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(54) Title: ß-ARRESTIN EFFECTORS AND COMPOSITIONS AND METHODS OF USE THEREOF

(57) Abstract: This application describes compounds acting as, for example, β-arrestin effectors and uses thereof, in, for example, the treatment of chronic and acute cardiovascular diseases.

β-Arrestin Effectors And Compositions And Methods Of Use Thereof

[0001] FIELD

[0002] This application relates to compounds acting as β-arrestin effectors. Such compounds may provide significant therapeutic benefit in the treatment of cardiovascular diseases, such as acute heart failure or acute hypertensive crisis.

[0003] BACKGROUND

[0004] Drugs targeting GPCRs have been developed based on a signaling paradigm in which stimulation of the receptor by an agonist (*e.g.*, angiotensin II) leads to activation of a heterotrimeric "G protein", which then leads to second messenger/down-stream signaling (*e.g.*, via diacylglycerol, inositol-triphosphate, calcium, *etc*…) and changes in physiological function (*e.g.*, blood pressure and fluid homeostasis). There is a need for additional drugs that target GPCRs for treatment of pathology associated with blood pressure and fluid homeostasis.

[0005] The foregoing description of related art is not intended in any way as an admission that any of the documents described therein, including pending United States patent applications, are prior art. Moreover, the description herein of any disadvantages associated with the described products, methods, and/or apparatus, is not intended to limit the embodiments or claims. Indeed, aspects of the embodiments may include certain features of the described products, methods, and/or apparatus without suffering from their described disadvantages.

[0006] SUMMARY OF THE INVENTION

[0007] Embodiments described herein provide compositions comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Zz is Arg or Met; wherein Aa is Tyr or D-Cys; wherein Xx is Pro, Ile, NMeIle, cyHex, cyPen, AA01, AA02, or AA03; wherein Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me, and wherein Yy is any amino acid residue, D-Ala, AA01, AA02, AA03, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or

peptide mimetics described in a). In some embodiments, the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 1-24 and 29-60. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Zz can also be lysine.

[0008] Embodiments described herein provide compositions comprising a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-Tyr-Pro-His-Pro-Yy (SEQ ID NO: 26), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 1, 4-10, and 60 .

[0009] Embodiments described herein provide compositions comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Zz-Val-Tyr-cyHex-His-Bb-Yy (SEQ ID NO: 27), wherein ZZ is arginine, lysine, or methionine; wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Bb is Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me and Yy is null. In some embodiments, the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 2, 11-17, 31-32, 34-39, 41-51, and 54-57.

[0010] Embodiments described herein provide compositions comprising a) a peptide or peptide mimetic selected from the group consisting of a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-Tyr-cyPen-His-Pro-Yy (SEQ ID NO: 28), wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 3, 18-24, and 59.

[0011] Embodiments described herein provide compositions comprising a peptide or peptide mimetic comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-Tyr-AA01-His-Pro-Yy (SEQ ID NO: 61), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the peptide or peptide mimetic comprises SEQ ID NO: 33 or SEQ ID NO: 40.

[0012] Embodiments described herein provide compositions comprising a peptide or peptide mimetic comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-Tyr-AA02-His-Pro-Yy (SEQ ID NO: 62), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine,

glutamic acid, tryptophan, proline, tyrosine, histidine, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine. In some embodiments, the peptide or peptide mimetic comprises SEQ ID NO: 29 or 30.

[0013] Embodiments described herein provide compositions comprising a peptide or peptide mimetic comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-D-Cys-Ile-His-Cys-Yy (SEQ ID NO: 63), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Yy is D-alanine or null. In some embodiments, the peptide or peptide mimetic comprises SEQ ID NO: 52 or 53. In some embodiments, the peptide or petide mimetics comprising SEQ ID NO: 63 form a cyclic peptide. The cyclic peptide can be formed by a disulfide bond being formed between the two cysteine (D-Cys and Cys) residues.

[0014] Embodiments described herein provide compositions comprising a peptide or peptide mimetic comprising a) a peptide or peptide mimetic comprising the sequence of Sar-Arg-Val-Tyr-NMeIle-His-Pro-Yy (SEQ ID NO: 64), wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative

positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Yy is D-alanine. In some embodiments, the peptide or peptide mimetic comprises SEQ ID NO: 58.

[0015] In some embodiments, the peptides or peptide mimetics described herein are provided as isolated or purified peptides or peptide mimetics. In some embodiments, the peptides or peptide mimetics are cyclic. In some embodiments, the cyclic peptides or peptide mimetics are formed by a disulfide bond. In some embodiments, the peptides or peptide mimetics are dimerized. In some embodiments, the peptides or peptide mimetics are trimerized.

[0016] Embodiments described herein provide methods of treating cardiovascular disorders comprising administering to a subject or subject in need thereof a therapeutically effective amount of one or more compositions, peptides, peptide mimetics, or pharmaceutical compositions described herein. In some embodiments, the cardiovascular disorder is chronic hypertension, hypertensive crisis, acute congestive heart failure, angina, acute myocardial infarction, left ventricular failure, cerebrovascular insufficiency, intracranial haemorrhage, heart failure, acute decompensated heart failure, essential hypertension, post-operative hypertension, hypertensive heart disease, hypertensive renal disease, renovascular hypertension, malignant hypertension, post-renal transplant patient stabilization, dilated cardiomyopathy, myocarditis, post-cardiac transplant patient stabilization, disorders associated with post-stent management, neurogenic hypertension, pre-eclampsia, abdominal aortic aneurysm, or any cardiovascular disorder with a hemodynamic component. In some embodiments, the cardiovascular disorder is an acute cardiovascular disorder. In some embodiments, the acute cardiovascular disorder is acute hypertensive crisis, toxemia of pregnancy, acute myocardial infarction, acute congestive heart failure, acute ischaemic heart disease, pulmonary hypertension, post-operative hypertension, migraine, retinopathy or post-operative cardiac/valve surgery.

[0017] Embodiments described herein provide methods of treating and/or preventing a viral infectious disease linked to AT1R comprising administering to a subject or subject in need thereof a

therapeutically effective amount of one or more compositions, peptides, peptide mimetics, or pharmaceutical compositions described herein.

[0018] Embodiments described herein provide methods of treating cardiovascular disorders comprising administering to a subject or subject in need thereof a therapeutically effective amount of one or more peptides or peptide mimetics comprising Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Zz is Arg or Met; wherein Aa is Tyr or D-Cys; wherein Xx is Pro, Ile, NMeIle, cyHex, cyPen, AA01, AA02, or AA03; wherein Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, AA01, AA02, AA03, or null. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Zz can also be lysine. In some embodiments, the one or more peptides or peptide mimetics comprises SEQ ID NO: 1-24 or 29-60.

[0019] Embodiments described herein provide methods of treating and/or preventing a viral infectious diseases linked to AT1R comprising administering to a subject or subject in need thereof a therapeutically effective amount of one or more peptides or peptide mimetics comprising Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Zz is Arg or Met; wherein Aa is Tyr or D-Cys; wherein Xx is Pro, Ile, NMeIle, cyHex, cyPen, AA01, AA02, or AA03; wherein Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me, wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, AA01, AA02, AA03, or null. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Zz can also be lysine. In some embodiments, the one or more peptides or peptide mimetics comprises SEQ ID NO: 1-24 or 29-60.

[0020] Embodiments described herein provide methods of agonizing β-arrestin comprising administering to a subject or subject in need thereof an effective amount of one or more peptides, peptide mimetics, compositions, or pharmaceutical compositions described herein.

[0021] Embodiments described herein provide compositions comprising: a) a peptide or peptide mimetic comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein

Aa is Tyr; wherein Xx is NMeIle, proline, cyHex, cyPen, AA01, AA02, AA03 or Ile; wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me, or Cys; wherein Yy is any amino acid residue, AA01, AA02, AA03, or null; wherein Zz is Arg or Met, provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or c) Fa peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a). In some embodiments, Zz can also be lysine. In some embodiments, Yy is null. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is D-alanine. In some embodiments, Xx is proline and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is cyHex and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is cyPen and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is N-methyl-isoleucine and Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is N-Methyl-isoleucine, Zz is arginine, Aa is tyrosine, Bb is proline, and Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

[0022] Embodiments described herein provide a pharmaceutical composition comprising one or more compounds (*i.e.* peptides or peptide mimetics) described herein and a pharmaceutically acceptable carrier. The compounds can be employed in any form, such as a solid or solution (*e.g.*, aqueous solution) as is described further below. The compound, for example, can be obtained and employed in a lyophilized form alone or with suitable additives.

[0023] Also provided are methods for treating cardiovascular disorders. Such methods comprise administering a therapeutically effective amount of one or more compounds described herein to a subject or a subject in need thereof.

[0024] DETAILED DESCRIPTION

[0025] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the embodiments disclosed belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present embodiments, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only not intended to be limiting. Other features and advantages of the embodiments will be apparent from the following detailed description and claims.

[0026] For the purposes of promoting an understanding of the embodiments described herein, reference will be made to certain embodiments and specific language will be used to describe the same. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present disclosure.

[0027] Before the present proteins, nucleotide sequences, peptides, *etc.*, and methods are described, it is understood that these embodiments are not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It also is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present embodiments or claims. The compositions described herein may include D amino acids, L amino acids, a racemic backbone of D and L amino acids, or any mixture thereof at each residue. That is, at each position, the residue may be a D amino acid residue or a L-amino acid residue and each position can be independently D or L of each other position, unless context dictates otherwise.

[0028] As used herein, the phrase "in need thereof" means that the animal or mammal has been identified or suspected as having a need for the particular method or treatment. In some embodiments, the identification can be by any means of diagnosis. In any of the methods and treatments described herein, the animal or mammal can be in need thereof. In some embodiments, the animal or mammal is in an environment or will be traveling to an environment in which a particular disease, disorder, or condition is prevalent.

[0029] As used herein, the term "subject," "individual" or "patient," used interchangeably, means any

animal, including mammals, such as mice, rats, other rodents, rabbits, dogs, cats, swine, cattle, sheep, horses, or primates, such as humans.

[0030] As used herein, the terms "a" or "an" means that "at least one" or "one or more" unless the context clearly indicates otherwise.

[0031] As used herein, the term "about" means that the numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical limitation is used, unless indicated otherwise by the context, "about" means the numerical value can vary by ±10% and remain within the scope of the disclosed embodiments. Where a numerical value is used with the term "about" the numerical value without the term "about" is also disclosed and can be used without the term "about."

[0032] As used herein, the term "animal" includes, but is not limited to, humans and non-human vertebrates such as wild, domestic, and farm animals.

[0033] As used herein, the terms "comprising" (and any form of comprising, such as "comprise", "comprises", and "comprised"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), or "containing" (and any form of containing, such as "contains" and "contain"), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0034] As used herein, the phrase "integer from X to Y" means any integer that includes the endpoints. That is, where a range is disclosed, each integer in the range including the endpoints is disclosed. For example, the phrase "integer from X to Y" discloses 1, 2, 3, 4, or 5 as well as the range 1 to 5.

[0035] As used herein, the term "mammal" means a rodent (i.e., a mouse, a rat, or a guinea pig), a monkey, a cat, a dog, a cow, a horse, a pig, or a human. In some embodiments, the mammal is a human.

[0036] As used herein, the phrase "therapeutically effective amount" means the amount of active compound or pharmaceutical agent that elicits the biological or medicinal response that is being sought in a tissue, system, animal, individual or human by a researcher, veterinarian, medical doctor or other clinician. The therapeutic effect is dependent upon the disorder being treated or the biological effect desired. As such, the therapeutic effect can be a decrease in the

severity of symptoms associated with the disorder and/or inhibition (partial or complete) of progression of the disorder, or improved treatment, healing, prevention or elimination of a disorder, or side-effects. The amount needed to elicit the therapeutic response can be determined based on the age, health, size and sex of the subject. Optimal amounts can also be determined based on monitoring of the subject's response to treatment.

[0037] As used herein, the terms "treat," "treated," or "treating" can refer to therapeutic treatment and/or prophylactic or preventative measures wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disorder or disease, or obtain beneficial or desired clinical results. For purposes of the embodiments described herein, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder or disease. Treatment can also include eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment. Thus, "treatment of a cardiovascular disorder" or "treating a cardiovascular disorder" means an activity that prevents, alleviates or ameliorates any of the primary phenomena or secondary symptoms associated with the cardiovascular disorder.

[0038] This application describes compounds, β-arrestin effectors. Without being bound by any particular theory, the compounds described herein act as agonists of β-arrestin/GRK-mediated signal transduction via the AT1 angiotensin receptor. Thus, these compounds modulate signaling pathways that provide significant therapeutic benefit in the treatment of, but not limited to, cardiovascular diseases such as acute heart failure and acute hypertensive crisis.

[0039] According to some embodiments, the compounds described herein comprise the following formula: Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Aa is tyrosine or D-cysteine; wherein Xx is proline, cyHex, cyPen, AA01, AA02, AA03, NMeIle, or Ile; wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me, or Cys; wherein Yy is any amino acid residue, AA01, AA02, AA03, or null; wherein Zz is Arg or Met. In some embodiments, Zz is can also be lysine. In some embodiments, Xx is proline. In some

embodiments, Xx is cyHex. In some embodiments, Xx is cyPen. In some embodiments, Xx is AA01. In some embodiments, Xx is AA02. In some embodiments, Xx is AA03. In some embodiments Xx is AA01, AA02, or AA03. In some embodiments, Xx is cyHex or CyPen. In some embodiments, XX is proline, isoleucine, or N-Methyl-isoleucine. In some embodiments, Xx is CyHex, proline, isoleucine, or N-Methyl-isoleucine. In some embodiments, Xx is proline or N-Methyl-isoleucine. In some embodiments, Xx is AA01 or AA02. In some embodiments, Xx is AA01 or AA03. In some embodiments, Xx is AA02 or AA03. In some embodiments, Yy is any naturally occurring eukaryotic or prokaryotic amino acid residue. In some embodiments, Yy is histidine, alanine, isoleucine, arginine, leucine, asparagine, lysine, aspartic acid, methionine, cysteine, phenylalanine, glutamic acid, threonine, glutamine, tryptophan, glycine, valine, ornithine, proline, selenocysteine, pyrrolysine, serine, taurine, or tyrosine. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is glycine. In some embodiments, Yy is alanine. In some embodiments, Yy is arginine. In some embodiments, Yy is asparagine. In some embodiments, Yy is aspartic acid. In some embodiments, Yy is cysteine. In some embodiments, Yy is glutamine. In some embodiments, Yy is glutamic acid. In some embodiments, Yy is isoleucine. In some embodiments, Yy is lysine. In some embodiments, Yy is methionine. In some embodiments, Yy is phenylalanine. In some embodiments, Yy is proline. In some embodiments, Yy is serine. In some embodiments, Yy is threonine. In some embodiments, Yy is tryptophan. In some embodiments, Yy is tyrosine. In some embodiments, Yy is valine. In some embodiments, Yy is not phenylalanine. In some embodiments, Yy is the D-form of the amino acid. In some embodiments, Yy is D-Ala. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me.

[0040] According to some embodiments, the compounds comprise the following formula: Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Aa is Tyr; wherein Xx is proline, cyHex, cyPen, AA01, AA02, AA03, NMeIle, or Ile; wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me, or Cys; wherein Yy is any amino acid residue, AA01, AA02, AA03 or null; and wherein Zz is Arg or Met. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In

some embodiments, Aa is Tyr, Zz is Arg; Xx is proline, cyHex, cyPen, AA01, AA02, AA03, NMeIle or Ile; wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me, or Cys; wherein Yy is any amino acid residue, AA01, AA02, or AA03, provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Aa is Tyr; Zz is Arg, and Xx is Pro, wherein Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me, or Cys; wherein Yy is any amino acid residue, D-Ala, AA01, AA02, or AA03. In some embodiments, Aa is Tyr; Zz is Arg; Xx is Pro; Bb is Pro; and Yy is any amino acid residue, AA01, AA02, or AA03. In some embodiments, Aa is Tyr, Zz is Arg, Xx is Pro, Bb is Pro, and Yy is Ala, Ile, Leu, Val, Thr, Ser, Met, or Phe. In some embodiments, Aa is Tyr and Zz is Met. In some embodiments, Aa is Tyr, Zz is Met, and Xx is cyHex. In some embodiments, Aa is Tyr, Zz is Met, and Xx is Pro. In some embodiments, Aa is Tyr, Zz is Met, Xx is Pro, and Yy is L-Ala or D-Ala. In some embodiments, Aa is Tyr and Zz is Lys.

[0041] In some embodiments, the compound comprises a sequence selected from the group consisting of SEQ ID NOs: 1-24 and 29-60. In some embodiments, the compound does not comprise SEQ ID NO.: 1. In some embodiments, the compound does not comprise SEQ ID NO.: 2. In some embodiments, the compound does not comprise SEQ ID NO.: 3. In some embodiments, the compound does not comprise SEQ ID NO.: 4. In some embodiments, the compound does not comprise SEQ ID NO.: 5. In some embodiments, the compound does not comprise SEQ ID NO.: 6. In some embodiments, the compound does not comprise SEQ ID NO.: 7. In some embodiments, the compound does not comprise SEQ ID NO.: 8. In some embodiments, the compound does not comprise SEQ ID NO.: 9. In some embodiments, the compound does not comprise SEQ ID NO.: 10. In some embodiments, the compound does not comprise SEQ ID NO.: 11. In some embodiments, the compound does not comprise SEQ ID NO.: 12. In some embodiments, the compound does not comprise SEQ ID NO.: 13. In some embodiments, the compound does not comprise SEQ ID NO.: 14. In some embodiments, the compound does not comprise SEQ ID NO.: 15. In some embodiments, the compound does not comprise SEQ ID NO.: 16. In some embodiments, the compound does not comprise SEQ ID NO.: 17. In some embodiments, the compound does not comprise SEQ ID NO.: 18. In some embodiments, the compound does not comprise SEQ ID NO.: 19. In some embodiments, the compound does not comprise SEQ ID NO.: 20. In some embodiments, the compound does not comprise SEQ ID NO.: 21. In some embodiments, the compound does not comprise SEQ ID NO.:22. In some embodiments, the compound does not comprise SEQ ID NO.: 23. In some embodiments, the

compound does not comprise SEQ ID NO.: 24. In some embodiments, the compound does not comprise SEQ ID NO.: 29. In some embodiments, the compound does not comprise SEQ ID NO.: 30. In some embodiments, the compound does not comprise SEQ ID NO.: 31. In some embodiments, the compound does not comprise SEQ ID NO.: 32. In some embodiments, the compound does not comprise SEQ ID NO.: 33. In some embodiments, the compound does not comprise SEQ ID NO.: 34. In some embodiments, the compound does not comprise SEQ ID NO.: 35. In some embodiments, the compound does not comprise SEQ ID NO.: 36. In some embodiments, the compound does not comprise SEQ ID NO.: 37. In some embodiments, the compound does not comprise SEQ ID NO.: 38. In some embodiments, the compound does not comprise SEQ ID NO.: 39. In some embodiments, the compound does not comprise SEQ ID NO.: 40. In some embodiments, the compound does not comprise SEQ ID NO.: 41. In some embodiments, the compound does not comprise SEQ ID NO.: 42. In some embodiments, the compound does not comprise SEQ ID NO.: 43. In some embodiments, the compound does not comprise SEQ ID NO.: 44. In some embodiments, the compound does not comprise SEQ ID NO.: 45. In some embodiments, the compound does not comprise SEQ ID NO.: 46. In some embodiments, the compound does not comprise SEQ ID NO.: 47. In some embodiments, the compound does not comprise SEQ ID NO.: 48. In some embodiments, the compound does not comprise SEQ ID NO.: 49. In some embodiments, the compound does not comprise SEQ ID NO.: 50. In some embodiments, the compound does not comprise SEQ ID NO.: 51. In some embodiments, the compound does not comprise SEQ ID NO.: 52. In some embodiments, the compound does not comprise SEQ ID NO.: 53. In some embodiments, the compound does not comprise SEQ ID NO.: 54. In some embodiments, the compound does not comprise SEQ ID NO.: 55. In some embodiments, the compound does not comprise SEQ ID NO.: 56. In some embodiments, the compound does not comprise SEQ ID NO.: 57. In some embodiments, the compound does not comprise SEQ ID NO.: 58. In some embodiments, the compound does not comprise SEQ ID NO.: 59. In some embodiments, the compound does not comprise SEQ ID NO.: 60. In some embodiments, the compound does not comprise a combination of more than one of the above in the same composition.

[0042] In some embodiments, Xx is proline and Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Xx is cyHex and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

In some embodiments, Xx is cyPen and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine.

[0043] In some embodiments a peptide or peptide mimetic comprising Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Zz is Arg or Met; wherein Aa is Tyr or D-Cys; wherein Xx is Pro, Ile, cyHex, cyPen, AA01, AA02, or AA03; wherein Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; wherein Yy is any amino acid residue, AA01, AA02, AA03, or null. In some embodiments, Zz can also be lysine. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is AA01, AA02, or AA03. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is null. In some embodiments, Xx is cyHex. In some embodiments, Xx is cyPen. In some embodiments, Xx is proline, N-methyl-isoleucine, or isoleucine, provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Xx is AA01. In some embodiments, Xx is AA02. In some embodiments, Xx is AA03. In some embodiments, Bb is Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. Is some embodiments, Bb is Pro or Cys. In some embodiments, Xx is proline and Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is cyHex and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is cyHex and Yy is AA01, AA02, or AA03. In some embodiments, Xx is cyHex and Yy is null. In some embodiments, Xx is cyPen and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is cyPen and Yy is AA01, AA02, or AA03. In some embodiments, Xx is cyPen and Yy is null. In some embodiments, when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me.

[0044] In some embodiments, Xx is AA01 and Yy is AA01, AA02, AA03, alanine, isoleucine,

leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is AA01 and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is AA02 and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is AA02 and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is AA03 and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is AA03 and Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Xx is Ile and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null. In some embodiments, Xx is Ile and Yy is D-alanine or null, provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, where Xx is Ile, Bb is L-cysteine or D-cysteine.

[0045] According to some embodiments, the compounds comprise the following formula: Sar-Arg-Val-Tyr-Pro-His-Pro-Yy (SEQ ID NO: 26), wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is D-alanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the compound comprises a sequence selected from the group consisting of SEQ ID NOs: 1, 4-10, and 60.

[0046] According to some embodiments, the compounds comprise the following formula: Sar-Zz-Val-Tyr-cyHex-His-Bb-Yy (SEQ ID NO: 27), wherein ZZ is arginine, lysine or methionine; Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null. In some embodiments, Bb is Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me. In some embodiments, Bb is Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me and Yy is null. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine,

methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the compound comprises a sequence selected from the group consisting of SEQ ID NOs: 2 and 11-17.

[0047] According to some embodiments, compounds comprising the following formula: Sar-Arg-Val-Tyr-cyPen-His-Pro-Yy (SEQ ID NO: 28), wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine are provided. In some embodiments, Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine. In some embodiments, Yy is glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the compound comprises a sequence selected from the group consisting of SEQ ID NOs: 3, 18-24, and 59.

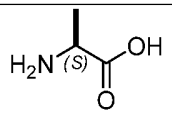
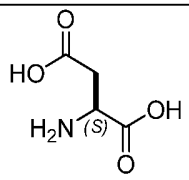
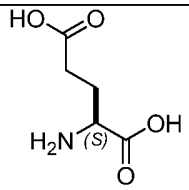
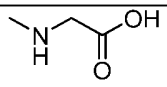
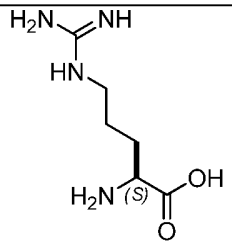
[0048] In some embodiments, compounds comprising the following formula Sar-Arg-Val-Tyr-AA01-His-Pro-Yy (SEQ ID NO: 61), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null are provided. In some embodiments, Yy is null. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the compound comprises SEQ ID NO: 33 or 40.

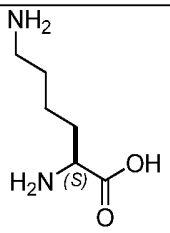
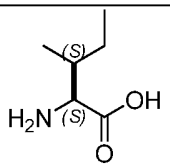
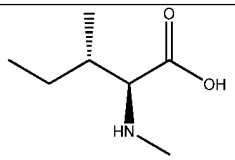
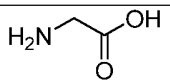
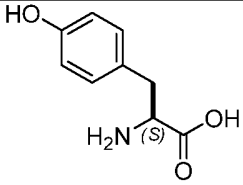
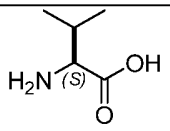
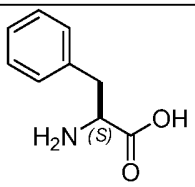
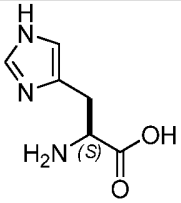
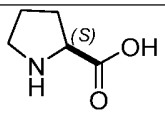
[0049] In some embodiments, compounds comprising the following formula Sar-Arg-Val-Tyr-AA02-His-Pro-Yy (SEQ ID NO: 62), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null are provided. In some embodiments, Yy is null. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, the compound comprises SEQ ID NO: 29 or 30.

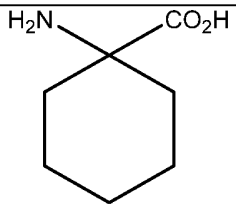
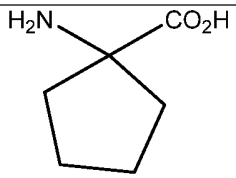
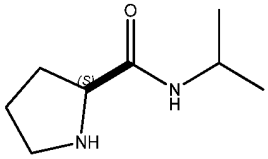
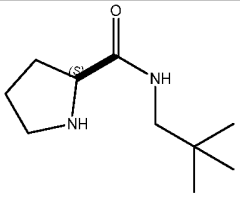
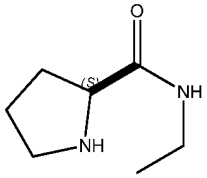
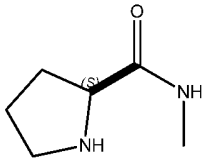
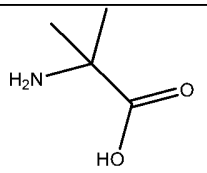
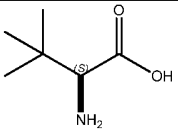
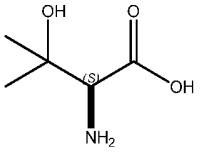
[0050] In some embodiments, compounds comprising the following formula Sar-Arg-Val-D-Cys-Ile-

His-Cys-Yy (SEQ ID NO: 63), wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null are provided. In some embodiments, Yy is null. In some embodiments, Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine. In some embodiments, Yy is D-alanine. In some embodiments, the compound comprises SEQ ID NO: 52 or 53. In some embodiments, the peptide or peptide mimetic comprises SEQ ID NO: 52 or 53. In some embodiments, the peptide or petide mimetics comprising SEQ ID NO: 63 form a cyclic peptide. The cyclic peptide can be formed by a disulfide bond being formed between the two cysteine (D-Cys and Cys) residues.

[0051] The definition of some of the abbreviations used herein are given below. Any abbreviation not explicitly defined herein is used in accordance with customary usage by one of skill in the art.

Abbreviation	Chemical name of amino acid or its analog	Structure of amino acid or its analog
Ala	L-Alanine	
Asp	L-Aspartic acid	
Glu	L-Glutamic acid	
Sar	Sarcosine	
Arg	L-Arginine	

Lys	L-Lysine	
ILe	L-Isoleucine	
NMeIle	N-Methyl-L-isoleucine	
Gly	Glycine	
Tyr	L-Tyrosine	
Val	L-Valine	
Phe	L-Phenylalanine	
His	L-Histidine	
Pro	L-Proline	

cyHex	1-aminocyclohexanecarboxylic acid	
cyPen	1-aminocyclopentanecarboxylic acid	
Pro-NH-i-Pr	(2S)-N-isopropylpyrrolidine-2-carboxamide	
Pro-NH-neopentyl	(2S)-N-neopentylpyrrolidine-2-carboxamide	
Pro-NH-Et	(2S)-N-ethylpyrrolidine-2-carboxamide	
Pro-NH-Me	(2S)-N-methylpyrrolidine-2-carboxamide	
AA01	2-amino-2-methylpropanoic acid	
AA02	(2S)-2-amino-3,3-dimethylbutanoic acid	
AA03	(2S)-2-amino-3-hydroxy-3-methylbutanoic acid	

[0052] Cyclic forms, cyclic truncated forms, cyclic truncated dimerized forms, and cyclic truncated

trimerized forms of the compounds of the above formulas may be prepared using any known method. A truncated form has one or more amino acid residues removed from either end, or both, of the peptides or mimetics described herein. The peptides may have 1 or 2 amino acids removed from each end independently. According to some embodiments, cyclic forms of the compounds of the above formulas may be prepared by bridging free amino and free carboxyl groups. According to some embodiments, formation of the cyclic compounds may be conducted conventionally by treatment with a dehydrating agent by means known in the art, with suitable protection if needed. According to some embodiments, the open chain (linear form) to cyclic form reaction may involve a trans to cis isomerization of the proline. According to some embodiments, the open chain (linear form) to cyclic form reaction may involve intramolecular-cyclization.

[0053] Examples of the compounds of the present embodiments include, but are not limited to, the compounds listed in Table 1 below.

[0054] Table 1.

SEQ ID#	Residues							
	X1	X2	X3	X4	X5	X6	X7	X8
1	Sar	Arg	Val	Tyr	Pro	His	Pro	Ala
2	Sar	Arg	Val	Tyr	cyHex	His	Pro	Ala
3	Sar	Arg	Val	Tyr	cyPen	His	Pro	Ala
4	Sar	Arg	Val	Tyr	Pro	His	Pro	Ile
5	Sar	Arg	Val	Tyr	Pro	His	Pro	Leu
6	Sar	Arg	Val	Tyr	Pro	His	Pro	Val
7	Sar	Arg	Val	Tyr	Pro	His	Pro	Thr
8	Sar	Arg	Val	Tyr	Pro	His	Pro	Ser
9	Sar	Arg	Val	Tyr	Pro	His	Pro	Met
10	Sar	Arg	Val	Tyr	Pro	His	Pro	Phe
11	Sar	Arg	Val	Tyr	cyHex	His	Pro	Ile
12	Sar	Arg	Val	Tyr	cyHex	His	Pro	Leu
13	Sar	Arg	Val	Tyr	cyHex	His	Pro	Val
14	Sar	Arg	Val	Tyr	cyHex	His	Pro	Thr
15	Sar	Arg	Val	Tyr	cyHex	His	Pro	Ser
16	Sar	Arg	Val	Tyr	cyHex	His	Pro	Met
17	Sar	Arg	Val	Tyr	cyHex	His	Pro	Phe
18	Sar	Arg	Val	Tyr	cyPen	His	Pro	Ile
19	Sar	Arg	Val	Tyr	cyPen	His	Pro	Leu
20	Sar	Arg	Val	Tyr	cyPen	His	Pro	Val
21	Sar	Arg	Val	Tyr	cyPen	His	Pro	Thr
22	Sar	Arg	Val	Tyr	cyPen	His	Pro	Ser
23	Sar	Arg	Val	Tyr	cyPen	His	Pro	Met
24	Sar	Arg	Val	Tyr	cyPen	His	Pro	Phe
29	Sar	Arg	Val	Tyr	AA02	His	Pro	Val

SEQ ID#	Residues							
	X1	X2	X3	X4	X5	X6	X7	X8
30	Sar	Arg	Val	Tyr	AA02	His	Pro	Thr
31	Sar	Arg	Val	Tyr	cyHex	His	Pro	AA03
32	Sar	Arg	Val	Tyr	cyHex	His	Pro	Gly
33	Sar	Arg	Val	Tyr	AA01	His	Pro	Val
34	Sar	Arg	Val	Tyr	cyHex	His	Pro	Cys
35	Sar	Arg	Val	Tyr	cyHex	His	Pro	AA01
36	Sar	Arg	Val	Tyr	cyHex	His	Pro	Asn
37	Sar	Arg	Val	Tyr	cyHex	His	Pro	His
38	Sar	Arg	Val	Tyr	cyHex	His	Pro	Gln
39	Sar	Arg	Val	Tyr	cyHex	His	Pro	Pro
40	Sar	Arg	Val	Tyr	AA01	His	Pro	Thr
41	Sar	Arg	Val	Tyr	cyHex	His	Pro	
42	Sar	Arg	Val	Tyr	cyHex	His	Pro	Arg
43	Sar	Arg	Val	Tyr	cyHex	His	Pro	Glu
44	Sar	Arg	Val	Tyr	cyHex	His	Pro	Asp
45	Sar	Met	Val	Tyr	cyHex	His	Pro	Ala
46	Sar	Arg	Val	Tyr	cyHex	His	Pro-NH-i-Pr	
47	Sar	Arg	Val	Tyr	cyHex	His	Pro-NH-neopentyl	
48	Sar	Arg	Val	Tyr	cyHex	His	Pro-NH-ethyl	
49	Sar	Arg	Val	Tyr	cyHex	His	Pro-NH-methyl	
50	Sar	Arg	Val	Tyr	cyHex	His	Pro	Lys
51	Sar	Lys	Val	Tyr	cyHex	His	Pro	Ala
52 ‡	Sar	Arg	Val	D-Cys	Ile	His	Cys	D-Ala
53 ‡	Sar	Arg	Val	D-Cys	Ile	His	Cys	
54	Sar	Arg	Val	Tyr	cyHex	His	Pro	Trp
55	Sar	Arg	Val	Tyr	cyHex	His	Pro	Tyr
56	Sar	Arg	Val	Tyr	cyHex	His	Pro	AA02
57	Sar	Arg	Val	Tyr	cyHex	His	Pro	D-Ala
58	Sar	Arg	Val	Tyr	NMeIle	His	Pro	Ala
59	Sar	Arg	Val	Tyr	cyPen	His	Pro	D-Ala
60	Sar	Arg	Val	Tyr	Pro	His	Pro	D-Ala

‡ Peptide can also be a cyclic peptide with a S-S Bridge

For the definition of the amino acid or its analogues, see the table of abbreviations.

[0055] Determining GPCR activity

[0056] The compounds of the embodiments are agonists of β-arrestin/GRK-mediated signal transduction via the AT1 angiotensin receptor. The ability of the compounds to effect G protein-mediated signaling may be measured using any assay known in the art used to detect G protein-mediated signaling or GPCR activity, or the absence of such signaling/activity. "GPCR activity" refers to the ability of a GPCR to transduce a signal. Such activity can be measured, *e.g.*, in a heterologous cell, by coupling a GPCR (or a chimeric GPCR) to a G-protein and a downstream effector such as PLC or adenylate cyclase, and measuring increases

in intracellular calcium (see, *e.g.*, Offermans & Simon, J. Biol. Chem. 270:15175 15180 (1995)). Receptor activity can be effectively measured by recording ligand-induced changes in $[Ca^{2+}]_i$ using fluorescent Ca^{2+}-indicator dyes and fluorometric imaging. A "natural ligand-induced activity" as used herein, refers to activation of the GPCR by a natural ligand of the GPCR. Activity can be assessed using any number of endpoints to measure the GPCR activity. For example, activity of a GPCR may be assessed using an assay such as calcium mobilization, *e.g.*, an Aequorin luminescence assay.

[0057] Generally, assays for testing compounds that modulate GPCR-mediated signal transduction include the determination of any parameter that is indirectly or directly under the influence of a GPCR, *e.g.*, a functional, physical, or chemical effect. It includes ligand binding, changes in ion flux, membrane potential, current flow, transcription, G-protein binding, gene amplification, expression in cancer cells, GPCR phosphorylation or dephosphorylation, signal transduction, receptor-ligand interactions, second messenger concentrations (*e.g.*, cAMP, cGMP, IP_3, DAG, or intracellular Ca^{2+}), *in vitro*, *in vivo*, and *ex vivo* and also includes other physiologic effects such as increases or decreases of neurotransmitter or hormone release; or increases in the synthesis of particular compounds, *e.g.*, triglycerides. Such parameters can be measured by any means known to those skilled in the art, *e.g.*, changes in spectroscopic characteristics (*e.g.*, fluorescence, absorbance, refractive index), hydrodynamic (*e.g.*, shape), chromatographic, or solubility properties, patch clamping, voltage-sensitive dyes, whole cell currents, radioisotope efflux, inducible markers, transcriptional activation of GPCRs; ligand binding assays; voltage, membrane potential and conductance changes; ion flux assays; changes in intracellular second messengers such as cAMP and inositol triphosphate (IP_3); changes in intracellular calcium levels; neurotransmitter release, and the like.

[0058] When a G protein receptor becomes active, it binds to a G protein (*e.g.*, Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. G protein-mediated signaling or GPCR activity may be measured using assay systems that are capable of detecting and/or measuring GTP binding and/or hydrolysis of GTP to GDP.

[0059] Gs stimulates the enzyme adenylyl cyclase. Gi (and Go), on the other hand, inhibit this enzyme. Adenylyl cyclase catalyzes the conversion of ATP to cAMP. Thus, constitutively activated GPCRs that couple the Gs protein are associated with increased cellular levels of

cAMP. On the other hand, activated GPCRs that couple the Gi (or Go) protein are associated with decreased cellular levels of cAMP. Thus, assays that detect cAMP can be utilized to determine if a candidate compound is, *e.g.*, an inverse agonist to the receptor (*i.e.*, such a compound would decrease the levels of cAMP). A variety of approaches known in the art for measuring cAMP can be utilized; one approach relies upon the use of anti-cAMP antibodies in an ELISA-based format. Another type of assay that can be utilized is a whole cell second messenger reporter system assay. Promoters on genes drive the expression of the proteins that a particular gene encodes. Cyclic AMP drives gene expression by promoting the binding of a cAMP-responsive DNA binding protein or transcription factor (CREB) which then binds to the promoter at specific sites called cAMP response elements and drives the expression of the gene. Reporter systems can be constructed which have a promoter containing multiple cAMP response elements before the reporter gene, *e.g.*, β-galactosidase or luciferase. Thus, a constitutively activated Gs-linked receptor causes the accumulation of cAMP that then activates the gene and expression of the reporter protein. The reporter protein such as β-galactosidase or luciferase can then be detected using standard biochemical assays.

[0060] Gq and Go are associated with activation of the enzyme phospholipase C, which in turn hydrolyzes the phospholipid PIP2, releasing two intracellular messengers: diacycloglycerol (DAG) and inositol 1,4,5-triphoisphate (IP3). Increased accumulation of IP3 is associated with activation of Gq- and Go-associated receptors. Assays that detect IP3 accumulation can be utilized to determine if a candidate compound is, *e.g.*, an inverse agonist to a Gq- or Go-associated receptor (*i.e.*, such a compound would decrease the levels of IP3). Gq-dependent receptors can also be examined using an AP1 reporter assay in that Gq-dependent phospholipase C causes activation of genes containing AP1 elements.

[0061] Samples or assays comprising GPCRs that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of inhibition. Control samples (untreated with inhibitors) are assigned a relative GPCR activity value of 100%. Inhibition of a GPCR is achieved when the GPCR activity value relative to the control is about 99%, 95%, 90%, 85%, 80%, 75%, 70%, 60%, 50%, or 25%. Activation of a GPCR is achieved when the GPCR activity value relative to the control (untreated with activators) is 110%, 150%, 200-500% (*i.e.*, two to five fold higher relative to the control), or 1000-3000% or higher.

[0062] The effects of the compounds upon the function of the GPCR polypeptides can be measured by

examining any of the parameters described above. Any suitable physiological change that affects GPCR activity can be used to assess the influence of a compound on the GPCRs and natural ligand-mediated GPCR activity. When the functional consequences are determined using intact cells or animals, one can also measure a variety of effects such as transmitter release, hormone release, transcriptional changes to both known and uncharacterized genetic markers (*e.g.*, northern blots), changes in cell metabolism such as cell growth or pH changes, and changes in intracellular second messengers such as Ca^{2+}, IP3 or cAMP.

[0063] For a general review of GPCR signal transduction and methods of assaying signal transduction, see, *e.g.*, Methods in Enzymology, vols. 237 and 238 (1994) and volume 96 (1983); Bourne *et al.*, Nature 10:349:117 27 (1991); Bourne *et al.*, Nature 348:125 32 (1990); Pitcher *et al.*, Annu. Rev. Biochem. 67:653 92 (1998).

[0064] Modulators of GPCR activity are tested using GPCR polypeptides as described above, either recombinant or naturally occurring. The protein can be isolated, expressed in a cell, expressed in a membrane derived from a cell, expressed in tissue or in an animal. For example, adipocytes, cells of the immune system, transformed cells, or membranes can be used to test the GPCR polypeptides described above. Modulation is tested using one of the *in vitro* or *in vivo* assays described herein. Signal transduction can also be examined *in vitro* with soluble or solid state reactions, using a chimeric molecule such as an extracellular domain of a receptor covalently linked to a heterologous signal transduction domain, or a heterologous extracellular domain covalently linked to the transmembrane and or cytoplasmic domain of a receptor. Furthermore, ligand-binding domains of the protein of interest can be used *in vitro* in soluble or solid state reactions to assay for ligand binding.

[0065] Ligand binding to a GPCR, a domain, or chimeric protein can be tested in a number of formats. Binding can be performed in solution, in a bilayer membrane, attached to a solid phase, in a lipid monolayer, or in vesicles. In some embodiments of an assay, the binding of the natural ligand to its receptor is measured in the presence of a candidate modulator. Alternatively, the binding of the candidate modulator may be measured in the presence of the natural ligand. Often, competitive assays that measure the ability of a compound to compete with binding of the natural ligand to the receptor are used. Binding can be tested by measuring, *e.g.*, changes in spectroscopic characteristics (*e.g.*, fluorescence, absorbance, refractive index), hydrodynamic (*e.g.*, shape) changes, or changes in chromatographic or solubility properties.

[0066] Receptor-G-protein interactions can also be used to assay for modulators. For example, in the absence of GTP, binding of an activator such as the natural ligand will lead to the formation of a tight complex of a G protein (all three subunits) with the receptor. This complex can be detected in a variety of ways, as noted above. Such an assay can be modified to search for inhibitors. For example, the ligand can be added to the receptor and G protein in the absence of GTP to form a tight complex. Inhibitors or antagonists may be identified by looking at dissociation of the receptor-G protein complex. In the presence of GTP, release of the α subunit of the G protein from the other two G protein subunits serves as a criterion of activation.

[0067] An activated or inhibited G-protein will in turn alter the properties of downstream effectors such as proteins, enzymes, and channels. The classic examples are the activation of cGMP phosphodiesterase by transducin in the visual system, adenylate cyclase by the stimulatory G-protein, phospholipase C by Gq and other cognate G proteins, and modulation of diverse channels by Gi and other G proteins. Downstream consequences such as generation of diacyl glycerol and IP3 by phospholipase C, and in turn, for calcium mobilization, *e.g.*, by IP3 (further discussed below) can also be examined. Thus, modulators can be evaluated for the ability to stimulate or inhibit ligand-mediated downstream effects. Candidate modulators may be assessed for the ability to inhibit calcium mobilization induced by nicotinic acid or a related compound that activates the receptor.

[0068] In other examples, the ability of a compound to inhibit GPCR activity can be determined using downstream assays such as measuring lipolysis in adipocytes, release of free fatty acids from adipose tissue, and lipoprotein lipase activity. This may be accomplished, for example, using a competition assay in which varying amounts of a compound are incubated with a GPCR.

[0069] Modulators may therefore also be identified using assays involving β-arrestin recruitment. β-arrestin serves as a regulatory protein that is distributed throughout the cytoplasm in unactivated cells. Ligand binding to an appropriate GPCR is associated with redistribution of β-arrestin from the cytoplasm to the cell surface, where it associates with the GPCR. Thus, receptor activation and the effect of candidate modulators on ligand-induced receptor activation, can be assessed by monitoring β-arrestin recruitment to the cell surface. This is frequently performed by transfecting a labeled β-arrestin fusion protein (*e.g.*, β-arrestin-green fluorescent protein (GFP)) into cells and monitoring its distribution using confocal microscopy (see, *e.g.*, Groarke *et al.*, J. Biol. Chem. 274(33):23263 69 (1999)).

[0070] Receptor internalization assays may also be used to assess receptor function. Upon ligand binding, the G-protein coupled receptor--ligand complex is internalized from the plasma membrane by a clathrin-coated vesicular endocytic process; internalization motifs on the receptors bind to adaptor protein complexes and mediate the recruitment of the activated receptors into clathrin-coated pits and vesicles. Because only activated receptors are internalized, it is possible to detect ligand-receptor binding by determining the amount of internalized receptor. In one assay format, cells are transiently transfected with radiolabeled receptor and incubated for an appropriate period of time to allow for ligand binding and receptor internalization. Thereafter, surface-bound radioactivity is removed by washing with an acid solution, the cells are solubilized, and the amount of internalized radioactivity is calculated as a percentage of ligand binding. See, *e.g.*, Vrecl *et al.*, Mol. Endocrinol. 12:1818 29 (1988) and Conway *et al.*, J. Cell Physiol. 189(3):341 55 (2001). In addition, receptor internalization approaches have allowed real-time optical measurements of GPCR interactions with other cellular components in living cells (see, *e.g.*, Barak *et al.*, Mol. Pharmacol. 51(2)177 84 (1997)). Modulators may be identified by comparing receptor internalization levels in control cells and cells contacted with candidate compounds.

[0071] Another technology that can be used to evaluate GPCR-protein interactions in living cells involves bioluminescence resonance energy transfer (BRET). A detailed discussion regarding BRET can be found in Kroeger *et al.*, J. Biol. Chem., 276(16):12736 43 (2001).

[0072] Receptor-stimulated guanosine 5'-O-(γ-Thio)-Triphosphate ([^{35}S]GTPγS) binding to G-proteins may also be used as an assay for evaluating modulators of GPCRs. [^{35}S]GTPγS is a radiolabeled GTP analog that has a high affinity for all types of G-proteins, is available with a high specific activity and, although unstable in the unbound form, is not hydrolyzed when bound to the G-protein. Thus, it is possible to quantitatively assess ligand-bound receptor by comparing stimulated versus unstimulated [^{35}S]GTPγS binding utilizing, for example, a liquid scintillation counter. Inhibitors of the receptor-ligand interactions would result in decreased [^{35}S]GTPγS binding. Descriptions of [^{35}S]GTPγS binding assays are provided in Traynor and Nahorski, Mol. Pharmacol. 47(4):848 54 (1995) and Bohn *et al.*, Nature 408:720 23 (2000).

[0073] The ability of modulators to affect ligand-induced ion flux may also be determined. Ion flux may be assessed by determining changes in polarization (*i.e.*, electrical potential) of the cell or membrane expressing a GPCR. One means to determine changes in cellular polarization is by measuring changes in current (thereby measuring changes in polarization) with voltage-clamp

and patch-clamp techniques, *e.g.*, the "cell-attached" mode, the "inside-out" mode, and the "whole cell" mode (see, *e.g.*, Ackerman *et al.*, New Engl. J. Med. 336:1575 1595 (1997)). Whole cell currents are conveniently determined using the standard methodology (see, *e.g.*, Hamil *et al.*, PFlugers. Archiv. 391:85 (1981). Other known assays include: radiolabeled ion flux assays and fluorescence assays using voltage-sensitive dyes (see, *e.g.*, Vestergarrd-Bogind *et al.*, J. Membrane Biol. 88:67 75 (1988); Gonzales & Tsien, Chem. Biol. 4:269 277 (1997); Daniel *et al.*, J. Pharmacol. Meth. 25:185 193 (1991); Holevinsky *et al.*, J. Membrane Biology 137:59 70 (1994)). Generally, the compounds to be tested are present in the range from 1 pM to 100 mM.

[0074] Assays for G-protein coupled receptors include, but are not limited to, cells that are loaded with ion or voltage sensitive dyes to report receptor activity. Assays for determining activity of such receptors can also use known agonists and antagonists for other G-protein coupled receptors and the natural ligands disclosed herein as negative or positive controls to assess activity of tested compounds. In assays for identifying modulatory compounds (*e.g.*, agonists, antagonists), changes in the level of ions in the cytoplasm or membrane voltage are monitored using an ion sensitive or membrane voltage fluorescent indicator, respectively. Among the ion-sensitive indicators and voltage probes that may be employed are those disclosed in the Molecular Probes 1997 Catalog. For G-protein coupled receptors, promiscuous G-proteins such as Gα15 and Gα16 can be used in the assay of choice (Wilkie *et al.*, Proc. Nat'l Acad. Sci. USA 88:10049 10053 (1991)). Such promiscuous G-proteins allow coupling of a wide range of receptors to signal transduction pathways in heterologous cells.

[0075] As noted above, receptor activation by ligand binding typically initiates subsequent intracellular events, *e.g.*, increases in second messengers such as IP3, which releases intracellular stores of calcium ions. Activation of some G-protein coupled receptors stimulates the formation of inositol triphosphate (IP3) through phospholipase C-mediated hydrolysis of phosphatidylinositol (Berridge & Irvine, Nature 312:315 21 (1984)). IP3 in turn stimulates the release of intracellular calcium ion stores. Thus, a change in cytoplasmic calcium ion levels, or a change in second messenger levels such as IP3 can be used to assess G-protein coupled receptor function. Cells expressing such G-protein coupled receptors may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable although not necessary to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as

EGTA, to distinguish fluorescence response resulting from calcium release from internal stores.

[0076] Other assays can involve determining the activity of receptors which, when activated by ligand binding, result in a change in the level of intracellular cyclic nucleotides, *e.g.*, cAMP or cGMP, by activating or inhibiting downstream effectors such as adenylate cyclase. In one embodiment, changes in intracellular cAMP or cGMP can be measured using immunoassays. The method described in Offermanns & Simon, J. Biol. Chem. 270:15175 15180 (1995) may be used to determine the level of cAMP. Also, the method described in Felley-Bosco *et al.*, Am. J. Resp. Cell and Mol. Biol. 11:159 164 (1994) may be used to determine the level of cGMP. Further, an assay kit for measuring cAMP and/or cGMP is described in U.S. Pat. No. 4,115,538, herein incorporated by reference.

[0077] In another embodiment, phosphatidyl inositol (PI) hydrolysis can be analyzed according to U.S. Pat. No. 5,436,128, herein incorporated by reference. Briefly, the assay involves labeling of cells with 3H-myoinositol for 48 or more hrs. The labeled cells are treated with a compound for one hour. The treated cells are lysed and extracted in chloroform-methanol-water after which the inositol phosphates are separated by ion exchange chromatography and quantified by scintillation counting. Fold stimulation is determined by calculating the ratio of counts per minute (cpm) in the presence of agonist to cpm in the presence of buffer control. Likewise, fold inhibition is determined by calculating the ratio of cpm in the presence of antagonist to cpm in the presence of buffer control (which may or may not contain an agonist).

[0078] In another embodiment, transcription levels can be measured to assess the effects of a test compound on ligand-induced signal transduction. A host cell containing the protein of interest is contacted with a test compound in the presence of the natural ligand for a sufficient time to effect any interactions, and then the level of gene expression is measured. The amount of time to effect such interactions may be empirically determined, such as by running a time course and measuring the level of transcription as a function of time. The amount of transcription may be measured by using any method known to those of skill in the art to be suitable. For example, mRNA expression of the protein of interest may be detected using northern blots or their polypeptide products may be identified using immunoassays. Alternatively, transcription based assays using reporter genes may be used as described in U.S. Pat. No. 5,436,128, herein incorporated by reference. The reporter genes can be, *e.g.*, chloramphenicol acetyltransferase, firefly luciferase, bacterial luciferase, β-galactosidase and alkaline phosphatase. Furthermore,

the protein of interest can be used as an indirect reporter via attachment to a second reporter such as green fluorescent protein (see, *e.g.*, Mistili & Spector, Nature Biotechnology 15:961 964 (1997)).

[0079] The amount of transcription is then compared to the amount of transcription in either the same cell in the absence of the test compound, or it may be compared with the amount of transcription in a substantially identical cell that lacks the protein of interest. A substantially identical cell may be derived from the same cells from which the recombinant cell was prepared but which had not been modified by introduction of heterologous DNA. Any difference in the amount of transcription indicates that the test compound has in some manner altered the activity of the protein of interest.

[0080] Samples that are treated with a GPCR antagonist are compared to control samples comprising the natural ligand without the test compound to examine the extent of modulation. Control samples (untreated with activators or inhibitors) are assigned a relative GPCR activity value of 100. Inhibition of a GPCR is achieved when the GPCR activity value relative to the control is about 99%, 95%, 90%, 85%, 80%, 75%, 70%, 60%, 50%, or 25%. Activation of a GPCR is achieved when the GPCR activity value relative to the control is 110%, 150%, 200-500%, or 1000-2000%.

[0081] ***Determining β-arrestin/GRK-mediated signal transduction***

[0082] The ability of the compounds to activate β-arrestin/GRK-mediated signal transduction via the AT1 angiotensin receptor may be measured using any assay known in the art used to detect β-arrestin/GRK-mediated signal transduction via the AT1 angiotensin receptor, or the absence of such signal transduction. Generally, activated GPCRs become substrates for kinases that phosphorylate the C-terminal tail of the receptor (and possibly other sites as well). Thus, an antagonist will inhibit the transfer of ^{32}P from gamma-labeled GTP to the receptor, which can be assayed with a scintillation counter. The phosphorylation of the C-terminal tail will promote the binding of arrestin-like proteins and will interfere with the binding of G-proteins. The kinase/arrestin pathway plays a key role in the desensitization of many GPCR receptors.

[0083] The proximal event in β-arrestin function mediated by GPCRs is recruitment to receptors following ligand binding and receptor phosphorylation by GRK's. Thus, measure of β-arrestin recruitment was used to determine ligand efficacy for β-arrestin function.

[0084] Peptides, Derivatives and mimetics

[0085] The terms "peptidyl" and "peptidic" as used throughout the specification and claims are intended to include active derivatives, variants, and/or mimetics of the peptides according to the present embodiments. Peptidic compounds are structurally similar bioactive equivalents of the peptides according to the present embodiments. By a "structurally similar bioactive equivalent" is meant a peptidyl compound with structure sufficiently similar to that of an identified bioactive peptide to produce substantially equivalent therapeutic effects. For example, peptidic compounds derived from the amino acid sequence of the peptide, or having an amino acid sequence backbone of the peptide, are considered structurally similar bioactive equivalents of the peptide.

[0086] The term "variant" refers to a protein or polypeptide in which one or more (*i.e.*, 1, 2, 3, 4, etc.) amino acid substitutions, deletions, and/or insertions are present as compared to the amino acid sequence of an protein or peptide and includes naturally occurring allelic variants or alternative splice variants of an protein or peptide. The term "variant" includes the replacement of one or more amino acids in a peptide sequence with a similar or homologous amino acid(s) or a dissimilar amino acid(s). Some variants include alanine substitutions at one or more of amino acid positions. Other substitutions include conservative substitutions that have little or no effect on the overall net charge, polarity, or hydrophobicity of the protein. Conservative substitutions are set forth in the table below. According to some embodiments, the peptides or peptide mimetics have at least 60%, 65%, 70%, 75%, 80%, 85%, 88%, 95%, 96%, 97%, 98% or 99% sequence identity with the amino acid or amino acid analogue sequences of embodiments described herein.

[0087] Conservative Amino Acid Substitutions

Basic:	arginine lysine histidine
Acidic:	glutamic acid aspartic acid
Uncharged Polar:	glutamine asparagine

	serine threonine tyrosine
Non-Polar:	phenylalanine tryptophan cysteine glycine alanine valine praline methionine leucine isoleucine

[0088] The table below sets out another scheme of amino acid substitution:

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

[0089] Other variants can consist of less conservative amino acid substitutions, such as selecting residues that differ more significantly in their effect on maintaining a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. The substitutions that in general are expected to have a more significant effect on function are those in which a) glycine and/or proline is substituted by another amino acid or is deleted or inserted; (b) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl, or alanyl; (c) a cysteine residue is substituted for (or by) any other residue; (d) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) a residue

having an electronegative charge, e.g., glutamyl or aspartyl; or (e) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having such a side chain, e.g., glycine. Other variants include those designed to either generate a novel glycosylation and/or phosphorylation site(s), or those designed to delete an existing glycosylation and/or phosphorylation site(s). Variants include at least one amino acid substitution at a glycosylation site, a proteolytic cleavage site and/or a cysteine residue. Variants also include proteins and peptides with additional amino acid residues before or after the protein or peptide amino acid sequence on linker peptides. The term "variant" also encompasses polypeptides that have the amino acid sequence of the proteins/peptides of the present embodiments with at least one and up to 25 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20) additional amino acids flanking either the 3' or 5' end of the amino acid sequence or both.

[0090] The term "variant" also refers to a protein that is at least 60 to 99 percent identical (*e.g.*, 60, 65, 70, 75, 80, 85, 90, 95, 98, 99, inclusive) in its amino acid sequence of the proteins of the present embodiments described herein as determined by standard methods that are commonly used to compare the similarity in position of the amino acids of two polypeptides. The degree of similarity or identity between two proteins can be readily calculated by known methods. Methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Variants will typically have one or more (*e.g.*, 2, 3, 4, 5, *etc.*) amino acid substitutions, deletions, and/or insertions as compared with the comparison protein or peptide, as the case may be.

[0091] Identity and similarity of related polypeptides can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York (1988); Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York (1993); Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey (1994); Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press (1987); Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York (1991); and Carillo et al., SIAM J. Applied Math., 48:1073 (1988).

[0092] In some embodiments, methods to determine identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are described in publicly available computer programs. In some embodiments, computer program

methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package, including GAP (Devereux et al., Nucl. Acid. Res., 12:387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis., BLASTP, BLASTN, and FASTA (Altschul et al., J. Mol. Biol., 215:403 410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra (1990)). The well-known Smith-Waterman algorithm may also be used to determine identity. To determine similarity between peptides, BLASTP can be used with default settings taking into account the small size of the peptides.

[0093] Certain alignment schemes for aligning two amino acid sequences may result in the matching of only a short region of the two sequences, and this small aligned region may have very high sequence identity even though there is no significant relationship between the two full-length sequences. Accordingly, in some embodiments, the selected alignment method (GAP program) will result in an alignment that spans at least 8, 10, 20, 30, 40, or 50 contiguous amino acids of the target polypeptide.

[0094] For example, using the computer algorithm GAP (Genetics Computer Group, University of Wisconsin, Madison, Wis.), two polypeptides for which the percent sequence identity is to be determined are aligned for optimal matching of their respective amino acids (the "matched span", as determined by the algorithm). A gap opening penalty (which is calculated as 3X the average diagonal; the "average diagonal" is the average of the diagonal of the comparison matrix being used; the "diagonal" is the score or number assigned to each perfect amino acid match by the particular comparison matrix) and a gap extension penalty (which is usually 1/10 times the gap opening penalty), as well as a comparison matrix such as PAM 250 or BLOSUM 62 are used in conjunction with the algorithm. A standard comparison matrix (see Dayhoff et al., Atlas of Protein Sequence and Structure, 5(3) (1978) for the PAM 250 comparison matrix; Henikoff et al., Proc. Natl. Acad. Sci USA, 89:10915 10919 (1992) for the BLOSUM 62 comparison matrix) is also used by the algorithm. In some embodiments, parameters for a polypeptide sequence comparison include the following: Algorithm: Needleman et al., J. Mol. Biol., 48:443 453 (1970); Comparison matrix: BLOSUM 62 from Henikoff et al., supra (1992); Gap Penalty: 12 Gap Length Penalty: 4 Threshold of Similarity: 0. The GAP program can be used with the above parameters. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps) using the GAP algorithm.

[0095] Other exemplary algorithms, gap opening penalties, gap extension penalties, comparison matrices, thresholds of similarity, etc. may be used by those of skill in the art, including those set forth in the Program Manual, Wisconsin Package, Version 9, September, 1997. The particular choices to be made will be apparent to those of skill in the art and will depend on the specific comparison to be made, such as DNA-to-DNA, protein-to-protein, protein-to-DNA; and additionally, whether the comparison is between given pairs of sequences (in which case GAP or BestFit are generally used) or between one sequence and a large database of sequences (in which case FASTA or BLASTA are used).

[0096] The compounds of the present embodiments include compounds having one of the general formulas described herein, in addition to derivatives and/or mimetics thereof.

[0097] The term "derivative" refers to a chemically modified protein or polypeptide that has been chemically modified either by natural processes, such as processing and other post-translational modifications, but also by chemical modification techniques, as for example, by addition of one or more polyethylene glycol molecules, sugars, phosphates, and/or other such molecules, where the molecule or molecules are not naturally attached to wild-type proteins. Derivatives include salts. Such chemical modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature, and they are well known to those of skill in the art. It will be appreciated that the same type of modification may be present in the same or varying degree at several sites in a given protein or polypeptide. Also, a given protein or polypeptide may contain many types of modifications. Modifications can occur anywhere in a protein or polypeptide, including the peptide backbone, the amino acid side-chains, and the amino or carboxyl termini. Modifications include, for example, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins, such as arginylation, and ubiquitination. See, for instance, Proteins-

-Structure And Molecular Properties, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993) and Wold, F., "Posttranslational Protein Modifications: Perspectives and Prospects," pgs. 1-12 in Posttranslational Covalent Modification Of Proteins, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter *et al.*, Meth. Enzymol. 182:626-646 (1990) and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging," Ann. N.Y. Acad. Sci. 663: 48-62 (1992). The term "derivatives" include chemical modifications resulting in the protein or polypeptide becoming branched or cyclic, with or without branching. Cyclic, branched and branched circular proteins or polypeptides may result from post-translational natural processes and may be made by entirely synthetic methods, as well.

[0098] According to some embodiments, the compounds may optionally include compounds wherein the N-terminus is derivatized to a —NRR^1 group; to a —NRC(=O)R group; to a —NRC(=O)OR group; to a —NRS(O)$_2$ R group; to a —NHC(=O)NHR group, where R and R^1 are hydrogen or lower alkyl with the proviso that R and R^1 are not both hydrogen; to a succinimide group; to a benzyloxycarbonyl-NH—(CBz—CH—) group; or to a benzyloxycarbonyl-NE— group having from 1 to 3 substituents on the phenyl ring selected from the group consisting of lower alkyl, lower alkoxy, chloro, and bromo.

[0099] According to some embodiments, the compounds may optionally include compounds wherein the C terminus is derivatized to —C(=O)R^2 where R^2 is selected from the group consisting of lower alkoxy, and —NR^3 R^4 where R^3 and R^4 are independently selected from the group consisting of hydrogen and lower alkyl.

[0100] The term "peptide mimetic" or "mimetic" refers to biologically active compounds that mimic the biological activity of a peptide or a protein but are no longer peptidic in chemical nature, that is, they no longer contain any peptide bonds (that is, amide bonds between amino acids). Here, the term peptide mimetic is used in a broader sense to include molecules that are no longer completely peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. Examples of peptide mimetics in this broader sense (where part of a peptide is replaced by a structure lacking peptide bonds) are described below. Whether completely or partially non-peptide, peptide mimetics according to the embodiments provide a spatial arrangement of reactive chemical moieties that closely resemble the three-dimensional arrangement of active groups in the peptide on which the peptide mimetic is based. As a result of this similar active-

site geometry, the peptide mimetic has effects on biological systems that are similar to the biological activity of the peptide.

[0101] The peptides and peptide mimetics include, but are not limited to having from about 8 to about 25, about 8 to about 20, about 8 to about 15, about 8 to about 12, about 8 to about 10, about 8 to about 9, about 9 to about 25, about 9 to about 20, about 9 to about 18, about 9 to about 15, about 9 to about 14, about 9 to about 12, about 10 to about 25, about 10 to about 20, about 10 to about 15, about 10 to about 14, about 10 to about 12, about 12 to about 25 or about 12 to about 20 amino acids or amino acid analogues in length. In some embodiments, the peptide or peptide mimetics comprise a spacer inserted between the residues of the peptides or peptide mimetics described herein. In some embodiments, the spacer is 1 to 3 amino acids or amino acid analogues inserted between one or more of the amino acids or amino acid analogues present in the peptides or peptide mimetics described herein. In some embodiments, the total length of the peptide comprising the spacer is from about 8 to about 25, about 8 to about 20, about 8 to about 15, about 8 to about 12, about 8 to about 10, about 8 to about 9, about 9 to about 25, about 9 to about 20, about 9 to about 18, about 9 to about 15, about 9 to about 14, about 9 to about 12, about 10 to about 25, about 10 to about 20, about 10 to about 15, about 10 to about 14, about 10 to about 12, about 12 to about 25 or about 12 to about 20 amino acids or amino acid analogues in length.

[0102] In some embodiments, the peptide mimetics of the embodiments are substantially similar in both three-dimensional shape and biological activity to the peptides described herein. According to some embodiments, peptide mimetics have protective groups at one or both ends of the compounds, and/or replacement of one or more peptide bonds with non-peptide bonds. Such modifications may render the compounds less susceptible to proteolytic cleavage than the compound itself. For instance, one or more peptide bonds can be replaced with an alternative type of covalent bond (*e.g.*, a carbon—carbon bond or an acyl bond). Peptide mimetics can also incorporate amino-terminal or carboxyl terminal blocking groups such as t-butyloxycarbonyl, acetyl, alkyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,-dinitrophenyl, thereby rendering the mimetic less susceptible to proteolysis. Non-peptide bonds and carboxyl- or amino-terminal blocking groups can be used singly or in combination to render the mimetic less susceptible to proteolysis than the corresponding peptide/compound. Additionally, substitution of D-amino acids for the normal

L-stereoisomer can be effected, *e.g.* to increase the half-life of the molecule.

[0103] Thus, according to some embodiments, the compounds may optionally include a pseudopeptide bond wherein one or more of the peptidyl [—C(=O)NR—] linkages (bonds) have been replaced by a non-peptidyl linkage such as —CH_2—NH—, —CH_2—S—, —CH_2—SO—, —CH_2—SO_2—, —NH—CO—, or —CH=CH— replacing a peptide bond (—CO—NH—). According to some embodiments, the compounds may optionally include a pseudopeptide bond wherein one or more of the peptidyl [—C(=O)NR—] linkages (bonds) have been replaced by a non-peptidyl linkage such as a —CH_2 -carbamate linkage [—CH_2—OC(=O)NR—]; a phosphonate linkage; a —CH_2 -sulfonamide [—CH_2—$S(O)_2$ NR—] linkage; a urea [—NHC(=O)NH—] linkage; a —CH_2 -secondary amine linkage; or an alkylated peptidyl linkage [—C(=O)NR^6— where R^6 is lower alkyl]. Some mimetics have from zero to all of the —C(=O)NH— linkages replaced by a pseudopeptide.

[0104] Examples of methods of structurally modifying a peptide known in the art to create a peptide mimetic include the inversion of backbone chiral centers leading to D-amino acid residue structures that may, particularly at the N-terminus, lead to enhanced stability for proteolytical degradation without adversely affecting activity. An example is given in the paper "Tritriated D-ala^1-Peptide T Binding", Smith C. S. *et al.*, Drug Development Res., 15, pp. 371-379 (1988). A second method is altering cyclic structure for stability, such as N to C interchain imides and lactames (Ede *et al.* in Smith and Rivier (Eds.) "Peptides: Chemistry and Biology", Escom, Leiden (1991), pp. 268-270). An example of this is given in conformationally restricted thymopentin-like compounds, such as those disclosed in U.S. Pat. No. 4,457,489 (1985), Goldstein, G. *et al.*, the disclosure of which is incorporated by reference herein in its entirety. A third method is to substitute peptide bonds in the peptide by pseudopeptide bonds that confer resistance to proteolysis. The synthesis of peptides containing pseudopeptide bonds such as —CH_2—NH—, —CH_2—S—, —CH_2—SO—, —CH_2—SO_2—, —NH—CO— or —CH=CH— is performed either by solution methods or in a combined procedure with solid-phase synthesis using standard methods of organic chemistry. Thus, for example, the introduction of the —CH_2—NH— bond is accomplished by preparing in solution the aldehyde Fmoc-NH-CHR-CHO according to the technique described by FEHRENTZ and CASTRO (Synthesis, 676-678, 1983) and condensing it with the growing peptide chain, either on a solid phase according to the technique described by SASAKI and COY (Peptides, 8, 119-121, 1988), or in solution.

[0105] Pharmaceutical Compositions/ Formulations

[0106] Pharmaceutical compositions for use in the embodiments described herein can be formulated by standard techniques using one or more physiologically acceptable carriers or excipients. In some embodiments, the formulations may contain a buffer and/or a preservative. The compounds and their physiologically acceptable salts and solvates can be formulated for administration by any suitable route, including via inhalation, topically, nasally, orally, parenterally (*e.g.*, intravenously, intraperitoneally, intravesically or intrathecally) or rectally in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the peptide, chosen route of administration and standard biological practice.

[0107] According to some embodiments, pharmaceutical compositions are provided comprising effective amounts of one or more compound(s) described herein together with, for example, pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or other carriers. Such compositions include diluents of various buffer content (*e.g.*, TRIS or other amines, carbonates, phosphates, amino acids, for example, glycinamide hydrochloride (especially in the physiological pH range), N-glycylglycine, sodium or potassium phosphate (dibasic, tribasic), *etc.* or TRIS-HCl or acetate), pH and ionic strength; additives such as detergents and solubilizing agents (*e.g.*, surfactants such as Pluronics, Tween 20, Tween 80 (Polysorbate 80), Cremophor, polyols such as polyethylene glycol, propylene glycol, *etc.*), anti-oxidants (*e.g.*, ascorbic acid, sodium metabisulfite), preservatives (*e.g.*, Thimersol, benzyl alcohol, parabens, *etc.*) and bulking substances (*e.g.*, sugars such as sucrose, lactose, mannitol, polymers such as polyvinylpyrrolidones or dextran, *etc.*); and/or incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, *etc.* or into liposomes. Hyaluronic acid may also be used. Such compositions can be employed to influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of a compound described herein. See, *e.g.*, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference. The compositions can, for example, be prepared in liquid form, or can be in dried powder, such as lyophilized form. Particular methods of administering such compositions are described *infra*.

[0108] Where a buffer is to be included in the formulations, the buffer is selected from the group consisting of sodium acetate, sodium carbonate, citrate, glycylglycine, histidine, glycine,

lysine, arginin, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate, and tris(hydroxymethyl)-aminomethan, or mixtures thereof. Each one of these specific buffers constitutes an alternative embodiment. In some embodiments, the buffer is glycylglycine, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium phosphate or mixtures thereof.

[0109] Where a pharmaceutically acceptable preservative is to be included in the formulations, the preservative is selected from the group consisting of phenol, m-cresol, methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, 2-phenoxyethanol, butyl p-hydroxybenzoate, 2-phenylethanol, benzyl alcohol, chlorobutanol, and thiomerosal, or mixtures thereof. Each one of these specific preservatives constitutes an alternative embodiment. In some embodiments, the preservative is phenol or m-cresol.

[0110] In some embodiments, the preservative is present in a concentration from about 0.1 mg/ml to about 50 mg/ml, in a concentration from about 0.1 mg/ml to about 25 mg/ml, or in a concentration from about 0.1 mg/ml to about 10 mg/ml.

[0111] The use of a preservative in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

[0112] In some embodiments, the formulation may further comprise a chelating agent where the chelating agent may be selected from salts of ethlenediaminetetraacetic acid (EDTA), citric acid, and aspartic acid, and mixtures thereof. Each one of these specific chelating agents constitutes an alternative embodiment.

[0113] In some embodiments, the chelating agent is present in a concentration from 0.1 mg/ml to 5 mg/ml. In some embodiments, the chelating agent is present in a concentration from 0.1 mg/ml to 2 mg/ml. In some embodiments, the chelating agent is present in a concentration from 2 mg/ml to 5 mg/ml.

[0114] The use of a chelating agent in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

[0115] In some embodiments, the formulation may further comprise a stabilizer selected from the

group of high molecular weight polymers or low molecular compounds where such stabilizers include, but are not limited to, polyethylene glycol (*e.g.* PEG 3350), polyvinylalcohol (PVA), polyvinylpyrrolidone, carboxymethylcellulose, different salts (*e.g.* sodium chloride), L-glycine, L-histidine, imidazole, arginine, lysine, isoleucine, aspartic acid, tryptophan, threonine and mixtures thereof. Each one of these specific stabilizers constitutes an alternative embodiment. In some embodiments, the stabilizer is selected from the group consisting of L-histidine, imidazole and arginine.

[0116] In some embodiments, the high molecular weight polymer is present in a concentration from 0.1 mg/ml to 50 mg/ml. In some embodiments, the high molecular weight polymer is present in a concentration from 0.1 mg/ml to 5 mg/ml. In some embodiments, the high molecular weight polymer is present in a concentration from 5 mg/ml to 10 mg/ml. In some embodiments, the high molecular weight polymer is present in a concentration from 10 mg/ml to 20 mg/ml. In some embodiments, the high molecular weight polymer is present in a concentration from 20 mg/ml to 30 mg/ml. In some embodiments, the high molecular weight polymer is present in a concentration from 30 mg/ml to 50 mg/ml.

[0117] In some embodiments, the low molecular weight compound is present in a concentration from 0.1 mg/ml to 50 mg/ml. In some embodiments, the low molecular weight compound is present in a concentration from 0.1 mg/ml to 5 mg/ml. In some embodiments, the low molecular weight compound is present in a concentration from 5 mg/ml to 10 mg/ml. In some embodiments, the low molecular weight compound is present in a concentration from 10 mg/ml to 20 mg/ml. In some embodiments, the low molecular weight compound is present in a concentration from 20 mg/ml to 30 mg/ml. In some embodiments, the low molecular weight compound is present in a concentration from 30 mg/ml to 50 mg/ml.

[0118] The use of a stabilizer in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

[0119] In some embodiments, the formulation may further comprise a surfactant where a surfactant may be selected from a detergent, ethoxylated castor oil, polyglycolyzed glycerides, acetylated monoglycerides, sorbitan fatty acid esters, poloxamers, such as 188 and 407, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene derivatives such as alkylated and alkoxylated derivatives (tweens, *e.g.* Tween-20, or Tween-80), monoglycerides or ethoxylated derivatives

thereof, diglycerides or polyoxyethylene derivatives thereof, glycerol, cholic acid or derivatives thereof, lecithins, alcohols and phospholipids, glycerophospholipids (lecithins, kephalins, phosphatidyl serine), glyceroglycolipids (galactopyransoide), sphingophospholipids (sphingomyelin), and sphingoglycolipids (ceramides, gangliosides), DSS (docusate sodium, docusate calcium, docusate potassium, SDS (sodium dodecyl sulfate or sodium lauryl sulfate), dipalmitoyl phosphatidic acid, sodium caprylate, bile acids and salts thereof and glycine or taurine conjugates, ursodeoxycholic acid, sodium cholate, sodium deoxycholate, sodium taurocholate, sodium glycocholate, N-Hexadecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, anionic (alkyl-aryl-sulphonates) monovalent surfactants, palmitoyl lysophosphatidyl-L-serine, lysophospholipids (*e.g.* 1-acyl-sn-glycero-3-phosphate esters of ethanolamine, choline, serine or threonine), alkyl, alkoxyl (alkyl ester), alkoxy (alkyl ether)-derivatives of lysophosphatidyl and phosphatidylcholines, *e.g.* lauroyl and myristoyl derivatives of lysophosphatidylcholine, dipalmitoylphosphatidylcholine, and modifications of the polar head group, that is cholines, ethanolamines, phosphatidic acid, serines, threonines, glycerol, inositol, and the positively charged DODAC, DOTMA, DCP, BISHOP, lysophosphatidylserine and lysophosphatidylthreonine, zwitterionic surfactants (*e.g.* N-alkyl-N,N-dimethylammonio-1-propanesulfonates, 3-cholamido-1-propyldimethylammonio-1-propanesulfonate, dodecylphosphocholine, myristoyl lysophosphatidylcholine, hen egg lysolecithin), cationic surfactants (quarternary ammonium bases) (*e.g.* cetyl-trimethylammonium bromide, cetylpyridinium chloride), non-ionic surfactants, polyethyleneoxide/polypropyleneoxide block copolymers (Pluronics/Tetronics, Triton X-100, Dodecyl β-D-glucopyranoside) or polymeric surfactants (Tween-40, Tween-80, Brij-35), fusidic acid derivatives--(*e.g.* sodium tauro-dihydrofusidate *etc.*), long-chain fatty acids and salts thereof C6-C12 (*e.g.* oleic acid and caprylic acid), acylcarnitines and derivatives, N_α -acylated derivatives of lysine, arginine or histidine, or side-chain acylated derivatives of lysine or arginine, N_α-acylated derivatives of dipeptides comprising any combination of lysine, arginine or histidine and a neutral or acidic amino acid, N_α-acylated derivative of a tripeptide comprising any combination of a neutral amino acid and two charged amino acids, or the surfactant may be selected from the group of imidazoline derivatives, or mixtures thereof. Each one of these specific surfactants constitutes an alternative embodiment.

[0120] The use of a surfactant in pharmaceutical compositions is well-known to the skilled person. For convenience reference is made to Remington: The Science and Practice of Pharmacy, 19th edition, 1995.

[0121] In some embodiments, pharmaceutically acceptable sweeteners comprise at least one intense sweetener such as saccharin, sodium or calcium saccharin, aspartame, acesulfame potassium, sodium cyclamate, alitame, a dihydrochalcone sweetener, monellin, stevioside or sucralose (4,1',6'-trichloro-4,1',6'-trideoxygalactosucrose), or saccharin, sodium or calcium saccharin, and optionally a bulk sweetener such as sorbitol, mannitol, fructose, sucrose, maltose, isomalt, glucose, hydrogenated glucose syrup, xylitol, caramel or honey.

[0122] Intense sweeteners are conveniently employed in low concentrations. For example, in the case of sodium saccharin, the concentration may range from 0.04% to 0.1% (w/v) based on the total volume of the final formulation, and, in some embodiments, is about 0.06% in the low-dosage formulations and about 0.08% in the high-dosage ones. The bulk sweetener can effectively be used in larger quantities ranging from about 10% to about 35%, or from about 10% to 15% (w/v).

[0123] The formulations may be prepared by conventional techniques, *e.g.* as described in Remington's Pharmaceutical Sciences, 1985 or in Remington: The Science and Practice of Pharmacy, 19th edition, 1995, where such conventional techniques of the pharmaceutical industry involve dissolving and mixing the ingredients as appropriate to give the desired end product.

[0124] The phrase "pharmaceutically acceptable" or "therapeutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and/or do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. As used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a State government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia (*e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985)) for use in animals, and more particularly in humans.

[0125] Administration of the compounds may be carried out using any method known in the art. For example, administration may be transdermal, parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intracerebroventricular, intrathecal, intranasal, aerosol, by suppositories, or oral administration. In some embodiments, a pharmaceutical composition can be for administration for injection, or for oral, pulmonary,

nasal, transdermal, ocular administration.

[0126] For oral administration, the peptide or a therapeutically acceptable salt thereof can be formulated in unit dosage forms such as capsules or tablets. The tablets or capsules may be prepared by conventional means with pharmaceutically acceptable excipients, including binding agents, for example, pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose; fillers, for example, lactose, microcrystalline cellulose, or calcium hydrogen phosphate; lubricants, for example, magnesium stearate, talc, or silica; disintegrants, for example, potato starch or sodium starch glycolate; or wetting agents, for example, sodium lauryl sulphate. Tablets can be coated by methods well known in the art. Liquid preparations for oral administration can take the form of, for example, solutions, syrups, or suspensions, or they can be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives, for example, suspending agents, for example, sorbitol syrup, cellulose derivatives, or hydrogenated edible fats; emulsifying agents, for example, lecithin or acacia; non-aqueous vehicles, for example, almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils; and preservatives, for example, methyl or propyl-p-hydroxybenzoates or sorbic acid. The preparations can also contain buffer salts, flavoring, coloring, and/or sweetening agents as appropriate. If desired, preparations for oral administration can be suitably formulated to give controlled release of the active compound.

[0127] For topical administration, the composition can be formulated in a pharmaceutically acceptable vehicle containing 0.1 to 10 percent or 0.5 to 5 percent, of the active compound(s). Such formulations can be in the form of a cream, lotion, sublingual tablet, aerosols and/or emulsions and can be included in a transdermal or buccal patch of the matrix or reservoir type as are conventional in the art for this purpose.

[0128] For parenteral administration, the compounds can be administered by either intravenous, subcutaneous, or intramuscular injection, in compositions with pharmaceutically acceptable vehicles or carriers. The compounds can be formulated for parenteral administration by injection, for example, by bolus injection or continuous infusion. Formulations for injection can be presented in unit dosage form, for example, in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and can contain formulatory agents, for example,

suspending, stabilizing, and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, for example, sterile pyrogen-free water, before use.

[0129] For administration by injection, it is common to use the compound(s) in solution in a sterile aqueous vehicle which may also contain other solutes such as buffers or preservatives as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic. In some embodiments, the pharmaceutical compositions may be formulated with a pharmaceutically acceptable carrier to provide sterile solutions or suspensions for injectable administration. In particular, injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspensions in liquid prior to injection or as emulsions. Suitable excipients are, for example, water, saline, dextrose, mannitol, lactose, lecithin, albumin, sodium glutamate, cysteine hydrochloride, or the like. In addition, if desired, the injectable pharmaceutical compositions may contain minor amounts of nontoxic auxiliary substances, such as wetting agents, pH buffering agents, and the like. If desired, absorption enhancing preparations (*e.g.*, liposomes) may be utilized. Suitable pharmaceutical carriers are described in "Remington's pharmaceutical Sciences" by E. W. Martin.

[0130] For administration by inhalation, the compounds may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, for example, gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the compound and a suitable powder base, for example, lactose or starch. For intranasal administration the compounds may be used, for example, as a liquid spray, as a powder or in the form of drops.

[0131] The compounds can also be formulated in rectal compositions, for example, suppositories or retention enemas, for example, containing conventional suppository bases, for example, cocoa butter or other glycerides.

[0132] Furthermore, the compounds can be formulated as a depot preparation. Such long-acting formulations can be administered by implantation (for example, subcutaneously or

intramuscularly) or by intramuscular injection. Thus, for example, the compounds can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0133] The compositions can, if desired, be presented in a pack or dispenser device that can contain one or more unit dosage forms containing the active ingredient. The pack can, for example, comprise metal or plastic foil, for example, a blister pack. The pack or dispenser device can be accompanied by instructions for administration.

[0134] ***Dosages***

[0135] The compounds may be administered to a patient at therapeutically effective doses to prevent, treat, or control diseases and disorders mediated, in whole or in part, by a GPCR-ligand interaction. Pharmaceutical compositions comprising one or more of compounds may be administered to a patient in an amount sufficient to elicit an effective protective or therapeutic response in the patient. An amount adequate to accomplish this is defined as "therapeutically effective dose" or "therapeutically effective amount."

[0136] Toxicity and therapeutic efficacy of such compounds can be determined, for example, by standard pharmaceutical procedures in cell cultures or experimental animals, for example, by determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and can be expressed as the ratio, LD50/ED50. Compounds that exhibit large therapeutic indices can be used. While compounds that exhibit toxic side effects can be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue to minimize potential damage to normal cells and, thereby, reduce side effects.

[0137] The data obtained from cell culture assays and animal studies can be used to formulate a dosage range for use in humans. In some embodiments, the dosage of such compounds is within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration. For any compound used in the methods, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to

achieve a circulating plasma concentration range that includes the IC50 (the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography (HPLC). In general, the dose equivalent of a modulator is from about 1 ng/kg to 10 mg/kg for a typical subject.

[0138] The amount and frequency of administration of the compounds and/or the pharmaceutically acceptable salts thereof will be regulated according to the judgment of the attending clinician considering such factors as age, condition and size of the patient as well as severity of the symptoms being treated. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition. In general it is contemplated that an effective amount would be from 0.001 mg/kg to 10 mg/kg body weight, and in particular from 0.01 mg/kg to 1 mg/kg body weight. More specifically it is contemplated that an effective amount would be to continuously infuse by intravenous administration from 0.01 micrograms/kg body weight/min to 100 micrograms/kg body weight/min for a period of 12 hours to 14 days. It may be appropriate to administer the required dose as two, three, four or more sub-doses at appropriate intervals throughout the day. Said sub-doses may be formulated as unit dosage forms, for example, containing 0.01 to 500 mg, and in particular 0.1 mg to 200 mg of active ingredient per unit dosage form.

[0139] In some embodiments, the peptide or mimetics is administered at a rate of about 0.5 μg/kg/min to about 20 μg/kg/min, about 0.5 μg/kg/min to about 15 μg/kg/min, about 0.5 μg/kg/min to about 10 μg/kg/min, about 0.5 μg/kg/min to about 5 μg/kg/min, about 0.5 μg/kg/min to about 4 μg/kg/min, about 0.5 μg/kg/min to about 3 μg/kg/min, about 0.5 μg/kg/min to about 2 μg/kg/min, about 0.5 μg/kg/min to about 1 μg/kg/min, about 1 μg/kg/min to about 2 μg/kg/min, about 1 μg/kg/min to about 3 μg/kg/min, about 1 μg/kg/min to about 4 μg/kg/min, about 1 μg/kg/min to about 5 μg/kg/min, about 1 μg/kg/min to about 10 μg/kg/min, about 1 μg/kg/min to about 15 μg/kg/min, about 1 μg/kg/min to about 15 μg/kg/min, about 1 μg/kg/min to about 20 μg/kg/min. In some embodiments, the peptide or peptide mimetic described herein is administered at a rate of about, or at least, 0.5 μg/kg/min, 1 μg/kg/min, 2 μg/kg/min, 3 μg/kg/min, 4 μg/kg/min, 5 μg/kg/min, 6 μg/kg/min, 7 μg/kg/min, 8

μg/kg/min, 9 μg/kg/min, 10 μg/kg/min, 15 μg/kg/min, or 20 μg/kg/min. The dose can be administered for about, or at least, 1-24 hours or any hourly increment in thereof, including the endpoints. In some embodiments, the dose is administered for about 1 to about 7 days, about 2 to about 7 days, about 3 to about 7 days, about 4 to about 7 days, about 5 to about 7 days, or about 6 to about 7 days. In some embodiments, the dose is administered for about 1, about 2, about 3, about 4, about 5, about 6, or about 7 days.

[0140] In some embodiments, the pharmaceutical preparation is in a unit dosage form. In such form, the preparation is subdivided into suitably sized unit doses containing appropriate quantities of the active component, *e.g.*, an effective amount to achieve the desired purpose. The quantity of active compound in a unit dose of preparation may be varied or adjusted from about 0.01 mg to about 1000 mg, from about 0.01 mg to about 750 mg, from about 0.01 mg to about 500 mg, and from about 0.01 mg to about 250 mg, according to the particular application. The actual dosage employed may be varied depending upon the requirements of the patient and the severity of the condition being treated. Determination of the proper dosage regimen for a particular situation is within the skill of the art. For convenience, the total dosage may be divided and administered in portions during the day as required.

[0141] ***Medical Use***

[0142] The compositions are useful for treating any cardiovascular disorder that will respond favorably to a decrease in blood pressure. These disorders include chronic hypertension, hypertensive crisis (an acute hypertensive emergency), acute congestive heart failure, angina, acute myocardial infarction, left ventricular failure, cerebrovascular insufficiency, and intracranial haemorrhage. Intravenous injection is one non-limiting method for treating acute cardiovascular disorders. Such a method would comprise administering a therapeutically effective amount of one or more compounds to a subject or subject in need thereof. Examples of acute cardiovascular disorders include, but are not limited to, hypertensive crisis, toxemia of pregnancy, and acute congestive heart failure.

[0143] ***Combination Therapies***

[0144] Also provided are methods of treating any cardiovascular or cardiorenal disorder by administering one or more of the compositions as described above in combination with other drugs for the treatment of cardiovascular and/or cardiorenal disorders. These other drugs include diuretics such as furosemide; vasodilators such as nitroglycerin, nitroprusside, brain

natriuretic peptide (BNP), or analogues thereof; inotropes such as dobutamine; angiotensin convertin enzyme (ACE) inhibitors such as captopril and enalapril; β blockers such as carvedilol and propranolol; angiotensin receptor blockers (ARBs) such as valsartan and candesartan; and/or aldosterone antagonists such as spironolactone.

[0145] In the combination therapies, one or more compounds or compositions are coadministered with one or more drugs for the treatment of cardiovascular and/or cardiorenal disorders to increase efficacy of treatment of cardiovascular and/or cardiorenal disorders and to reduce side effects associated with high doses of these therapeutics.

[0146] The combination therapies described above have synergistic and additive therapeutic effects. Synergy is defined as the interaction of two or more agents so that their combined effect is greater than the sum of their individual effects. For example, if the effect of drug A alone in treating a disease is 25%, and the effect of drug B alone in treating a disease is 25%, but when the two drugs are combined the effect in treating the disease is 75%, the effect of A and B is synergistic.

[0147] Additivity is defined as the interaction of two or more agents so that their combined effect is the same as the sum of their individual effects. For example, if the effect of drug A alone in treating a disease is 25%, and the effect of drug B alone in treating a disease is 25%, but when the two drugs are combined the effect in treating the disease is 50%, the effect of A and B is additive.

[0148] An improvement in the drug therapeutic regimen can be described as the interaction of two or more agents so that their combined effect reduces the incidence of adverse event (AE) of either or both agents used in co- therapy. This reduction in the incidence of adverse effects can be a result of, *e.g.*, administration of lower dosages of either or both agent used in the co-therapy. For example, if the effect of Drug A alone is 25% and has an adverse event incidence of 45% at labeled dose; and the effect of Drug B alone is 25% and has an adverse event incidence of 30% at labeled dose, but when the two drugs are combined at lower than labeled doses of each, if the overall effect is 35% (an improvement, but not synergistic or additive) and the adverse incidence rate is 20%, there is an improvement in the drug therapeutic regimen.

[0149] **EXAMPLES**

[0150] The following examples are illustrative, but not limiting, of the methods and compositions

described herein. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in therapy and that are obvious to those skilled in the art are within the spirit and scope of the embodiments.

[0151] ***Example 1: Synthesis of Compounds***

[0152] Peptides and intermediates described herein were prepared by the solid-phase method of peptide synthesis. (cf. R. Merrifield J. Am. Chem. Soc. 1964, 85, 2149; M. Bodansky, "Principles of Peptide Synthesis." Springer-Verlag, 1984.) The peptide synthesis and purification procedures employed were standard methods well described in the art, including, but not limited to, amino acid coupling procedures, wash steps, deprotection procedures, resin cleavage procedures, and ion exchange and HPLC purification methods using commercial automated peptide synthesizers and commercially available resins and protected amino acids. More specifically, the peptides were synthesized from their C-terminus by stepwise addition of Fmoc-protected amino acids (pre-activated or in situ activated) and deprotection of the Fmoc group with piperidine to an acid labile linker attached to an insoluble support resin. Following synthesis, the resin bound peptide was side chain-deprotected and detached from the resin with trifluoroacetic acid and cation scavengers. Peptides were purified by aqueous extraction or by precipitation from organic solvents such as ether or t-butyl methyl ether followed by centrifugation and decanting and/or by HPLC and lyophilization.

[0153] ***Example 2: B-arrestin recruitment assay***

[0154] The proximal event in β-arrestin function mediated by GPCRs is recruitment to receptors following ligand binding and receptor phosphorylation by GRK's. Thus, the measure of β-arrestin recruitment was used to determine ligand efficacy for β-arrestin function.

[0155] B-arrestin-2 recruitment to the human and rat angiotensin 2 type 1 receptor (human AT1R and rat AT1aR, respectively) was measured with the PathHunter™ β-arrestin assay (DiscoveRx Corporation, Fremont CA). Cells, plasmid(s), and detection reagent(s) were purchased from DiscoveRx, and assays were performed per manufacturer's instructions. Human AT1R and rat AT1aR were cloned into the pCMV-ProLink vector, verified by sequencing, and transfected into PathHunter β-arrestin HEK293 cells. Stably transfected clonal cell lines were selected with Hygromycin and G418. These clonal cell lines were used for all experiments.

[0156] For assays, 4,000-8,000 cells were seeded per well in 384-well microplates "HiBase" small-

volume plate in volumes of 20 uL and grown overnight in the incubator (37° C, 5% CO2, saturated humidity). Peptides were dissolved in DMSO to a concentration of 10 mM. Peptides were then further diluted in assay buffer (Hank's balanced salt solution with 20 mM HEPES) to add peptide to the cells to reach final concentrations ranging from 100 μM to 1 pM. Cells were then incubated for 60 minutes at 37°C in 5% CO_2, followed by addition of 2 μL of PathHunter Detection Reagent to each well. The microplates were then incubated at room temperature for 60 minutes, and then luminescence was measured using a PHERAstar Plus microplate reader from BMG Labtech. B-arrestin-2 recruitment to receptors was measured as relative luminescence intensity expressed in arbitrary units. Results are displayed in Table 2below.

[0157] ***Example 3: IP1 accumulation assay***

[0158] A secondary measure of G protein coupling efficacy was also performed. IP3 is generated by activation of phospholipase C by Gα-q. IP3 is degraded to IP1, which can be forced to accumulate in cells by blocking degradation with lithium chloride. Thus we measured accumulation of IP1 to determine ligand efficacy for G protein activation.

[0159] IP1 accumulation generated by human and rat angiotensin 2 type 1 receptor (human AT1R and rat AT1aR, respectively) was measured with IP-One Tb kits purchased from Cisbio and used per the manufacturer's instructions. Clonal stably transfected cell lines expressing human AT1TR or rat AT1aR were used for all experiments.

[0160] For assays, 4,000 -8,000 cells were seeded per well in 384-well small-volume microplates "HiBase" small-volume plate in volumes of 20 uL and allowed to grow overnight at 37°C in 5% CO_2. Cell growth media was then replaced with stimulation buffer supplied by Cisbio containing 50 mM lithium chloride. Peptides TRV0111318-336;468-471;479-482;546-548;847-860 through to TRV0111879-885 were dissolved in DMSO to a concentration of 10 mM. For agonist detection, peptides were then further diluted in stimulation buffer to add peptide to the cells to reach final concentrations ranging from 100 uM to 1 pM. Following addition of peptides, cells were incubated at 37°C in 5% CO_2 for 30 minutes and then lysed with 4 uL of pre-mixed HTRF IP-One reagents diluted per manufacturer's instructions (Cisbio). Microplates were incubated for 60-90 minutes at room temperature and then time-resolved fluorescence intensities were measured using a PHERAstar Plus microplate reader from BMG Labtech. IP1 accumulation was measured as change in ratio of time-resolved

fluorescent intensities measured at 665 nm and 620 nm. Results are displayed in Tables 2 and 3 below.

[0161] **Table 2: Biological activity.**

	IP1 G-protein assay					
Identifier or SEQ ID NO:	human AT1R			rat AT1aR		
	pEC50	EC50 (nM)	Span	pEC50	EC50 (nM)	Span
hAngII	9.2	0.6	103	9.2	0.6	104
losartan	>9.2	>10000	<103	>9.2	<0.6	<104
SEQ ID NO: 1	>9.2	>10000	n/a	<9.2	>0.6	<104
SEQ ID NO: 2	<9.2	10000	<103	>9.2	>0.6	n/a
SEQ ID NO: 3	>9.2	>10000	n/a	>9.2	>0.6	n/a
SEQ ID NO:4	<9.2	2.5	<103	>9.2	>0.6	n/a
SEQ ID NO:5	<9.2	3.2	<103	<9.2	>0.6	<104
SEQ ID NO:6	<9.2	63.1	<103	<9.2	>0.6	<104
SEQ ID NO: 7	>9.2	>10000	n/a	>9.2	>0.6	n/a
SEQ ID NO:8	<9.2	6310	<103	>9.2	>0.6	n/a
SEQ ID NO:9	<9.2	5.0	<103	<9.2	>0.6	<104
SEQ ID NO:10	<9.2	1.3	<103	<9.2	>0.6	<104
SEQ ID NO:11	<9.2	>1.3	<103	>9.2	>0.6	n/a
SEQ ID NO:12	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 13	>9.2	>1.3	n/a	>9.2	>0.6	n/a
SEQ ID NO: 14	>9.2	>1.3	n/a	>9.2	>0.6	n/a
SEQ ID NO: 15	>9.2	>1.3	n/a	<9.2	>0.6	<104
SEQ ID NO:16	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO:17	<9.2	<1.3	<103	<9.2	>0.6	>104
SEQ ID NO:18	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO:19	<9.2	>1.3	<103	>9.2	>0.6	n/a
SEQ ID NO:20	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 21	>9.2	>1.3	n/a	<9.2	>0.6	<104
SEQ ID NO:22	>9.2	>1.3	n/a	<9.2	>0.6	<104
SEQ ID NO:23	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO:24	<9.2	<1.3	>103	<9.2	>0.6	<104
SEQ ID NO: 29	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 30	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 31	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 32	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 33	<9.2	>1.3	<103	N.Q.	N.Q.	
SEQ ID NO: 34	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 35	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 36	N.Q.	N.Q.		N.Q.	N.Q.	

SEQ ID NO: 37	N.Q.	N.Q.		<9.2	>0.6	<104
SEQ ID NO: 38	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 39	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 40	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 41	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 42	<9.2	>1.3	<103	N.Q.	N.Q.	
SEQ ID NO: 43	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 44	N.Q.	N.Q.		<9.2	>0.6	<104
SEQ ID NO: 45	<9.2	>1.3	<103	N.Q.	N.Q.	
SEQ ID NO: 46	<9.2	>1.3	<103	N.Q.	N.Q.	
SEQ ID NO: 47	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 48	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 49	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 50	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 51	<9.2	>1.3	<103	N.Q.	N.Q.	
SEQ ID NO: 52	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 53	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 54	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 55	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 56	<9.2	>1.3	<103	<9.2	>0.6	<104
SEQ ID NO: 57	Inactive	Inactive		Inactive	Inactive	
SEQ ID NO: 58	Inactive	Inactive		<9.2	>0.6	<104
SEQ ID NO: 59	Inactive	Inactive		<9.2	>0.6	<104
SEQ ID NO: 60	<9.2	>1.3	<103	<9.2	>0.6	<104

Span (relative to hAngII); N.Q.=not quantified

Table 3

	beta-arrestin2 assay					
Identifier or SEQ ID NO:	human AT1R			rat AT1aR		
	pEC50	**EC50 (nM)**	**Span**	**pEC50**	**EC50 (nM)**	**Span**
hAngII	8.5	3.2	101	8.5	3.2	105
losartan	<8.5	>3.2	<101	Inactive	Inactive	
SEQ ID NO: 1	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 2	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 3	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:4	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:5	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:6	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 7	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:8	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:9	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:10	<8.5	>3.2	<101	8.5	3.2	>105
SEQ ID NO:11	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:12	<8.5	>3.2	<101	<8.5	>3.2	<105

SEQ ID NO: 13	<8.5	>3.2	103	<8.5	>3.2	<105
SEQ ID NO: 14	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 15	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:16	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:17	<8.5	>3.2	>101	8.5	3.2	<105
SEQ ID NO:18	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:19	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:20	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 21	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:22	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:23	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO:24	<8.5	>3.2	>101	<8.5	>3.2	<105
SEQ ID NO: 29	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 30	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 31	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 32	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 33	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 34	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 35	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 36	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 37	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 38	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 39	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 40	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 41	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 42	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 43	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 44	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 45	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 46	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 47	<8.5	>3.2	<101	N.Q.	N.Q.	
SEQ ID NO: 48	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 49	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 50	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 51	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 52	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 53	N.Q.	N.Q.		N.Q.	N.Q.	
SEQ ID NO: 54	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 55	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 56	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 57	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 58	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 59	<8.5	>3.2	<101	<8.5	>3.2	<105
SEQ ID NO: 60	<8.5	>3.2	<101	<8.5	>3.2	<105

Span (relative to hAngII); N.Q.=not quantified

[0162] ***Example 4: Calcium mobilization assay***

[0163] G protein efficacy can be measured in many ways. GPCRs that couple to the G_q subclass of heterotrimeric g proteins activate a wide array of signal transduction when activated by agonists. One of the most commonly measured pathways is activation of phospholipase C by Galpha-q, which cleaves phosphatidylinositol bisphosphate to release IP_3; IP_3 in turn releases calcium to the cytosol from intracellular stores via the IP_3 receptor. Thus we measured intracellular free calcium to determine ligand efficacy for G protein activation.

[0164] Intracellular free calcium generated by human and rat angiotensin 2 type 1 receptor (human AT1R and rat AT1aR, respectively) was measured with Fluo-4 NW kits purchased from Invitrogen and used per the manufacturer's instructions. Clonal stably transfected cell lines expressing human AT1TR or rat AT1aR were used for all experiments.

[0165] For assays, 25,000 cells were seeded per well in 96-well microplates in volumes of 90 uL and allowed to grow overnight at 37°C in 5% CO_2. Fluo-4 NW dye was mixed with probenecid and assay buffer (Hank's balanced salt solution with 20 mM HEPES), and cell growth media was replace with this mixture, followed by incubation for 30-45 minutes at 37°C in 5% CO_2. Peptides were dissolved in deionized water to a concentration of 1mM. Peptides were then further diluted in assay buffer (Hank's balanced salt solution with 20 mM HEPES) to add peptide to the cells to reach final concentrations ranging from 10 uM to 1 pM. Peptide was added to cells while fluorescence intensity was measured using a NOVOstar microplate reader purchased from BMG Labtech. Calcium mobilization was measured as relative fluorescence intensity expressed as fold over basal at 5 seconds and 20 seconds after ligand addition.

[0166] ***Example 5: Evaluation of Peptides in Normal Rats. (Prophetic Example)***

[0167] The effects of the peptides described herein on vascular and cardiac function are tested by i.v. infusion at doses ranging from 0.1 – 10 μg/kg/min in preliminary dosing experiments in normal anesthetized rats. Various hemodynamic measurements are made including mean arterial pressure, heart rate and pressure volume relationships. The peptides are expected to produce a dose-dependent decrease in mean arterial pressure with little to no effect on HR. In addition, the peptides are expected to increase the slope of the end systolic pressure volume relationship and preserves pre-recruitable stroke work, resulting in preservation of stroke volume in the background of a drop in vasoconstriction.

[0168] ***Example 6: Evaluation of Peptides in a Paced Dog Model of Acute Heart Failure. (Prophetic Example)***

[0169] The peptides described herein (0.01, 0.1, 1, 10 and 100 mcg/kg/min dose escalation, 30 minutes each dose) are dosed in the paced heart failure model. In the paced dog model, pacemakers are implanted and the dog hearts are paced for ten days at a rate of 240 beats per minute, resulting in reduced left ventricular systolic function, right-side congestion, and an elevation in the renin-angiotensin system activity. In the heart failure dogs one or more of the peptides are expected to produce a dose-dependent decrease in mean arterial pressure, systemic vascular resistance, pulmonary capillary wedge pressure, and right arterial pressure, and cardiac output is expected to be preserved in these animals. At the level of the kidney, there is expected to be a dose-dependent increase in renal blood flow resulting in a significant drop in renal vascular resistance. Urine sodium excretion may modestly increase with urine output, urine potassium, and glomerular filtration rate being maintained.

[0170] While some embodiments have been described with reference to particular examples, those skilled in the art recognize that various modifications may be made to the embodiments without departing from the spirit and scope thereof.

[0171] All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety.

What is claimed is:

1. A peptide or peptide mimetic comprising comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein:
Zz is Arg or Met;
Aa is Tyr or D-Cys;
Xx is Pro, NMeIle, Ile, cyHex, cyPen, AA01, AA02, or AA03;
Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me;
Yy is any amino acid residue, D-Ala, AA01, AA02, AA03, or null;
provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me.

2. The peptide or peptide mimetic of claim 1, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine.

3. The peptide or peptide mimetic of claim 1, wherein Yy is AA01, AA02, or AA03.

4. The peptide or peptide mimetic of claim 1, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

5. The peptide or peptide mimetic of claim 1, wherein Yy is null.

6. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is cyHex.

7. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is cyPen.

8. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is proline, N-Methyl-isoleuceine, or isoleucine.

9. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is AA01.

10. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is AA02.

11. The peptide or peptide mimetic of any one of claims 1-5, wherein Xx is AA03.

12. The peptide or peptide mimetic of any one of claims 1-11, wherein Bb is Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me.

13. The peptide or peptide mimetic of any one of claims 1-11, wherein Bb is Pro or Cys.

14. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is proline and Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

15. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is cyHex and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

16. The peptide or peptide mimetic of claim 15, wherein Yy is AA01, AA02, or AA03.

17. The peptide or peptide mimetic of claim 15, wherein Yy is null.

18. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is cyPen and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

19. The peptide or peptide mimetic of claim 18, wherein Yy is AA01, AA02, or AA03.

20. The peptide or peptide mimetic of claim 18, wherein Yy is null.

21. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is AA01 and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

22. The peptide or peptide mimetic of claim 21, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

23. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is AA02 and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

24. The peptide or peptide mimetic of claim 23, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

25. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is AA03 and Yy is AA01, AA02, AA03, alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

26. The peptide or peptide mimetic of claim 25, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, or phenylalanine.

27. The peptide or peptide mimetic of any one of claims 1, 2, 12, or 13, wherein Xx is Ile or NMeIle and Yy is AA01, AA02, AA03, alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, or null.

28. The peptide or peptide mimetic of claim 27, wherein Yy is D-alanine or null.

29. The peptide or peptide mimetic of claims 27 or 28, wherein Bb is cysteine.

30. The peptide or peptide mimetic of any one of claims 1-29, wherein Zz is arginine.

31. The peptide or peptide mimetic of any one of claims 1-29, wherein Zz is methionine.

32. The peptide or peptide mimetic of any one of claims 1-31, wherein Aa is tyrosine.

33. The peptide or peptide mimetic of any one of claims 1-31, wherein Aa is D-cysteine.

34. The peptide or peptide mimetic of claim 1, wherein the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 1-24 and 29-60.

35. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-Tyr-Pro-His-Pro-Yy (SEQ ID NO: 26),
wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine;

36. The peptide or peptide mimetic of claim 35, wherein the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 1, 4-10, and 60.

37. A peptide or peptide mimetic comprising the sequence of
Sar-Zz-Val-Tyr-cyHex-His-Bb-Yy (SEQ ID NO: 27), wherein:
ZZ is lysine, arginine or methionine,
Bb is Pro, Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; and
Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null;

38. The peptide or peptide mimetic of claim 38, wherein Bb is Pro-NH-i-Pr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me and Yy is null.

39. The peptide or peptide mimetic of claim 37, wherein the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 2, 11-17, 31-32, 34-39, 41-50, and 54-57.

40. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-Tyr-cyPen-His-Pro-Yy (SEQ ID NO: 28),
wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine;

41. The peptide or peptide mimetic of claim 40, wherein the peptide or peptide mimetic comprises a sequence selected from the group consisting of SEQ ID NOs: 3, 18-24, and 59.

42. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-Tyr-AA01-His-Pro-Yy (SEQ ID NO: 61),
wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null;

43. The peptide or peptide mimetic of claim 42, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine.

44. The peptide or peptide mimetic of claim 42, wherein the peptide or peptide mimetic comprises SEQ ID NO: 33 or SEQ ID NO: 40.

45. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-Tyr-AA02-His-Pro-Yy (SEQ ID NO: 62),
wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null;

46. The peptide or peptide mimetic of claim 45, wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine.

47. The peptide or peptide mimetic of claim 45, wherein the peptide or peptide mimetic comprises SEQ ID NO: 29 or 30.

48. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-D-Cys-Ile-His-Cys-Yy (SEQ ID NO: 63),
wherein Yy is alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, or null;

49. The peptide or peptide mimetic of claim 48, wherein Yy is D-Alanine or null.

50. A peptide or peptide mimetic comprising the sequence of
Sar-Arg-Val-Tyr-NMeIle-His-Pro-Yy (SEQ ID NO: 64),
wherein Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, or histidine.

51. The peptide or peptide mimetic of claim 50, wherein Yy is D-Alanine.

52. The peptide or peptide mimetic of claim 50, wherein the peptide or peptide mimetic comprises SEQ ID NO: 58.

53. A composition comprising:
a) a peptide or peptide mimetic of any one of claims 1-52;
b) a peptide or peptide mimetic wherein the members of the sequence of the peptide or peptide mimetic maintain their relative positions as they appear in the sequence described in a), wherein spacers of 1 to 3 amino acids or amino acid analogues are inserted between one or more of the amino acids or amino acid analogues as described in a) and wherein the total length of the peptide or peptide mimetic is from 8 to 25 amino acids and/or amino acid analogues; or
c) a peptide or peptide mimetic that is at least 85% identical to the peptide or peptide mimetics described in a).

54. The peptide or peptide mimetic of any one of claims 1-52 or the composition of claim 53, wherein the peptide or peptide mimetic is cyclic.

55. The peptide or peptide mimetic of any one of claims 1-52 or the composition of claim 53, wherein the peptide or peptide mimetic is dimerized.

56. The peptide or peptide mimetic of any one of claims 1-52 or the composition of claim 53,wherein the peptide or peptide mimetic is trimerized.

57. A pharmaceutical composition comprising one or more peptides or peptide mimetics of any one of claims 1-55 and a pharmaceutically acceptable carrier.

58. The pharmaceutical composition of claim 57, wherein the pharmaceutically acceptable carrier is pure sterile water, phosphate buffered saline or an aqueous glucose, solution.

59. A method of treating cardiovascular disorders comprising administering to a subject or subject in need thereof a therapeutically effective amount of one or more peptides, peptide mimetics, or compositions of any one of claims claim 1-57.

60. The method of claim 59 wherein the cardiovascular disorder is chronic hypertension, hypertensive crisis, acute congestive heart failure, angina, acute myocardial infarction, left ventricular failure, cerebrovascular insufficiency, intracranial haemorrhage, heart failure, acute decompensated heart failure, essential hypertension, post-operative hypertension, hypertensive heart disease, hypertensive renal disease, renovascular hypertension, malignant hypertension, post-renal transplant patient stabilization, dilated cardiomyopathy, myocarditis, post-cardiac transplant patient stabilization, disorders associated with post-stent management, neurogenic hypertension, pre-eclampsia, abdominal aortic aneurysm, or any cardiovascular disorder with a hemodynamic component.

61. The method of claim 59 wherein the cardiovascular disorder is an acute cardiovascular disorder.

62. The method of claim 61 wherein the acute cardiovascular disorder is acute hypertensive crisis, toxemia of pregnancy, acute myocardial infarction, acute congestive heart failure, acute ischaemic heart disease, pulmonary hypertension, post-operative hypertension, migraine, retinopathy or post-operative cardiac/valve surgery.

63. A method of treating a viral infectious disease linked to AT1R comprising: administering to a subject or subject in need thereof a therapeutically effective amount of one or more peptides, peptide mimetics, or compositions of any one of claims claim 1-57.

64. A method of treating cardiovascular disorders comprising: administering to a subject or subject in need thereof a therapeutically effective amount of one or more peptides or peptide mimetics comprising Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein:

Zz is Arg or Met;

Aa is Tyr or D-Cys;

Xx is Pro, NMeIle, Ile, cyHex, cyPen, AA01, AA02, or AA03;

Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me; and

Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, AA01, AA02, AA03, or null,

provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, or Pro-NH-Me.

65. The method of claim 64, wherein the one or more peptides or peptide mimetics are selected from the group consisting of SEQ ID NOs: 1-24 and 29-60.

66. The method of claim 64 wherein the cardiovascular disorder is chronic hypertension, hypertensive crisis, acute congestive heart failure, angina, acute myocardial infarction, left ventricular failure, cerebrovascular insufficiency, intracranial haemorrhage, heart failure, acute decompensated heart failure, essential hypertension, post-operative hypertension, hypertensive heart disease, hypertensive renal disease, renovascular hypertension, malignant hypertension, post-renal transplant patient stabilization, dilated cardiomyopathy, myocarditis, post-cardiac transplant patient stabilization, disorders associated with post-stent management, neurogenic hypertension, pre-eclampsia, abdominal aortic aneurysm, or any cardiovascular disorder with a hemodynamic component.

67. The method of claim 64, wherein the cardiovascular disorder is an acute cardiovascular disorder.

68. A method of treating a viral infectious diseases linked to AT1R comprising:
administering to a subject or subject in need thereof a therapeutically effective amount of one or more a peptides or peptide mimetics comprising

Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein:

Zz is Arg or Met;

Aa is Tyr or D-Cys;

Xx is Pro, NMeIle, Ile, cyHex, cyPen, AA01, AA02, or AA03;

Bb is Pro, Cys, Pro-NH-iPr, Pro-NH-neopentyl, Pro-NH-Et, Pro-NH-Me; and

Yy is alanine, D-alanine, isoleucine, leucine, valine, threonine, serine, methionine, phenylalanine, glycine, aspartic acid, lysine, asparagine, glutamic acid, tryptophan, proline, tyrosine, histidine, AA01, AA02, AA03, or null,

provided that when Aa is Tyr and Xx is Ile, Bb is not Pro, Pro-NH-iPr, Pro-NH-

neopentyl, Pro-NH-Et, or Pro-NH-Me.

69. The method of claim 68, wherein the one or more peptides or peptide mimetics are selected from the group consisting of SEQ ID NOs: 1-24 and 29-60.

70. A method of agonizing β-arrestin comprising: administering to a subject or subject in need thereof an effective amount of one or more compositions of one or more peptides, peptide mimetics, or compositions of any one of claims 1-58.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/23808

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/08; A61K 38/04 (2013.01)
USPC - 514/21.7, 530/328
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)
USPC- 514/21.7, 530/328

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 514/1.1; 530/326, 530/329

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google, Google Scholar: Arrestin-2, ?-arrestin, beta arrestin, TRV120027, sarcosine, Sar-ARVYPHPA, N-methylglycine, Sar-Arg-Val-Tyr-Ile-His-Pro-D-Ala, Sar-Ala-Arg-Val-Tyr-Pro-His-Pro-Ala, angiotensin antagonist, biased ligand. GenCore 6.4.1: SEQ ID NO: 1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/077339 A2 (YAMASHITA, et al.) 08 July 2010 (08.07.2010) para [0042], Table 1, SEQ ID NO: 1-57	1-4, 8, 34
A	US 2010/0184701 A1 (Yamashita, et al.) 22 July 2010 (22.07.2010) Table 1, SEQ ID NO: 1-57	1-4, 8, 34
A	Violin, et al. Selectively engaging b-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. The Journal of Pharmacology and Experimental Therapeutics 2010, 335(3):572-579	1-4, 8, 34
A	Violin, et al. Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol Sci. 2007, 28(8):416-22	1-4, 8, 34
A	Kenakin. Functional selectivity and biased receptor signaling. J Pharmacol Exp Ther. 2011, 336(2):296-302	1-4, 8, 34
A	Dell'Italia. Translational success stories: angiotensin receptor 1 antagonists in heart failure. Circ Res. 2011 Aug 5;109(4):437-52	1-4, 8, 34
A	DeWire, et al. Biased ligands for better cardiovascular drugs: dissecting G-protein-coupled receptor pharmacology. Circ Res. 2011, 109(2):205-16	1-4, 8, 34
A	Rajagopal, et al. Quantifying Ligand Bias at Seven-Transmembrane Receptors. Molecular Pharmacology 2011, 80(3):367-377	1-4, 8, 34

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
06 June 2013 (06.06.2013)	19 JUN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/23808

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

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

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

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

3. ☒ Claims Nos.: 12-33, 53-63, 70
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-11, 34-52 drawn to a peptide or peptide mimetic comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25). The first invention (claims 1-4, 8, 34) to be searched free is restricted to SEQ ID NO:1. Should an additional fee be paid, Applicant is invited to elect an additional SEQ ID NO to be searched, e.g., Seq ID No: 2. Exact claims to be searched will depend on the election. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group will result in only the first claimed invention to be searched/examined.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional 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 additional fees, this Authority did not invite payment of additional fees.

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

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 8, 34, restricted to SEQ ID NO:1

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

International application No.
PCT/US 13/23808

********** Supplemental Sheet **********

In Continuation of Box III. Observations where unity of invention is lacking:

Group II+: claims 64-69, drawn to a method of treatment comprising administering to a subject a therapeutically effective amount of one or more peptides or peptide mimetics comprising SEQ ID NO: 25. The first invention is restricted to SEQ ID NO:1. Should an additional fee be paid, Applicant is invited to elect this or an additional SEQ ID NO to be searched, e.g., Seq Id No: 2. Exact claims to be searched will depend on the election. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group will result in only the first claimed invention to be searched/examined.

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group II+ includes the special technical feature of treating disorders comprising administration the compositions of claim 1, not required by Group I+.

The inventions of Groups I+ and II+ share the technical feature of a peptide or peptide mimetic comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25). However, this shared technical feature does not represent a contribution over prior art as being anticipated by a paper titled "Selectively engaging b-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance" by Violin, et al. (The Journal of Pharmacology and Experimental Therapeutics 2010, 335(3):572-579) (hereinafter "Violin") that discloses a peptide comprising the sequence of Sar-Zz-Val-Aa-Xx-His-Bb-Yy (SEQ ID NO: 25), wherein Zz is Arg, Aa is Tyr, Xx is Ile, Bb is Pro, Yy is D-Ala (Abstract, TRV120027, Sar-Arg-Val-Tyr-Ile-His-Pro-DAla-OH). As the shared technical feature peptide was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

The inventions of Group II+ share the technical feature of a method comprising administering to a subject a therapeutically effective amount of one or more a peptides or peptide mimetics comprising SEQ ID NO: 25. However, this shared technical feature does not represent a contribution over prior art as being anticipated by Violin disclosing administering to a subject a therapeutically effective amount of said peptide (pg 573, col 2, "(pg 573, col 2, para 7, "Telmisartan and TRV120027 were dosed to anesthetized (sodium pentobarbital)/ventilated healthy male rats"; see Fig 5 for effective amount). As the shared technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Therefore, Groups I+-II+ lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

摘要

本申請描述了充當例如β -抑制蛋白效應器的化合物和其在例如治療慢性和急性心血管疾病方面的用途。