antagonists, CCK receptor agonists (*e.g.*, loxiglumide), NKl receptor antagonists, NK3 receptor antagonists, norepinephrine-serotonin reuptake inhibitors (NSRI), vanilloid and cannabanoid receptor agonists, and sialorphin. Analgesics agents in the various classes are described in the literature.

Among the useful analgesic polypeptides are sialorphin-related polypeptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO:), including: VQHNPR (SEQ ID NO:); VRQHNPR (SEQ ID NO:); VRGQHNPR (SEQ ID NO:); VRGPQHNPR (SEQ ID NO:); VRGPRQHNPR (SEQ ID NO:); VRGPRRQHNPR (SEQ ID NO:); and RQHNPR (SEQ ID NO:). 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 polypeptides described herein in a co-therapy or linked to the polypeptides described herein, *e.g.*,

by a covalent bond. Sialophin and related polypeptides are described in U.S. Patent 6,589,750; U.S. 20030078200 Al; and WO 02/051435 A2.

Opioid receptor antagonists and agonists can be administered with the GCRA peptides described herein in co-therapy or linked to the agent described herein, *e.g.*, by a covalent bond. For example, opioid receptor antagonists such as naloxone, naltrexone, methyl nalozone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, and nor-binaltorphimine are thought to be useful in the treatment of IBS. It can be useful to formulate opioid antagonists of this type is a delayed and sustained release formulation such that initial release of the antagonist is in the mid to distal small intestine and/or ascending colon. Such antagonists are described in WO 01/32180 A2. Enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-homoserine) is an agonist of the mu and delta opioid receptors and is thought to be useful for increasing intestinal motility {Eur. J. Pharm. 219:445, 1992), and this polypeptide can be used in conjunction with the polypeptides described herein. Also useful is trimebutine which is thought to bind to mu/delta/kappa opioid receptors and activate release of motilin and modulate the release of gastrin, vasoactive intestinal polypeptide, gastrin and glucagons. Kappa opioid receptor agonists such as fedotozine, asimadoline, and ketocyclazocine, and compounds described in WO03/097051 and WO05/007626 can be used with or linked to the polypeptides described herein. In addition, mu opioid receptor agonists such as morphine, diphenyloxylate, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH 2; WO 01/019849 Al) and loperamide can be used.

Tyr-Arg (kyotorphin) is a dipeptide that acts by stimulating the release of met-enkephalins to elicit an analgesic effect (J. Biol. Chem 262:8165, 1987). Kyotorphin can be used with or linked to the GCRA peptides described herein.

Chromogranin-derived polypeptide (CgA 47-66; *See, e.g.*, Ghia et al. 2004 Regulatory polypeptides 119:199) can be used with or linked to the GCRA peptides described herein.

CCK receptor agonists such as caerulein from amphibians and other species are useful analgesic agents that can be used with or linked to the GCRA peptides described herein.

Conotoxin polypeptides represent a large class of analgesic polypeptides that act at voltage gated calcium channels, NMDA receptors or nicotinic receptors. These polypeptides can be used with or linked to the polypeptides described herein.

Peptide analogs of thymulin (FR Application 2830451) can have analgesic activity and can be used with or linked to the polypeptides described herein.

CCK (CCKa or CCKb) receptor antagonists, including loxiglumide and dexloxiglumide (the R- isomer of loxiglumide) (WO 88/05774) can have analgesic activity and can be used with or linked to the polypeptides described herein.

Other useful analgesic agents include 5-HT4 agonists such as tegaserod (Zelnorm®), mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lirexapride. Such agonists are described in: EP1321 142 Al, WO 03/053432A1, EP 505322 Al, EP 505322 Bl, US 5,510,353, EP 507672 Al, EP 507672 Bl, and US 5,273,983.

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 6087,091, US 6,136,786, WO 93/13128 Al, EP 1336409 Al, EP 835126 Al, EP 835126 Bl, US 5,795,864, US 5,891,849, US 6,054,429, WO 97/01351 Al, can be used with or linked to the polypeptides described herein.

Various antagonists of the NK-I, NK-2, and NK-3 receptors (for a review see Giardina et al. 2003.Drugs 6:758) can be can be used with or linked to the polypeptides described herein.

NKl 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 Al, US 20010006972 Al, US 20030109417 Al, WO 01/52844 Al, can be used with or linked to the polypeptides described herein.

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) can be used with or linked to the polypeptides described herein.

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 Al, WO 97/21680 Al, 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) can be used with or linked to the polypeptides described herein.

Norepinephrine-serotonin reuptake inhibitors (NSRI) such as milnacipran and related compounds described in WO 03/077897 A1 can be used with or linked to the polypeptides described herein.

Vanilloid receptor antagonists such as arvanil and related compouds described in WO 01/64212 A1 can be used with or linked to the polypeptides described herein.

The analgesic polypeptides and compounds can be administered with the polypeptides and agonists described herein (simultaneously or sequentially). The analgesic agents can also be covalently linked to the polypeptides and agonists described herein to create therapeutic conjugates. Where the analgesic is a polypeptide and is covalently linked to an agent described herein the resulting polypeptide may also include at least one trypsin cleavage site. When present within the polypeptide, the analgesic polypeptide may be preceded by (if it is at the carboxy terminus) or followed by (if it is at the amino terminus) a trypsin cleavage site that allows release of the analgesic polypeptide.

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

Agents to Treat Gastrointestinal Disorders

Examples of additional therapeutic agents to treat gastrointestinal and other disorders include agents to treat constipation (*e.g.*, a chloride channel activator such as the bicylic fatty acid, Lubiprostone (formerly known as SPI-0211; Sucampo Pharmaceuticals, Inc.; Bethesda, MD), a laxative (*e.g.* a bulk-forming laxative (e.g. nonstarch polysaccharides, Colonel Tablet (polycarbophil calcium), Plantago Ovata®, Equalactin® (Calcium Polycarbophil)), fiber (*e.g.* FIBERCON® (Calcium Polycarbophil), an osmotic laxative, a stimulant laxative (such as diphenylmethanes (*e.g.* bisacodyl), anthraquinones (*e.g.* cascara, senna), and surfactant laxatives (*e.g.* castor oil, docusates), an emollient/lubricating agent (such as mineral oil, glycerine, and docusates), MiraLax (Braintree Laboratories, Braintree MA), dexloxiglumide (Forest Laboratories, also known as CR 2017 Rottapharm (Rotta Research Laboratorium SpA)), saline laxatives, enemas, suppositories, and CR 3700 (Rottapharm (Rotta Research Laboratorium SpA); acid reducing agents such as proton pump inhibitors (*e.g.*, omeprazole (Prilosec®), esomeprazole (Nexium®), lansoprazole (Prevacid®), pantoprazole (Protonix®) and rabeprazole (Aciphex®)) and Histamine H2 -receptor antagonist (also known as H2 receptor blockers including

cimetidine, ranitidine, famotidine and nizatidine); prokinetic agents including itopride, octreotide, bethanechol, metoclopramide (Reglan®), domperidone (Motilium®), erythromycin (and derivatives thereof) or cisapride (propulsid®); Prokineticin polypeptides homologs, variants and chimeras thereof including those described in US 7,052,674 which can be used with or linked to the polypeptides described herein; pro-motility agents such as the vasostatin-derived polypeptide, chromogranin A (4-16) (*See, e.g.*, Ghia et al. 2004 Regulatory polypeptides 121:31) or motilin agonists (*e.g.*, GM-611 or mitemcinal fumarate) or nociceptin/Orphanin FQ receptor modulators (US20050169917); other peptides which can bind to and/or activate GC-C including those described in US20050287067; complete or partial 5HT (*e.g.* 5HTl, 5HT2, 5HT3, 5HT4) receptor agonists or antagonists (including 5HT1A antagonists (*e.g.* AGI-OOl (AGI therapeutics), 5HT2B antagonists (*e.g.* PGN 1091 and PGNl 164 (Pharmagene Laboratories Limited), and 5HT4 receptor agonists (such as tegaserod (ZELNORM®), prucalopride, mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lirexapride). Such agonists/modulators are described in: EP1321142 Al, WO 03/053432A1, EP 505322 Al, EP 505322 Bl, US 5,510,353, EP 507672 Al, EP 507672 Bl, US 5,273,983, and US 6,951,867); 5HT3 receptor agonists such as MKC-733; and 5HT3 receptor antagonists such as DDP-225 (MCI-225; Dynogen Pharmaceuticals, Inc.), cilansetron (Calmactin®), alosetron (Lotronex®), Ondansetron HCl (Zofran®), Dolasetron (ANZEMET®), palonosetron (Aloxi®), Granisetron (Kytril®), YM060(ramosetron; Astellas Pharma Inc.; ramosetron may be given as a daily dose of 0.002 to 0.02 mg as described in EP01588707) and ATI-7000 (Aryx Therapeutics, Santa Clara CA); muscarinic receptor agonists; anti-inflammatory agents; antispasmodics including but not limited to anticholinergic drugs (like dicyclomine (*e.g.* Colimex®, Formulex®, Lomine®, Protylol®, Visceral®, Spasmoban®, Bentyl®, Bentylol®), hyoscyamine (*e.g.* IB-Stat®, Nulev®, Levsin®, Levbid®, Levsinex Timecaps®, Levsin/SL®, Anaspaz®, A-Spas S/L®, Cystospaz®, Cystospaz-M®, Donnamar®, Colidrops Liquid Pediatric®, Gastrosed®, Hyco Elixir®, Hyosol®, Hyospaz®, Hyosyne®, Losamine®, Medispaz®, Neosol®, Spacol®, Spasdel®, Symax®, Symax SL®), Donnatal (*e.g.* Donnatal Extentabs®), clidinium (e.g. Quarzan, in combination with Librium = Librax), methantheline (*e.g.* Banthine), Mepenzolate (*e.g.* Cantil), homatropine (*e.g.* hycodan, Homapin), Propantheline bromide (*e.g.* Pro-Banthine), Glycopyrrolate (*e.g.* Robinul®, Robinul Forte®), scopolamine (*e.g.* Transderm-Scop®, Transderm-V®), hyosine-N-butylbromide (*e.g.*

Buscopan®), Pirenzepine (*e.g.* Gastrozepin®) Propantheline Bromide (*e.g.* Propanthel®), dicycloverine (*e.g.* Merbentyl®), glycopyrronium bromide (*e.g.* Glycopyrrolate®), hyoscine hydrobromide, hyoscine methobromide, methanthelinium, and octatropine); peppermint oil; and direct smooth muscle relaxants like cimetropium bromide, mebeverine (DUSPATAL®, DUSPATALIN®, COLOFAC MR®, COLOTAL®), otilonium bromide (octilonium), pinaverium (e.g. Dicetel® (pinaverium bromide; Solvay S. A.)), Spasfon® (hydrated phloroglucinol and trimethylphloroglucinol)and trimebutine (including trimebutine maleate (Modulon®); antidepressants, including but not limited to those listed herein, as well as tricyclic antidepressants like amitriptyline (Elavil®), desipramine (Norpramin®), imipramine (Tofranil®), amoxapine (Asendin®), nortriptyline; the selective serotonin reuptake inhibitors (SSRTs) like paroxetine (Paxil®), fluoxetine (Prozac®), sertraline (Zoloft®), and citralopram (Celexa®); and others like doxepin (Sinequan®) and trazodone (Desyrel®); centrally-acting analgesic agents such as opioid receptor agonists, opioid receptor antagonists (e.g., naltrexone); agents for the treatment of Inflammatory bowel disease; agents for the treatment of Crohn's disease and/or ulcerative colitis (*e.g.*, alequel (Enzo Biochem, Inc.; Farmingsale, NY), the anti-inflammatory polypeptide RDP58 (Genzyme, Inc.; Cambridge, MA), and TRAFICET-EN™ (ChemoCentryx, Inc.; San Carlos, CA); agents that treat gastrointestinal or visceral pain; agents that increase cGMP levels (as described in US20040121994) like adrenergic receptor antagonists, dopamine receptor agonists and PDE (phosphodiesterase) inhibitors including but not limited to those disclosed herein; purgatives that draw fluids to the intestine (*e.g.*, VISICOL®, a combination of sodium phosphate monobasic monohydrate and sodium phosphate dibasic anhydrate); Corticotropin Releasing Factor (CRF) receptor antagonists (including NBI-34041 (Neurocrine Biosciences, San Diego, CA), CRH9-41, astressin, R121919 (Janssen Pharmaceutica), CP154,526, NBI-27914, Antalarmin, DMP696 (Bristol-Myers Squibb) CP-316,311 (Pfizer, Inc.), SB723620 (GSK), GW876008 (Neurocrine/Glaxo Smith Kline), ONO-2333Ms (Ono Pharmaceuticals), TS-041 (Janssen), AAG561 (Novartis) and those disclosed in US 5,063,245, US 5,861,398, US20040224964, US20040198726, US20040176400, US20040171607, US20040110815, US20040006066, and US20050209253); glucagon- like polypeptides (glp-1) and analogues thereof (including exendin-4 and GTP-010 (Gastrotech Pharma A)) and inhibitors of DPP-IV (DPP-IV mediates the inactivation of glp-1); tofisopam, enantiomerically-pure R-tofisopam, and pharmaceutically-acceptable salts thereof (US

20040229867); tricyclic anti-depressants of the dibenzothiazepine type including but not limited to Dextofisopam® (Vela Pharmaceuticals), tianeptine (Stablon®) and other agents described in US 6,683,072; (E)-4 (1 ,3bis(cyclohexylmethyl)-l ,2,34,-tetrahydro-2,6-diono-9H-purin-8-yl)cinnamic acid nonaethylene glycol methyl ether ester and related compounds described in WO 02/067942; the probiotic PROBACTRIX® (The BioBalance Corporation; New York, NY) which contains microorganisms useful in the treatment of gastrointestinal disorders; antidiarrheal drugs including but not limited to loperamide (Imodium, Pepto Diarrhea), diphenoxylate with atropine (Lomotil, Lomocot), cholestyramine (Questran, Cholybar), atropine (Co-Phenotrope, Diarsed, Diphenoxylate, Lofene, Logen, Lonox, Vi-Atro, atropine sulfate injection) and Xifaxan® (rifaximin; Salix Pharmaceuticals Ltd), TZP-201(Tranzyme Pharma Inc.), the neuronal acetylcholine receptor (nAChR) blocker AGI-004 (AGI therapeutics), and bismuth subsalicylate (Pepto-bismol); anxiolytic drugs including but not limited toAtivan (lorazepam), alprazolam (Xanax®), chlordiazepoxide/clidinium (Librium®, Librax®), clonazepam (Klonopin®), clorazepate (Tranxene®), diazepam (Valium®), estazolam (ProSom®), flurazepam (Dalmane®), oxazepam (Serax®), prazepam (Centrax®), temazepam (Restoril®), triazolam (Halcion®; Bedelix® (Montmorillonite beidellitic; Ipsen Ltd), Solvay SLV332 (ArQule Inc), YKP (SK Pharma), Asimadoline (Tioga Pharmaceuticals/Merck), AGI-003 (AGI Therapeutics); neurokinin antagonists including those described in US20060040950; potassium channel modulators including those described in US7,002,015; the serotonin modulator AZD7371 (AstraZeneca Plc); M3 muscarinic receptor antagonists such as darifenacin (Enablex; Novartis AG and zamifenacin (Pfizer); herbal and natural therapies including but not limited to acidophilus, chamomile tea, evening primrose oil, fennel seeds,wormwood, comfrey, and compounds of Bao-Ji-Wan (magnolol, honokiol, imperatorin, and isoimperatorin) as in US6923992; and compositions comprising lysine and an anti-stress agent for the treatment of irritable bowel syndrome as described in EPO 1550443.

Insulin and Insulin Modulating Agents

The GCRA 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).

The GCRA 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 agonistsdescribed herein can be used in combitherapy with SYMLIN® (pramlintide acetate) and Exenatide® (synthetic exendin-4; a 39 aa polypeptide).

Agents for the Treatment of Postoperative Ileus

The GCRA peptides described herein can also be used in combination therapy with agents (*e.g.*, Entereg™ (alvimopan; formerly called ado lor/ ADL 8-2698), conivaptan and related agents describe in US 6,645,959) used for the treatment of postoperative ileus and other disorders.

Anti-Hypertensive Agents

The GCRA 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, epirenone, and the like; (2) beta-adrenergic blockers such as acebutolol, atenolol, betaxolol, bevantolol, bisoprolol, bopindolol, carteolol, carvedilol, celiprolol, esmolol, indenolol, metaprolol, nadolol, nebivolol, penbutolol, pindolol, propanolol, 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, nimodepine, nisoldipine, nitrendipine, manidipine, pranidipine, and verapamil, and the like; (4) angiotensin converting enzyme (ACE) inhibitors such as benazepril; captopril; ceranapril; cilazapril; delapril; enalapril; enalopril; fosinopril; imidapril; lisinopril; losinopril; moexipril;

quinapril; quinaprilat; ramipril; perindopril; perindropril; 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, pratosartan, 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 XENOlO, 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), epirenone, and the like); β-adrenergic blockers such as Amiodarone (Cordarone, Pacerone), bunolol hydrochloride (CAS RN 31969-05-8, Parke-Davis), acebutolol (±N-[3-Acetyl-4-[2-hydroxy-3-[(1 methylethyl)amino]propoxy]phenyl]-butanamide, or (±)-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,l-[(l,l-dimethylethyl)amino]-3-[[4-4(4-morpholinyl)-l,2,5-thiadiazol-3-yl]oxy]-, hemihydrate, (S)-, CAS RN 91524-16-2), timolol maleate (S)-l -[(1,1 -dimethylethyl) amino]-3-[[4- (4-morpholinyl)-l,2,5-thiadiazol -3- yl] oxy]-2-propanol (Z)-2-butenedioate (1 :1) salt, CAS RN 26921-17-5), bisoprolol (2-Propanol, l-[4-[[2-(l-methylethoxy)ethoxy]-methyl]phenoxyl]-3-[(l-meth- ylethyl)amino]-, (±), CAS RN 66722-44-9), bisoprolol fumarate (such as (±)-l-[4-[[2-(l-Methylethoxy) ethoxy]methyl]phenoxy]-3-[(l-methylethyl)amino]-2-propanol (E) -2-butenedioate (2:1) (salt), *e.g.*, Zebeta™ , Lederle Consumer), nebivalol (2H-l-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, l-[4-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-[l-methylethyl)amino]-, hydrochloride, A.A.S. RN 63686-79-3), dexpropranolol hydrochloride (2-Propanol,l-[l-methylethy)-amino]-3-(l-naphthalenyloxy)-hydrochloride (CAS RN 13071-11-9), diacetolol hydrochloride (Acetamide, N-[3-acetyl-4-[2-hydroxy-3-[(l-methyl-ethyl)amino]propoxy] [phenyl]-, monohydrochloride CAS RN 69796-04-9), dilevalol hydrochloride (Benzamide, 2-hydroxy-5-[l-hydroxy-2-[l-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), flestolol sulfate (Benzoic acid, 2-fluro-,3-[[2-[aminocarbonyl)amino]- - dimethylethyl]amino]-2-hydroxypropyl ester, (+)- sulfate (1 :1) (salt), CAS RN 88844-73-9; metalol hydrochloride (Methanesulfonamide, N-[4-[l-hydroxy-2-(methylamino)propyl][phenyl]-, monohydrochloride CAS RN 7701-65-7), metoprolol 2-Propanol, l-[4-(2- methoxyethyl)phenoxy]-3-[l-methylethyl)amino]-; CAS RN 37350-58-6), metoprolol tartrate (such as 2-Propanol, l-[4-(2-methoxyethyl)phenoxy]-3-[(l-methylethyl)amino]-, *e.g.*, Lopressor®, Novartis), pamatolol sulfate (Carbamic acid, [2-[4-[2-hydroxy-3-[(l- methylethyl)amino]propoxyl]phenyl]-ethyl]-, methyl ester, (±) sulfate (salt) (2:1), CAS RN 59954-01-7), penbutolol sulfate (2-Propanol, l-(2-cyclopentylphenoxy)-3-[l,l-

dimethyle- thyl)amino] 1 , (S)-, sulfate (2:1) (salt), CAS RN 38363-32-5), practolol (Acetamide, N-[4-[2- hydroxy-3-[(l-methylethyl)amino]-propoxy]phenyl]-, CAS RN 6673-35-4;) tiprenolol hydrochloride (Propanol, l-[(l-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)-l ,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)-l,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 is 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)-l,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate- , *e.g.* Plendil® Extended-Release, AstraZeneca LP), nilvadipine (3,5-Pyridinedicarboxylic acid, 2-cyano-l,4-dihydro-6-methyl-4-(3-nitrophenyl)-,3-methyl 5-(l-methylethyl) ester, also see US3799934), nifedipine (such as 3, 5 -pyridinedicarboxylic acid,l,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, dimethyl ester, *e.g.*, Procardia XL® Extended Release Tablets, Pfizer), diltiazem hydrochloride (such as l,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 benzeneacetronitrile, (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-[(lE)-3-(l,l-dimethylethoxy)-3-oxo- l-propenyl]phenyl]-l,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,l l-Dihydrodibenzo(b,e)thiep- in-l l-yl)-4-(p- fluorophenyl)-l-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-[[l-(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 l-[(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), enalopril, enaloprilat, fosinopril, ((such as L-proline, 4-cyclohexyl-l-[[[2-methyl- l-(l-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-l-[[(R)-[(lS)-2-methyl-l-(l-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-[[(lS)- 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 (Hoechsst) disclosed in EP 79022 and Curr. Ther. Res. 40:74 (1986), perindopril erbumine (such as 2S,3aS,7aS- 1 -[(S)-N-[(S)- 1 -Carboxybutyljalanyljhexahydro^-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-([l-ethoxycarbonyl-3-phenyl-(lS)-propyl]amino)-2,3,4,5-tetrahydro-2-ox- o-l-(3S)-benzazepine-l

acetic acid HCl, see U.K. Patent No. 2103614), CGS 16,617 (Ciba- Geigy, 3(S)-[[(lS)-5-amino-l-carboxypentyl]amino]-2,3,4,- 5-tetrahydro-2-oxo-lH-l- 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 (phosphonamidates); 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), nicotinyl 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 l,4:3,6-dianhydro-D-glucitol 2,5-dinitrate *e.g.*, Isordil® Titradose®, Wyeth- Ayerst), sosorbide mononitrate (such as l,4:3,6-dianhydro-D-glucito- 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 dimethoxypheny l)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-(l-methyl-2-pyrrolidinylidene)- Pyrrolidine, 2-[(3,4-dimethoxyphenethyl)imino]- 1 -methyl- l-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(l,2-Propanediol, 3-chloro-, dinitrate (7CI, 8CI, 9CI) CAS RN 2612-33-1), dipyridamole Ethanol, 2,2',2",2"'-[(4,8-di-l-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]-Nisoldipine3,5-Pyridinedicarboxylic acid, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-, methyl 2-methylpropyl ester CAS RN 63675-72-9), nifedipine3,5-Pyridinedicarboxylic acid, l,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-(l -methylethyl)- CAS RN 52-53-9) and the like; angiotensin II receptor antagonists such as, aprosartan, zolasartan, olmesartan, pratosartan, FI6828K, RNH6270, candesartan (1 H-Benzimidazole-7-carboxylic acid, 2-ethoxy-l-[[2'-(lH-tetrazol-5-yl)[l,l'-biphenyl]4-yl]methyl]- CAS RN 139481-59-7), candesartan cilexetil ((+/-)-l-(cyclohexylcarbonyloxy)ethyl-2-ethoxy-l-[[2'-(lH-tetrazol-5-yl)biphenyl-4-yl]-lH-benzimidazole carboxylate, CAS RN 145040-37-5, US5703110 and US5196444), eprosartan (3-[l-4-carboxyphenylmethyl)-2-n-butyl-imidazol-5-yl]-(2-thienylmethyl) propenoic acid, US5185351 and US5650650), irbesartan (2-n-butyl-3- [[2'-(lh-tetrazol-5-yl)biphenyl-4-yl]methyl] 1 ,3-diazazspiro[4,4]non-l-en-4-one, US5270317 and US5352788), losartan (2-N-butyl-4-chloro-5-hydroxymethyl-l-[(2'-(lH-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole, potassium salt, US5138069, US5153197 and US5128355), tasosartan (5,8-dihydro-2,4-dimethyl-8-[(2'-(lH-tetrazol-5-yl)[l,r-biphenyl]4-yl)methyl]-pyrido[2,3-d]pyrimidin-7(6H)-one, US5149699), telmisartan (4'-[(1 ,4-dimethyl-2'-propyl-(2,6'-bi-lH-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'-(lH-tetrazol-5-yl)biphenyl-4-yl)methyl]valine, US5399578), EXP-3137 (2-N-butyl-4-chloro-l-[(2'-(lH-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole-5-carboxylic acid, US5138069, US5153197 and US5128355), 3-(2'-(tetrazol-

5-yl)-l,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[l,2-a]pyridin-2-yl]-benzimidazol-l-yl]-methyl]-l,r-biphenyl]-2- carboxylic acid, 2-butyl-6-(l-methoxy-l-methylethyl)-2-[2'-)lH-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-l-[(2'-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole-carboxylic acid, 2-butyl-4-chloro-l-[[2'-(lH-tetrazol-5- yl) [1 , 1 ' -biphenyl] -4-yl]methyl]- 1 H-imidazole-5 -carboxylic acid- 1 -(ethoxycarbonyl-oxy)ethyl ester potassium salt, dipotassium 2-butyl-4-(methylthio)-l-[[2-[[[(propylamino)carbonyl]amino]-sulfonyl](1,l '-biphenyl)-4-yl]methyl]-l H-imidazole-5 -carboxylate, methyl-2-[[4-butyl-2-methyl-6-oxo-5-[[2'-(lH-tetrazol-5-yl)-[1,l '-biphenyl]-4-yl]methyl]-l-(6H)- pyrimidinyl]methyl]-3-thiophencarboxylate, 5-[(3,5-dibutyl-lH-1,2,4-triazol-l-yl)methyl]-2-[2- (1 H-tetrazol-5 -ylphenyl)]pyridine, 6-butyl-2-(2-phenylethyl)-5 [[2'-(1 H-tetrazol-5 -yl)[1,1 '- biphenyl]-4-methyl]pyrimidin-4-(3H)-one D,L lysine salt, 5-methyl-7-n-propyl-8-[[2'-(lH- tetrazol-5-yl)biphenyl-4-yl]methyl]-[1 ,2,4]-triazolo[1 ,5-c]pyrimidin-2(3H)-one, 2,7-diethyl-5- [[2'-(5-tetrazoly)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'-(lH-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'(lH-tetrazol-5-yl)-1,l '-biphenyl-4-yl]methoxy]pyridine, 2-ethoxy-l-[[2'-(5-oxo-2,5-dihydro- 1 ,2,4-oxadiazol-3 -yl)biphenyl-4-yl]methyl] - 1 H-benzimidazole-7-carboxylic acid, 1 - [N-(2 ' -(1 H- tetrazol-5-yl)biphenyl-4-yl-methyl)-N-valerolylaminomethyl)cyclopentane- 1 -carboxylic acid, 7- methyl-2n-propyl-3-[[2' lH-tetrazol-5-yl)biphenyl-4-yl]methyl]-3H-imidazo[4,5-6]pyridine, 2- [5-[(2-ethyl-5,7-dimethyl-3H-imidazo[4,5-b]pyridine-3-yl)methyl]-2-quinolinyl]sodium benzoate, 2-butyl-6-chloro-4-hydroxymethyl-5 -methyl-3 -[[2'-(1 H-tetrazol-5 -yl)biphenyl-4-yl]methyl]pyridine, 2- [[[2-butyl- 1 - [(4-carboxyphenyl)methyl] - 1 H-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-l,3-dihydro-3-[[6-[2-(lH-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'(lH-tetrazol-5-yl)biphenyl-4-yl]methyl]-lH,4H-l,3,4a,8a-tetrazacyclopentanaphthalene-9-one, 4-[l-[2'-(1,2,3,4-tetrazol-5-yl)biphen-4-yl)methylamino]-5,6,7,8-tetrahydro-2-trifylquinazoline, 2-(2-chlorobenzoyl)imino-5-ethyl-3-[2'-(lH-tetrazole-5-yl)biphenyl-4-

yl)methyl-l,3,4-thiadiazoline, 2-[5-ethyl-3-[2-(lH-tetrazole-5-yl)biphenyl-4-yl]methyl-l,3,4-thiazoline-2-ylidene]aminocarbonyl-l-cyclopentenecarboxylic acid dipotassium salt, and 2-butyl-4-[N-methyl-N-(3 -methylcrotonoyl)amino] - 1 - [[2 ' -(1 H-tetrazol-5 -yl)biphenyl-4-yl]methyl]- 1 H- imidzole-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, WO9210179, 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 (±)-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-lH-imidazol-2-yl)methyl](4-methylphenyl)amino]-, monomethanesulfonate (salt) CAS RN 65-28-1), solypertine tartrate (5H-l,3-Dioxolo[4,5-f]indole, 7-[2-[4-(2-methoxyphenyl)-l-piperazinyl]ethyl]-, (2R,3R)-2,3-dihydroxybutanedioate (1 :1) CAS RN 5591-43-5), zolertine hydrochloride (Piperazine, l-phenyl4-[2-(lH-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, XENOlO, 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 (SPPlOO; Novartis/Speedel); angiopoietin-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, l-[4-[2 (cyclopropylmethoxy)ethyl]phenoxy]-3-[(l- methylethyl)amino]-, hydrochloride CAS RN 63659-19-8), butoprozine hydrochloride (Methanone, [4-[3(dibutylamino)propoxy]phenyl](2-ethyl-3-indolizinyl)-, monohydrochloride CAS RN 62134-34-3), cinepazet maleatel-Piperazineacetic acid, 4-[l-oxo-3-(3,4,5- trimethoxyphenyl)-2-propenyl]-, ethyl ester, (2Z)-2-butenedioate (1 :1) CAS RN 50679-07-7), tosifen (Benzenesulfonamide, 4-methyl-N-[[[(lS)-l-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-meth-oxyphenoxy)propyl]-, dihydrochloride CAS RN 95635-56-6); tosifen (Benzenesulfonamide, 4-methyl-N-[[[(lS)-l-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 -l,2,4-benzothiadiazine-7- sulfonamide 1,1-dioxide, *e.g.*, the combination as, e.g., Aldoril® Tablets available from Merck), methyldopa-chlorothiazide (such as 6-chloro-2H-l, 2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide and methyldopa 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-(l-hydroxy-3-oxo-l-isoindolinyl) benzenesulfonamide), *e.g.*, Combipres®, Boehringer Ingelheim), clonidine hydrochloride (such as 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride, e.g., Catapres®, Boehringer Ingelheim), clonidine (lH-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.

Agents for the Treatment of Respiratory Disorders

The GCRA 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 AMOl® , 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) β2-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 Hl 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, astamizole, bromopheniramine, bromopheniramine maleate, carbinoxamine, carebastine, cetirizine, chlorpheniramine, chloropheniramine 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, pyrilamine, ranitidine, temelastine, terfenadine, trimeprazine, tripelennamine, 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: guafenesin, guaicolsulfate, 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, pirprofen, pranoprofen, sudoxicam, tenoxican, 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.

Anti-obesity agents

The GCRA peptides described herein can be used in combination therapy with an anti-obesity agent. Suitable such agents include, but are not limited to: 1 lβ HSD-I (11-beta hydroxy steroid dehydrogenase type 1) inhibitors, such as BVT 3498, BVT 2733, 3-(l-adamantyl)-4-ethyl-5-(ethylthio)- 4H-l,2,4-triazole, 3-(l-adamantyl)-5-(3,4,5- trimethoxyphenyl)-4-methyl-4H-l,2,4-triazole, 3- adamantanyl-4,5,6,7,8,9,10,11,12,3a- decahydro-l,2,4-triazolo[4,3-a][l 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; 5HTIa 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-l,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 inhibtors, 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-(IH-imidazol-4- yl)propyl N-(4-pentenyl)carbamate), clobenpropit, iodophenpropit, imoproxifan, GT2394 (Gliatech), and A331440, O-[3-(IH-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, USUS5512565, 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; serotoninergic 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, EPOl 12987, EPOl 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, DEl 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, diparnfylline, 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 (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 phytanic acid, 4-[(E)-2-(5, 6,7,8- tetrahydro-5,5,8,8-tetramethyl-2-napthalenyl)-l-propenyl]benzoic acid (TTNPB), retinoic acid, and those disclosed in WO99/00123; β3 (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)-l -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,l-a]isoindol-5-ol) such as Sanorex®, Novartis or Mazanor®, Wyeth Ayerst), phenylpropanolamine (or Benzenemethanol, alpha-(l-aminoethyl)-, hydrochloride), phentermine ((or Phenol, 3-[[4,5-duhydro-lH-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-lH-indenol[2,l-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, sercloremine, 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, DGATl (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)/glicentin/glucagon gene family and/or those of the adrenomedullin/amylin/calcitonin gene related polypeptide (CGRP) gene family includingGLP-1 (glucagon- like polypeptide 1) agonists (e.g. (1) exendin-4, (2) those GLP-l molecules described in US20050130891 including GLP- 1(7-34), GLP-l(7-35), GLP-l(7-36) or

GLP-l(7-37) in its C-terminally carboxylated or amidated form or as modified GLP-1 polypeptides and modifications thereof including those described in paragraphs 17-44 of US20050130891, and derivatives derived from GLP-l-(7- 34)COOH and the corresponding acid amide are employed which have the following general formula: R-NH-HAEGTFTSDVSYLEGQAAKEFIAWLVK-$CONH_2$ 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-1), 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.

Anti-Diabetic Agents

The GCRA peptides described herein can be used in therapeutic combination with one or more anti-diabetic agents, including but not limited to: PPARγ 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 (Mitsibishi disclosed in 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, US6303640, US6329404, US5994554, W097/10813, 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 Glucovance™, Bristol-Myers Squibb); buformin (Imidodicarbonimidic diamide, N-butyl-); etoformine (1-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, 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, 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 acetohexamide (*e.g.* Dymelor, Eli Lilly), carbutamide, chlorpropamide (*e.g.* Diabinese®, Pfizer), gliamilide (Pfizer), gliclazide (*e.g.* Diamcron, Servier 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.* Pranidin®, Novo 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]-alpha-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 Al -3688, and the compounds disclosed in US4451455, US4623714, and US4273765; SGLT2 inhibtors including those disclosed in US6414126 and US6515117; an aP2 inhibitor such as disclosed in US6548529; insulin secreatagogues such as linogliride, A-4166, forskilin, dibutyrl 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, deriglidole, 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; PPARα/γ dual agonists such as AR-HO39242 (Aztrazeneca), 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), tesaglitzar (Astrazeneca), reglitazar

(JTT-501) and those disclosed in WO99/16758, WO99/19313, WO99/20614, WO99/38850, 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, such as those disclosed in WO03/015774; retinoid modulators such as those disclosed in WO03/000249; GSK 3β/GSK 3 inhibitors such as 4-[2-(2-bromophenyl)-4-(4-fluorophenyl-lH-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 compounds disclosed in WOO 1/94300, WO02/20530, WO03/037864, and pharmaceutically acceptable salts or esters thereof; ATP consumption promotors 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 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; PPARδ agonists such as GW 501516, GW 590735, and compounds disclosed in JP10237049 and WO02/14291; dipeptidyl peptidase IV (DP-IV) inhibitors, such as 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-l,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, 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,

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® (pramlintide acetate); and glycokinase activators such as those disclosed in US2002103199 (fused heteroaromatic compounds) and WO02/48106 (isoindolin-1-one-substituted propionamide compounds).

Phosphodiesterase inhibitors

The GCRA peptides described herein can be used in combination therapy with a phosphodiesterase inhibitor. PDE inhibitors are those 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 intracelluiar concentration of c AMP and/or 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. By way of example, those PDE inhibitors may be mentioned such as are described and/or claimed in the following patent applications and patents: DE1470341, DE210843S, DE2123328, DE2305339, DE2305575, DE2315801, DE2402908, DE2413935, DE2451417, DE2459090, DE2646469, DE2727481, DE2825048, DE2837161, DE2845220, DE2847621, DE2934747, DE3021792, DE3038166, DE3044568, EP000718, EP0008408, EP0010759, EP0059948, EP0075436, EP0096517, EPOl 12987, EPOl 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, DEI 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).

Anti- Uterine Contractions Agents

The GCRA peptides described herein can be used in combination therapy (for example, in order to decrease or inhibit uterine contractions) with a tocolytic agent including but not limited to beta-adrenergic agents, magnesium sulfate, prostaglandin inhibitors, and calcium channel blockers.

Anti- Neoplastic Agents

The GCRA peptides described herein can be used in combination therapy with an antineoplastic agents including but not limited to 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.

The GCRA peptides described herein can be used in combination therapy (for example as in a chemotherapeutic composition) with an antiviral and monoclonal antibody therapies.

Agents to treat Congestive Heart Failure

The GCRA peptides described herein can be used in combination therapy (for example, in prevention/treatment of congestive heart failure or another method described herein) with the partial agonist of the nociceptin receptor ORL1 described by Dooley et al. (The Journal of Pharmacology and Experimental Therapeutics, 283 (2): 735-741, 1997). The agonist is a hexapeptide having the amino acid sequence Ac- RYY (RK) (WI) (RK)-NH2 ("the Dooley polypeptide"), where the brackets show allowable variation of amino acid residue. Thus Dooley polypeptide can include but are not limited to KYYRWR, RYYRWR, KWRYYR, RYYRWK, RYYRWK (all-D amin acids), RYYRIK, RYYRIR, RYYKIK, RYYKIR, RYYKWR, RYYKWK, RYYRWR, RYYRWK, RYYRIK, RYYKWR, RYYKWK, RYYRWK and KYYRWK, wherein the amino acid residues are in the L-form unless otherwise specified. The GCRA peptides described herein can also be used in combination therapy with polypeptide conjugate modifications of the Dooley polypeptide described in WO0198324.

DOSAGE

Dosage levels of active ingredients in a pharmaceutical composition can also be varied so as to achieve a transient or sustained concentration of the compound in a subject, especially in and

around the site of inflammation or disease area, and to result in the desired response. It is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired effect and to gradually increase the dosage until the desired effect is achieved. It will be understood that the specific dose level for any particular subject will depend on a variety of factors, including body weight, general health, diet, natural history of disease, route and scheduling of administration, combination with one or more other drugs, and severity of disease.

An effective dosage of the composition will typically be between about 1 μg and about 10 mg per kilogram body weight, preferably between about 10 μg 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.

The guanylate cyclase receptor agonists used in the methods described above may be administered 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. Agonists may be administered as either the sole active agent or in combination with other drugs, *e.g.*, an inhibitor of cGMP-dependent phosphodiesterase and anti-inflammatory agent. 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.

Dosage levels of the GCR agonist for use in methods of this invention typically are from about 0.001 mg to about 10,000 mg daily, preferably from about 0.005 mg to about 1,000 mg daily. On the basis of mg/kg daily dose, either given in single or divided doses, dosages typically range from about 0.001/75 mg/kg to about 10,000/75 mg/kg, preferably from about 0.005/75 mg/kg to about 1,000/75 mg/kg.

The total daily dose of each inhibitor can be administered to the patient in a single dose, or in multiple subdoses. Typically, subdoses 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. Doses can be in immediate release form or sustained release form sufficiently effective to obtain the desired control over the medical condition.

The dosage regimen to prevent, treat, give relief from, or ameliorate a medical condition or disorder, or to otherwise protect against or treat a medical condition with the combinations and compositions of the present invention is selected in accordance with a variety of factors.

These factors include, but are not limited to, the type, age, weight, sex, diet, and medical condition of the subject, the severity of the disease, the route of administration, pharmacological considerations such as the activity, efficacy, pharmacokinetics and toxicology profiles of the particular inhibitors employed, whether a drug delivery system is utilized, and whether the inhibitors are administered with other active ingredients. Thus, the dosage regimen actually employed may vary widely and therefore deviate from the preferred dosage regimen set forth above.

EXAMPLES

EXAMPLE 1: SYNTHESIS AND PURIFICATION OF GCRA PEPTIDES

The GCRA peptides were synthesized using standard methods for solid-phase peptide synthesis. Either a Boc/Bzl or Fmoc/tBu protecting group strategy was seleceted depending upon the scale of the peptide to be produced. In the case of smaller quantities, it is possible to get the desired product using an Fmoc/tBu protocol, but for larger quantities (1 g or more), Boc/Bzl is superior.

In each case the GCRA peptide was started by either using a pre-loaded Wang (Fmoc) or Merrifield (Boc) or Pam (Boc) resin. For products with C-terminal Leu, Fmoc-Leu-Wang (D-1115) or Boc-Leu-Pam resin (D-1230) or Boc-Leu-Merrifield (D-1030) Thus, for peptides containing the C-terminal d-Leu, the resin was Fmoc-dLeu-Wang Resin (D-2535) and Boc-dLeu-Merrifield, Boc-dLeu-Pam-Resin (Bachem Product D-1230 and D-1590, respectively) (SP-332 and related analogs). For peptides produced as C-terminal amides, a resin with Ramage linker (Bachem Product D-2200) (Fmoc) or mBHA (Boc) (Bachem Product D-1210 was used and loaded with the C-terminal residue as the first synthetic step.

Fmoc-tBu Overview

Each synthetic cycle consisted deprotection with 20% piperidine in DMF. Resin washes were accomplished with alternating DMF and IpOH to swell and shrink the resin, respectively. Peptide synthesis elongated the chain from the C-terminus to the N-terminus. Activation chemistry for each amino acid was with HBTU/DIEA in a 4 fold excess for 45 minutes. In automated chemistries, each amino acid was double coupled to maximize the coupling efficiency. To insure the correct position of disulfide bonds, the Cys residues were introduced as Cys(Acm) at positions 15 and 7. Cys(Trt) was positioned at Cys4 and Cys12. This protecting

group strategy yields the correct topoisomer as the dominant product (75:25). (For enterotoxin analogs, a third disulfide bond protecting group (Mob) was utilized).

For peptides containing C-terminal Aeea (aminoethyloxyethyloxyacetyl) groups, these were coupled to a Ramage amide linker using the same activation chemistry above by using an Fmoc-protected Aeea derivative. The Cys numbering in these cases remains the same and the positioning of the protecting groups as well. For the peptides containing the N-terminal extension of Aeea, the Cys residue numbering will be increased by three Cys4 becomes Cys7, Cys12 becomes Cys15; Cys7 becomes Cys10 and Cys 15 becomes Cys18. The latter pair is protected with Acm and the former pair keeps the Trt groups.

For analogs containing D-amino acid substitutions, these were introduced directly by incorporating the correctly protected derivative at the desired position using the same activation chemistry described in this document. For Fmoc strategies, Fmoc-dAsn(Trt)-OH, Fmoc-dAsn(Xan)-OH, Fmoc-dAsp(tBu)-OH, Fmoc-dGlu(tBu)-OH and for Boc strategies, Boc-dAsn(Xan)-OH, Boc-dAsn(Trt)-OH, Boc-dAsp(Chx), Boc-dAsp(Bzl)-OH, Boc-dGlu(Chx)-OH and Boc-dGlu(Bzl)-OH would be utilized.

Each peptide is cleaved from the solid-phase support using a cleavage cocktail of TFA:H2O:Trisisopropylsilane (8.5:0.75:0.75) ml/g of resin for 2 hr at RT. The crude deprotected peptide is filtered to remove the spent resin beads and precipitated into ice-cold diethylether.

Each disulfide bonds was introduced orthogonally. Briefly, the crude synthetic product was dissolved in water containing NH_4OH to increase the pH to 9. Following complete solubilization of the product, the disulfide bond was made between the Trt deprotected Cys residues by titration with H_2O_2. The monocyclic product was purified by RP-HPLC. The purified mono-cyclic product was subsequently treated with a solution of iodine to simultaneously remove the Acm protecting groups and introduce the second disulfide bond.

For enterotoxin analogs, the Mob group was removed via treatment of the dicyclic product with TFA 85% containing 10% DMSO and 5% thioanisole for 2 hr at RT.

Each product was then purified by RP-HPLC using a combination buffer system of TEAP in H2O versus MeCN, followed by TFA in H2O versus MeCN. Highly pure fractions were combined and lyophilized. The final product was converted to an Acetate salt using either ion exchange with Acetate loaded Dow-Ex resin or using RP-HPLC using a base-wash step with NH_4OAc followed by 1% AcOH in water versus MeCN.

It is also possible to prepare enterotoxin analogs using a random oxidation methodology using Cys(Trt) in Fmoc or Cys(McB) in Boc. Following cleavage, the disulfide bonds can be formed using disulfide interchange redox pairs such as glutathione (red/ox) and/or cysteine/cystine. This process will yield a folded product that the disulfide pairs must be determined as there would be no way of knowing their position directly.

Boc-Bzl Process

Peptide synthesis is initiated on a Merrifield or Pam pre-loaded resin or with mBHA for peptides produced as C-terminal amides. Each synthetic cycle consists of a deprotection step with 50% TFA in MeCL2. The resin is washed repetitively with MeCl2 and MeOH. The TFA salt formed is neutralized with a base wash of 10% TEA in MeCl2. The resin is washed with MeCl2 and MeOH and lastly with DMF prior to coupling steps. A colorimetric test is conducted to ensure deprotection. Each coupling is mediated with diisopropyl carbodiimide with HOBT to form the active ester. Each coupling is allowed to continue for 2 hr at RT or overnight on difficult couplings. Recouplings are conducted with either Uronium or Phosphonium reagents until a negative colorimetric test is obtained for free primary amines. The resin is then washed with DMF, MeCl2 and MeOH and prepared for the next solid-phase step. Cys protection utilizes Cys(Acm) at positions 7 and 15, and Cys(MeB) at Cys 4 and Cys12.

Cleavage and simultaneous deprotection is accomplished by treatment with HF using anisole as a scavenger (9:1:1) ml:ml:g (resin) at 0°C for 60 min. The peptide is subsequently extracted from the resin and precipitated in ice cold ether. The introduction of disulfide bonds and purification follows the exact same protocol described above for the *Fmoc-produced* product.

EXAMPLE 2: *IN VITRO* PROTEOLYTIC STABILITY USING SIMULATED GASTRIC FLUID (SGF) DIGESTION

The stability of SP-304 in the presence of simulated gastric fluid (SGF) was determined. SP-304 (final concentration of 8.5 mg/ml) was incubated in SGF (Proteose peptone (8.3 g/liter; Difco), D-Glucose (3.5 g/liter; Sigma), NaCl (2.05 g/liter; Sigma), KH_2PO_4 (0.6 g/liter; Sigma), $CaCl_2$ (0.11 g/liter), KCl (0.37 g/liter; Sigma), Porcine bile (final 1 X concentration 0.05 g/liter; Sigma) in PBS, Lysozyme (final 1 X concentration 0.10 g/liter; Sigma) in PBS, Pepsin (final 1 X concentration 0.0133 g/liter; Sigma) in PBS). SGF was made on the day of the experiment and

the pH was adjusted to 2.0 ± 0.1 using HCl or NaOH as necessary. After the pH adjustment, SGF is filter sterilized with 0.22 μm membrane filters. SP-304 (final concentration of 8.5 mg/ml) was incubated in SGF at 37°C for 0, 15, 30, 45, 60 and 120 min, respectively, in triplicate aliquots. Following incubations, samples were snap frozen in dry ice then stored in a -80°C freezer until assayed in duplicate.

Figure 1A is a bar chart showing the biological activity of SP-304 after incubation with SGF for times as indicated. The activity at 0 min was taken as 100%. The data are an average of triplicates ± SD for each data point. The data demonstrate that SP-304 is not sensitive to digestion with SGF. In addition, the data also suggest that the activity of SP-304 is not affected by exposure to the acidic pH of the SGF.

These results were further confirmed by the HPLC analyses of the samples after digestion with SGF. Here, aliquots of samples from all digestions were analyzed using a previously developed method for analyzing SP-304 peptide using HPLC. Samples from the SGF digestions were diluted to give a final concentration 0.17 mg/mL of SP-304. Figure 1B shows HPLC chromatographs of SP-304 samples after incubation with SGF at indicated times. The major peak of SP-304 did not change following digestion with SGF, indicating that the peptide was resistant to SGF digestion.

EXAMPLE 3: *IN VITRO* PROTEOLYTIC STABILITY USING SIMULATED INTESTINAL FLUID (SIF) DIGESTION

The stability of SP-304 was also evaluated after incubation with simulated intestinal fluid (SIF). SIF solution was prepared by the method as described in the United States Pharmacopoeia, 24th edition, p2236. The recipe to prepare SIF solution was as described below. The SIF solution contained NaCl (2.05 g/liter; Sigma), KH_2PO_4 (0.6 g/liter; Sigma), $CaCl_2$ (0.11 g/liter), KCl (0.37 g/liter; Sigma), and Pacreatin 10 mg/ml. The pH was adjusted to 6 and the solution was filter sterilized. A solution of SP-304 (8.5 mg/ml) was incubated in SGF at 37°C for 0, 30, 60, 90, 120, 150 and 300 min respectively, in triplicate aliquots. Following incubations, samples were removed and snap frozen with dry ice and stored in a -80°C freezer until assayed in duplicate. Figure 2A is a bar chart showing the ability of SP-304, after incubation in SIF for times as indicated, to stimulate cGMP synthesis in T84 cells. The cGMP stimulation activity at 0 min was taken as 100%. The data are an average of 3 triplicates ± SD.

The data indicated that the biological activity of SP-304 is reduced by 30% following digestion with SIF. This could be due to degradation of the peptide. Hence, samples after digestion with SIF were further analyzed by HPLC.

The integrity of SP-340 peptide exposed to SIF was evaluated by HPLC by essentially using the method described for SGF digestion. Figure 2B is a schematic representation of the results of HPLC chromatographic analyses of SP-304 samples after incubation with heat-inactivated SIF for 300 min, and SIF for 120 min, respectively. The major peak of SP-304, which elutes at 16.2 min was converted into another peak at 9.4 min and a few minor peptide peaks. Thus, it was important to find out structures of the metabolites of SP-304 produced after digestion with SIF. SP-304 peptide was incubated with SIF for various times and the peptide digestion products were isolated and subjected to structure elucidation by MS analysis.

Figure 3 is a schematic representation of the possible metabolites of SP-304. The major degradation products involve N and D clipped from the N-terminus and L from the C-terminus of SP304. However, there was only 30% reduction in biological activity, implying that one or more of the degradation products were also biologically active. To address this possibility, several truncated peptides were synthesized and evaluated for their abilities to stimulate cGMP synthesis in T84 cells (Figure 4).

Figure 4 shows data from the analyses of various peptides in the T84 cell cGMP stimulation assay (essentially as described in Shailubhai, *et al.*, Cancer Research 60, 5151-5157 (2000) . Briefly, 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) and pre-incubated at 37°C for 10 minutes with 250 µl of DMEM containing 50 mM HEPES (pH 7.4) and 1 mM isobutyl methylxanthine (IBMX). Monolayers of T84 cells were then incubated with 250 µl of DMEM containing 50 mM HEPES (pH 7.4) containing one of the peptides shown in the Figure 4 at a concentration of 1.0 µM for 30 min. After the 30 min incubation, the medium was aspirated and the reaction was terminated by the addition of 3% perchloric acid. Following centrifugation and the addition of NaOH (0.1 N) to neutralize the pH, intracellular cGMP levels were determined in lysates using a cGMP ELISA kit (Cat. No. 581021 ; Cayman Chemical, Ann Arbor, MI). Samples were run in duplicates incubations and each sample was run as duplicates in ELISA test.

The data suggest that the leucine (L) residue at the C-terminus of SP-304 contributes to the biological potency of the peptide. For example, there was considerable reduction in potency when L was deleted from SP-304, as in SP-338. Similarly, the peptides SP-327, SP-329 and SP-331, without L at the C-terminal, also showed 20-25% reduction in biological potency as compared to their counterpart peptides with L at the C-terminus, as in SP-326, SP-328 and SP-330 peptides. In addition, results also suggest that amino acid residues at the N-terminus might also be important for stability and/or potency of the peptides. Based on these results, several new peptides were synthesized with D-forms of amino acids replacing the corresponding L-forms at the C- and N-termini of the peptides. These peptides were evaluated for their abilities to stimulate cGMP synthesis in T84 cells as shown in Figure 5.

The results presented in Figure 5 suggest that substitution of L-amino acids with D-amino acids at the C- and N-termini did not significantly alter their potency. Peptides SP-332, SP-333 and SP-335 showed comparable ability to stimulate cGMP synthesis in T84 cells. On the other hand, the substitution of L-leucine with D-leucine at the 6^{th} position in SP-337 resulted in a complete loss in its ability to stimulate cGMP synthesis in T84 cells. These results suggest that the amino acid residues Asn, Asp and Glu at the N-terminus and Leu at the C-terminus can be replaced with their respective D- amino acid forms. However, the leucine at the 6^{th} position can not be replaced with its D-form.

Figure 7 (A-F) shows the stabilities of peptides SP-332, SP-333 and SP-304 when incubated with SIF for two hours. The results demonstrated that the peptide SP-333, which has D-Asn at the N-terminus and D-Leu at the C-terminus, was virtually completely resistant to digestion with SIF (Figure 7F), and remained virtually 100% biologically active after a two hour incubation in SIF (Figure 7A). The peptide SP-332 with D-Leu at the C-terminus showed some reduction in potency following the 120 min incubation with SIF (Figure 7B). However, the HPLC analyses of SP-332 did not reveal any degradation of the peptide (Figure 7E), suggesting that these peptides are completely resistant to proteoysis by SIF. On the other hand, the peptide SP-304 lost about 30% of its potency following digestion with SIF for just one hour (Figure 7C). The HPLC analysis of SP-304 following SIF incubation confirmed its degradation (Figure 7D). These results suggest that the peptide SP-304 undergoes proteolysis following incubation with SIF, whereas substitution of L-Asn with D-Asn at the N-terminus plus the substitution of L-Leu

with D-Leu at the C-terminus protects SP-333 against digestion with SIF. Thus, the peptide SP-333 appears more stable and potent as a drug candidate.

EXAMPLE 4: CYCLIC cGMP STIMULATION ASSAYS

The ability of the GCRA peptide to bind to and activate the intestinal GC-C receptor was tested by usingT 84 human colon carcinoma cell line. Human T84 colon carcinoma cells were obtained from the American Type Culture Collection. 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 µg/ml streptomycin. The cells were fed fresh medium every third day and split at a confluence of approximately 80%.

Biological activity of the GCRA peptides was assayed as previously reported (Shailubhai, *et al.*, Cancer Research 60, 5151-5157 (2000)). 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 GCRA peptides (0.1 nM to 10 .mu.M) 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, Mich.).

Figure 6 shows results from the experiments evaluating potency of peptides that are similar to the *E. coli* enterotoxin ST peptide in the cGMP stimulation assay (as above). Among these the peptides SP-353 and SP-354 were found to be quite potent to stimulate cGMP synthesis in T84 cells. Particularly, the peptide SP-353 that has Ser residue at the 6^{th} position was found to be the most potent among the peptides tested. The peptide SP-355 that has D-Tyr at the C-terminus showed potency markedly less than the other peptides.

EXAMPLE 5: PEGGYLATED PEPTIDES

An additional strategy to render peptides more resistant towards digestion by digestive proteases is to peggylate them at the N- and C-terminus. The peptide SP-333 was peggylated with the aminoethyloxy-ethyloxy-acetic acid (Aeea) group at the C-terminus (SP-347) or at the N-terminus (SP-350) or at both termini (SP-343). Cyclic GMP synthesis in T84 cells was measured by the method as described above.

The peptides SP-347 and SP-350 showed potencies comparable to SP-333 in their abilities to stimulate cGMP synthesis in T84 cells. However, peptide SP-343 was considerably less potent as compared to the other peptides tested. The poor activity of SP-343 might be due to the considerable steric hindrance afforded by the large Aeea groups at both termini.

EXAMPLE 6: COMBINATION OF GUANYLATE CYCLASE AGONISTS WITH PHOSPHODIESTERASE INHIBITORS

Regulation of intracellular concentrations of cyclic nucleotides (*i.e.*, cAMP and cGMP) and thus, signaling via these second messengers, has been generally considered to be governed by their rates of production versus their rates of destruction within cells. Thus, levels of cGMP in tissues and organs can also be regulated by the levels of expression of cGMP-specific phosphodiesterases (cGMP-PDE), which are generally overexpressed in cancer and inflammatory diseases. Therefore, a combination consisting of an agonist of GC-C with an inhibitor of cGMP-PDE might produce synergistic effect on levels of cGMP in the target tissues and organs.

Sulindac Sulfone (SS) and Zaprinast (ZAP) are two of the known inhibitors of cGMP-PDE and have been shown to induce apoptosis in cancer cells via a cGMP-dependent mechanism. SS and ZAP in combination with SP304 or SP-333 was evaluated to see if these PDE inhibitors had any synergistic effect on intracellular accumulation of cGMP (Fig. 9-12). As the data shows, SS at concentration of 100 μM did not enhance intracellular accumulation of cGMP. However, the combination SS with SP304 stimulated cGMP production several fold more then the stimulation by SP304 used alone. This synergistic effect on cGMP levels was more pronounced when SP304 were used at 0.1 μM concentration (Fig 10). Similar observations were made when SP304 or SP333 were used in combination with ZAP (Fig 10, Fig 11 and Fig 12). These results suggest that the intracellular levels of cGMP are stabilized because SS inhibits cGMP-PDE that might be responsible for depletion of intracellular cGMP. Thus, the approach to use a combination of GC-C agonist with a cGMP-PDE inhibitor is attractive.

For the results shown in Figure 9, cyclic GMP synthesis in T84 cells was assessed essentially as described in Shailubhai et al., Cancer Research 60, 5151-5157 (2000). Briefly, 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) and pre-incubated at 37°C for 10 minutes with 250 μl of DMEM containing 50 mM HEPES (pH 7.4) and 1 mM isobutyl methylxanthine (IBMX).

Monolayers of T84 cells were then incubated with 250 µl of DMEM containing 50 mM HEPES (pH 7.4) containing SP-304 or PDE inhibitors either alone or in combinations, as indicated below in the following experimental sets: 1) Control; 2) SP-304 (0.1 µM); 3) Sulindac Sulfone (100 µM); 4) Zaprinast (100 µM); 5) SP-304 (0.1 µM) + Sulindac Sulfone (100 µM); and 6) SP-304 (0.1 µM) + Zaprinast (100 µM). After the 30 min incubation, the medium was aspirated and the reaction was terminated by the addition of 3% perchloric acid. Following centrifugation and the addition of NaOH (0.1 N) to neutralize the pH, intracellular cGMP levels were determined in lysates using a cGMP ELISA kit (Cat. No. 581021 ; Cayman Chemical, Ann Arbor, MI). Samples were run in duplicates incubations and each sample was run as duplicates in ELISA test.

For the results shown in Figure 10, the method used was same as the one used for Fig. 9 except that the monolayers of T84 cells were incubated with 500 µl of DMEM containing 50 mM HEPES (pH 7.4) containing SP-304 (0.1 or 1.0 µM) or increasing concentrations of PDE inhibitors (0 to 750 µM) either alone or in combination with SP-304. After the 30 min incubation, the medium was aspirated and the reaction was terminated by the addition of 3% perchloric acid. Following centrifugation and the addition of NaOH (0.1 N) to neutralize the pH, intracellular cGMP levels were determined in lysates using a cGMP ELISA kit (Cat. No. 581021; Cayman Chemical, Ann Arbor, MI). Samples were run as triplicates in ELISA test.

For the results shown in Figure 11, the method used was same as the one used for Fig. 10 except that the monolayers of T84 cells were incubated with 500 µl of DMEM containing 50 mM HEPES (pH 7.4) containing SP-3333 (0.1 or 1.0 µM) or increasing concentrations of ZAP (0 to 500 µM) either alone or in combination with SP-333. After the 30 min incubation, the medium was aspirated and the reaction was terminated by the addition of 3% perchloric acid. Following centrifugation and the addition of NaOH (0.1 N) to neutralize the pH, intracellular cGMP levels were determined in lysates using a cGMP ELISA kit (Cat. No. 581021 ; Cayman Chemical, Ann Arbor, MI). Samples were run as triplicates in ELISA test.

For the results shown in Figure 12, the method used was same as the one used for Fig. 10 except that the monolayers of T84 cells were incubated with 500 µl of DMEM containing 50 mM HEPES (pH 7.4) containing SP-333 (0.1 µM) or increasing concentrations of Sulindac Sulfone (0 to 500 µM) either alone or in combination with SP-333. After the 30 min incubation, the medium was aspirated and the reaction was terminated by the addition of 3% perchloric acid.

Following centrifugation and the addition of NaOH (0.1 N) to neutralize the pH, intracellular cGMP levels were determined in lysates using a cGMP ELISA kit (Cat. No. 581021 ; Cayman Chemical, Ann Arbor, MI). Samples were run as triplicates using the ELISA test.

EXAMPLE 7: AN ORAL RANGE-FINDING TOXICITY STUDY IN CYNOMOLGUS MONKEYS.

The objective of the study is to determine the toxicity of the GRCA peptides according to the invention following a single oral gavage administration to the cynomolgus monkey and to allow assessment of reversibility of any changes following a minimum 7-day observation/washout period. Each GRCA peptide according to the invention will be given at two different dose levels.

Experimental Design

The test (e.g., the GRCA peptides according to the invention) and control/vehicle article will be administered in three phases separated by a minimum 7-day observation period. Each phase will consist of a single oral gavage administration to female cynomolgus monkeys as indicated in the tables below:

Phase 1:

Eight non-naive female cynomolgus monkeys will be transferred from the ITR Spare Monkey colony and assigned to four dose groups as follows:

Group Number	Group Designation	Study Days	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals (Females)
1	Control/Vehicle	1	0	0	10	2
		4				
2	Test Peptides	1	1	0.1	10	2
		4				
		4				

Following completion of the Phase 1 dosing, all monkeys will be observed for 33 days. Upon completion of the observation period, all monkeys will be transferred back to the ITR Spare Monkey Colony.

Phase 2:

The same eight non-naïve female cynomolgus monkeys as previously used in Phase 1 will be transferred from the ITR Sparc Monkey colony and assigned to four dose groups as follows:

Group Number	Group Designation	Study Day	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals (Females)
1	Control/Vehicle	1	10	1	10	2
2	Test Peptides	1	10	1	10	2

Following completion of the Phase 2 dosing, all monkeys will be observed for a minimum of 7 days.

Route of Administration

The oral route of administration has been chosen because it is a preferred human therapeutic route.

Preparation of Test and Control /Vehicle Articles

The test and control/vehicle articles will be prepared fresh on the day of dosing in cold distilled water (maintained in an ice water bath). A sufficient amount of test article powder will be added to the appropriate amount of distilled water in order to achieve the desired concentration. The dose formulations will be mixed by simple inversion.

Analysis of Test Article Concentration and Stability in the Dose Formulations

For possible confirmation of the concentration and stability of the test article in the formulations, representative samples will be taken from the middle of each concentration, including the control/vehicle article on the first day of dosing of each group, as indicated below. Samples will be collected immediately after preparation on Day 1 and again after dosing is completed on that day and will be stored frozen (approximately 80°C nominal) in 20 mL screw cap vials. Therefore, the remaining dose formulation vials will be returned to the Pharmacy Department as soon as possible after completion of dosing.

Group 1: 1.5 mL in duplicate from the middle on Day 1 (pre-dose and post-dose).

Group 2: 1.5 mL in duplicate from the middle on Day 1 (pre-dose and post-dose).

Group 3: 1.5 mL in duplicate from the middle on Day 1 (pre-dose and post-dose).

Group 4: 1.5 mL in duplicate from the middle on Day 1 (pre-dose and post-dose).

The formulations will be maintained cold in an ice water bath during all sampling procedures.

The formulations will be stirred continuously with a stir bar for a minimum of 15 minutes prior to sampling.

The samples will be retained frozen (approximately -80°C nominal) at ITR until requested by the Sponsor to be shipped to a laboratory designated by the Sponsor for analysis. The samples can be discarded once it is determined by the analyst and Study Director that they are no longer needed. These samples' disposition will be recorded in the raw data.

If analyzed, a Dose Formulation report will be prepared by the Principal Investigator (Formulation analysis) and will be provided to ITR for inclusion in the final report.

Test System

Species/Strain:	Cynomolgus Monkey *(Macaca Fasicularis)*
Source:	orldwide Primates Inc., P.O. Box 971279 Miami, Florida, 33187, USA *and* Covance Research Products Inc. P.O. Box 549 Alice, Texas, 78333, USA
Total No. of monkeys on study:	8 non-naive females
Body Weight Range:	2-4 kg at onset of treatment
Age Range at Start:	Young adult at onset of treatment
Acclimation Period:	The animals will be transferred from ITR's spare monkey colony. They are therefore, considered to be fully acclimated to the laboratory environment.

The actual age and body weight ranges will be noted in the final report.

Administration of the Test and Control/Vehicle Articles

The test and control/vehicle articles will be administered by oral gavage administration using a gavage tube attached to a syringe in three Phases separated by a minimum 7-day

observation/washout period. Each dosing session will consist of a single oral gavage administration. The gavage tube will be flushed with 3 mL of reverse osmosis water immediately following administration of the dose formulation in order to ensure that the entire dose volume has been delivered to the animal. The dose volume will be 10 mL/kg for all animals, including controls. The actual volume administered to each monkey on Day 1 of each Phase will be calculated using the Day -1 body weights of each Phase.

Dosing formulations will be maintained cold during dose administration by placing them in an ice water bath.

The dosing formulations must be placed on a stir plate for a minimum of 15 minutes prior to the start of dosing and maintained on the stir plate throughout the dosing procedure.

The dosing formulations must be used within 2 hours of preparation.

Clinical Observations

Cage-side clinical signs (ill health, behavioral changes etc.) will be recorded as indicated below except on detailed clinical examination days, where the morning cage-side clinical signs will be replaced by a detailed clinical examination (DCE). During regular cage side clinical signs and detailed examinations, particular attention will be paid to stools with respect to amount of stools produced, description of stools, etc.

Cage side clinical signs will be performed as follows:

During the pretreatment period and during the 7-day (minimum) observation periods: Three times per day with a minimum of 3 hours between each occasion.

On the dosing day of Phase 1: pre-dose, 2, 4, 6, 8 and 24 hours post-dosing

On the dosing day of Phase 2: pre-dose, continuously for the first 4 hours post-dose and at 6, 8 and 24 hours post-dosing

On the dosing day of Phase 3: pre-dose, continuously for the first 4 hours post-dose and at 6, 8 and 24 hours post-dosing

A detailed clinical examination of each monkey will be performed once at the time of animal transfer and once weekly thereafter.

Animals whose health status is judged to warrant additional evaluation will be examined by a Clinical Veterinarian, or a technician working under the supervision of the Clinical Veterinarian. Any veterinarian-recommended treatments will only be performed once agreement has been obtained from the Study Director. Where possible, the Sponsor will be consulted prior to administration of therapeutic drugs.

Body weights will be recorded for all animals once daily from the day of transfer through to the end of the study.

Food consumption will be recorded for all animals once daily from the day of transfer through to the end of the study.

Cages will be cleaned prior to the start of the daily food consumption to ensure no food cookies remain in the cage. Monkeys will be fed 7 cookies before 12pm and 7 cookies after 12pm. The sum of the total number of cookies given for the day will be recorded.

The next morning, a visual check will be performed to see how many cookies are left in the cage. The number of whole cookies remaining in the food hopper or on the tray will be recorded. The number of whole cookies left will be subtracted from the total number of cookies given in order to calculate the number of cookies eaten.

EXAMPLE 8: SUCKLING MOUSE MODEL OF INTESTINAL SECRETION (SUMI ASSAY)

The GCRA peptides described herein can be tested for their ability to increase intestinal secretion using a suckling mouse model of intestinal secretion. In this model a GCRA peptide is administered to suckling mice that are between seven and nine days old. After the mice are sacrificed, the gastrointestinal tract from the stomach to the cecum is dissected ("guts"). The remains ("carcass") as well as the guts are weighed and the ratio of guts to carcass weight is calculated. If the ratio is above 0.09, one can conclude that the test compound increases intestinal secretion. Controls for this assay may include wild-type SP-304, ST polypeptide and Zelnorm®.

Phenylbenzoquinone-induced writhing model

The PBQ-induced writhing model can be used to assess pain control activity of the GCRA peptide described herein. This model is described by Siegmund et al. (1957 Proc. Soc. Exp. Bio. Med. 95:729-731). Briefly, one hour after oral dosing with a test compound, e.g., a GCRA peptide, morphine or vehicle, 0.02% phenylbenzoquinone (PBQ) solution (12.5 mL/kg)

is injected by intraperitoneal route into the mouse. The number of stretches and writhings are recorded from the 5th to the 10th minute after PBQ injection, and can also be counted between the 35th and 40th minute and between the 60th and 65th minute to provide a kinetic assessment. The results are expressed as the number of stretches and writhings (mean ± SEM) and the percentage of variation of the nociceptive threshold calculated from the mean value of the vehicle-treated group. The statistical significance of any differences between the treated groups and the control group is determined by a Dunnett's test using the residual variance after a one-way analysis of variance ($P < 0.05$) using SigmaStat Software.

EXAMPLE 9 : PHARMACOKINETIC PROPERTY DETERMINATION OF GCRA PEPTIDES

Serum samples are extracted from the whole blood of exposed (mice dosed orally or intravenously with GCRA peptides (s) described herein) and control mice, then injected directly (10 mL) onto an in-line solid phase extraction (SPE) column (Waters Oasis HLB 25μm column, 2.0 x 15mm direct connect) without further processing. The sample on the SPE column is washed with a 5% methanol, 95% dH_2O solution (2.1 mL/min, 1.0 minute), then loaded onto an 0 analytical column using a valve switch that places the SPE column in an inverted flow path onto the analytical column (Waters Xterra MS C8 5μm IS column, 2.1 x 20mm). The sample is eluted from the analytical column with a reverse phase gradient (Mobile Phase A: 10 mM ammonium hydroxide in dH_2O, Mobile Phase B: 10 mM ammonium hydroxide in 80% acetonitrile and 20% methanol; 20% B for the first 3 minutes then ramping to 95% B over 4 min. and holding for 2 5 min., all at a flow rate of 0.4 mL/min.). At 9.1 minutes, the gradient returns to the initial conditions of 20%B for 1 min. polypeptide is eluted from the analytical column and is detected by triple-quadrapole mass spectrometry (MRM, 764 (+2 charge state)>182 (+1 charge state) Da; cone voltage = 30V; collision = 20 eV; parent resolution = 2 Da at base peak; daughter resolution = 2 Da at base peak). Instrument response is converted into concentration units by comparison with a standard curve using known amounts of chemically synthesized polypeptide(s) prepared and injected in mouse plasma using the same procedure.

Similarly, pharmacokinetic properties are determined in rats using LCMS methodology. Rat plasma samples containing the GCRA peptide are extracted using a Waters Oasis MAX 96 well solid phase extraction (SPE) plate. A 200 μL volume of rat plasma is mixed with 200 μL of ^{13}Cg, ^{15}N -labeled polypeptide in the well of a prepared SPE plate. The samples are drawn

through the stationary phase with 15 mm Hg vacuum. All samples are rinsed with 200 μL of 2% ammonium hydroxide in water followed by 200 μL of 20% methanol in water. The samples are eluted with consecutive 100 μL volumes of 5/20/75 formic acid/water/methanol and 100 μL 5/15/80 formic acid/water/methanol. The samples are dried under nitrogen and resuspended in 100 μL of 20% methanol in water. Samples are analyzed by a Waters Quattro Micro mass spectrometer coupled to a Waters 1525 binary pump with a Waters 2777 autosampler. A 40 μL volume of each sample is injected onto a Thermo Hypersil GOLD C18 column (2.1x50 mm, 5 um). polypeptide is eluted by a gradient over 3 minutes with acetonitrile and water containing 0.05% trifluoroacetic acid. The Quattro Micro mass spectrometer is run in multiple reaction monitoring (MRM) mode using the mass transitions of, for example 764>182 or 682>136. Using this methodology, polypeptide is dosed orally and by IV to rats at 10 mg/kg. Pharmacokinetic properties including area under the curve and bioavailabilty are determined.

EXAMPLE 10: DIURESIS RELATED EXPERIMENTS EFFECT ON DIURESIS AND NATRIURESIS

The effect of GCRA peptides described herein on diuresis and natriuresis can be determined using methodology similar to that described in WO06/001931 (examples 6 (p. 42) and 8 (p.45)). Briefly, the polypeptide/agonist described herein (180-pmol) is infused for 60 min into a group of 5 anesthetized mice or primates. Given an estimated rat plasma volume of 10 mL, the infusion rate is approximately 3 pmol/mL/min. Blood pressure, urine production, and sodium excretion are monitored for approximately 40 minutes prior to the infusion, during the infusion, and for approximately 50 minutes after the infusion to measure the effect of the GCRA peptides on diuresis and natriuresis. For comparison, a control group of five rats is infused with regular saline. Urine and sodium excretion can be assessed. Dose response can also be determined. polypeptide/GC-C agonist described herein is infused intravenously into mice or primates over 60 minutes. Urine is collected at 30 minute intervals up to 180 minutes after termination of polypeptide/GC-C agonist infusion, and urine volume, sodium excretion, and potassium excretion are determined for each collection interval. Blood pressure is monitored continuously. For each dose a dose-response relationship for urine volume, sodium and potassium excretion can be determined. Plasma concentration of the polypeptide/GC-agonist is also determined before and after iv infusion.

Mouse or Primate Diuresis Experiment: Once an appropriate level of anesthesia has been achieved, a sterile polyurethane catheter is inserted into the urethra and secured using 1 - 2 drops of veterinary bond adhesive applied to urethra/catheter junction. Animals are then dosed with either vehicle or test article via the intravenous or intraperitoneal route. Animals are allowed to regain consciousness, and the volume of urine excreted over a 1-5 hour duration is recorded periodically for each rat.

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Wc claim:

1. A peptide consisting essentially of the amino acid sequence of any one of SEQ ID NO:2-54 and 57-98.

2. A pharmaceutical composition in unit dose comprising a guanylate cyclase receptor agonist peptide having the sequence of any one of NO:2-54 and 56-94 present in a therapeutically effective amount and a pharmacetical carrier, excipient or diluent.

3. The peptide of claim 1, wherein said peptide is SEQ ID NO: 8, 9, 10, 58 or 59.

4. The pharmaceutical composition of claim 2, wherein said peptide is SEQ ID NO: 8, 9, 10, 58 or 59.

5. The peptide of claim 1, wherein said peptide is SEQ ID NO: 45-54 and said peptide increases cGMP production in a cell and wherein said peptide is not SEQ ID NO:1.

6. The pharmaceutical composition of claim 2, wherein said peptide is SEQ ID NO: 45-54, and said peptide increases cGMP production in a cell and wherein said peptide is not SEQ ID NO:1.

7. The peptide of claim 1, wherein said peptide is SEQ ID NO: 87-98, and said peptide increases cGMP production in a cell and wherein said peptide is not SEQ ID NO:55 or 56.

8. The pharmaceutical composition of claim 2, wherein said peptide is SEQ ID NO: 87-98, and said peptide increases cGMP production in a cell and wherein said peptide is not SEQ ID NO:55 or 56.

9. The pharmaceutical composition of any one of claims claim 2, 4, 6, or 8, wherein the unit dose form is selected from the group consisting of a tablet, a capsule, a solution or inhalation formulation.

10. A method for preventing or treating a condition selected from the group consisting of Ulcerative Colitis, Irritable bowel syndrome (IBS), non-ulcer dyspepsia chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, constipation associated with use of opiate pain killers, gastroesophageal reflux disease (GERD), post surgical constipation, gastroparesis, constipation associated with neuropathic disorders, heartburn, poor gastrointestinal motility , congestive heart failure, hypertension, benign prostatic hyperplasia (BPH), colon cancer, lung cancer, bladder cancer, liver cancer,

salivary gland cancer or skin cancer, bronchitis, tissue inflammation, organ inflammation, respiratory inflammation, asthma, COPD comprising administering toa patient in need thereof, an effective dosage of a guanylate cyclase receptor agonist having the sequence of any one of NO:2-54 and 56-94.

11. The method of claim 10, wherein said peptide is SEQ ID NO: 8, 9, 10, 58 or 59.

12. A method of claim 11 or 12, further comprising administering an effective dose of inhibitor of a cGMP-specific phosphodiesterase.

13. The method of claim 12, 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.

14. The method of claim 12, wherein said cGMP-dependent phosphodiesterase inhibitor is selected from the group consisting of suldinac sulfone, zaprinast, and motapizone, vardenifil, and suldenifil.

15. The method of claim 12, futher comprising administering an effective does of at least one anti-inflammatory agent.

16. The method of claim12, wherein an anti-inflammatory agent is a steroid or nonsteroid anti-inflammatory drug (NISAIDS).

17 The use of any one of the peptides having the sequence of any one of SEQ ID NO:2-54 and 56-94 in the manufacture of a medicament for the treatment of a human disease.

18. The useof claim 17, wherein said peptide is SEQ ID NO: 8, 9, 10, 58 or 59.

19. A method of increasing cGMP production in a cell comprising contacting said cell with a peptide selected from the group consisting of the amino acid sequence of SEQ ID NO:2-54 and 57-98.

20. The method of claim 19, further comprising contacting said cell with a phosphodiesterase inhibitor.

21. The method of claim 20, wherein said cGMP-dependent phosphodiesterase inhibitor is selected from the group consisting of suldinac sulfone, zaprinast, and motapizone, vardenifil, and suldenifil.

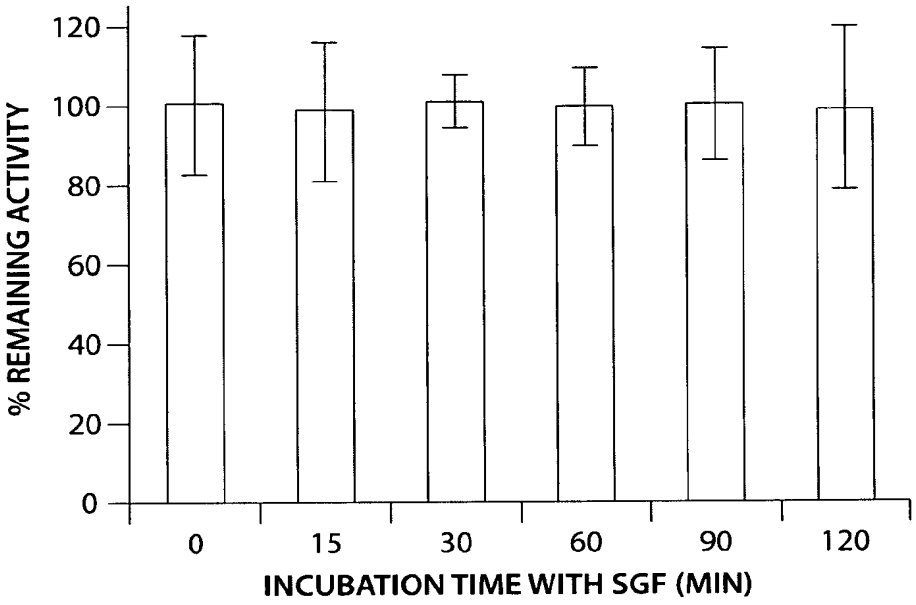
120
100
80
60
40
20
0
% REMAINING ACTIVITY
0
15
30
60
90
120
INCUBATION TIME WITH SGF (MIN)

Fig. 1A

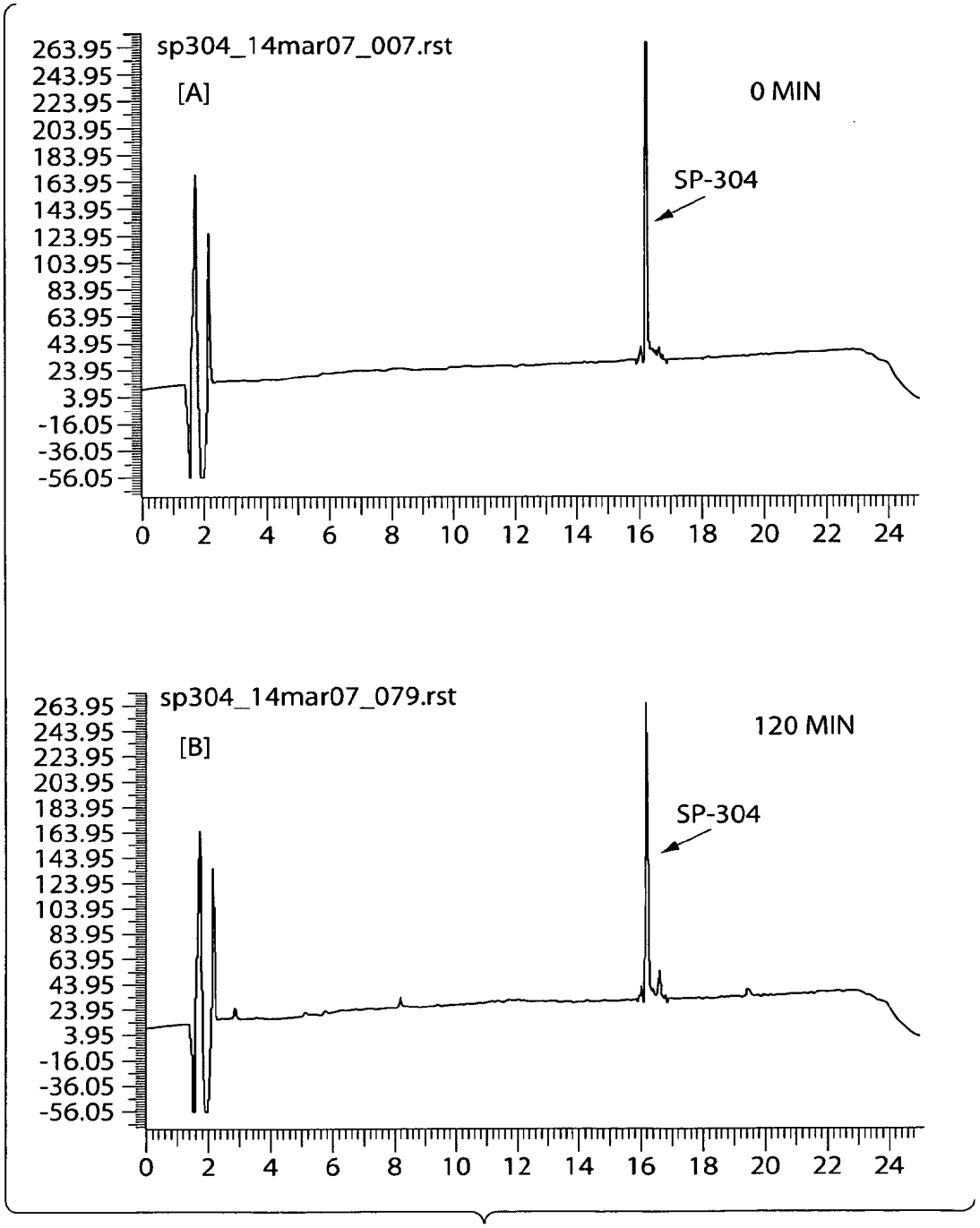
sp304_14mar07_007.rst
[A]
0 MIN
SP-304
263.95
243.95
223.95
203.95
183.95
163.95
143.95
123.95
103.95
83.95
63.95
43.95
23.95
3.95
-16.05
-36.05
-56.05
0 2 4 6 8 10 12 14 16 18 20 22 24
sp304_14mar07_079.rst
[B]
120 MIN
SP-304
263.95
243.95
223.95
203.95
183.95
163.95
143.95
123.95
103.95
83.95
63.95
43.95
23.95
3.95
-16.05
-36.05
-56.05
0 2 4 6 8 10 12 14 16 18 20 22 24

Fig. 1B

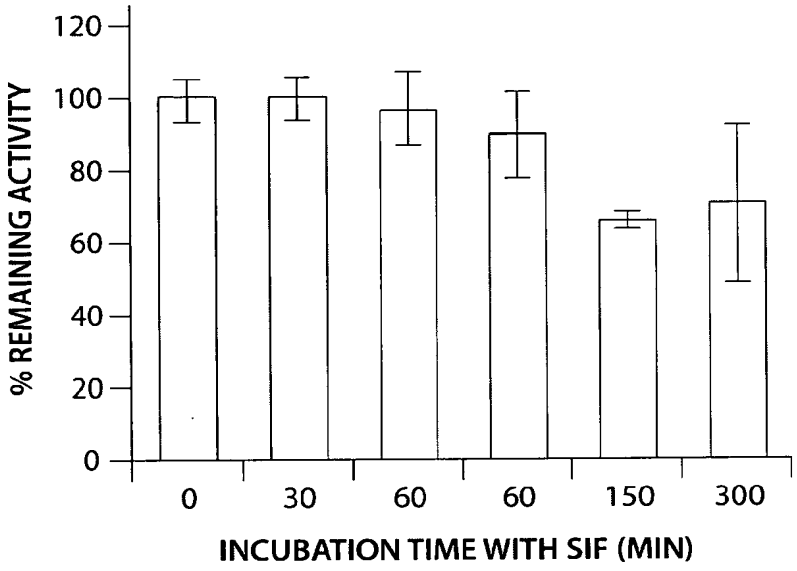
120
100
80
60
40
20
0
% REMAINING ACTIVITY
0
30
60
60
150
300
INCUBATION TIME WITH SIF (MIN)

Fig. 2A

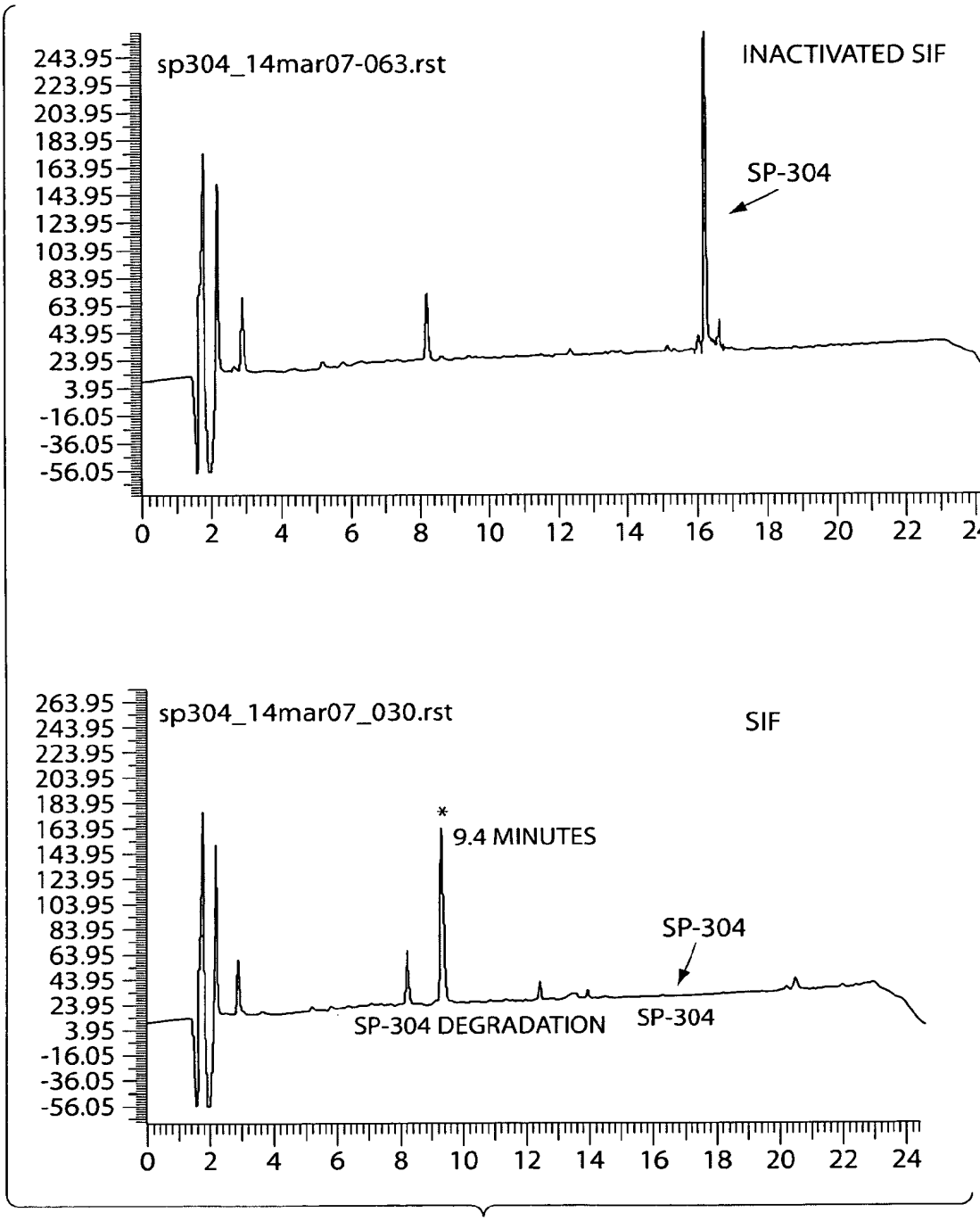
243.95
223.95
203.95
183.95
163.95
143.95
123.95
103.95
83.95
63.95
43.95
23.95
3.95
-16.05
-36.05
-56.05
sp304_14mar07-063.rst
INACTIVATED SIF
SP-304
0 2 4 6 8 10 12 14 16 18 20 22 24
263.95
243.95
223.95
203.95
183.95
163.95
143.95
123.95
103.95
83.95
63.95
43.95
23.95
3.95
-16.05
-36.05
-56.05
sp304_14mar07_030.rst
SIF
*
9.4 MINUTES
SP-304
SP-304 DEGRADATION
SP-304
0 2 4 6 8 10 12 14 16 18 20 22 24

Fig. 2B

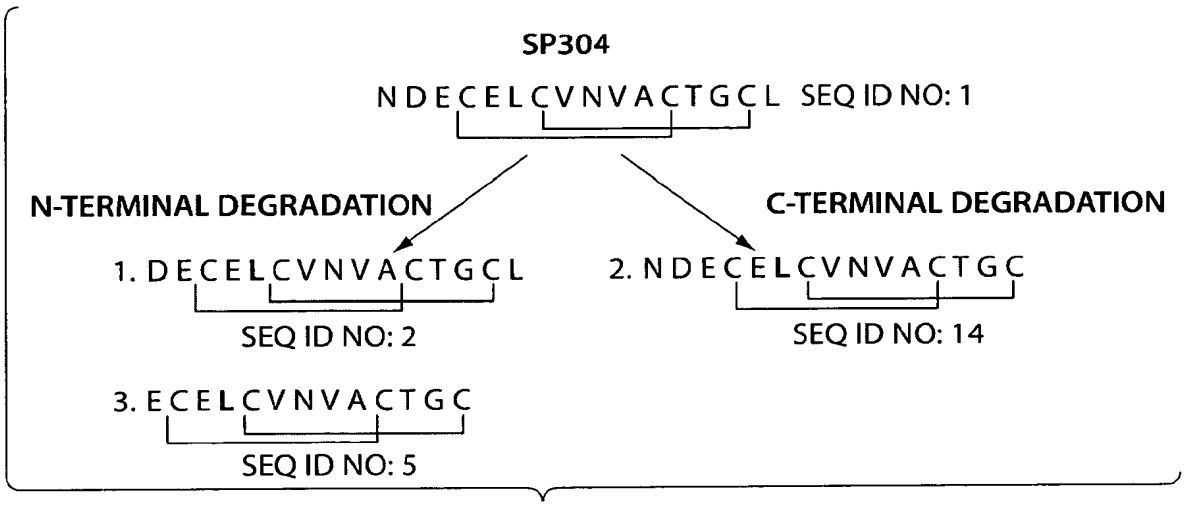
SP304
N D E C E L C V N V A C T G C L SEQ ID NO: 1
N-TERMINAL DEGRADATION
C-TERMINAL DEGRADATION
1. D E C E L C V N V A C T G C L
SEQ ID NO: 2
2. N D E C E L C V N V A C T G C
SEQ ID NO: 14
3. E C E L C V N V A C T G C
SEQ ID NO: 5

Fig. 3

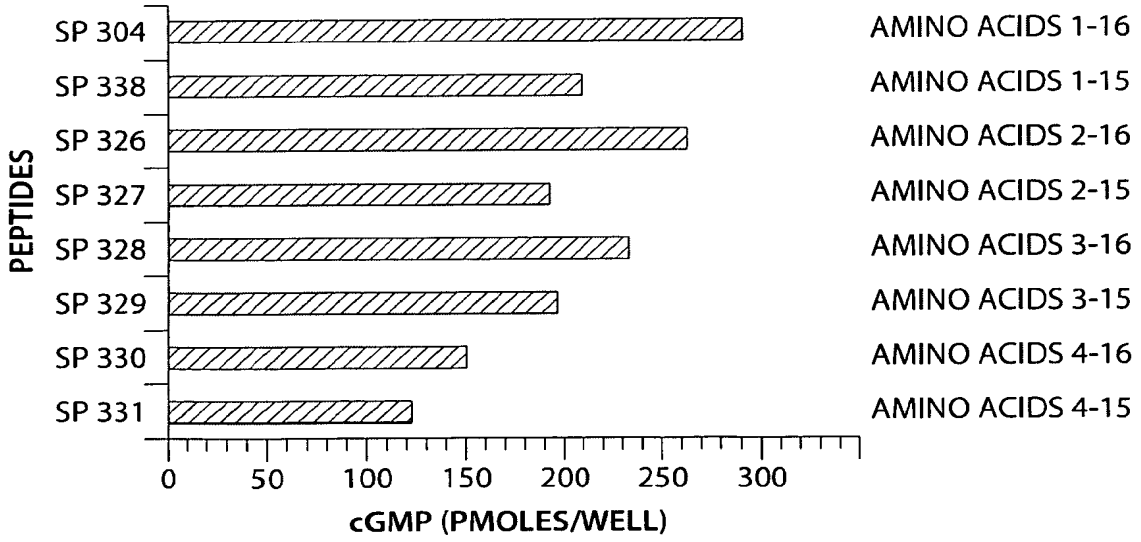
PEPTIDES
SP 304
SP 338
SP 326
SP 327
SP 328
SP 329
SP 330
SP 331
AMINO ACIDS 1-16
AMINO ACIDS 1-15
AMINO ACIDS 2-16
AMINO ACIDS 2-15
AMINO ACIDS 3-16
AMINO ACIDS 3-15
AMINO ACIDS 4-16
AMINO ACIDS 4-15
0
50
100
150
200
250
300
cGMP (PMOLES/WELL)

Fig. 4

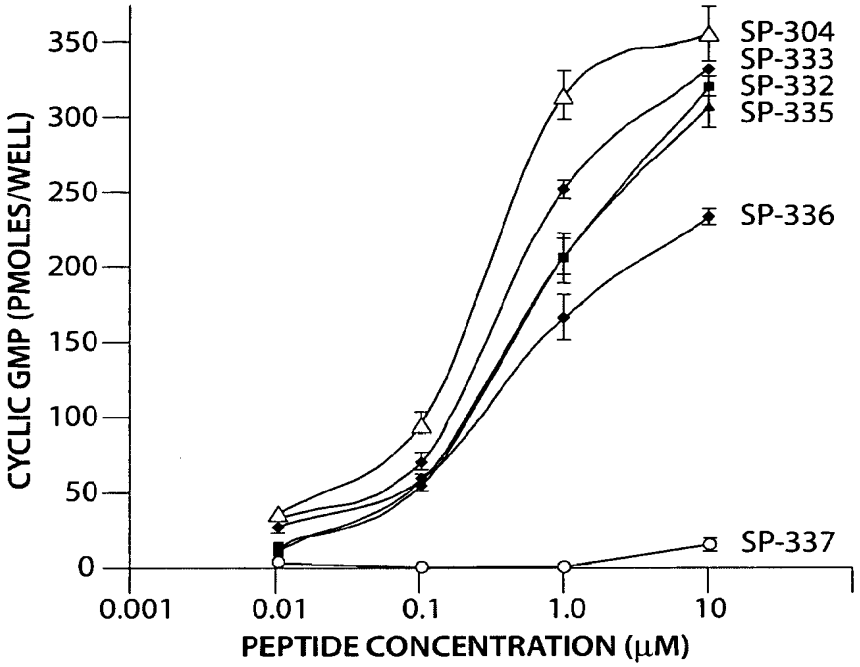
CYCLIC GMP (PMOLES/WELL)
350
300
250
200
150
100
50
0
0.001
0.01
0.1
1.0
10
PEPTIDE CONCENTRATION (μM)
SP-304
SP-333
SP-332
SP-335
SP-336
SP-337

Fig. 5

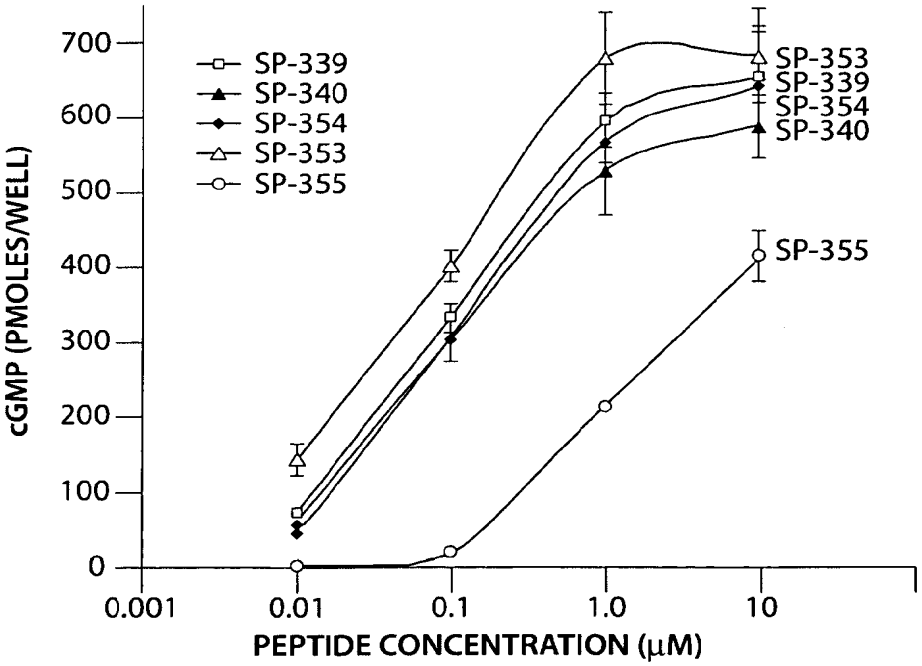
cGMP (PMOLES/WELL)
700
600
500
400
300
200
100
0
0.001
0.01
0.1
1.0
10
PEPTIDE CONCENTRATION (μM)
SP-339
SP-340
SP-354
SP-353
SP-355
SP-353
SP-339
SP-354
SP-340
SP-355

Fig. 6

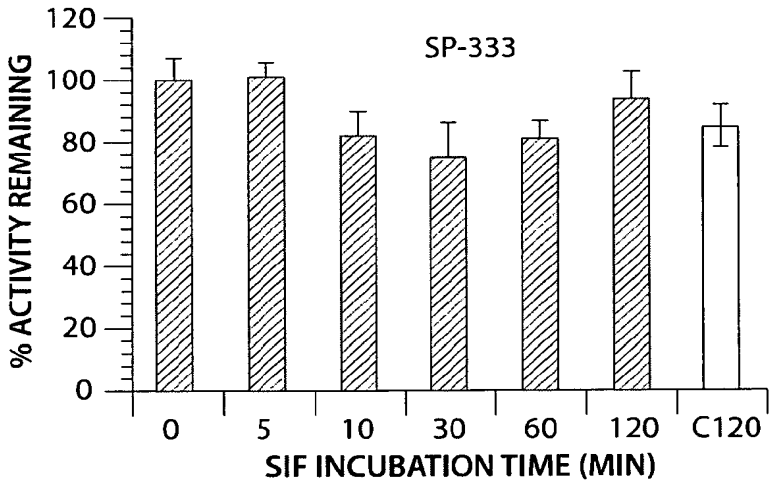
SP-333
% ACTIVITY REMAINING
120
100
80
60
40
20
0
0
5
10
30
60
120
C120
SIF INCUBATION TIME (MIN)

Fig. 7A

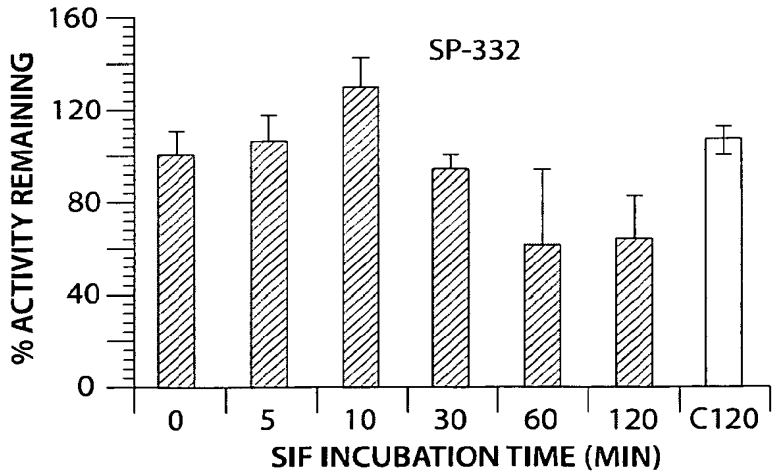
SP-332
% ACTIVITY REMAINING
160
120
80
40
0
0
5
10
30
60
120
C120
SIF INCUBATION TIME (MIN)

Fig. 7B

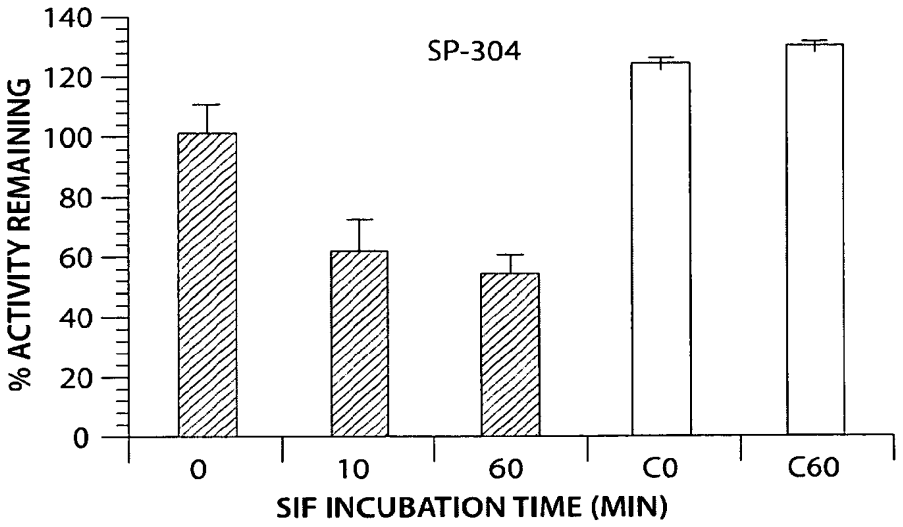

Fig. 7C

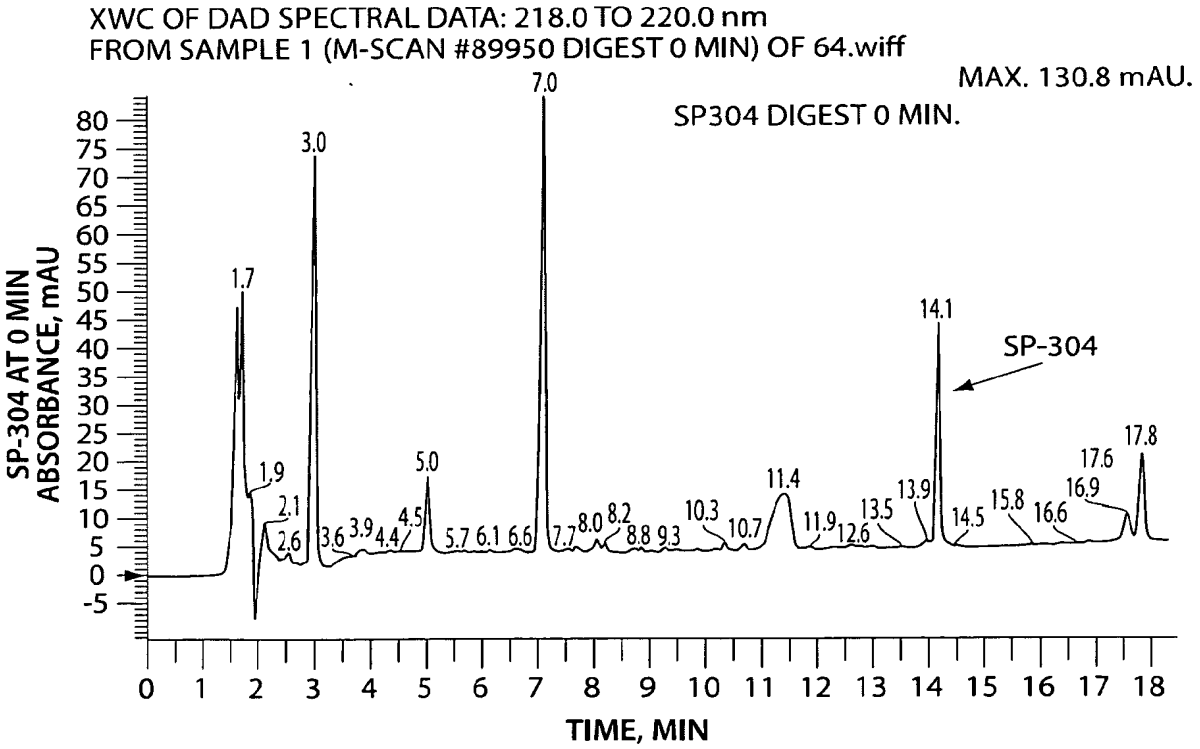

Fig. 7D-1

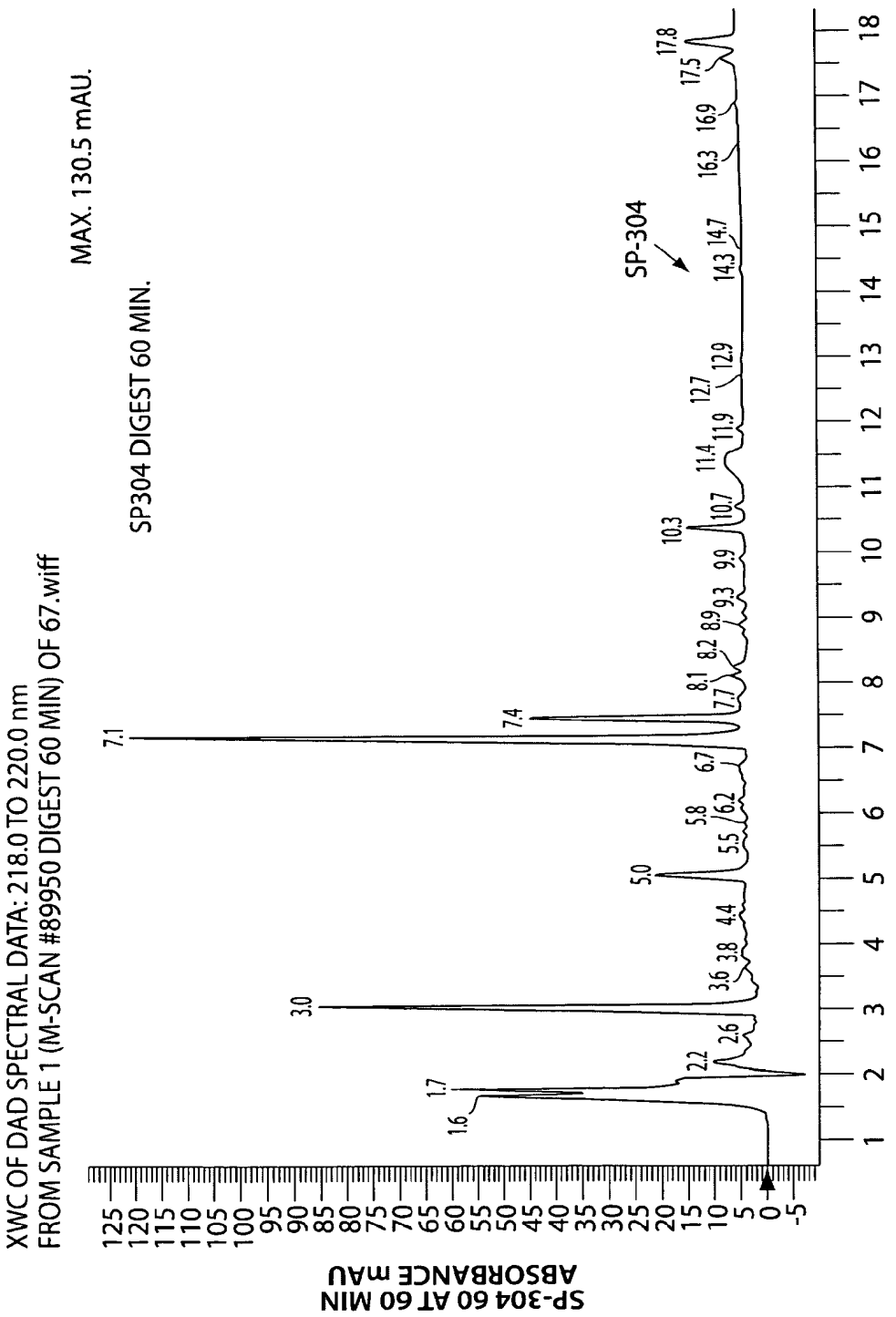
XWC OF DAD SPECTRAL DATA: 218.0 TO 220.0 nm
FROM SAMPLE 1 (M-SCAN #89950 DIGEST 60 MIN) OF 67.wiff
SP304 DIGEST 60 MIN.
MAX. 130.5 mAU.
SP-304
SP-304 60 AT 60 MIN
ABSORBANCE mAU
TIME, MIN

Fig. 7D-2

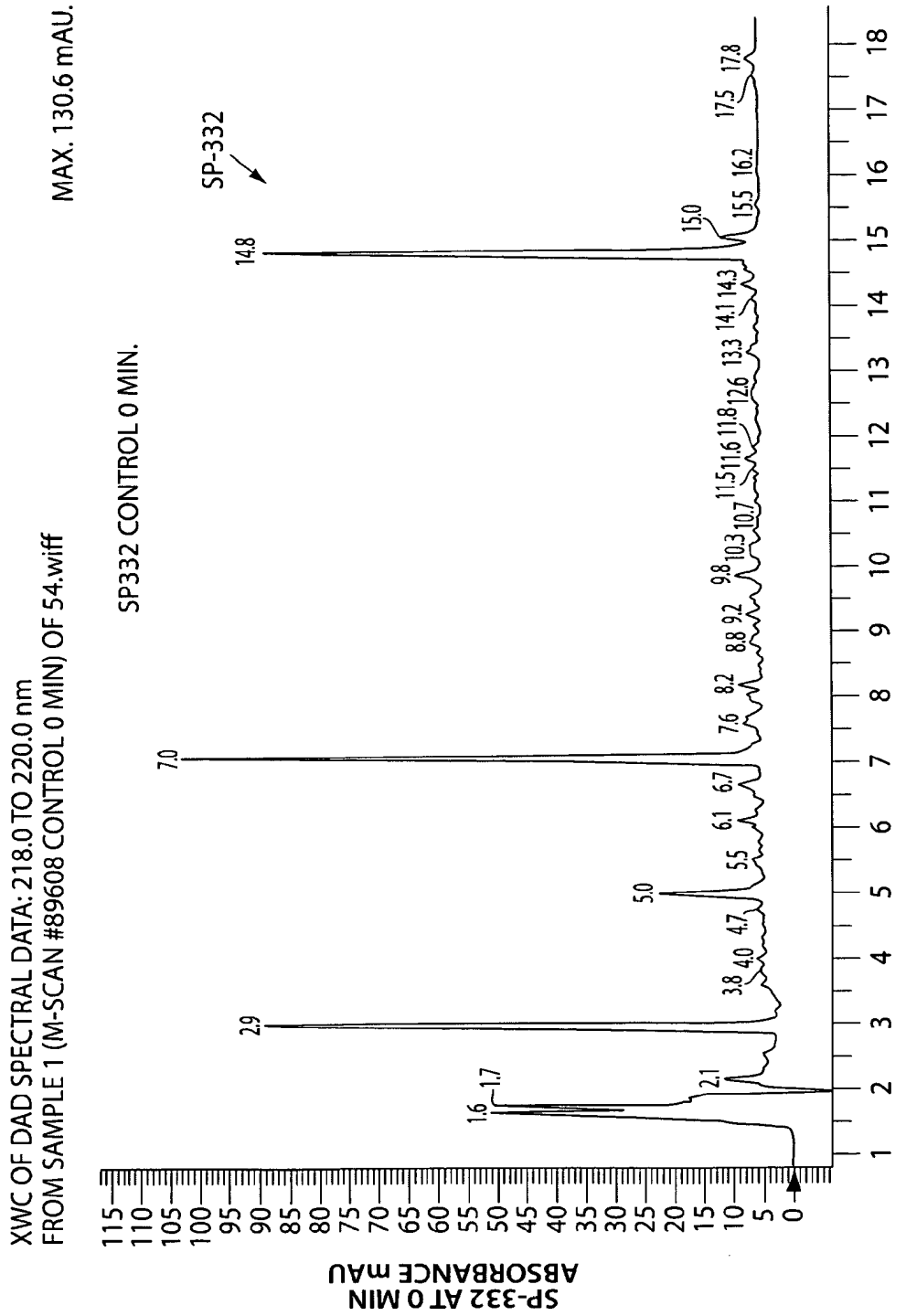
XWC OF DAD SPECTRAL DATA: 218.0 TO 220.0 nm
FROM SAMPLE 1 (M-SCAN #89608 CONTROL 0 MIN) OF 54.wiff
SP332 CONTROL 0 MIN.
MAX. 130.6 mAU.
SP-332
SP-332 AT 0 MIN
ABSORBANCE mAU
TIME, MIN
1.6
1.7
2.1
2.9
3.8
4.0
4.7
5.0
5.5
6.1
6.7
7.0
7.6
8.2
8.8
9.2
9.8
10.3
10.7
11.5
11.6
11.8
12.6
13.3
14.1
14.3
14.8
15.0
15.5
16.2
17.5
17.8

Fig. 7E-1

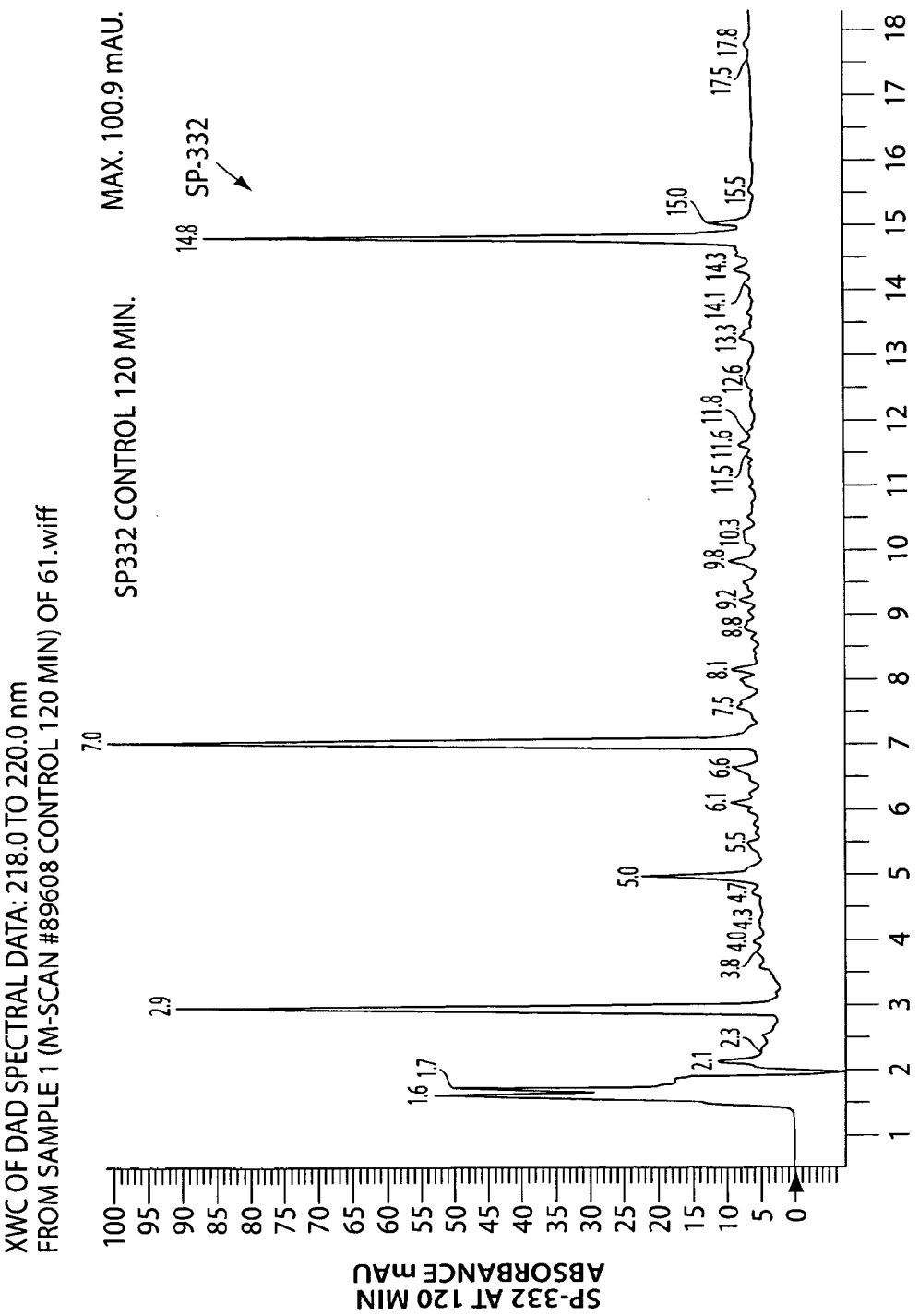
XWC OF DAD SPECTRAL DATA: 218.0 TO 220.0 nm
FROM SAMPLE 1 (M-SCAN #89608 CONTROL 120 MIN) OF 61.wiff
SP332 CONTROL 120 MIN.
MAX. 100.9 mAU.
SP-332
SP-332 AT 120 MIN
ABSORBANCE mAU
TIME, MIN
1.6 1.7 2.1 2.3 2.9 3.8 4.0 4.3 4.7 5.0 5.5 6.1 6.6 7.0 7.5 8.1 8.8 9.2 9.8 10.3 11.5 11.6 11.8 12.6 13.3 14.1 14.3 14.8 15.0 15.5 17.5 17.8

Fig. 7E-2

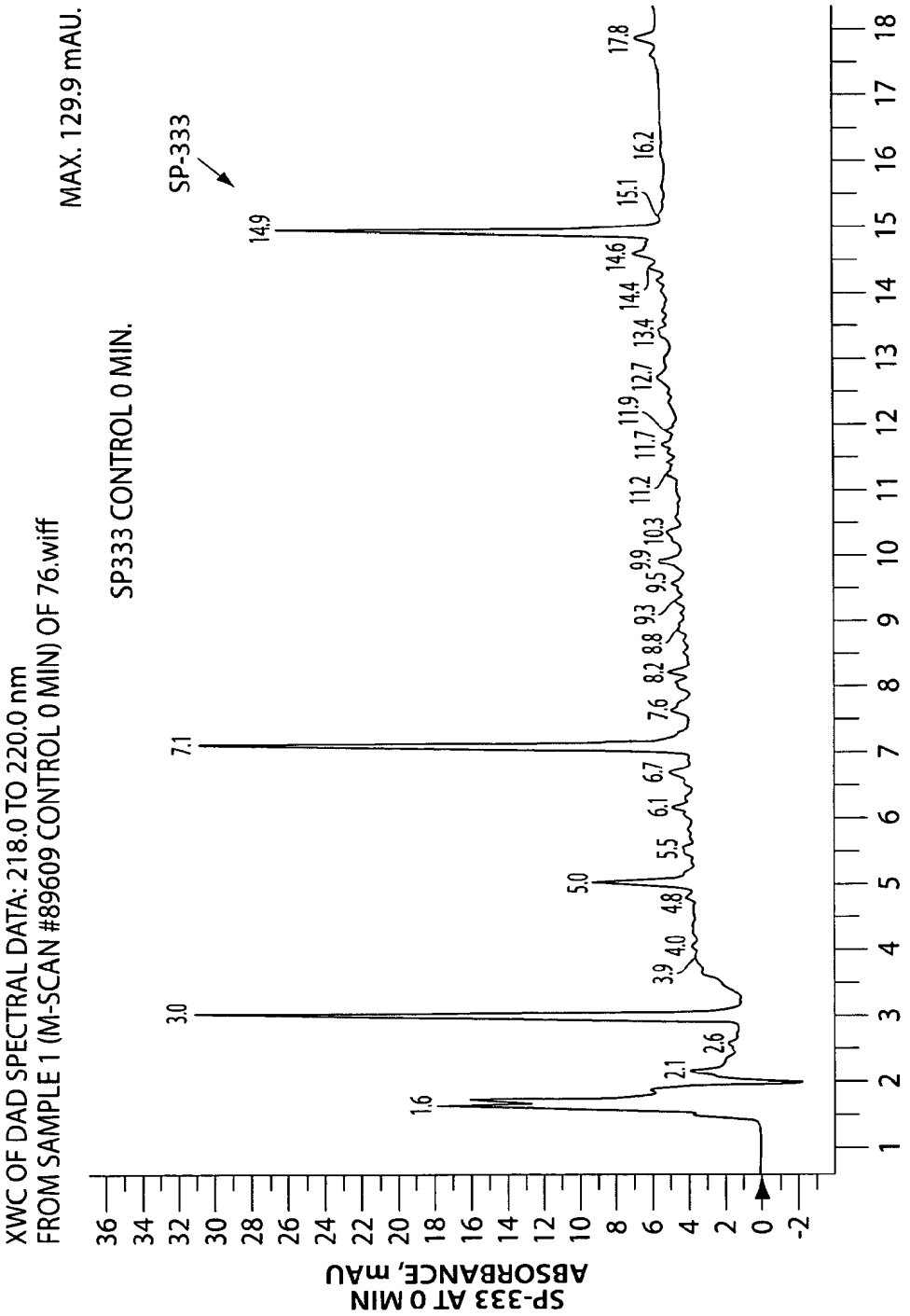
XWC OF DAD SPECTRAL DATA: 218.0 TO 220.0 nm
FROM SAMPLE 1 (M-SCAN #89609 CONTROL 0 MIN) OF 76.wiff
SP333 CONTROL 0 MIN.
MAX. 129.9 mAU.
SP-333
SP-333 AT 0 MIN
ABSORBANCE, mAU
TIME, MIN
1.6
2.1
2.6
3.0
3.9
4.0
4.8
5.0
5.5
6.1
6.7
7.1
7.6
8.2
8.8
9.3
9.5
9.9
10.3
11.2
11.7
11.9
12.7
13.4
14.4
14.6
14.9
15.1
16.2
17.8

Fig. 7F-1

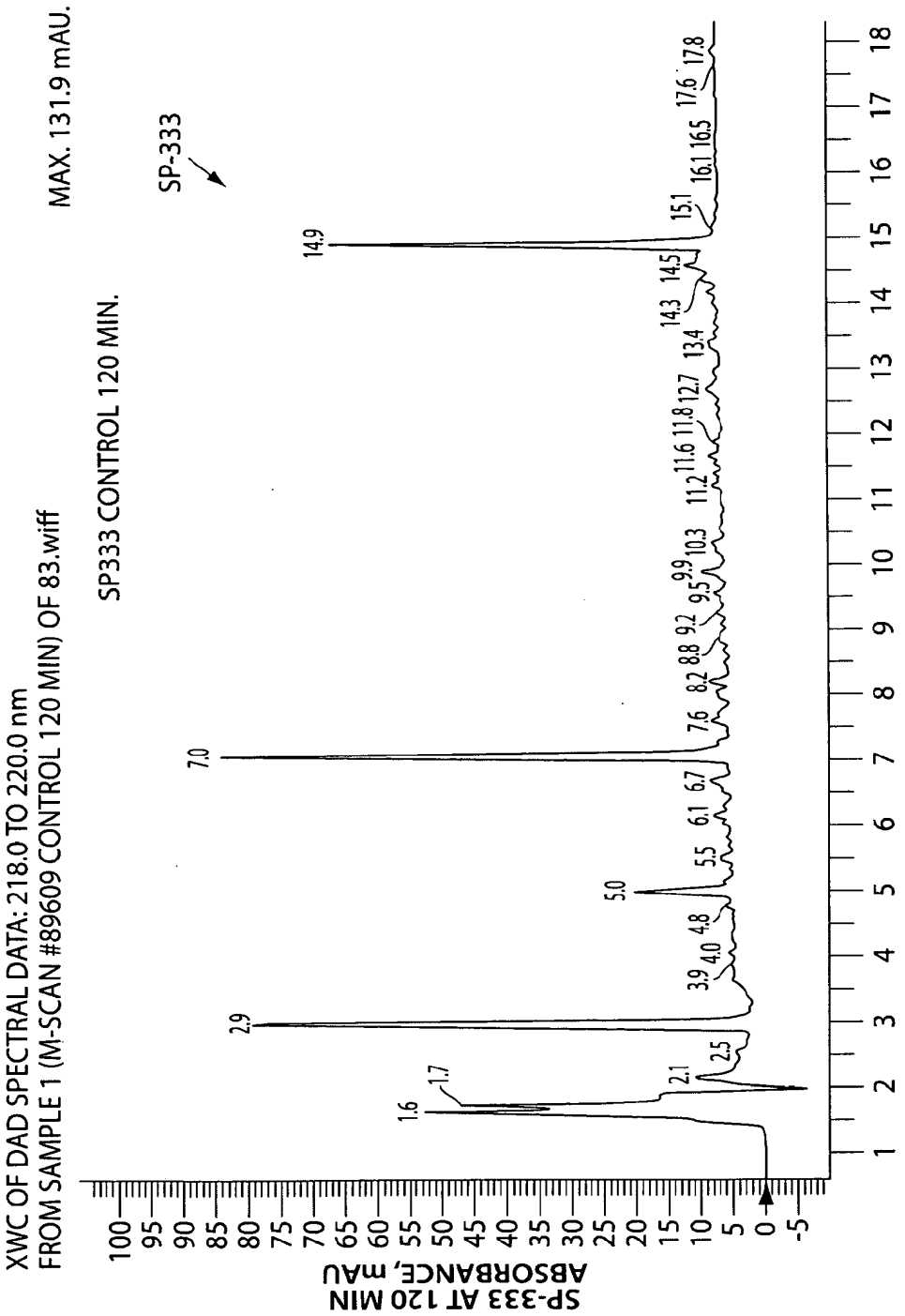
XWC OF DAD SPECTRAL DATA: 218.0 TO 220.0 nm
FROM SAMPLE 1 (M-SCAN #89609 CONTROL 120 MIN) OF 83.wiff
SP333 CONTROL 120 MIN.
MAX. 131.9 mAU.
SP-333
SP-333 AT 120 MIN
ABSORBANCE, mAU
TIME, MIN

Fig. 7F-2

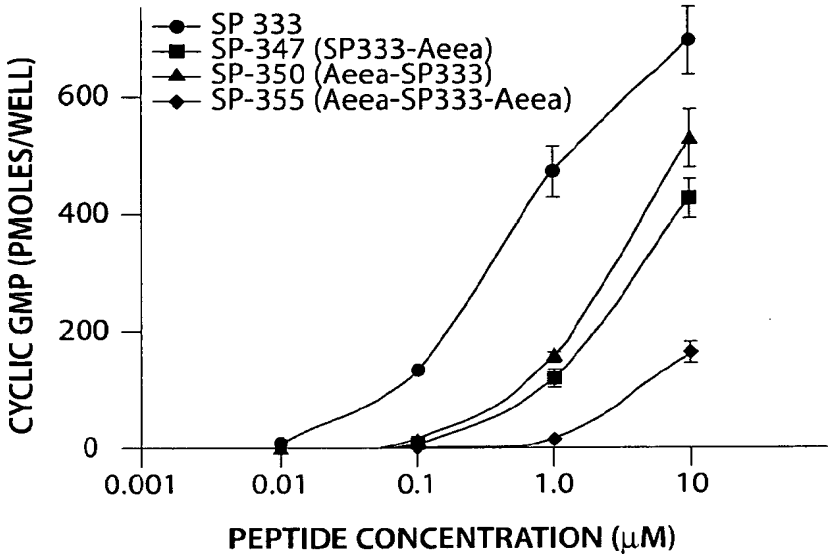
SP 333
SP-347 (SP333-Aeea)
SP-350 (Aeea-SP333)
SP-355 (Aeea-SP333-Aeea)
CYCLIC GMP (PMOLES/WELL)
600
400
200
0
0.001
0.01
0.1
1.0
10
PEPTIDE CONCENTRATION (μM)

Fig. 8

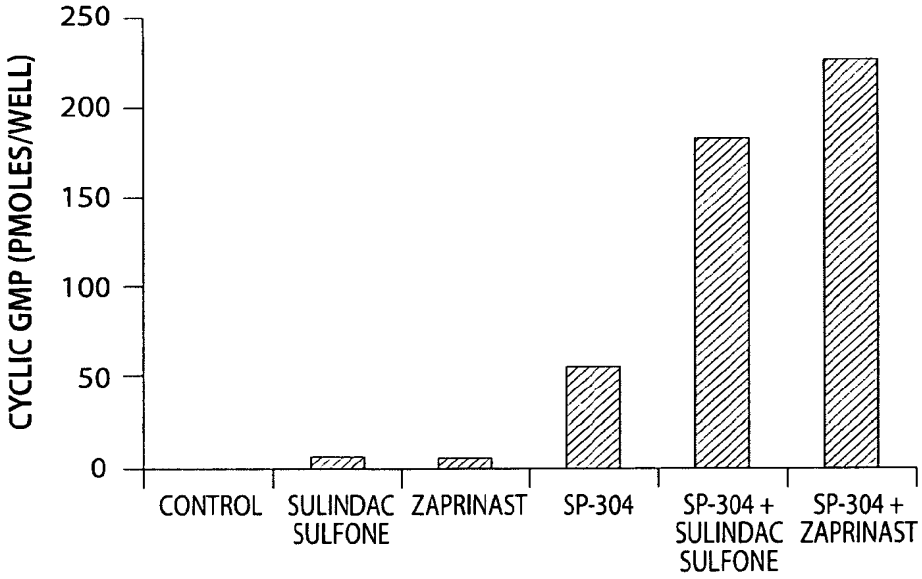
CYCLIC GMP (PMOLES/WELL)
250
200
150
100
50
0
CONTROL
SULINDAC SULFONE
ZAPRINAST
SP-304
SP-304 + SULINDAC SULFONE
SP-304 + ZAPRINAST

Fig. 9

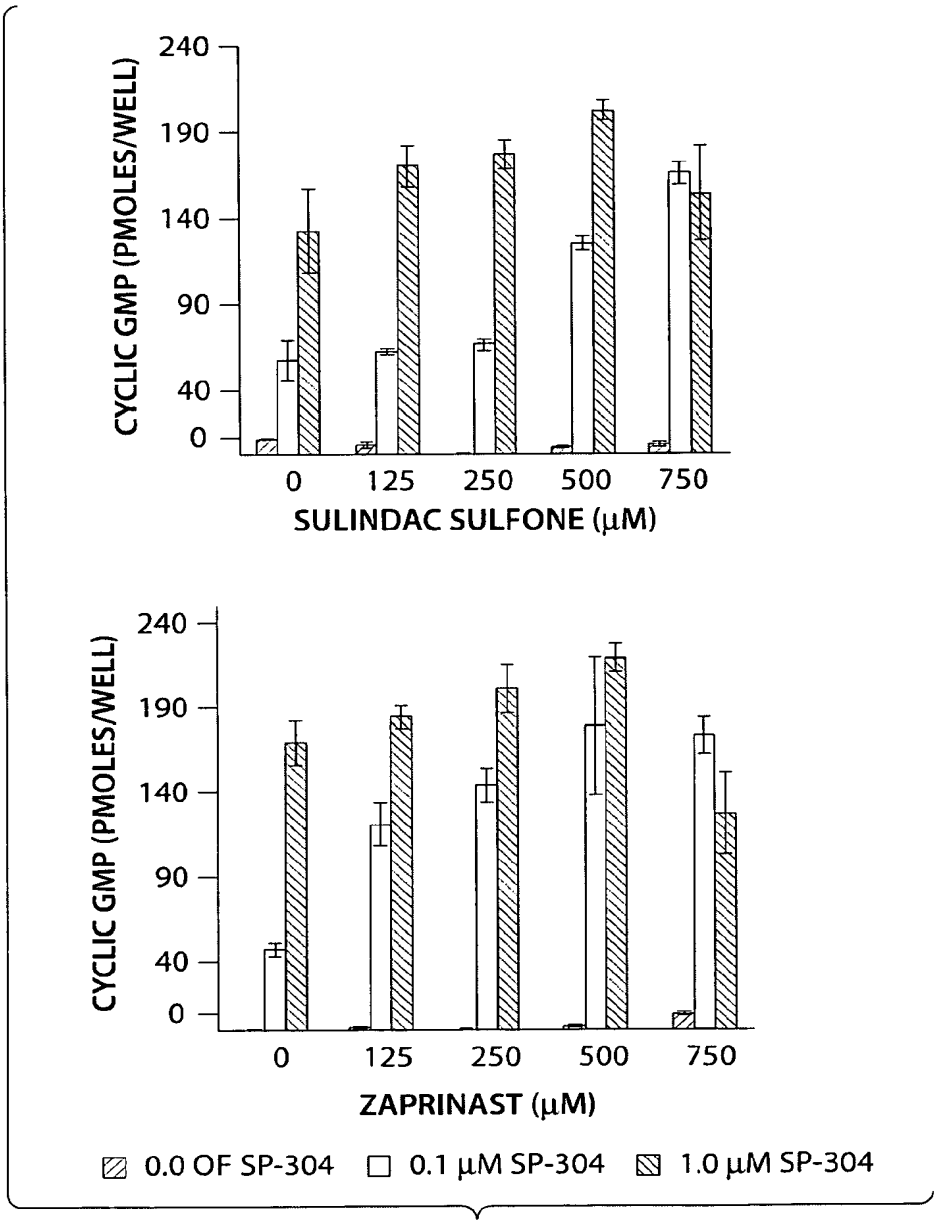
CYCLIC GMP (PMOLES/WELL)
240
190
140
90
40
0
0
125
250
500
750
SULINDAC SULFONE (μM)
CYCLIC GMP (PMOLES/WELL)
240
190
140
90
40
0
0
125
250
500
750
ZAPRINAST (μM)
0.0 OF SP-304
0.1 μM SP-304
1.0 μM SP-304

Fig. 10

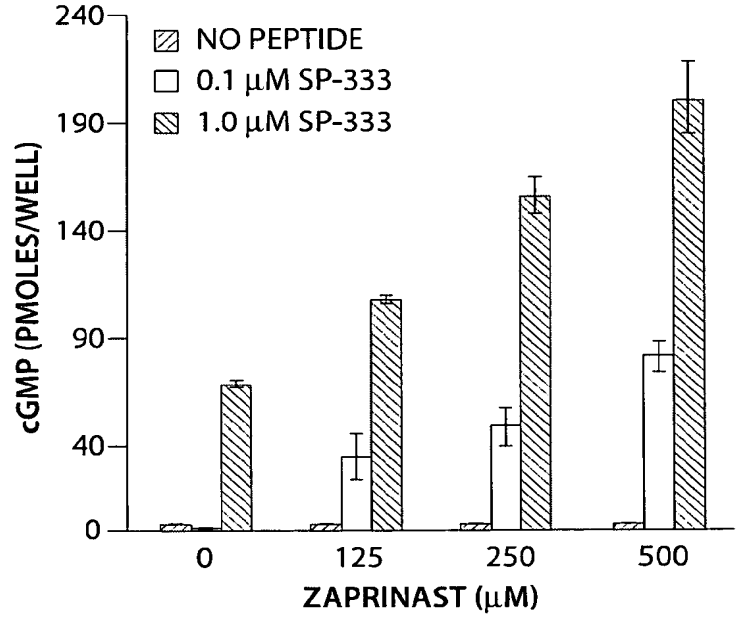
240
190
140
90
40
0
cGMP (PMOLES/WELL)
NO PEPTIDE
0.1 μM SP-333
1.0 μM SP-333
0
125
250
500
ZAPRINAST (μM)

Fig. 11

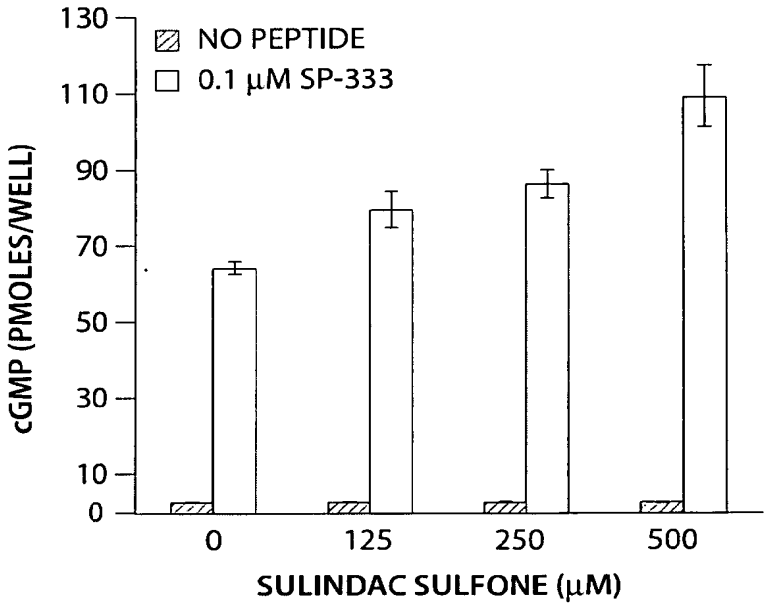
130
110
90
70
50
30
10
0
cGMP (PMOLES/WELL)
NO PEPTIDE
0.1 μM SP-333
0
125
250
500
SULINDAC SULFONE (μM)

Fig. 12

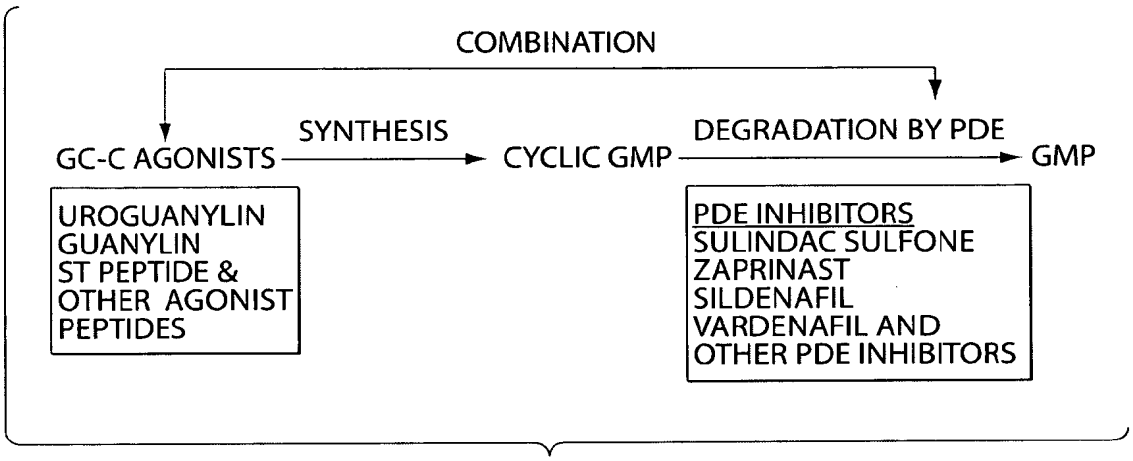
COMBINATION
SYNTHESIS
DEGRADATION BY PDE
GC-C AGONISTS
CYCLIC GMP
GMP
UROGUANYLIN
GUANYLIN
ST PEPTIDE &
OTHER AGONIST
PEPTIDES
PDE INHIBITORS
SULINDAC SULFONE
ZAPRINAST
SILDENAFIL
VARDENAFIL AND
OTHER PDE INHIBITORS

Fig. 13

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(74) Agents: **ELRIFI, Ivor, R.** et al.; Mintz, Levin, Cohn, Ferris, Glovsky And Popeo,P.C., One Financial Center, Boston, MA 02111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2009/149278 A1

(54) Title: AGONISTS OF GUANYLATE CYCLASE USEFUL FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS, INFLAMMATION, CANCER AND OTHER DISORDERS

(57) Abstract: The invention provides novel guanylate cyclase-C agonist peptides and their use in the treatment of human diseases including gastrointestinal disorders, inflammation or cancer (*e.g.*, a gastrointestinal cancer). The peptides can be administered either alone or in combination with an inhibitor of cGMP-dependent phosphodiesterase. The gastrointestinal disorder may be classified as either irritable bowel syndrome, constipation, or excessive acidity etc. The gastrointestinal disease may be classified as either inflammatory bowel disease or other GI condition including Crohn's disease and ulcerative colitis, and cancer.

AGONISTS OF GUANYLATE CYCLASE USEFUL FOR THE TREATMENT OF GASTROINTESTINAL DISORDERS, INFLAMMATION, CANCER AND OTHER DISORDERS

RELATED APPLICATIONS

This application claims the benefit of U.S.S.N. 61/058,888, filed June 4, 2008 the contenst of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to the therapeutic use of guanylate cyclase C (GC-C) 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 inflammation, cancer and other disorders, particularly of the gastrointestinal tract and the lung.

BACKGROUND OF THE INVENTION

Uroguanylin, guanylin and bacterial ST peptides are structurally related peptides that 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 subsequent enhancement of transepithelial secretion of chloride lead 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). Thus, the cGMP-mediated activation of CFTR and the downstream signaling plays an important role in normal functioning of gut physiology. Therefore, any abnormality in this process could potentially lead to gastrointestinal disorders such as irritable bowel syndrome, inflammatory bowel disease, excessive acidity and cancer (25, 26).

The process of epithelial renewal involves the proliferation, migration, differentiation, 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.

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 by maintaining the balance between proliferation and apoptosis in cells lining GI mucosa. Therefore, any disruption in this renewal process, due to reduced production of uroguanylin and/or guanylin can lead to GI inflammation and cancer (25, 26). This is consistent with previously published data in WO 01/25266, which suggest 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, recent data also suggest that uroguanylin also binds to a currently unknown receptor, which is distinct from GC-C receptor (3,4). Knockout mice lacking this guanylate cyclase receptor show resistance to ST peptides in the intestine, but effects of uroguanylin and ST peptides 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). Thus, it is not clear if the anti-colon cancer and anti-inflammatory activities of uroguanylin and its analogs are mediated through binding to one or both of these receptors.

Inflammatory bowel disease is a general name given to a group of disorders that cause intestines to become inflamed, characterized by red and swollen tissue. Gastrointestinal (GI) inflammation can be a chronic condition and often leads to GI cancer (14). Examples of such inflammatory bowel diseases (IBD) include Crohn's disease and ulcerative colitis (UC). It is estimated that as many as 1,000,000 Americans are afflicted with IBD, 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.

Crohn's disease is a serious inflammatory disease that predominantly effects ileum and colon, but can also occur in other sections of the GI tract, whereas UC is exclusively an inflammatory disease of the colon, the large intestine (15). 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, UC affects only the innermost lining (mucosa) of the colon in a continuous manner (16). Depending on which portion of the GI tract is involved, Crohn's disease may be referred to as ileitis, regional enteritis, colitis, etc. Crohn's disease and UC differ from spastic colon or irritable bowel syndrome, which are motility disorders of the GI tract.

While the precise cause of IBD is not known, it is believed that the disruption of the process of continual renewal of GI mucosa may be involved in disease (17,18). The renewal process of the GI lining is an efficient and dynamic process involving the continual proliferation and replenishment of unwanted damaged cells. Proliferation rates of cells lining the GI mucosa are very high, second only to the hematopoietic system. Thus, the balance between proliferation and apoptosis is important to the maintenance of the homeostasis of the GI mucosa (19,20).

Necrotizing enterocolitis (NEC) is a devastating inflammatory condition of the gastrointestinal tract that afflicts 10% of premature infants born weighing less than 1500 grams. Despite modern medical advances, the etiology remains elusive, and morbidity and mortality is unacceptably high, with as many as 10–30% of affected infants succumbing to the disease. Although the pathophysiology is incompletely understood, it is known that prematurity, formula feeding, intestinal ischemia, and bacterial colonization are important risk factors. It has been suggested that these risk factors initiate the activation of the pro-inflammatory response that ultimately leads to bowel necrosis, and in some cases multi-organ dysfunction syndrome, and death. Multiple inflammatory mediators have been identified that might contribute to this final common pathway. Several of the pro- and anti-inflammatory molecules have been studied in

detail in animal models, in humans, and *in vitro*, including IL-6, IL-8, and IL-10 as well as nitric oxide, oxygen free radicals, and numerous others. Previously, we reported that SP-304 ameliorates GI inflammation in experimental models of murine colitis, possibly through downregulation of pro-inflammatory cytokines such as IL-4, IL-5, IL-17, IL-23 and TNF-a. (Shailubhai et al, 2007 and 2008). Therefore, GC-C agonists such as uroguanylin, guanylin, E.coli enterotoxin ST peptides and their analogs might be used to prevent, control and treat NEC. GC-C agonists may be given either in drinking water or in mother's milk to treat NEC in newborne babies.

GI homeostasis depends on both proliferation and programmed cellular death (apoptosis) of epithelial cells lining the gut mucosa. Hence, 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. It has become increasingly apparent that the control of cell death is an equally, if not more, important regulator of cell number and proliferation index (19,20). Reduced rates of apoptosis are often associated with abnormal growth, inflammation, and neoplastic transformation. Thus, both decreased proliferation and/or increased cell death may reduce cell number, whereas increased proliferation and/or reduced cell death may increase the proliferation index of intestinal tissue (20), which may lead to GI inflammatory diseases and cancer.

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 intestine, colon, and rectum, it may metastasize and spread to such localities as bone, lymph nodes, liver, lung, peritoneum, ovaries, and 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

Irritable bowel syndrome (IBS) and chronic idiopathic constipation are pathological conditions that can cause a great deal of intestinal discomfort and distress but unlike the IBD diseases such as ulcerative colitis and Crohn's disease, 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 (IBD), celiac disease and irritable bowel syndrome (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 (27). Serotonin (5-hydroxytryptamine [5-HT]) is a key modulator of gut function and is known to play a major role in pathophysiology of IBS. It has been shown that the activity of 5-HT is regulated by cGMP (28). Therefore, based on this observation as well as other effects of cGMP, we believe that GC-C agonists will be useful in the treatment of IBS.

Given the prevalence of inflammatory conditions in Western societies and the attendant risk of developing cancerous lesions from inflamed tissue, particularly intestinal tissue, a need exists to improve the treatment options for inflammatory conditions, particularly of the gastrointestinal tract.

SUMMARY OF THE INVENTION

The present invention is based upon the development of agonists of guanylate cyclase receptor. The agonists are analogs of uroguanylin and bacterial ST peptides and have superior properties such as for example high resistance to degradation at the N-terminus and C-terminus from carboxypeptidases and/or by other proteolytic enzymes present in the stimulated human intestinal juices and human gastric juices.

The peptides of the invention 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 gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity. Gastointestinal disorders include for example, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease (GERD), ileus inflammation

(*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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. 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.

In one aspect, the present invention is directed to a peptide consisting essentially of the amino acid sequence of, SEQ ID NOs: 2-8 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: 2-8 or if its activation of cellular cGMP production is reduced by more than 50% compared to a control peptide such as SEQ ID NO:1. 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 instant peptide sequences comprise at least 12 amino acid residues, preferably between 12 and 26 amino acids in length.

The peptides may be in a pharmaceutical composition in unit dose form, together with one or more pharmaceutically acceptable carrier, excipients or diluents. 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, shrinkage of polyps or tumors, a reduction in metastatic lesions, etc.

In yet another aspect, an invention provides administering to said patient an effective dose of an inhibitor of cGMP-specific phosphodiesterase (cGMP-PDE) either concurrently or sequentially with said guanylate cyclase receptor agonist. The cGMP-PDE inhibitor include for example suldinac sulfone, zaprinast, and motapizone, vardenifil, and sildenafil. In addition, GC-C agonist peptides may be used in combination with inhibitors of cyclic nucleotide transporters.

Optionally, anti-inflammatory agents are also administered. Anti-inflammatory agents include for example steroids and non-steroidal anti-inflammatory drugs (NSAIDS).

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

DETAILED DESCRIPTION

The present invention is based upon the development of agonists of guanylate cyclase-C (GC-C). The agonists are analogs of uroguanylin and have superior properties such as for example high resistance to degradation at the N-terminus and C-terminus from carboxypeptidases and/or by other proteolytic enzymes such as those present in the stimulated human intestinal fluid (SIF)and simulated human gastric fluid (SGF). Specifically, these peptides contain a d-amino acid at the amino- terminus and the carboxyl terminus. Additionally these peptides are modified as to mask the carboxyl-terminal carboxylic acid with an amide. Thus, the peptide is protected on both termini from degradation by proteases present in SIF and SGF. Examples of such a peptide include SP-363, SP-365, SP-367 and SP-373 shown in Table I.

The GC-C is expressed on various cells including on gastrointestinal epithelial cells, and on extra-intestinal tissues including kidney, lung, pancreas, pituitary, adrenal, developing liver, heart and male and female reproductive tissues (reviewed in Vaandrager 2002 Mol Cell Biochem 230:73-83). The GC-C is a key regulator of fluid and electrolyte balance in the intestine and kidney. In the intestine, when stimulated, the GC-C causes an increase in intestinal epithelial cGMP. This increase in cGMP causes a decrease in water and sodium absorption and an increase in chloride and potassium ion secretion, leading to changes in intestinal fluid and electrolyte transport and increased intestinal motility.

The gualylate cyclase-C agonists according to the invention include SEQ ID NO:2-8 and are summarized below in Table I. The gualylate cyclase-C agonists according to the invention are collectively refered to herein as "GCRA peptides".

Table I GCRA Peptides

Name	Structure	SEQ ID NO:
SP304	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-333	$dAsn^{1}$-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}-$dLeu^{16}$	2
SP-363	$dAsn^{1}$-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-$AMIDE^{16}$	3
SP-364	$dAsn^{1}$-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}-$dSer^{16}$	4
SP-365	$dAsn^{1}$-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-$AMIDE^{16}$	5
SP-366	$dAsn^{1}$-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}-$dTyr^{16}$	6
SP-367	$dAsn^{1}$-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-$AMIDE^{16}$	7
SP-373	$Pyglu^{1}$-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-$AMIDE^{16}$	8

The GCRA peptides described herein bind the guanylate cyclase C (GC-C) and stimulate intracellular production of cyclic guanosine monophosphate (cGMP). Optionally, the GCRA peptides induce apoptosis. In some aspects, the GCRA peptides stimulate intracellular cGMP production at higher levels than naturally occurring GC-C agonists (*e.g.*, uroguanylin, guanylin, and ST peptides) and/or SP-304. For example, the GCRA peptides of the invention stimulate 5, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to naturally occurring GC-C angonists and/or SP-304. The terms induced and stimulated are used interchangeably throughout the specification. The GCRA peptides described herein are more stable than naturally occurring GC-C agonists and/or SP-304. By more stable it is meant that the peptide degrade less and/or more slowly in simulated gastrointestinal fluid and/or simulatd intestinal fluid compared to naturally occurring GC-C angonists and/or SP-304. For example, the GCRA peptide of the invention degrade 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GC-C angonists and/or SP-304.

The GCRA peptides described herein have therapeutic value in the treatment of a wide variety of disorders and conditions including for example gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), 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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

As used herein, the term "guanylate cyclase C (GC-C)" refers to the class of guanylate cyclase C receptor on any cell type to which the inventive agonist peptides or natural agonists described herein bind. As used herein, "intestinal guanylate cyclase receptor" is found exclusively on epithelial cells lining the GI mucosa. Uroguanylin, guanylin, and ST peptides are expected to bind to these receptors and may induce apoptosis. The possibility that there may be different receptors for each agonist peptide is not excluded. Hence, the term refers to the class of guanylate cyclase receptors on epithelial cells lining the GI mucosa.

As used herein, the term "GCR agonist" is meant to refer to peptides and/or other compounds that bind to an intestinal guanylate cyclase C and stimulate fluid and electrolyte transport. This term also covers fragments and pro-peptides that bind to GC-C and stimulate fluid and water secretion.

As used herein, the term "substantially equivalent" is meant to refer 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.

Addition of carriers (*e.g.*, phosphate-buffered saline or PBS) and other components to the composition of the present invention is well within the level of skill in this art. In addition to the compound, such compositions may contain pharmaceutically acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake. Other formulations, such

as microspheres, nanoparticles, liposomes, and immunologically-based systems may also be used in accordance with the present invention. Other examples include formulations with polymers (*e.g.*, 20% w/v polyethylene glycol) or cellulose, or enteric formulations.

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 cytoplasmic phospholipase A2 (cPLA2), cyclooxygenase-2 (COX-2) and possibly 5-lipoxygenase (5-LO) 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 gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), 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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema) . Lung Disorders include for example COPD and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

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 agents 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 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 (21-24).

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, induces apoptosis in target cells. Therefore, administration of the novel peptides defined by SEQ ID NO:2-8, as shown in Table I are useful in eliminating or, at least retarding, the onset of gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), non-ulcer dyspepsia, chronic intestinal pseudo-obstruction, functional dyspepsia, colonic pseudo-obstruction, duodenogastric reflux, gastroesophageal reflux disease (GERD), ileus inflammation (*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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

Uroguanylin is a circulating peptide hormone with natriuretic activity and has been found to stimulate fluid and electrolyte transport in a manner similar to another 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.

GCRA PEPTIDES

In one aspect, the invention provides a GCRA peptide. The GCRA peptides are analogues uroguanylin and bacterial ST peptide. No particular length is implied by the term "peptide". In some embodiments, the GCRA peptide is less than 25 amino acids in length, *e.g.,* less than or equal to 20, 15, 14, 13, 12, 11, 10, or 5 amino acid in length.

The GCRA peptides 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 an D retro-inverso peptide by synthesizing a reverse of the sequence for the corresponding native L-amino acid sequence. For example a GCRA peptide includes the sequence of SEQ ID NO: SEQ ID NO:2-8.

By inducing cGMP production is meant that the GCRA peptide induces the production of intracellular cGMP. Intracellular cGMP is measured by methods known in the art. For example, the GCRA peptide of the invention stimulate 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared to naturally occurring GC-C angonists. Optionally, the GCRA peptides of the invention of the invention stimulate 5%, 10%, 20%, 30%, 40%, 50% , 75%, 90% or more intracellular cGMP compared SP-304 (SEQ ID NO:1). In further embodiments, the GCRA peptide stimulates apoptosis, *e.g.*, programmed cell death or activate the cystic fibrosis transmembrane conductance regulator (CFTR). In some embodimenst the GCRA peptides described herein are more stable than naturally occurring GC-C agonists and/or SP-304 (SEQ ID NO:1). By more stable it is meant that the peptide degrade less and/or more slowly in simulated gastric fluid and/or simulated ntestinal fluid compared to naturally occurring GC-C angonists and/or SP-304. For example, the GCRA peptide of the invention degrade 2%, 3%, 5%, 10%, 15%, 20%, 30%, 40%, 50% , 75%, 90% or less compared to naturally occurring GC-C angonists and/or SP-304.

As used herein, the term "AMIDE" is meant to denote that the terminal carboxylic acid is replaced with an amide group, i.e., the terminal COOH is replaced with $CONH_2$.

In certain embodiments, one or more amino acids of the GCRA peptides can be replaced by a non-naturally occurring amino acid or a naturally or non-naturally occurring amino acid analog. There are many amino acids beyond the standard 20 (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and VaI). 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-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.

With regard to non-naturally occurring amino acids or naturally and non-naturally occurring amino acid analogs, a number of substitutions in the polypeptide and agonists described herein are possible alone or in combination.

For example, glutamine residues can be substituted with gamma-Hydroxy-Glu or gamma- Carboxy-Glu. 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-(l-Cyclopentenyl)-Ala; beta-Cyclopentyl-Ala; beta-Cyclopropyl-Ala; beta-Quinolyl-Ala; beta-(2-Thiazolyl)-Ala; beta-(Triazole-l-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. 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 Alanine residues can be substituted with 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. 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).

Further examples of unnatural 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, alkynl, 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, ^{13}C, ^{15}N, or ^{18}O); 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 ρ-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 phosphonoserine; a phosphonotyrosine; 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 (Carbobenzoxyl); 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. Exempary GCRA peptides which include a n0n-

naturally occurring amino acid include for example SP-368 and SP-369.

In some embodiments, an amino acid can be replaced by a naturally-occurring, non-essential amino acid, *e.g.*, taurine.

Alternatively, the GCRA peptides are cyclic peptides. GCRA cyclic peptide are 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 aspects the GCRA peptides are [4,12; 7,15] bicycles.

In some GCRA peptides one or both members of one or both 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 JPept Protein Res 42:249) or diaminopropionic acid (Smith et al. 1978 J Med Chem 2 1:117) to form alternative internal cross-links at the 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)NHCH 2- or -CH2NHCH(O)CH 2-), 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(-CH 2CH=CHCH 2-), an ether linkage (-CH2CH2OCH2- or -CH2OCH2CH2-), a thioether linkage (-CH2CH2SCH2- or - CH2SCH2CH2-), an amine linkage (-CH2CH2NHCH2- or -CH2NHCH 2CH2-) or a thioamide linkage (-CH2CH(S)HNHCH 2- or -CH2NHCH(S)CH 2-). For example, Ledu et al. (Proc Nat'l Acad. Sci. 100:11263-78, 2003) describe methods for preparing lactam and amide cross-links. Exemplary GCRA peptides which include a lactam bridge include for example SP-370.

The GCRA peptides can 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 (0-CH 2 or CH2-O); an ethylene bond (CH2-CH2); a thioamide bond (C(S)-NH); a trans-olefine bond (CH=CH); a fiuoro substituted trans-olefme 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 GCRA peptides can be modified using standard modifications. Modifications may occur at the amino (N-), carboxy (C-) terminus, internally or a combination of any of the preceeding. 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), stearoylation, succinylation, sulfurylation and cyclisation (via disulfide bridges or amide cyclisation), and modification by Cys3 or Cys5. The GCRA peptides described herein may also be modified by 2, 4-dinitrophenyl (DNP), DNP-lysine, modification by 7-Amino-4-methyl- coumarin (AMC), flourescein, NBD (7-Nitrobenz-2-Oxa-l,3-Diazole), p-nitro-anilide, rhodamine B, EDANS (5-((2-aminoethyl)amino)naphthalene-l- sulfonic acid), dabcyl, dabsyl, dansyl, texas red, FMOC, and Tamra (Tetramethylrhodamine). The GCRA 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.

Also included in the invention are peptides that biologically or functional equivalent to the peptides described herein. The term "biologically equivalent" or functional equivalent" is intended to mean that the compositions of the present invention are capable of demonstrating some or all of the cGMP production modulatory effects.

GCRA peptides can also include derivatives of GCRA peptides which are intended to include hybrid and modified forms of GCRA peptides in which certain amino acids have been

deleted or replaced and modifications such as where one or more amino acids have been changed to a modified amino acid or unusual amino acid and modifications such as glycosylation so long the modified form retains the biological activity of GCRA peptides. By retaining the biological activity, it is meant that cGMP and or apoptosis is induced by the GCRA peptide, although not necessarily at the same level of potency as that of a naturally-occurring GCRA peptide identified.

Preferred variants are those that have conservative amino acid substitutions made at one or more predicted non-essential amino acid residues. A "conservative amino acid 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). Thus, a predicted nonessential amino acid residue in a GCRA polypeptide is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a GCRA coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened to identify mutants that retain activity.

Also included within the meaning of substantially homologous is any GCRA peptide which may be isolated by virtue of cross-reactivity with antibodies to the GCRA peptide.

PREPARATION OF GCRA PEPTIDES

GCRA peptides are easily prepared using modern cloning techniques, or may be synthesized by solid state methods or by site-directed mutagenesis. A GCRA peptide may include dominant negative forms of a polypeptide.

Chemical synthesis may generally be performed using standard solution phase or solid phase peptide synthesis techniques, 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.

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.

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.

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.

Alternatively the GCRA peptides are produced by modern cloning techniques For example, the GCRA 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 GCRA 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.

The sequence encoding a GCRA 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.

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.

The protein coding sequence that includes a GCRA 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.

Genetic constructs and methods suitable for production of immature and mature forms of the GCRA 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.

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.

THERAPEUTIC METHODS

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated that is mediated by guanylate cyclase receptor agonists. Disorders mediated by the guanylate cyclase receptor agonists include gastrointestinal disorders, inflammatory disorders, lung disorders, cancer, cardiac disorders, eye disorders, oral disorders, blood disorders, liver disorders, skin disorders, prostate disorders, endocrine disorders, increasing gastrointestinal motility and obesity.Gastointestinal disorders include for example, irritable bowel syndrome (IBS), necrotizing enterocolitis (NEC), 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 , osteoporosis drugs; post surigical constipation, constipation associated with neuropathic disorders. 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); pancreatic inflammation (*e.g.*, pancreatis), lung inflammation (*e.g.*, bronchitis or asthma) or skin inflammation (*e.g.*, psoriasis, eczema) . Lung Disorders include for example chronic obstructive pulmonary disease (COPD), and fibrosis. Cancer includes tissue and organ carcinogenesis including metatases 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. Cardiac disorders include for example, congestive heart failure, trachea cardia hypertension, high cholesterol, or high tryglycerides. Liver disorders include for example cirrhosis and fibrosis. 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.

The term "treatment" refers to reducing or alleviating symptoms in a subject, preventing symptoms from worsening or progressing, and/or preventing disease in a subject who is free therefrom. For a given subject, improvement in a symptom, its worsening, regression, or progression may be determined by any objective or subjective measure. Efficacy of the treatment may be measured as an improvement in morbidity or mortality (*e.g.*, lengthening of 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.

Intracellular cGMP induced by exposing, *e.g.*, contacting a tissue (*e.g.*, gastrointestinals tissue) or cell with GCRA agonists. GC-C receptors are expressed throughout the GI tract starting from esophagus, duodenum, jejunum, ilium, caecum and colon. Human colon cancer cell lines (T81, CaCo-2 and HT-29) also express GC-C receptors. By inducing is meant an increase in cGMP production compared to a tissue or cell that has not been in contact with GCRA peptide or variant. Tissues or cells are directly contacted with a GCRA peptide or variant. Alternatively, the GCRA peptide or variant is administered systemically. GCRA peptide or variant are administered in an amount sufficient to increase intracellular cGMP concentration. cGMP production is measured by a cell-based assay known in the art (25).

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 GCRA peptide. The GCRA 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 10 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.

The GCRA peptides can be administered alone or in combination with other agents. For example the GCRA peptides can be administered in combination with inhibitors of cGMP dependent phosphodiesterase, such as, for example, suldinac sulfone, zaprinast, motapizone, vardenafil or sildenifil; one or more other chemotherapeutic agents; or anti-inflammatory drugs such as, for example, steroids or non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin.

Combination therapy can be achieved by administering two or more agents, *e.g.*, a GCRA 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.

The GCRA 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.

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.

Combination therapy can also include the administration of one of the GC-C agonist with azothioprine and/or other immunomodulating agents. The immunomodulating agents may include small molecule drugs and biologics such as Remicade, Humaira, Cimzia etc.

Combination therapy can also include the administration of two or more agents via different routes or locations. For example, (a) one agent is administered orally and another agents is administered intravenously or (b) one agent is administered orally and another is administered locally. In each case, the agents can either simultaneously or sequentially. Approximated dosages for some of the combination therapy agents described herein are found in the "BNF Recommended Dose" column of tables on pages 11-17 of WO01/76632 (the data in the tables being attributed to the March 2000 British National Formulary) and can also be found in other standard formularies and other drug prescribing directories. For some drugs, the customary presecribed dose for an indication will vary somewhat from country to country.

The GCRA peptides, alone or in combination, can be combined with any pharmaceutically acceptable carrier or medium. Thus, they can be combined with materials that do not produce an adverse, allergic or otherwise unwanted reaction when administered to a patient. The carriers or mediums used can include solvents, dispersants, coatings, absorption promoting agents, controlled release agents, and one or more inert excipients (which include starches, polyols, granulating agents, microcrystalline cellulose (*e.g.* celphere, Celphere beads®), diluents, lubricants, binders, disintegrating agents, and the like), etc. If desired, tablet dosages of the disclosed compositions may be coated by standard aqueous or nonaqueous techniques.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as

ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (*e.g.*, a GCRA agonist) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. Such as mannitol, fructooligosaccharides, polyethylene glycol and other excepients. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid,

collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Pat. No. 4,522,811, incorporated fully herein by reference.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit 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.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Compositions of the present invention may also optionally include other therapeutic ingredients, anti-caking agents, preservatives, sweetening agents, colorants, flavors, desiccants, plasticizers, dyes, glidants, anti-adherents, anti-static agents, surfactants (wetting agents), anti-oxidants, film- coating agents, and the like. Any such optional ingredient must be compatible with the compound described herein to insure the stability of the formulation.

The composition may contain other additives as needed, including for exanple lactose, glucose, fructose, galactose, trehalose, sucrose, maltose, raffnose, 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.

Examples of excipients for use as the pharmaceutically acceptable carriers and the pharmaceutically acceptable inert carriers and the aforementioned additional ingredients include, but are not limited to binders, fillers, disintegrants, lubricants, anti-microbial agents, 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), 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, dextrates, 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, polacrilin potassium, sodium starch glycolate, potato or tapioca starch, other starches, pre-gelatinized starch, clays, other algins, other celluloses, gums (like gellan), low-substituted hydroxypropyl cellulose, or mixtures thereof, LUBRICANTS: calcium stearate, magnesium stearate, mineral oil, light mineral oil, glycerin, sorbitol, mannitol, polyethylene glycol, other glycols, stearic acid, sodium lauryl sulfate, sodium stearyl fumarate, 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, 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.

The formulation can also include other excipients and categories thereof including but not limited to L-histidine, Pluronic®, Poloxamers (such as Lutrol® and Poloxamer 188), ascorbic acid, glutathione, permeability enhancers (*e.g.* lipids, sodium cholate, acylcarnitine, salicylates, mixed bile salts, fatty acid micelles, chelators, fatty acid, surfactants, medium chain glycerides), protease inhibitors *(e.g.* soybean trypsin inhibitor, organic acids), pH lowering agents and absorption enhancers effective to promote bioavailability (including but not limited to those described in US6086918 and US5912014), 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); spheronization 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.

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).

The agents either in their free form or as a salt can be combined with a polymer such as polylactic-glycoloic acid (PLGA), poly-(I)-lactic-glycolic-tartaric acid (P(I)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. Such formulations can be used to implants that release a polypeptide or another agent over a period of a few days, a few weeks or several months depending on the polymer, the particle size of the polymer, and the size of the implant (*See, e.g.*, U.S. 6,620,422). Other sustained release formulations and polymers for use in 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 9726015, WO 97/04744, and US200200 19446. In such sustained release formulations microparticles (Delie and Blanco-Prieto 2005 Molecule 10:65-80) of polypeptide are combined with microparticles of polymer. One or more sustained release implants can be placed in the large intestine, the small intestine or both. U.S. 6,011,0 1 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 releaseof 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.

The GCRA peptideds 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 hyrdratable 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); US5,866,619 and US6,368,629 (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-Eudragrit)); 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).

The GCRA 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. 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.

The GCRA peptides described herein can be formulated in an osmotic device including the ones disclosed in US4,503,030, US5,609,590 and US5,358,502. US4,503,030 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.

EXEMPLARY AGENTS FOR COMBINATION THERAPY

Analgesic Agents

The GCRA peptides described herein can be used in combination therapy with an analgesic agent, *e.g.*, an analgesic compound or an analgesic polypeptide. These polypeptides and compounds can be administered with the GCRA peptides described herein (simultaneously or sequentially). They can also be optionally covalently linked or attached to an agent described herein to create therapeutic conjugates. Among the useful analgesic agents are: Ca channel blockers, 5HT receptor antagonists (for example 5HT3, 5HT4 and 5HTl receptor antagonists),

opioid receptor agonists (loperamide, fedotozine, and fentanyl), NKl receptor antagonists, CCK receptor agonists (*e.g.*, loxiglumide), NKl receptor antagonists, NK3 receptor antagonists, norepinephrine-serotonin reuptake inhibitors (NSRI), vanilloid and cannabanoid receptor agonists, and sialorphin. Analgesics agents in the various classes are described in the literature.

Among the useful analgesic polypeptides are sialorphin-related polypeptides, including those comprising the amino acid sequence QHNPR (SEQ ID NO:), including: VQHNPR (SEQ ID NO:); VRQHNPR (SEQ ID NO:); VRGQHNPR (SEQ ID NO:); VRGPQHNPR (SEQ ID NO:); VRGPRQHNPR (SEQ ID NO:); VRGPRRQHNPR (SEQ ID NO:); and RQHNPR (SEQ ID NO:). 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 polypeptides described herein in a co-therapy or linked to the polypeptides described herein, *e.g.*, by a covalent bond. Sialophin and related polypeptides are described in U.S. Patent 6,589,750; U.S. 20030078200 Al; and WO 02/051435 A2.

Opioid receptor antagonists and agonists can be administered with the GCRA peptides described herein in co-therapy or linked to the agent described herein, *e.g.*, by a covalent bond. For example, opioid receptor antagonists such as naloxone, naltrexone, methyl nalozone, nalmefene, cypridime, beta funaltrexamine, naloxonazine, naltrindole, and nor-binaltorphimine are thought to be useful in the treatment of IBS. It can be useful to formulate opioid antagonists of this type is a delayed and sustained release formulation such that initial release of the antagonist is in the mid to distal small intestine and/or ascending colon. Such antagonists are described in WO 01/32180 A2. Enkephalin pentapeptide (HOE825; Tyr-D-Lys-Gly-Phe-L-homoserine) is an agonist of the mu and delta opioid receptors and is thought to be useful for increasing intestinal motility {Eur. J. Pharm. 219:445, 1992), and this polypeptide can be used in conjunction with the polypeptides described herein. Also useful is trimebutine which is thought to bind to mu/delta/kappa opioid receptors and activate release of motilin and modulate the release of gastrin, vasoactive intestinal polypeptide, gastrin and glucagons. Kappa opioid receptor agonists such as fedotozine, asimadoline, and ketocyclazocine, and compounds described in WO03/097051 and WO05/007626 can be used with or linked to the polypeptides described herein. In addition, mu opioid receptor agonists such as morphine, diphenyloxylate, frakefamide (H-Tyr-D-Ala-Phe(F)-Phe-NH 2; WO 01/019849 Al) and loperamide can be used.

Tyr-Arg (kyotorphin) is a dipeptide that acts by stimulating the release of met-enkephalins to elicit an analgesic effect (J. Biol. Chem 262:8165, 1987). Kyotorphin can be used with or linked to the GCRA peptides described herein.

Chromogranin-derived polypeptide (CgA 47-66; *See, e.g.*, Ghia et al. 2004 Regulatory polypeptides 119:199) can be used with or linked to the GCRA peptides described herein.

CCK receptor agonists such as caerulein from amphibians and other species are useful analgesic agents that can be used with or linked to the GCRA peptides described herein.

Conotoxin polypeptides represent a large class of analgesic polypeptides that act at voltage gated calcium channels, NMDA receptors or nicotinic receptors. These polypeptides can be used with or linked to the polypeptides described herein.

Peptide analogs of thymulin (FR Application 2830451) can have analgesic activity and can be used with or linked to the polypeptides described herein.

CCK (CCKa or CCKb) receptor antagonists, including loxiglumide and dexloxiglumide (the R- isomer of loxiglumide) (WO 88/05774) can have analgesic activity and can be used with or linked to the polypeptides described herein.

Other useful analgesic agents include 5-HT4 agonists such as tegaserod (Zelnorm®), mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lirexapride. Such agonists are described in: EP1321 142 Al, WO 03/053432A1, EP 505322 Al, EP 505322 Bl, US 5,510,353, EP 507672 Al, EP 507672 Bl, and US 5,273,983.

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 6087,091, US 6,136,786, WO 93/13128 Al, EP 1336409 Al, EP 835126 Al, EP 835126 Bl, US 5,795,864, US 5,891,849, US 6,054,429, WO 97/01351 Al, can be used with or linked to the polypeptides described herein.

Various antagonists of the NK-I, NK-2, and NK-3 receptors (for a review see Giardina et al. 2003.Drugs 6:758) can be can be used with or linked to the polypeptides described herein.

NKl 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 Al, US 20010006972 Al, US

20030109417 Al, WO 01/52844 Al, can be used with or linked to the polypeptides described herein.

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) can be used with or linked to the polypeptides described herein.

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 Al, WO 97/21680 Al, 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) can be used with or linked to the polypeptides described herein.

Norepinephrine-serotonin reuptake inhibitors (NSRI) such as milnacipran and related compounds described in WO 03/077897 Al can be used with or linked to the polypeptides described herein.

Vanilloid receptor antagonists such as arvanil and related compouds described in WO 01/64212 Al can be used with or linked to the polypeptides described herein.

The analgesic polypeptides and compounds can be administered with the polypeptides and agonists described herein (simultaneously or sequentially). The analgesic agents can also be covalently linked to the polypeptides and agonists described herein to create therapeutic conjugates. Where the analgesic is a polypeptide and is covalently linked to an agent described herein the resulting polypeptide may also include at least one trypsin cleavage site. When present within the polypeptide, the analgesic polypeptide may be preceded by (if it is at the carboxy terminus) or followed by (if it is at the amino terminus) a trypsin cleavage site that allows release of the analgesic polypeptide.

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

Agents to Treat Gastrointestinal Disorders

Examples of additional therapeutic agents to treat gastrointestinal and other disorders include agents to treat constipation (*e.g.*, a chloride channel activator such as the bicylic fatty acid, Lubiprostone (formerly known as SPI-0211; Sucampo Pharmaceuticals, Inc.; Bethesda, MD), a laxative (*e.g.* a bulk-forming laxative (*e.g.* nonstarch polysaccharides, Colonel Tablet

(polycarbophil calcium), Plantago Ovata®, Equalactin® (Calcium Polycarbophil)), fiber (*e.g.* FIBERCON® (Calcium Polycarbophil), an osmotic laxative, a stimulant laxative (such as diphenylmethanes (*e.g.* bisacodyl), anthraquinones (*e.g.* cascara, senna), and surfactant laxatives (*e.g.* castor oil, docusates), an emollient/lubricating agent (such as mineral oil, glycerine, and docusates), MiraLax (Braintree Laboratories, Braintree MA), dexloxiglumide (Forest Laboratories, also known as CR 2017 Rottapharm (Rotta Research Laboratorium SpA)), saline laxatives, enemas, suppositories, and CR 3700 (Rottapharm (Rotta Research Laboratorium SpA); acid reducing agents such as proton pump inhibitors (*e.g.*, omeprazole (Prilosec®), esomeprazole (Nexium®), lansoprazole (Prevacid®), pantoprazole (Protonix®) and rabeprazole (Aciphex®)) and Histamine H2 -receptor antagonist (also known as H2 receptor blockers including cimetidine, ranitidine, famotidine and nizatidine); prokinetic agents including itopride, octreotide, bethanechol, metoclopramide (Reglan®), domperidone (Motilium®), erythromycin (and derivatives thereof) or cisapride (propulsid®); Prokineticin polypeptides homologs, variants and chimeras thereof including those described in US 7,052,674 which can be used with or linked to the polypeptides described herein; pro-motility agents such as the vasostatin-derived polypeptide, chromogranin A (4-16) (*See, e.g.*, Ghia et al. 2004 Regulatory polypeptides 121:31) or motilin agonists (*e.g.*, GM-611 or mitemcinal fumarate) or nociceptin/Orphanin FQ receptor modulators (US20050169917); other peptides which can bind to and/or activate GC-C including those described in US20050287067; complete or partial 5HT (*e.g.* 5HTl, 5HT2, 5HT3, 5HT4) receptor agonists or antagonists (including 5HT1A antagonists (*e.g.* AGI-OOl (AGI therapeutics), 5HT2B antagonists (*e.g.* PGN 1091 and PGNl 164 (Pharmagene Laboratories Limited), and 5HT4 receptor agonists (such as tegaserod (ZELNORM®), prucalopride, mosapride, metoclopramide, zacopride, cisapride, renzapride, benzimidazolone derivatives such as BIMU 1 and BIMU 8, and lirexapride). Such agonists/modulators are described in: EP1321142 Al, WO 03/053432A1, EP 505322 Al, EP 505322 Bl, US 5,510,353, EP 507672 Al, EP 507672 Bl, US 5,273,983, and US 6,951,867); 5HT3 receptor agonists such as MKC-733; and 5HT3 receptor antagonists such as DDP-225 (MCI-225; Dynogen Pharmaceuticals, Inc.), cilansetron (Calmactin®), alosetron (Lotronex®), Ondansetron HCl (Zofran®), Dolasetron (ANZEMET®), palonosetron (Aloxi®), Granisetron (Kytril®), YM060(ramosetron; Astellas Pharma Inc.; ramosetron may be given as a daily dose of 0.002 to 0.02 mg as described in EP01588707) and ATI-7000 (Aryx Therapeutics, Santa Clara CA); muscarinic receptor agonists;

anti-inflammatory agents; antispasmodics including but not limited to anticholinergic drugs (like dicyclomine (*e.g.* Colimex®, Formulex®, Lomine®, Protylol®, Visceral®, Spasmoban®, Bentyl®, Bentylol®), hyoscyamine (*e.g.* IB-Stat®, Nulev®, Levsin®, Levbid®, Levsinex Timecaps®, Levsin/SL®, Anaspaz®, A-Spas S/L®, Cystospaz®, Cystospaz-M®, Donnamar®, Colidrops Liquid Pediatric®, Gastrosed®, Hyco Elixir®, Hyosol®, Hyospaz®, Hyosyne®, Losamine®, Medispaz®, Neosol®, Spacol®, Spasdel®, Symax®, Symax SL®), Donnatal (*e.g.* Donnatal Extentabs®), clidinium (*e.g.* Quarzan, in combination with Librium = Librax), methantheline (*e.g.* Banthine), Mepenzolate (*e.g.* Cantil), homatropine (*e.g.* hycodan, Homapin), Propantheline bromide (*e.g.* Pro-Banthine), Glycopyrrolate (*e.g.* Robinul®, Robinul Forte®), scopolamine (*e.g.* Transderm-Scop®, Transderm-V®), hyosine-N-butylbromide (*e.g.* Buscopan®), Pirenzepine (*e.g.* Gastrozepin®) Propantheline Bromide (*e.g.* Propanthel®), dicycloverine (*e.g.* Merbentyl®), glycopyrronium bromide (*e.g.* Glycopyrrolate®), hyoscine hydrobromide, hyoscine methobromide, methanthelinium, and octatropine); peppermint oil; and direct smooth muscle relaxants like cimetropium bromide, mebeverine (DUSPATAL®, DUSPATALIN®, COLOFAC MR®, COLOTAL®), otilonium bromide (octilonium), pinaverium (*e.g.* Dicetel® (pinaverium bromide; Solvay S. A.)), Spasfon® (hydrated phloroglucinol and trimethylphloroglucinol)and trimebutine (including trimebutine maleate (Modulon®); antidepressants, including but not limited to those listed herein, as well as tricyclic antidepressants like amitriptyline (Elavil®), desipramine (Norpramin®), imipramine (Tofranil®), amoxapine (Asendin®), nortriptyline; the selective serotonin reuptake inhibitors (SSRTs) like paroxetine (Paxil®), fluoxetine (Prozac®), sertraline (Zoloft®), and citralopram (Celexa®); and others like doxepin (Sinequan®) and trazodone (Desyrel®); centrally-acting analgesic agents such as opioid receptor agonists, opioid receptor antagonists (*e.g.*, naltrexone); agents for the treatment of Inflammatory bowel disease; agents for the treatment of Crohn's disease and/or ulcerative colitis (*e.g.*, alequel (Enzo Biochem, Inc.; Farmingsale, NY), the anti-inflammatory polypeptide RDP58 (Genzyme, Inc.; Cambridge, MA), and TRAFICET-EN™ (ChemoCentryx, Inc.; San Carlos, CA); agents that treat gastrointestinal or visceral pain; agents that increase cGMP levels (as described in US20040121994) like adrenergic receptor antagonists, dopamine receptor agonists and PDE (phosphodiesterase) inhibitors including but not limited to those disclosed herein; purgatives that draw fluids to the intestine (*e.g.*, VISICOL®, a combination of sodium phosphate monobasic monohydrate and sodium phosphate

dibasic anhydrate); Corticotropin Releasing Factor (CRF) receptor antagonists (including NBI-34041 (Neurocrine Biosciences, San Diego, CA), CRH9-41, astressin, R121919 (Janssen Pharmaceutica), CP154,526, NBI-27914, Antalarmin, DMP696 (Bristol-Myers Squibb) CP-316,311 (Pfizer, Inc.), SB723620 (GSK), GW876008 (Neurocrine/Glaxo Smith Kline), ONO-2333Ms (Ono Pharmaceuticals), TS-041 (Janssen), AAG561 (Novartis) and those disclosed in US 5,063,245, US 5,861,398, US20040224964, US20040198726, US20040176400, US20040171607, US20040110815, US20040006066, and US20050209253); glucagon- like polypeptides (glp-1) and analogues thereof (including exendin-4 and GTP-010 (Gastrotech Pharma A)) and inhibitors of DPP-IV (DPP-IV mediates the inactivation of glp-1); tofisopam, enantiomerically-pure R-tofisopam, and pharmaceutically-acceptable salts thereof (US 20040229867); tricyclic anti-depressants of the dibenzothiazepine type including but not limited to Dextofisopam® (Vela Pharmaceuticals), tianeptine (Stablon®) and other agents described in US 6,683,072; (E)-4 (1 ,3bis(cyclohexylmethyl)-1 ,2,34,-tetrahydro-2,6-diono-9H-purin-8-yl)cinnamic acid nonaethylene glycol methyl ether ester and related compounds described in WO 02/067942; the probiotic PROBACTRIX® (The BioBalance Corporation; New York, NY) which contains microorganisms useful in the treatment of gastrointestinal disorders; antidiarrheal drugs including but not limited to loperamide (Imodium, Pepto Diarrhea), diphenoxylate with atropine (Lomotil, Lomocot), cholestyramine (Questran, Cholybar), atropine (Co-Phenotrope, Diarsed, Diphenoxylate, Lofene, Logen, Lonox, Vi-Atro, atropine sulfate injection) and Xifaxan® (rifaximin; Salix Pharmaceuticals Ltd), TZP-201(Tranzyme Pharma Inc.), the neuronal acetylcholine receptor (nAChR) blocker AGI-004 (AGI therapeutics), and bismuth subsalicylate (Pepto-bismol); anxiolytic drugs including but not limited toAtivan (lorazepam), alprazolam (Xanax®), chlordiazepoxide/clidinium (Librium®, Librax®), clonazepam (Klonopin®), clorazepate (Tranxene®), diazepam (Valium®), estazolam (ProSom®), flurazepam (Dalmane®), oxazepam (Serax®), prazepam (Centrax®), temazepam (Restoril®), triazolam (Halcion®; Bedelix® (Montmorillonite beidellitic; Ipsen Ltd), Solvay SLV332 (ArQule Inc), YKP (SK Pharma), Asimadoline (Tioga Pharmaceuticals/Merck), AGI-003 (AGI Therapeutics); neurokinin antagonists including those described in US20060040950; potassium channel modulators including those described in US7,002,015; the serotonin modulator AZD7371 (AstraZeneca Plc); M3 muscarinic receptor antagonists such as darifenacin (Enablex; Novartis AG and zamifenacin (Pfizer); herbal and natural therapies including but not limited to

acidophilus, chamomile tea, evening primrose oil, fennel seeds,wormwood, comfrey, and compounds of Bao-Ji-Wan (magnolol, honokiol, imperatorin, and isoimperatorin) as in US6923992; and compositions comprising lysine and an anti-stress agent for the treatment of irritable bowel syndrome as described in EPO 1550443.

Insulin and Insulin Modulating Agents

The GCRA 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).

The GCRA 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 agonistsdescribed herein can be used in combitherapy with SYMLIN® (pramlintide acetate) and Exenatide® (synthetic exendin-4, a 39 aa polypeptide).

Agents for the Treatment of Postoperative Ileus

The GCRA peptides described herein can also be used in combination therapy with agents (*e.g.*, Entereg™ (alvimopan; formerly called ado lor/ ADL 8-2698), conivaptan and related agents describe in US 6,645,959) used for the treatment of postoperative ileus and other disorders.

Anti-Hypertensive Agents

The GCRA 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, epirenone, and the like; (2) beta-adrenergic blockers such as acebutolol, atenolol, betaxolol, bevantolol, bisoprolol, bopindolol, carteolol, carvedilol, celiprolol, esmolol, indenolol, metaprolol, nadolol, nebivolol, penbutolol, pindolol, propanolol, 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, nimodepine, nisoldipine, nitrendipine, manidipine, pranidipine, and verapamil, and the like; (4) angiotensin converting enzyme (ACE) inhibitors such as benazepril; captopril; ceranapril; cilazapril; delapril; enalapril; enalopril; fosinopril; imidapril; lisinopril; losinopril; moexipril; quinapril; quinaprilat; ramipril; perindopril; perindropril; 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, pratosartan, 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 XENOlO, 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 396-01-0)), and aldosterone antagonists (*e.g.* spironolactone (CAS Number 52-01-7), epirenone, and the like); β-adrenergic blockers such as Amiodarone (Cordarone, Pacerone), bunolol hydrochloride (CAS RN 31969-05-8, Parke-Davis), acebutolol (±N-[3-Acetyl-4-[2-hydroxy-3-[(1 methylethyl)amino]propoxy]phenyl]-butanamide, or (±)-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,l-[(l,l-dimethylethyl)amino]-3-[[4-4(4-morpholinyl)-l,2,5-thiadiazol-3-yl]oxy]-, hemihydrate, (S)-, CAS RN 91524-16-2), timolol maleate (S)-l -[(1,1 -dimethylethyl) amino]-3-[[4- (4-morpholinyl)-l,2,5-thiadiazol -3- yl] oxy]-2-propanol (Z)-2-butenedioate (1 :1) salt, CAS RN 26921-17-5), bisoprolol (2-Propanol, l-[4-[[2-(l-methylethoxy)ethoxy]-methyl]phenoxyl]-3-[(l-meth- ylethyl)amino]-, (±), CAS RN 66722-44-9), bisoprolol fumarate (such as (±)-l-[4-[[2-(l-Methylethoxy) ethoxy]methyl]phenoxy]-3-[(l-methylethyl)amino]-2-propanol (E) -2-butenedioate (2:1) (salt), *e.g.*, Zebeta™ , Lederle Consumer), nebivalol (2H-l-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, l-[4-[2-(cyclopropylmethoxy)ethoxy]phenoxy]-3-[l-methylethyl)amino]-, hydrochloride, A.A.S. RN 63686-79-3), dexpropranolol hydrochloride (2-Propanol,l-[l-methylethy)-amino]-3-(l-naphthalenyloxy)-hydrochloride (CAS RN 13071-11-9), diacetolol hydrochloride (Acetamide, N-[3-acetyl-4-[2-hydroxy-3-[(l-methyl-ethyl)amino]propoxy] [phenyl]-, monohydrochloride CAS RN 69796-04-9), dilevalol hydrochloride (Benzamide, 2-hydroxy-5-[l-hydroxy-2-[l-methyl-3-phenylpropyl)amino]ethyl]-, monohydrochloride, CAS RN 75659-08-4), exaprolol