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(54) **Title**: ORAL FORMULATIONS OF ANGIOTENSIN

(57) **Abstract**: The present invention provides various formulations for oral delivery of angiotensin peptides.

ORAL FORMULATIONS OF ANGIOTENSIN

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Patent Application Serial No. 61/701,972, filed on September 17, 2012, the disclosure of which is hereby incorporated in its entirety.

BACKGROUND

[0002] Oral delivery is typically a desired route of administration because it is more convenient and involves less patient discomfort as compared to injection, nasal administration and other administration routes. Oral administration of peptides, however, is generally difficult because peptides are susceptible to degradation. Oral administration of short peptides like angiotensins tends to be even more problematic because short peptides typically lack secondary or tertiary structures and therefore are more susceptible to proteolytic enzymes of both the stomach and intestines. These enzymes can quickly degrade a short peptide, rendering it inactive before it can be absorbed into the bloodstream.

SUMMARY OF THE INVENTION

[0003] The present invention provides compositions and methods for effective oral delivery of an angiotensin peptide. In particular, the present invention provides various oral formulations that preserve stability of an angiotensin peptide and enhance its absorption to the blood stream. As a result, an angiotensin peptide delivered according to the present invention may achieve extended half-life and therapeutically effective bioavailability.

[0004] In one aspect, the present invention provides a solid dosage form for oral administration including (a) an angiotensin (1-7) peptide, (b) at least one pharmaceutically acceptable pH-lowering agent, (c) at least one absorption enhancer effective to promote bioavailability of the angiotensin (1-7) peptide, and (d) a protective vehicle.

[0005] In some embodiments, a suitable solid dosage form is a capsule or tablet.

[0006] In some embodiments, a suitable pH-lowering agent is citric acid. In some embodiments, the citric acid is present in an amount greater than about 200 mg (e.g., greater than

about 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg). In some embodiments, the citric acid is present in an amount greater than about 20% (e.g., greater than 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%) of the total weight of the solid dosage form.

[0007] In some embodiments, a suitable pH-lowering agent is tartaric acid.

[0008] In some embodiments, a suitable absorption enhancer is an acylcarnitine. In some embodiments, the acylcarnitine is lauroyl carnitine. In some embodiments, the lauroyl carnitine is present in an mount ranging from about 20-200 mg (e.g., ranging from 20-150 mg, 20-100 mg, 20-90 mg, 20-80 mg, 50-200 mg, 50-150 mg, 50-100 mg, 50-90 mg, 50-80 mg). In some embodiments, the lauroyl carnitine is present in an amount of approximately 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg, or 200 mg. In some embodiments, the lauroyl carnitine is present in an mount ranging from about 2-20% (e.g., 2-15%, 2-10%, 2-7.5%, 5-20%, 5-15%, 5-10%, 5-7.5%) of the total weight of the solid dosage form. In some embodiments, the lauroyl carnitine is present in an amount of or greater than approximately 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% of the total weight of the solid dosage form. In some embodiments, the lauroyl carnitine is present in an amount of or less than approximately 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5% of the total weight of the solid dosage form.

[0009] In some embodiments, a suitable protective vehicle is an enteric coat. In some embodiments, the protective vehicle constitutes an amount of or less than approximately 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5% of the total weight of the solid dosage form.

[0010] In some embodiments, a solid dosage form according to the present invention further comprises one or more excipients. In particular embodiments, the one or more excipients are selected from fillers such as PROSOLV®, disintegrants such as POLYPLASDONE™ crospovidone, glidants such as silicon dioxide or lubricants such as sodium stearyl fumarate.

[0011] In some embodiments, a solid dosage form according to the invention further comprises captopril.

[0012] In some embodiments, a suitable solid dosage form has a total weight ranging from about 500-1500 (e.g., from about 500-1200 mg, 500-1000 mg, 600-1500 mg, 600-1200 mg, 600-1000 mg, 700-1500 mg, 700-1200 mg, 700-1000 mg, 800-1500 mg, 800-1200 mg, 800-1000 mg). In some embodiments, a suitable solid dosage form has a total weight of or greater than about 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, or 1500 mg. In some embodiments, a suitable solid dosage form has a total weight of or less than about 2000 mg, 1900 mg, 1800 mg, 1700 mg, 1600 mg, 1500 mg, 1400 mg, 1300 mg, 1200 mg, 1100 mg, 1000 mg, 900 mg, 800 mg, 700 mg, 600 mg, or 500 mg.

[0013] In some embodiments, an angiotensin (1-7) peptide is present in an amount ranging from about 10-1000 mg (e.g., about 10-900 mg, 10-800 mg, 10-700 mg, 10-600 mg, 10-500 mg, 100-1000 mg, 100-900 mg, 100-800 mg, 100-700 mg, 100-600 mg, 100-500 mg, 100-400 mg, 100-300 mg, 200-1000 mg, 200-900 mg, 200-800 mg, 200-700 mg, 200-600 mg, 200-500 mg, 200-400 mg, 300-1000 mg, 300-900 mg, 300-800 mg, 300-700 mg, 300-600 mg, 300-500 mg). In some embodiments, an angiotensin (1-7) peptide is present in an amount of or greater than about 10 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg. In some embodiments, an angiotensin (1-7) peptide is present in an amount of or less than about 1000 mg, 950 mg, 900 mg, 850 mg, 800 mg, 750 mg, 700 mg, 650 mg, 600 mg, 550 mg, 500 mg, 450 mg, 400 mg, 350 mg, 300 mg, 250 mg, 200 mg, 150 mg, or 100 mg.

[0014] In particular embodiments, present invention provides a solid dosage form for oral administration including (a) an angiotensin (1-7) peptide, (b) citric acid, (c) lauroyl carnitine, and (d) a protective vehicle. In certain embodiments, the citric acid is present in an amount great than 500 mg and the lauroyl carnitine is present in an amount ranging from 50-100 mg.

[0015] In certain embodiments, the solid dosage form is a capsule or tablet. In certain embodiments, a suitable protective vehicle is an enteric coat.

[0016] In various embodiments, an angiotensin (1-7) peptide comprises the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1).

[0017] In various embodiments, an angiotensin (1-7) peptide is a functional equivalent of SEQ ID NO:1. In some embodiments, the functional equivalent is a linear peptide. In some

embodiments, the linear peptide comprises a sequence that includes at least four amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least four amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide comprises a sequence that includes at least five amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least five amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the linear peptide comprises a sequence that includes at least six amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least six amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7). In some embodiments, the at least four, five or six amino acids, respectively, further maintain their relative spacing as they appear in the naturally-occurring Angiotensin (1-7).

[0018] In some embodiments, the linear peptide contains 4-25 amino acids (e.g., 4-20, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7 amino acids). In some embodiments, the linear peptide contains 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids.

[0019] In some embodiments, the linear peptide is a fragment of the naturally-occurring Angiotensin (1-7).

[0020] In some embodiments, the linear peptide contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Angiotensin (1-7).

[0021] In particular embodiments, the linear peptide has an amino acid sequence of Asp^1-Arg^2-Nle^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:2).

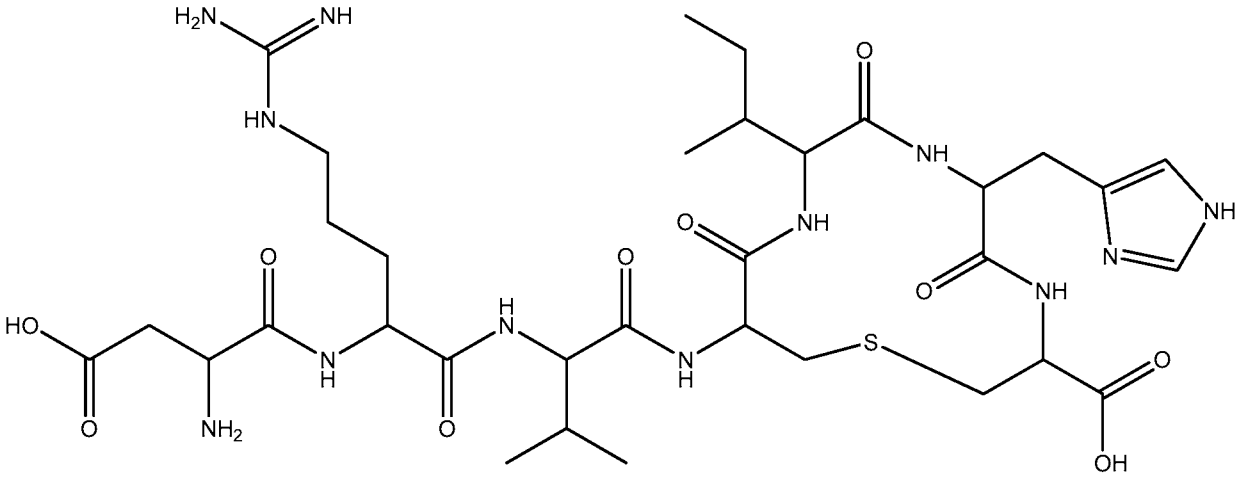
[0022] In particular embodiments, the linear peptide has an amino acid sequence of Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7 (SEQ ID NO:6).

[0023] In some embodiments, the functional equivalent is a cyclic peptide. In some embodiments, the cyclic peptide comprises a linkage between amino acids. In some embodiments, the linkage is located at residues corresponding to positions Tyr^4 and Pro^7 in naturally-occurring Angiotensin (1-7). In some embodiments, the linkage is a thioether bridge.

[0024] In particular embodiments, the cyclic peptide comprises an amino acid sequence otherwise identical to the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1).

[0025] In certain embodiments, the cyclic peptide comprises a norleucine (Nle) replacing position Val^3 in naturally-occurring Angiotensin (1-7).

[0026] In certain embodiments, the cyclic peptide is a 4,7-cyclized angiotensin (1-7) with the following formula:



[0027] In various embodiments, the angiotensin (1-7) peptide comprises one or more chemical modifications to increase protease resistance, serum stability and/or bioavailability. In some embodiments, the one or more chemical modifications comprise pegylation.

[0028] The present invention further provides methods for administering an oral formulation described herein.

[0029] As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0030] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed

description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

BRIEF DESCRIPTION OF THE DRAWING

[0031] The drawing is for illustration purposes only, not for limitation.

[0032] FIG. 1 depicts exemplary results illustrating total exposure of angiotensin (1-7) represented by area under the curve (AUC) compared between the various routes of administration.

DEFINITIONS

[0033] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0034] *Animal:* As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans, at any stage of development. In some embodiments, "animal" refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0035] *Approximately or about:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0036] *Bioavailability:* As used herein, the term "bioavailability" generally refers to the percentage of the administered dose that reaches the blood stream of a subject.

[0037] *Biologically active*: As used herein, the phrase "biologically active" refers to a characteristic of any agent that has activity in a biological system, and particularly in an organism. For instance, an agent that, when administered to an organism, has a biological effect on that organism, is considered to be biologically active. In particular embodiments, where a peptide is biologically active, a portion of that peptide that shares at least one biological activity of the peptide is typically referred to as a "biologically active" portion. In certain embodiments, a peptide has no intrinsic biological activity but that inhibits the effects of one or more naturally-occurring angiotensin compounds is considered to be biologically active.

[0038] *Carrier or diluent*: As used herein, the terms "carrier" and "diluent" refers to a pharmaceutically acceptable (e.g., safe and non-toxic for administration to a human) carrier or diluting substance useful for the preparation of a pharmaceutical formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0039] *Dosage form:* As used herein, the terms "dosage form" and "unit dosage form" refer to a physically discrete unit of a therapeutic agent for the patient to be treated. Each unit contains a predetermined quantity of active material calculated to produce the desired therapeutic effect. It will be understood, however, that the total dosage of the composition will be decided by the attending physician within the scope of sound medical judgment.

[0040] *Dosing regimen:* A "dosing regimen" (or "therapeutic regimen"), as that term is used herein, is a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regime comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, the therapeutic agent is administered continuously over a predetermined period. In some embodiments, the therapeutic agent is administered once a day (QD) or twice a day (BID).

[0041] *Excipient*: As used herein , the term "excipient" refers to any inert substance added to a drug and/or formulation for the purposes of improving its physical qualities (i.e. consistency), pharmacokinetic properties (i.e. bioavailability), pharmacodynamic properties and combinations thereof.

[0042] *Functional equivalent or functional derivative*: As used herein, the term "functional equivalent" or "functional derivative" denotes, in the context of a functional derivative of an amino acid sequence, a molecule that retains a biological activity that is substantially similar to that of the original sequence. A functional derivative or equivalent may be a natural derivative or is prepared recombinantly or synthetically. Exemplary functional derivatives include amino acid sequences having substitutions, deletions, or additions of one or more amino acids, provided that the biological activity of the protein is conserved. The substituting amino acid desirably has chemico-physical properties which are similar to that of the substituted amino acid. Desirable similar chemico-physical properties include, similarities in charge, bulkiness, hydrophobicity, hydrophilicity, and the like.

[0043] *Improve, increase, or reduce*: As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same form of disease as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0044] *In vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0045] *In vivo*: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* systems).

[0046] *Isolated*: As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 95%, about 98%, about 99%, substantially 100%, or 100% of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, substantially 100%, or 100% pure. As used herein, a substance is "pure" if it is substantially free of other components. As used herein, the term "isolated cell" refers to a cell not contained in a multi-cellular organism.

[0047] *Peptide*: The term "peptide" as used herein refers a sequential chain of amino acids linked together via peptide bonds. Typically, the term is used to refer to an amino acid chain of short length, but one of ordinary skill in the art will understand that the term is not limited to any particular length chains and can refer to a minimal chain comprising two amino acids linked together via a peptide bond. Typically, however, a peptide refers to an amino acid chain of or less than 50, 45, 40, 35, 30, 25, 20, 15, 10 amino acids. As is known to those skilled in the art, peptides may be processed and/or modified.

[0048] *Pharmaceutically acceptable:* As used herein, the term "pharmaceutically-acceptable" refers to any entity or composition that does not produce an undesirable allergic or antigenic response when administered to a subject.

[0049] *Protein*: The term "protein" as used herein refers to one or more polypeptides that function as a discrete unit. If a single polypeptide is the discrete functioning unit and does not require permanent or temporary physical association with other polypeptides in order to form the discrete functioning unit, the terms "polypeptide" and "protein" may be used interchangeably. If the discrete functional unit is comprised of more than one polypeptide that physically associate with one another, the term "protein" refers to the multiple polypeptides that are physically coupled and function together as the discrete unit.

[0050] *Stability:* As used herein, the term "stable" refers to the ability of the therapeutic agent to maintain its therapeutic efficacy (e.g., all or the majority of its intended biological activity and/or physiochemical integrity) over extended periods of time. The stability of a therapeutic agent, and the capability of the pharmaceutical composition to maintain stability of such therapeutic agent, may be assessed over extended periods of time (e.g., for at least 1, 3, 6, 12, 18, 24, 30, 36 months or more). In certain embodiments, pharmaceutical compositions described herein have been formulated such that they are capable of stabilizing, or alternatively slowing or preventing the degradation, of one or more therapeutic agents formulated therewith. In the context of a formulation a stable formulation is one in which the therapeutic agent therein essentially retains its physical and/or chemical integrity and biological activity upon storage and during processes (such as freeze/thaw, mechanical mixing and lyophilization).

[0051] *Subject*: As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre and post natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0052] *Substantially*: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0053] *Therapeutically effective amount*: As used herein, the term "therapeutically effective amount" of a therapeutic agent means an amount that is sufficient, when administered to a subject suffering from or susceptible to a disease, disorder, and/or condition, to treat, diagnose, prevent, and/or delay the onset of the symptom(s) of the disease, disorder, and/or condition. It will be appreciated by those of ordinary skill in the art that a therapeutically

effective amount is typically administered via a dosing regimen comprising at least one unit dose.

[0054] *Treating*: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0055] Other features, objects, and advantages of the present invention are apparent in the detailed description that follows. It should be understood, however, that the detailed description, while indicating embodiments of the present invention, is given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art from the detailed description.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0056] Among other things, the present invention provides formulations of angiotensin (1-7) (Ang-(1-7)) suitable for oral administration to a subject. Such administration could be for a variety of reasons including treatment of a disease, disorder or condition.

[0057] In some embodiments, a solid dosage form for oral administration is provided including (a) an angiotensin (1-7) peptide, (b) at least one pharmaceutically acceptable pH-lowering agent, (c) at least one absorption enhancer effective to promote bioavailability of the angiotensin (1-7) peptide, and (d) a protective vehicle.

[0058] In some embodiments, the solid dosage form is a capsule or tablet. Various methods and ingredients for making oral formulations are known in the art and it is expected that one of skill would be able to determine which of these methods and ingredients will be compatible with the invention as described in this specification. Such methods and ingredients are also contemplated as within the scope of the present invention.

[0059] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Angiotensin (1-7) peptides

[0060] As used herein, the term "angiotensin (1-7) peptide" refers to both naturally-occurring Angiotensin (1-7) and any functional equivalent, analogue or derivative of naturally-occurring Angiotensin (1-7). As used herein, "peptide" and "polypeptide" are interchangeable terms and refer to two or more amino acids bound together by a peptide bond. As used herein, the terms "peptide" and "polypeptide" include both linear and cyclic peptide. The terms "angiotensin-(1-7)", "Angiotensin-(1-7)", and "Ang-(1-7)" are used interchangeably.

Naturally-occurring Angiotensin (1-7)

[0061] Naturally-occurring Angiotensin (1-7) (also referred to as Ang-(1-7)) is a seven amino acid peptide shown below:

Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1)

It is part of the renin-angiotensin system and is converted from a precursor, also known as Angiotensinogen, which is an α-2-globulin that is produced constitutively and released into the circulation mainly by the liver. Angiotensinogen is a member of the serpin family and also known as renin substrate. Human angiotensinogen is 452 amino acids long, but other species have angiotensinogen of varying sizes. Typically, the first 12 amino acids are the most important for angiotensin activity:

Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}-Val^{11}-Ile^{12} (SEQ ID NO:4)

[0062] Different types of angiotensin may be formed by the action of various enzymes. For example, Angiotensin (1-7) is generated by action of Angiotensin-converting enzyme 2 (ACE 2).

[0063] Ang-(1-7) is an endogenous ligand for Mas receptors. Mas receptors are G-protein coupled receptor containing seven transmembrane spanning regions. As used herein, the term "angiotensin-(1-7) receptor' encompasses the G Protein-Coupled Mas Receptors.

[0064] As used herein, the term "naturally-occurring Angiotensin (1-7)" includes any Angiotensin (1-7) peptide purified from natural sources and any recombinantly produced or chemically synthesized peptides that have an amino acid sequence identical to that of the naturally-occurring Angiotensin (1-7).

Functional equivalents, analogs or derivatives of Ang-(1-7)

[0065] In some embodiments, an angiotensin (1-7) peptide suitable for the present invention is a functional equivalent of naturally-occurring Ang-(1-7). As used herein, a functional equivalent of naturally-occurring Ang-(1-7) refers to any peptide that shares amino acid sequence identity to the naturally-occurring Ang-(1-7) and retain substantially the same or similar activity as the naturally-occurring Ang-(1-7). For example, in some embodiments, a functional equivalent of naturally-occurring Ang-(1-7) described herein has pro-angiogenic activity as determined using methods described herein or known in the art, or an activity such as nitric oxide release, vasodilation, improved endothelial function, antidiuresis, or one of the other properties discussed herein, that positively impacts angiogenesis. In some embodiments, a functional equivalent of naturally-occurring Ang-(1-7) described herein can bind to or activate an angiotensin-(1-7) receptor (e.g., the G protein-coupled Mas receptor) as determined using various assays described herein or known in the art. In some embodiments, a functional equivalent of Ang-(1-7) is also referred to as an angiotensin (1-7) analogue or derivative, or functional derivative.

[0066] Typically, a functional equivalent of angiotensin (1-7) shares amino acid sequence similarity to the naturally-occurring Ang-(1-7). In some embodiments, a functional equivalent of Ang-(1-7) according to the invention contains a sequence that includes at least 3 (e.g., at least 4, at least 5, at least 6, at least 7) amino acids from the seven amino acids that appear in the naturally-occurring Ang-(1-7), wherein the at least 3 (e.g., at least 4, at least 5, at least 6, or at least 7) amino acids maintain their relative positions and/or spacing as they appear in the naturally-occurring Ang-(1-7).

[0067] In some embodiments, a functional equivalent of Ang-(1-7) also encompass any peptide that contain a sequence at least 50% (e.g., at least 60%, 70%, 80%, or 90%) identical to the amino acid sequence of naturally-occurring Ang-(1-7). Percentage of amino acid sequence identity can be determined by alignment of amino acid sequences. Alignment of amino acid

sequences can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. Preferably, the WU-BLAST-2 software is used to determine amino acid sequence identity (Altschul *et al.*, Methods in Enzymology 266, 460-480 (1996); http://blast.wustl/edu/blast/README.html). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=11. HSP score (S) and HSP S2 parameters are dynamic values and are established by the program itself, depending upon the composition of the particular sequence, however, the minimum values may be adjusted and are set as indicated above.

[0068] In some embodiments, a functional equivalent, analogue or derivative of Ang-(1-7) is a fragment of the naturally-occurring Ang-(1-7). In some embodiments, a functional equivalent, analogue or derivative of Ang-(1-7) contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Ang-(1-7). Ang-(1-7) functional equivalents, analogues or derivatives can be made by altering the amino acid sequences by substitutions, additions, and/or deletions. For example, one or more amino acid residues within the sequence of the naturally-occurring Ang-(1-7) (SEQ ID NO:1) can be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitution for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the positively charged (basic) amino acids include arginine, lysine, and histidine. The nonpolar (hydrophobic) amino acids include leucine, isoleucine, alanine, phenylalanine, valine, proline, tryptophane, and methionine. The uncharged polar amino acids include serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The negatively charged (acid) amino acids include glutamic acid and aspartic acid. The amino acid glycine may be included in either the nonpolar amino acid family or the uncharged (neutral) polar amino acid family. Substitutions made within a family of amino acids are generally understood to be conservative substitutions. For example, the amino acid sequence of a peptide inhibitor can be modified or substituted.

[0069] Examples of Ang-(1-7) functional equivalents, analogues and derivatives are described in the section entitled "Exemplary Angiotensin(1-7) Peptides" below.

[0070] An angiotensin-(1-7) peptide can be of any length. In some embodiments, an angiotensin-(1-7) peptide according to the present invention can contain, for example, from 4-25 amino acids (e.g., 4-20, 4-15, 4-14, 4-13, 4-12, 4-11, 4-10, 4-9, 4-8, 4-7 amino acids). In some embodiments, the linear peptide contains 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids.

[0071] In some embodiments, an angiotensin-(1-7) peptide contains one or more modifications to increase protease resistance, serum stability and/or bioavailability. In some embodiments, suitable modifications are selected from pegylation, acetylation, glycosylation, biotinylation, substitution with D-amino acid and/or un-natural amino acid, and/or cyclization of the peptide.

[0072] As used herein, the term "amino acid," in its broadest sense, refers to any compound and/or substance that can be incorporated into a polypeptide chain. In certain embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In certain embodiments, an amino acid is a naturally-occurring amino acid. In certain embodiments, an amino acid is a synthetic or un-natural amino acid (e.g., α,α-disubstituted amino acids, N-alkyl amino acids); in some embodiments, an amino acid is a d-amino acid; in certain embodiments, an amino acid is an l-amino acid. "Standard amino acid" refers to any of the twenty standard amino acids commonly found in naturally occurring peptides including both l- and d- amino acids which are both incorporated in peptides in nature. "Nonstandard" or "unconventional amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. As used herein, "synthetic or un-natural amino acid" encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and/or substitutions. Amino acids, including carboxy- and/or amino-terminal amino acids in peptides, can be modified by methylation, amidation, acetylation, and/or substitution with other chemical groups that can change the peptide's circulating half-life without adversely affecting its activity. Examples of unconventional or un-natural amino acids include, but are not limited to, citrulline, ornithine, norleucine, norvaline, 4-(*E*)-butenyl-4(*R*)-methyl-N-methylthreonine (MeBmt), N-methyl-

leucine (MeLeu), aminoisobutyric acid, statine, and N-methyl-alanine (MeAla). Amino acids may participate in a disulfide bond. The term "amino acid" is used interchangeably with "amino acid residue," and may refer to a free amino acid and/or to an amino acid residue of a peptide. It will be apparent from the context in which the term is used whether it refers to a free amino acid or a residue of a peptide.

[0073] In certain embodiments, angiotensin-(1-7) peptides contain one or more L-amino acids, D-amino acids, and/or un-natural amino acids.

[0074] In addition to peptides containing only naturally occurring amino acids, peptidomimetics or peptide analogs are also encompassed by the present invention. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. The non-peptide compounds are termed "peptide mimetics" or peptidomimetics (Fauchere et al., *Infect. Immun.* 54:283-287 (1986); Evans et al., *J. Med. Chem.* 30:1229-1239 (1987)). Peptide mimetics that are structurally related to therapeutically useful peptides and may be used to produce an equivalent or enhanced therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to the paradigm polypeptide (i.e., a polypeptide that has a biological or pharmacological activity) such as naturally-occurring receptor-binding polypeptides, but have one or more peptide linkages optionally replaced by linkages such as $–CH_2NH–$, $–CH_2S–$, $–CH_2–CH_2–$, –CH=CH– (cis and trans), $–CH_2SO–$, $–CH(OH)CH_2–$, $–COCH_2–$ etc., by methods well known in the art (Spatola, Peptide Backbone Modifications, Vega Data, 1(3):267 (1983); Spatola et al. *Life Sci.* 38:1243-1249 (1986); Hudson et al. *Int. J. Pept. Res.* 14:177-185 (1979); and Weinstein. B., 1983, Chemistry and Biochemistry, of Amino Acids, Peptides and Proteins, Weinstein eds, Marcel Dekker, New-York,). Such peptide mimetics may have significant advantages over naturally-occurring polypeptides including more economical production, greater chemical stability, enhanced pharmacological properties (e.g., half-life, absorption, potency, efficiency, etc.), reduced antigenicity and others.

[0075] Ang-(1-7) peptides also include other types of peptide derivatives containing additional chemical moieties not normally part of the peptide, provided that the derivative retains the desired functional activity of the peptide. Examples of such derivatives include (1) N-acyl derivatives of the amino terminal or of another free amino group, wherein the acyl group may be

an alkanoyl group (e.g., acetyl, hexanoyl, octanoyl) an aroyl group (e.g., benzoyl) or a blocking group such as F-moc (fluorenylmethyl–O–CO–); (2) esters of the carboxy terminal or of another free carboxy or hydroxyl group; (3) amide of the carboxy-terminal or of another free carboxyl group produced by reaction with ammonia or with a suitable amine; (4) phosphorylated derivatives; (5) derivatives conjugated to an antibody or other biological ligand and other types of derivatives; and (6) derivatives conjugated to a polyethylene glycol (PEG) chain.

[0076] Ang-(1-7) peptides may be obtained by any method of peptide synthesis known to those skilled in the art, including synthetic (e.g., exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, classical solution synthesis, native-chemical ligation) and recombinant techniques. For example, the peptides or peptides derivatives can be obtained by solid phase peptide synthesis, which in brief, consist of coupling the carboxyl group of the C-terminal amino acid to a resin (e.g., benzhydrylamine resin, chloromethylated resin, hydroxymethyl resin) and successively adding N-alpha protected amino acids. The protecting groups may be any such groups known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. Such solid phase synthesis has been disclosed, for example, by Merrifield, *J. Am. Chem. Soc.* 85: 2149 (1964); Vale et al., *Science* 213:1394-1397 (1981), in U.S. Patent Numbers 4, 305, 872 and 4,316, 891, Bodonsky et al. *Chem. Ind.* (London), 38:1597 (1966); and Pietta and Marshall, *Chem. Comm.* 650 (1970) by techniques reviewed in Lubell et al. "Peptides" Science of Synthesis 21.11, *Chemistry of Amides*. Thieme, Stuttgart, 713-809 (2005). The coupling of amino acids to appropriate resins is also well known in the art and has been disclosed in U.S. Patent Number 4,244,946. (Reviewed in Houver-Weyl, *Methods of Organic Chemistry.* Vol E22a. Synthesis of Peptides and Peptidomimetics, Murray Goodman, Editor-in-Chief, Thieme. Stuttgart. New York 2002).

[0077] Unless defined otherwise, the scientific and technological terms and nomenclature used herein have the same meaning as commonly understood by a person of ordinary skill to which this invention pertains. Generally, the procedures of cell cultures, infection, molecular biology methods and the like are common methods used in the art. Such standard techniques can be found in reference manuals such as, for example, Ausubel *et al., Current Protocols in Molecular Biology*, Wiley Interscience, New York, 2001; and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, N.Y., 2001.

[0078] During any process of the preparation of an Ang-(1-7) peptide, it may be desirable to protect sensitive reactive groups on any of the molecule concerned. This may be achieved by means of conventional protecting groups such as those described in Protective Groups In Organic Synthesis by T.W. Greene & P.G.M. Wuts, 1991, John Wiley and Sons, New-York; and Peptides: chemistry and Biology by Sewald and Jakubke, 2002, Wiley-VCH, Wheinheim p.142. For example, alpha amino protecting groups include acyl type protecting groups (e.g., trifluoroacetyl, formyl, acetyl), aliphatic urethane protecting groups (e.g., t-butyloxycarbonyl (BOC), cyclohexyloxycarbonyl), aromatic urethane type protecting groups (e.g., fluorenyl-9-methoxy-carbonyl (Fmoc), benzyloxycarbonyl (Cbz), Cbz derivatives) and alkyl type protecting groups (e.g., triphenyl methyl, benzyl). The amino acids side chain protecting groups include benzyl (for Thr and Ser), Cbz (Tyr, Thr, Ser, Arg, Lys), methyl ethyl, cyclohexyl (Asp, His), Boc (Arg, His, Cys) etc. The protecting groups may be removed at a convenient subsequent stage using methods known in the art.

[0079] Further, Ang-(1-7) peptides may be synthesized according to the FMOC protocol in an organic phase with protective groups. Desirably, the peptides are purified with a yield of 70% with high-pressure liquid chromatography (HPLC) on a C18 chromatography column and eluted with an acetonitrile gradient of 10-60%. The molecular weight of a peptide can be verified by mass spectrometry (reviewed in Fields, G.B. "Solid-Phase Peptide Synthesis" *Methods in Enzymology.* Vol. 289, Academic Press, 1997).

[0080] Alternatively, Ang-(1-7) peptides may be prepared in recombinant systems using, for example, polynucleotide sequences encoding the polypeptides. It is understood that a polypeptide may contain more than one of the above-described modifications within the same polypeptide.

[0081] While peptides may be effective in eliciting a biological activity *in vitro*, their effectiveness *in vivo* might be reduced by the presence of proteases. Serum proteases have specific substrate requirements. The substrate must have both L-amino acids and peptide bonds for cleavage. Furthermore, exopeptidases, which represent the most prominent component of the protease activity in serum, usually act on the first peptide bond of the peptide and require a free N-terminus (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). In light of this, it is often advantageous to use modified versions of peptides. The modified peptides retain the structural

characteristics of the original L-amino acid peptides that confer the desired biological activity of Ang-(1-7) but are advantageously not readily susceptible to cleavage by protease and/or exopeptidases.

[0082] Systematic substitution of one or more amino acids of a consensus sequence with D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. Thus, a peptide derivative or peptidomimetic of the present invention may be all L, all D or mixed D, L peptide, in either forward or reverse order. The presence of an N-terminal or C-terminal D-amino acid increases the *in vivo* stability of a peptide since peptidases cannot utilize a D-amino acid as a substrate (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). Reverse-D peptides are peptides containing D-amino acids, arranged in a reverse sequence relative to a peptide containing L-amino acids. Thus, the C-terminal residue of an L-amino acid peptide becomes N-terminal for the D-amino acid peptide, and so forth. Reverse D-peptides retain the same secondary conformation and therefore similar activity, as the L-amino acid peptides, but are more resistant to enzymatic degradation *in vitro* and *in vivo*, and thus can have greater therapeutic efficacy than the original peptide (Brady and Dodson, *Nature* 368:692-693 (1994); Jameson et al., *Nature* 368:744-746 (1994)). Similarly, a reverse-L peptide may be generated using standard methods where the C-terminus of the parent peptide becomes takes the place of the N-terminus of the reverse-L peptide. It is contemplated that reverse L-peptides of L-amino acid peptides that do not have significant secondary structure (e.g., short peptides) retain the same spacing and conformation of the side chains of the L-amino acid peptide and therefore often have the similar activity as the original L-amino acid peptide. Moreover, a reverse peptide may contain a combination of L- and D-amino acids. The spacing between amino acids and the conformation of the side chains may be retained resulting in similar activity as the original L-amino acid peptide.

[0083] Another effective approach to confer resistance to peptidases acting on the N-terminal or C-terminal residues of a peptide is to add chemical groups at the peptide termini, such that the modified peptide is no longer a substrate for the peptidase. One such chemical modification is glycosylation of the peptides at either or both termini. Certain chemical modifications, in particular N-terminal glycosylation, have been shown to increase the stability of peptides in human serum (Powell et al., *Pharm. Res.* 10:1268-1273 (1993)). Other chemical modifications which enhance serum stability include, but are not limited to, the addition of an N-

terminal alkyl group, consisting of a lower alkyl of from one to twenty carbons, such as an acetyl group, and/or the addition of a C-terminal amide or substituted amide group. In particular, the present invention includes modified peptides consisting of peptides bearing an N-terminal acetyl group and/or a C-terminal amide group.

[0084] Substitution of non-naturally-occurring amino acids for natural amino acids in a subsequence of the peptides can also confer resistance to proteolysis. Such a substitution can, for instance, confer resistance to proteolysis by exopeptidases acting on the N-terminus without affecting biological activity. Examples of non-naturally-occurring amino acids include α,α -disubstituted amino acids, N-alkyl amino acids, C-α-methyl amino acids, β-amino acids, and β-methyl amino acids. Amino acids analogs useful in the present invention may include, but are not limited to, β-alanine, norvaline, norleucine, 4-aminobutyric acid, orithine, hydroxyproline, sarcosine, citrulline, cysteic acid, cyclohexylalanine, 2-aminoisobutyric acid, 6-aminohexanoic acid, t-butylglycine, phenylglycine, o-phosphoserine, N-acetyl serine, N-formylmethionine, 3-methylhistidine and other unconventional amino acids. Furthermore, the synthesis of peptides with non-naturally-occurring amino acids is routine in the art.

[0085] In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods well known in the art (Rizo and Gierasch, *Ann. Rev. Biochem.* 61:387-418 (1992)). For example, constrained peptides may be generated by adding cysteine residues capable of forming disulfide bridges and, thereby, resulting in a cyclic peptide. Cyclic peptides can be constructed to have no free N- or C-termini. Accordingly, they are not susceptible to proteolysis by exopeptidases, although they may be susceptible to endopeptidases, which do not cleave at peptide termini. The amino acid sequences of the peptides with N-terminal or C-terminal D-amino acids and of the cyclic peptides are usually identical to the sequences of the peptides to which they correspond, except for the presence of N-terminal or C-terminal D-amino acid residue, or their circular structure, respectively.

Cyclic Peptides

[0086] In some embodiments, a functional equivalent, analogue or derivative of naturally-occurring Ang-(1-7) is a cyclic peptide. As used herein, a cyclic peptide has an intramolecular covalent bond between two non-adjacent residues. The intramolecular bond may

be a backbone to backbone, side-chain to backbone or side-chain to side-chain bond (i.e., terminal functional groups of a linear peptide and/or side-chain functional groups of a terminal or interior residue may be linked to achieve cyclization). Typical intramolecular bonds include disulfide, amide and thioether bonds. A variety of means for cyclizing polypeptides are well known in the art, as are many other modifications that can be made to such peptides. For a general discussion, see International Patent Publication Nos. WO 01/53331 and WO 98/02452, the contents of which are incorporated herein by reference. Such cyclic bonds and other modifications can also be applied to the cyclic peptides and derivative compounds of this invention.

[0087] Cyclic peptides as described herein may comprise residues of L-amino acids, D-amino acids, or any combination thereof. Amino acids may be from natural or non-natural sources, provided that at least one amino group and at least one carboxyl group are present in the molecule; α- and β-amino acids are generally preferred. Cyclic peptides may also contain one or more rare amino acids (such as 4-hydroxyproline or hydroxylysine), organic acids or amides and/or derivatives of common amino acids, such as amino acids having the C-terminal carboxylate esterified (e.g., benzyl, methyl or ethyl ester) or amidated and/or having modifications of the N-terminal amino group (e.g., acetylation or alkoxycarbonylation), with or without any of a wide variety of side-chain modifications and/or substitutions (e.g., methylation, benzylation, t-butylation, tosylation, alkoxycarbonylation, and the like). Suitable derivatives include amino acids having an N-acetyl group (such that the amino group that represents the N-terminus of the linear peptide prior to cyclization is acetylated) and/or a C-terminal amide group (i.e., the carboxy terminus of the linear peptide prior to cyclization is amidated). Residues other than common amino acids that may be present with a cyclic peptide include, but are not limited to, penicillamine, β,β-tetramethylene cysteine, β,β-pentamethylene cysteine, β-mercaptopropionic acid, β,β-pentamethylene-β-mercaptopropionic acid, 2-mercaptobenzene, 2-mercaptoaniline, 2-mercaptoproline, ornithine, diaminobutyric acid, α-aminoadipic acid, m-aminomethylbenzoic acid and α,β-diaminopropionic acid.

[0088] Following synthesis of a linear peptide, with or without N-acetylation and/or C-amidation, cyclization may be achieved by any of a variety of techniques well known in the art. Within one embodiment, a bond may be generated between reactive amino acid side chains. For example, a disulfide bridge may be formed from a linear peptide comprising two thiol-containing

residues by oxidizing the peptide using any of a variety of methods. Within one such method, air oxidation of thiols can generate disulfide linkages over a period of several days using either basic or neutral aqueous media. The peptide is used in high dilution to minimize aggregation and intermolecular side reactions. Alternatively, strong oxidizing agents such as I_2 and $K_3Fe(CN)_6$ can be used to form disulfide linkages. Those of ordinary skill in the art will recognize that care must be taken not to oxidize the sensitive side chains of Met, Tyr, Trp or His. Within further embodiments, cyclization may be achieved by amide bond formation. For example, a peptide bond may be formed between terminal functional groups (i.e., the amino and carboxy termini of a linear peptide prior to cyclization). Within another such embodiment, the linear peptide comprises a D-amino acid. Alternatively, cyclization may be accomplished by linking one terminus and a residue side chain or using two side chains, with or without an N-terminal acetyl group and/or a C-terminal amide. Residues capable of forming a lactam bond include lysine, ornithine (Orn), α-amino adipic acid, m-aminomethylbenzoic acid, α,β-diaminopropionic acid, glutamate or aspartate. Methods for forming amide bonds are generally well known in the art. Within one such method, carbodiimide-mediated lactam formation can be accomplished by reaction of the carboxylic acid with DCC, DIC, ED AC or DCCI, resulting in the formation of an O-acylurea that can be reacted immediately with the free amino group to complete the cyclization. Alternatively, cyclization can be performed using the azide method, in which a reactive azide intermediate is generated from an alkyl ester via a hydrazide. Alternatively, cyclization can be accomplished using activated esters. The presence of electron withdrawing substituents on the alkoxy carbon of esters increases their susceptibility to aminolysis. The high reactivity of esters of p-nitrophenol, N-hydroxy compounds and polyhalogenated phenols has made these "active esters" useful in the synthesis of amide bonds. Within a further embodiment, a thioether linkage may be formed between the side chain of a thiol-containing residue and an appropriately derivatized α-amino acid. By way of example, a lysine side chain can be coupled to bromoacetic acid through the carbodiimide coupling method (DCC, EDAC) and then reacted with the side chain of any of the thiol containing residues mentioned above to form a thioether linkage. In order to form dithioethers, any two thiol containing side-chains can be reacted with dibromoethane and diisopropylamine in DMF.

Exemplary Angiotensin-(1-7) Peptides

[0089] In certain aspects, the invention provides linear angiotensin-(1-7) peptides. As discussed above, the structure of naturally-occurring Ang-(1-7) is as follows:

Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1)

[0090] The peptides and peptide analogs of the invention can be generally represented by the following sequence:

Xaa^1-Xaa^2-Xaa^3-Xaa^4-Xaa^5-Xaa^6-Xaa^7 (SEQ ID NO:5),

or a pharmaceutically acceptable salt thereof.

[0091] Xaa^1 is any amino acid or a dicarboxylic acid. In certain embodiments, Xaa^1 is Asp, Glu, Asn, Acpc (1-aminocyclopentane carboxylic acid), Ala, Me_2Gly (N,N-dimethylglycine), Pro, Bet (betaine, 1-carboxy-N,N,N-trimethylmethanaminium hydroxide), Glu, Gly, Asp, Sar (sarcosine) or Suc (succinic acid). In certain such embodiments, Xaa^1 is a negatively-charged amino acid, such as Asp or Glu, typically Asp.

[0092] Xaa^2 is Arg, Lys, Ala, Cit (citrulline), Orn (ornithine), acetylated Ser, Sar, D-Arg and D-Lys. In certain embodiments, Xaa^2 is a positively-charged amino acid such as Arg or Lys, typically Arg.

[0093] Xaa^3 is Val, Ala, Leu, Nle (norleucine), Ile, Gly, Lys, Pro, HydroxyPro (hydroxyproline), Aib (2-aminoisobutyric acid), Acpc or Tyr. In certain embodiments, Xaa^3 is an aliphatic amino acid such as Val, Leu, Ile or Nle, typically Val or Nle.

[0094] Xaa^4 is Tyr, Tyr(PO_3), Thr, Ser, homoSer (homoserine), azaTyr (aza-α^1-homo-L-tyrosine) or Ala. In certain embodiments, Xaa^4 is a hydroxyl-substituted amino acid such as Tyr, Ser or Thr, typically Tyr.

[0095] Xaa^5 is Ile, Ala, Leu, norLeu, Val or Gly. In certain embodiments, Xaa^5 is an aliphatic amino acid such as Val, Leu, Ile or Nle, typically Ile.

[0096] Xaa^6 is His, Arg or 6-NH_2-Phe (6-aminophenylalaine). In certain embodiments, Xaa^6 is a fully or partially positively-charged amino acid such as Arg or His.

[0097] Xaa^7 is Cys, Pro or Ala.

[0098] In certain embodiments, one or more of Xaa^1-Xaa^7 is identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^7 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7).

[0099] In certain embodiments, Xaa^3 is Nle. When Xaa^3 is Nle, one or more of Xaa^1-Xaa^2 and Xaa^{4-7} are optionally identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^2 and Xaa^{4-7} are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^2 and Xaa^{4-7} are identical to the corresponding amino acid in naturally-occurring Ang-(1-7), resulting in the amino acid sequence: Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:2).

[0100] In certain embodiments, the peptide has the amino acid sequence Asp-Arg-Nle-Tyr-Ile-His-Pro (SEQ ID NO:2).

[0101] In certain embodiments, the peptide has the amino acid sequence Asp-Arg-Val-Ser-Ile-His-Cys (SEQ ID NO:6) or Asp-Arg-Val-ser-Ile-His-Cys (SEQ ID NO:3).

[0102] In some embodiments, a linear angiotensin (1-7) peptide as used herein is a peptide having a sequence of Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8-His^9 (SEQ ID NO: 23), which is identical to the sequence of Ang(1-9). In some embodiments, an angiotensin (1-7) peptide is a derivative of Ang (1-9). For exemplary Ang (1-9) peptides, including Ang(1-9) derivatives, see U.S. Patent Publication 2012/0172301, the disclosure of which is hereby incorporated by reference.

[0103] In some embodiments, a linear angiotensin (1-7) peptide is a peptide with an amino acid sequence of Ala^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO: 24). Additional sequences derived from SEQ ID NO: 24 may be found in European Patent Application 2,264,048, the disclosure of which is hereby incorporated by reference.

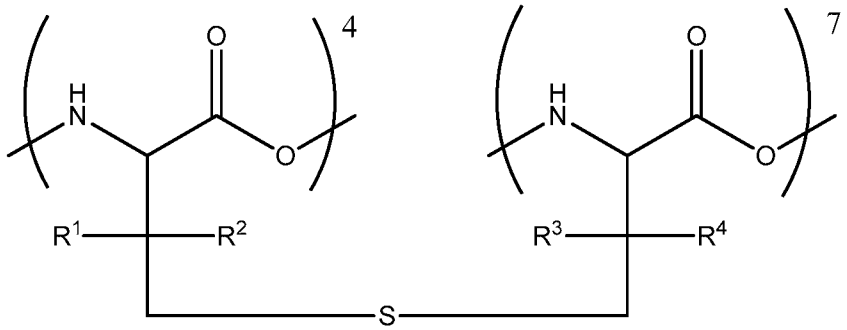
[0104] Further contemplated are variants of the linear peptides described herein, wherein the variants maintain one or more functional properties of the comparator peptide. Variants may

have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of the exemplary linear peptides described herein.

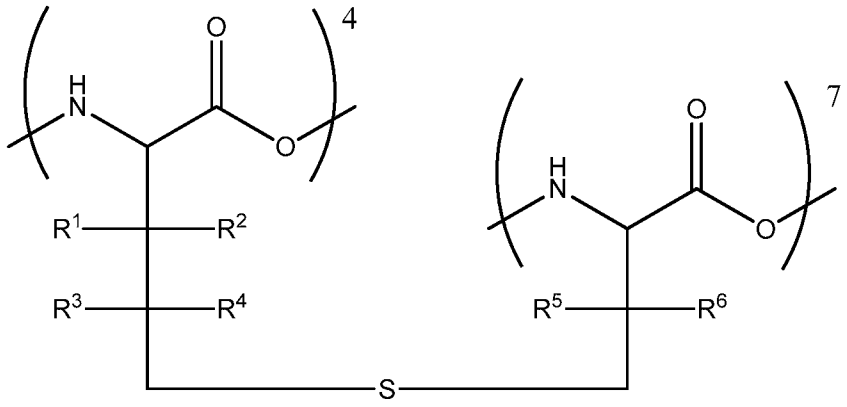
Exemplary Cyclic Angiotensin (1-7) Peptides

[0105] In certain aspects, the invention provides a cyclic angiotensin-(1-7) (Ang-(1-7)) peptide analog comprising a linkage, such as between the side chains of amino acids corresponding to positions Tyr^4 and Pro^7 in Ang. These peptide analogs typically comprise 7 amino acid residues, but can also include a cleavable sequence. As discussed in greater detail below, the invention includes fragments and analogs where one or more amino acids are substituted by another amino acid (including fragments).

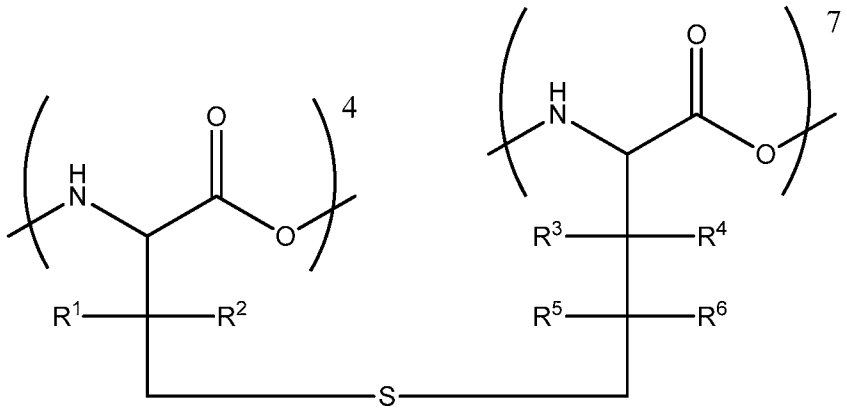
[0106] Although the following section describes aspects of the invention in terms of a thioether bond linking residues at the 4- and 7-positions, it should be understood that other linkages (as described above) could replace the thioether bridge and that other residues could be cyclized. A thioether bridge is also referred to as a monosulfide bridge or, in the case of Ala-S-Ala, as a lanthionine bridge. Thioether bridge-containing peptides can be formed by two amino acids having one of the following formulas:



Formula (I)



Formula (II)



Formula (III)

[0107] In these formulae, R^1, R^2, R^3, R^4, R^5 and R^6 are independently -H, an alkyl (e.g., C_1-C_6 alkyl, C_1-C_4 alkyl) or an aralkyl group, where the alkyl and aralkyl groups are optionally substituted with one or more halogen, -OH or –NRR' groups (where R and R' are independently –H or C_1-C_4 alkyl). In certain embodiments, R^1, R^2, R^3, R^4, R^5 and R^6 are each independently -H or -CH_3, such where all are –H.

[0108] In certain embodiments, the invention provides an Ang analog or derivative comprising a thioether bridge according to formula (I). Typically, R^1, R^2, R^3 and R^4 are independently selected from -H and -CH_3. Peptides comprising a thioether bridge according to formula (I) can be produced, for example, by lantibiotic enzymes or by sulfur extrusion of a disulfide. In one example, the disulfide from which the sulfur is extruded can be formed by D-

cysteine in position 4 and L-cysteine in position 7 or by D-cysteine in position 4 and L-penicillamine in position 7 (see, e.g., Galande, Trent and Spatola (2003) *Biopolymers* 71, 534-551).

[0109] In other embodiments, the linkage of the two amino acids can be the bridges depicted in Formula (II) or Formula (III). Peptides comprising a thioether bridge according to Formula (II) can be made, for example, by sulfur extrusion of a disulfide formed by D-homocysteine in position 4 and L-cysteine in position 7. Similarly, peptides comprising a thioether bridge as in Formula (III) can be made, for example, by sulfur extrusion of a disulfide formed by D-cysteine in position 4 and L-homocysteine in position 7.

[0110] As discussed above, the Ang analogs and derivatives of the invention vary in length and amino acid composition. The Ang analogs and derivatives of the invention preferably have biological activity or are an inactive precursor molecule that can be proteolytically activated (such as how angiotensin(I), with 10 amino acids, is converted to active fragments by cleavage of 2 amino acids). The size of an Ang analog or derivative can vary but is typically between from about 5 to 10 amino acids, as long as the "core" pentameric segment comprising the 3-7 Nle-thioether-ring structure is encompassed. The amino acid sequence of an analog or derivative of the invention can vary, typically provided that it is biologically active or can become proteolytically activated. Biological activity of an analog or derivative can be determined using methods known in the art, including radioligand binding studies, *in vitro* cell activation assays and *in vivo* experiments. See, for example, Godeny and Sayeski, (2006) *Am. J. Physiol. Cell. Physiol.* 291:C1297-1307; Sarr *et al.*, *Cardiovasc. Res.* (2006) 71:794-802; and Koziarz *et al.*, (1933) *Gen. Pharmacol.* 24:705- 713.

[0111] Ang analogs and derivatives where only the length of the peptide is varied include the following:

a 4,7-cyclized analog designated [Cyc^{4-7}]Ang-(1-7), which is derived from natural Ang-(1-7) (Asp^{1}-Arg^{2}-Val^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}, SEQ ID NO:7).

a 4,7-cyclized analog designated [Nle^{3}, Cyc^{4-7}]Ang-(1-10), which is derived from natural Angiotensin I (Ang-(1-10)) (Asp^{1}-Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8}-His^{9}-Leu^{10}, SEQ ID NO:8);

a 4,7-cyclized analog designated [Nle^3, Cyc^{4-7}]Ang-(1-8), which is derived from natural Angiotensin II (Ang-(1-8)) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:9);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(2-8), which is derived from natural Angiotensin III (Ang-(2-8)) (Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:10);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(3-8), which is derived from natural Angiotensin IV (Ang-(3-8)) (Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8, SEQ ID NO:11);

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(1-7) derived from natural Ang-(1-7) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7, SEQ ID NO:12); and

a 4,7-cyclised analog designated [Nle^3, Cyc^{4-7}]Ang-(1-9) derived from natural Ang-(1-9) (Asp^1-Arg^2-Nle^3-Cyc^4-Ile^5-His^6-Cyc^7-Phe^8-His^9, SEQ ID NO:13).

These analogs can have one of the thioether bridges shown in Formulae (I)-(III) as the Cyc^{4-7} moiety, for example, where Cyc^4 and Cyc^7 are represented by Formula (I), such as where R^1-R^4 are each –H or $–CH_3$, typically -H.

[0112] As compared to the amino acid sequence of the natural angiotensin peptide, the amino acids at positions 4 and 7 of the Cyc^{4-7} analog are modified to allow introduction of the thioether-ring structures shown above. In addition to the length of the Ang analogs, the amino acids at positions other than 3, 4 and 7 can be the same or different from the naturally-occurring peptide, typically provided that the analog retains a biological function. For analogs of inactive precursors, like [Cyc^{4-7}]Ang-(l-10), biological function refers to one or both of an analog's susceptibility to angiotensin-converting enzymes that can cleave it to a biologically active fragment (e.g. Ang-(l-8) or Ang-(l-7)) or the biological activity of the fragment itself. In certain embodiments, an Ang analog or derivative of the invention has no intrinsic function but inhibits the effects of one or more naturally-occurring angiotensin compounds.

[0113] In certain embodiments, an Ang analog of the invention is represented by Formula (IV):

Xaa^1-Xaa^2-Xaa^3-Cyc^4-Xaa^5-Xaa^6-Cyc^7 (IV, SEQ ID NO:14)

[0114] Xaa^1 is any amino acid, but typically a negatively-charged amino acid such as Glu or Asp, more typically Asp.

[0115] Xaa^2 is a positively-charged amino acid such as Arg or Lys, typically Arg.

[0116] Xaa^3 is an aliphatic amino acid, such as Leu, Ile or Val, typically Val.

[0117] Cyc^4 forms a thioether bridge in conjunction with Cyc^7. Cyc^4 can be a D-stereoisomer and/or a L-stereoisomer, typically a D-stereoisomer. Examples of Cyc^4 (taken with Cyc^7) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0118] Xaa^5 is an aliphatic amino acid, such as Leu, Ile or Val, typically Ile.

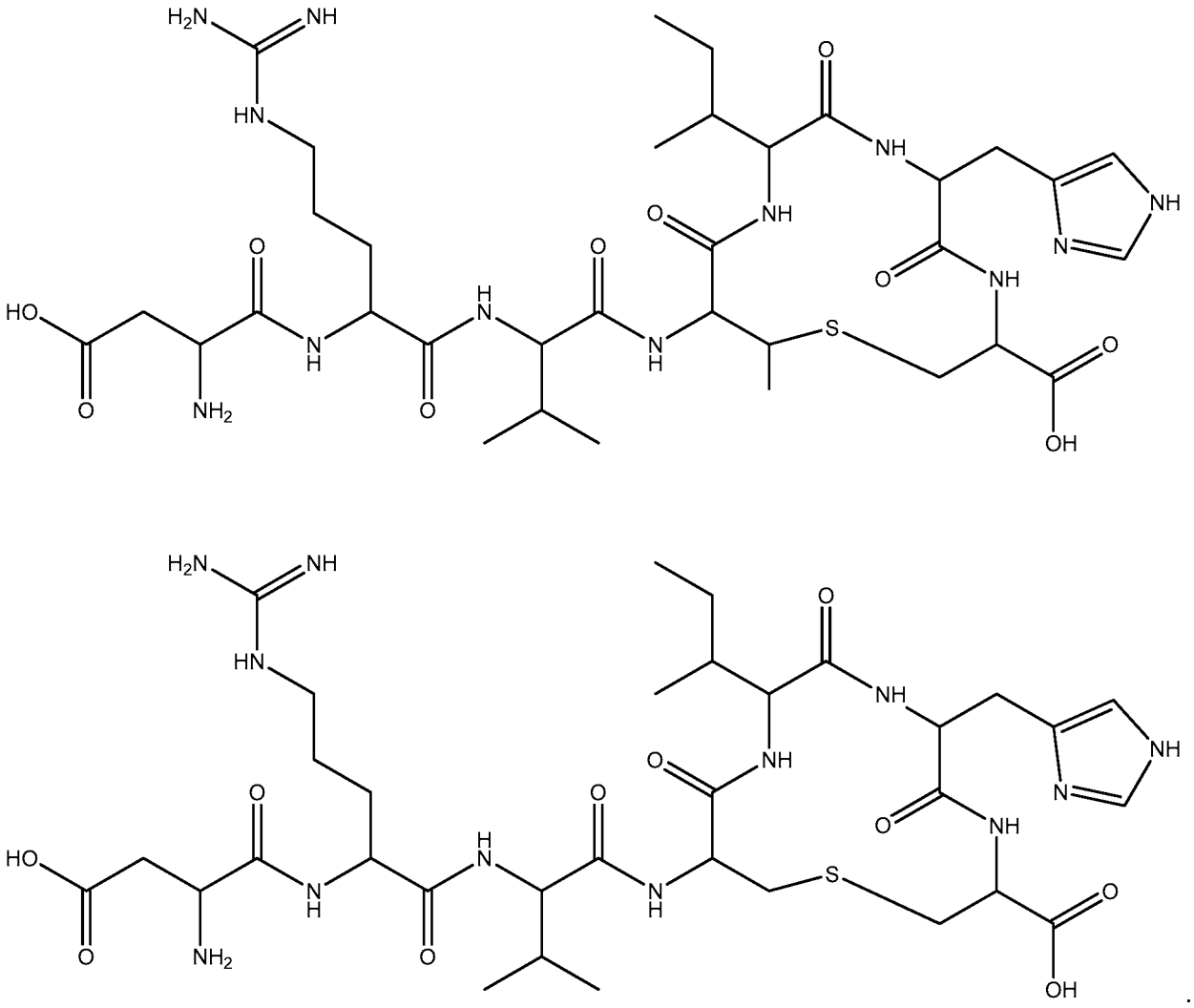
[0119] Xaa^6 is His.

[0120] Cyc^7 forms a thioether bridge in conjunction with Cyc^4, such as in Formula (I), (II) or (III). Cyc^7 can be a D-stereoisomer and/or a L-stereoisomer, typically a L-stereoisomer. Examples of Cyc^7 (taken with Cyc^4) are shown in Formulas (I), (II), (III) and (IVIII). Typically, the R groups in FormulaeFormulas (I), (II),) and (III) and (IV) are –H or $–CH_3$, especially –H.

[0121] In certain embodiments, one or more of Xaa^1-Xaa^6 (excluding Cyc^4 and Cyc^7) is identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In certain such embodiments, all but one or two of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7). In other embodiments, all of Xaa^1-Xaa^6 are identical to the corresponding amino acid in naturally-occurring Ang-(1-7).

[0122] In certain embodiments, Cyc^4 and Cyc^7 are independently selected from Abu (2-aminobutyric acid) and Ala (alanine), where Ala is present in at least one position. Thus, cyclic analogs can have a thioether linkage formed by -Ala^4-S-Ala^7- (Formula (I), where R^1-R^4 are each -H); -Ala^4-S-Abu^7- (Formula (I): R^1-R^3 are -H and R^4 is $-CH_3$) or -Abu^4-S-Ala^7- (Formula (I): R^1, R^3 and R^4 are –H and R^2 is $–CH_3$). Specific examples of cyclic analogs comprise a -Abu^4-S-Ala^7- or -Ala^4-S-Ala^7- linkage.

[0123] In certain embodiments, the invention provides an Ang-(1-7) analog with a thioether-bridge between position 4 and position 7 having the amino acid sequence Asp-Arg-Val-Abu-Ile-His-Ala (SEQ ID NO:15) or the amino acid sequence Asp-Arg-Val-Ala-Ile-His-Ala (SEQ ID NO:16), which are represented by the following structural diagrams:



[0124] In certain embodiments, an Ang analog or derivative of the invention is represented by Formula (IV):

Xaa^1-Xaa^2-Nle^3-Cyc^4-Xaa^5-Xaa^6-Cyc^7-Xaa^8-Xaa^9-Xaa^{10} (IV, SEQ ID NO:17)

As discussed above, one or more of Xaa^1, Xaa^2, Xaa^8, Xaa^9 and Xaa^{10} are absent in certain embodiments. For example, (1) Xaa^{10} is absent, (2) Xaa^9 and Xaa^{10} are absent, (3) Xaa^8, Xaa^9 and Xaa^{10} are absent, (4) Xaa^1 is absent, (5) Xaa^1 and Xaa^{10} are absent, (6) Xaa^1, Xaa^9 and Xaa^{10} are absent, (7) Xaa^1, Xaa^8, Xaa^9 and Xaa^{10} are absent, (8) Xaa^1 and Xaa^2 are absent, (9) Xaa^1, Xaa^2 and Xaa^{10} are absent, (10) Xaa^1, Xaa^2, Xaa^9 and Xaa^{10} are absent, or (11) Xaa^1, Xaa^2, Xaa^8, Xaa^9 and Xaa^{10} are absent. For each of these embodiments, the remaining amino acids have the values described below.

[0125] Xaa^1, when present, is any amino acid, but typically a negatively charged amino acid such as Glu or Asp, more typically Asp.

[0126] Xaa^2, when present, is a positively charged amino acid such as Arg or Lys, typically Arg.

[0127] Nle^3 is norleucine.

[0128] Cyc^4 forms a thioether bridge in conjunction with Cyc^7. Cyc^4 can be a D-stereoisomer and/or a L-stereoisomer, typically a D-stereoisomer. Examples of Cyc^4 (taken with Cyc^7) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0129] Xaa^5 is an aliphatic amino acid, such as Leu, Nle, Ile or Val, typically Ile.

[0130] Xaa^6 is His.

[0131] Cyc^7 forms a thioether bridge in conjunction with Cyc^4, such as in Formula (I), (II) or (III). Cyc^7 can be a D-stereoisomer and/or a L-stereoisomer, typically a L-stereoisomer. Examples of Cyc^7 (taken with Cyc^4) are shown in Formulas (I), (II) and (III). Typically, the R groups in Formulae (I), (II) and (III) are –H or $–CH_3$, especially –H.

[0132] Xaa^8, when present, is an amino acid other than Pro, typically Phe or Ile. In certain embodiments, Ile results in an inhibitor of Ang(1-8). In certain embodiments, Phe maintains the biological activity of Ang(1-8) or Ang(1-10).

[0133] Xaa^9, when present, is His.

[0134] Xaa^{10}, when present, is an aliphatic residue, for example, Ile, Val or Leu, typically Leu.

[0135] In certain embodiments, one or more of Xaa^1-Xaa^{10} (excluding Nle^3, Cyc^4 and Cyc^7) is identical to the corresponding amino acid in naturally-occurring Ang (including Ang-(1-7), Ang(1-8), Ang(1-9), Ang(1-10), Ang(2-7), Ang(2-8), Ang(2-9), Ang(2-10), Ang(3-8), Ang(3-9) and Ang(3-10). In certain such embodiments, all but one or two of Xaa^1-Xaa^{10} (for those present) are identical to the corresponding amino acid in naturally-occurring Ang. In other embodiments, all of Xaa^1-Xaa^{10} (for those present) are identical to the corresponding amino acid in naturally-occurring Ang.

[0136] In certain embodiments, Cyc^4 and Cyc^7 are independently selected from Abu (2-aminobutyric acid) and Ala (alanine), where Ala is present at at least one position. Thus, encompassed are cyclic analogs comprising a thioether linkage formed by -Ala^4-S-Ala^7- (Formula (I), where R^1-R^4 are each -H); -Ala^4-S-Abu^7- (Formula (I): R^1-R^3 are -H and R^4 is -CH_3) or -Abu^4-S-Ala^7- (Formula (I): R^1, R^3 and R^4 are –H and R^2 is –CH_3). Specific cyclic analogs comprise a -Abu^4-S-Ala^7- or -Ala^4-S-Ala^7- linkage.

[0137] In particular, the invention provides an Ang-(l-7) analog or derivative with a thioether-bridge between position 4 and position 7 having the amino acid sequence Asp-Arg-Nle-Abu-Ile-His-Ala (SEQ ID NO:18) or the amino acid sequence Asp-Arg-Nle-Ala-Ile-His-Ala (SEQ ID NO:19).

[0138] In another aspect, the invention provides an Ang-(l-8) analog or derivative with a thioether-bridge between position 4 and position 7 having Ang-(l-8) antagonistic activity, in particular an Ang(l-8) analog or derivative having the amino acid sequence Asp-Arg-Nle-Abu-Ile-His-Ala-Ile (SEQ ID NO:20), the amino acid sequence Asp-Arg-Nle-Ala-Ile-His-Ala-Ile (SEQ ID NO:21) or the amino acid sequence Asp-Arg-Nle-Abu-Ile-His-Ala-Ile (SEQ ID NO:22).

[0139] An alkyl group is a straight chained or branched non-aromatic hydrocarbon that is completely saturated. Typically, a straight chained or branched alkyl group has from 1 to about 20 carbon atoms, preferably from 1 to about 10. Examples of straight chained and branched alkyl groups include methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, tert-butyl, pentyl, hexyl, pentyl and octyl. A C1-C4 straight chained or branched alkyl group is also referred to as a "lower alkyl" group.

[0140] An aralkyl group is an alkyl group substituted by an aryl group. Aromatic (aryl) groups include carbocyclic aromatic groups such as phenyl, naphthyl, and anthracyl, and heteroaryl groups such as imidazolyl, thienyl, furyl, pyridyl, pyrimidyl, pyranyl, pyrazolyl, pyrrolyl, pyrazinyl, thiazolyl, oxazolyl, and tetrazolyl. Aromatic groups also include fused polycyclic aromatic ring systems in which a carbocyclic aromatic ring or heteroaryl ring is fused to one or more other heteroaryl rings. Examples include benzothienyl, benzofuryl, indolyl, quinolinyl, benzothiazole, benzoxazole, benzimidazole, quinolinyl, isoquinolinyl and isoindolyl.

[0141] Angiotensin (1-7) peptides, including derivatives and analogs, may be present in varying amounts in various embodiments. For example, an angiotensin (1-7) peptide is present in an amount ranging from about 10-1000 mg (e.g., about 20 mg – 1,000 mg, 30 mg – 1,000 mg, 40 mg – 1,000 mg, 50 mg – 1,000 mg, 60 mg – 1,000 mg, 70 mg – 1,000 mg, 80 mg – 1,000 mg, 90 mg – 1,000 mg, about 10-900 mg, 10-800 mg, 10-700 mg, 10-600 mg, 10-500 mg, 100-1000 mg, 100-900 mg, 100-800 mg, 100-700 mg, 100-600 mg, 100-500 mg, 100-400 mg, 100-300 mg, 200-1000 mg, 200-900 mg, 200-800 mg, 200-700 mg, 200-600 mg, 200-500 mg, 200-400 mg, 300-1000 mg, 300-900 mg, 300-800 mg, 300-700 mg, 300-600 mg, 300-500 mg, 400 mg – 1,000 mg, 500 mg – 1,000 mg, 100 mg - 900 mg, 200 mg – 800 mg, 300 mg – 700 mg, 400 mg – 700 mg, and 500 mg – 600 mg). In some embodiments, an angiotensin (1-7) peptide is present in an amount of or greater than about 10 mg, 50 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg. In some embodiments, an angiotensin (1-7) peptide is present in an amount of or less than about 1000 mg, 950 mg, 900 mg, 850 mg, 800 mg, 750 mg, 700 mg, 650 mg, 600 mg, 550 mg, 500 mg, 450 mg, 400 mg, 350 mg, 300 mg, 250 mg, 200 mg, 150 mg, or 100 mg.

[0142] In some embodiments, a cyclic angiotensin (1-7) peptide is a cyclized Ang (1-9) peptide or a cyclized peptide comprising SEQ ID NO: 24.

[0143] Further contemplated are variants of the cyclic peptides described herein, wherein the variants maintain one or more functional properties of the comparator peptide. Cyclized variants may have a sequence at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity to any of the sequences of the exemplary cyclic peptides described herein.

pH-Lowering Agents

[0144] It is contemplated that a pH-lowering agent suitable for the present invention include any pharmaceutically acceptable pH-lowering agent, or combination of pH-lowering agents, that are a) not toxic to the gastrointestinal tract, b) are capable of either delivering hydrogen ions or capable of inducing higher hydrogen ion content from the local environment, and/or c) that are capable of being orally administered in an amount sufficient to lower the local intestinal pH below the pH optima for proteases found there. Various tests may be used to determine if a pH-lowering agent is suitable for the present invention and what amount is

appropriate. For example, a pH-lowering agent or combination of pH-lowering agents is suitable for the present invention if, a particular amount when added to a solution of 10 milliliters of 0.1M sodium bicarbonate lowers the pH of the solution to no higher than 5.5, 4.7, or 3.5. In some embodiments, an amount of pH-lowering agent or agents may be added to lower pH, in a solution of 10 milliliters of 0.1M sodium bicarbonate, to no higher than 3.4, 3.2, 3.0, or 2.8.

[0145] In some embodiments, a suitable pH-lowering agent or agents include at least one pH-lowering agent that has a pKa no higher than 4.2 (e.g., no higher than 4.0, 3.8, 3.6, 3.4, 3.2, 3.0 or 2.8). Exemplary pH-lowering agents suitable for the present invention include, but are not limited to, carboxylic acids such as acetylsalicylic, acetic, ascorbic, citric, fumaric, glucuronic, glutaric, glyceric, glycocolic, glyoxylic, isocitric, isovaleric, lactic, maleic, oxaloacetic, oxalosuccinic, propionic, pyruvic, succinic, tartaric, and valeric; aluminum chloride; zinc chloride; acid salts of amino acids (or derivatives thereof) including acid salts of acetylglutamic acid, alanine, arginine, asparagine, aspartic acid, betaine, carnitine, carnosine, citrulline, creatine, glutamic acid, glycine, histidine, hydroxylysine, hydroxyproline, hypotaurine, isoleucine, leucine, lysine, methylhistidine, norleucine, ornithine, phenylalanine, proline, sarcosine, serine, taurine, threonine, tryptophan, tyrosine, and valine; certain phosphate esters including fructose 1,6 diphosphate and glucose 1,6 diphosphate may also be appropriate pH-lowering agents in certain embodiments. In particular embodiments, citric acid or tartaric acid is used as pH-lowering agent.

[0146] The quantity required of any particular pH-lowering agent or combination of pH-lowering agents may vary. Typically, suitable amount may be determined using various tests known in the art and described herein (for example, using pH-lowering test in a solution of 10 milliliters of 0.1M sodium bicarbonate described above). As non-limiting examples, suitable amount of a pH lowering agent used in a formulation according to the present invention may be an amount of or greater than about 100 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 425 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675, mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, or 1,000 mg. In other embodiments, the amount of citric acid used may exceed 1,000 mg.

[0147] In some embodiments, a suitable amount of a pH lowering agent (e.g., citric acid or tartaric acid) used may be measured as a percent of the total weight of a particular dosage

form. As non-limiting examples, a suitable amount of a pH lowering agent used may be an amount of or greater than about 10% (e.g., of or greater than 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%) of the total weight of a solid dosage form.

Absorption Enhancers

[0148] In various embodiments, a formulation of the invention has one or more absorption enhancers. As used herein, an absorption enhancer refers to an agent that increase the solubility of other components in either the aqueous or lipophilic environment into which they are released and/or enhance the uptake of an active peptide (e.g., an angiotensin (1-7) peptide) across the intestinal wall. In some embodiments, an absorption enhancer is referred to as a solubility enhancer and/or an uptake enhancer.

[0149] In some embodiments, it is possible to have a mixture of absorption enhancers wherein some provide enhanced solubility, some provide enhanced uptake, and some provide both. It is possible to have various numbers of absorption enhancers in a given embodiment including, without limitation, one, two, three, four, five, six, seven, eight, nine, or ten absorption enhancers.

[0150] Surface active agents are an example of useful absorption enhancers with properties of both solubility enhancers and uptake enhancers. In some embodiment, when surface active agents are used as absorption enhancers, they may be free flowing powders for facilitating the mixing and loading of capsules during the manufacturing process. In other embodiments when a surface active agent is used to increase the bioavailability of an angiotensin (1-7) peptide, the surface active agent may be selected from the group consisting of (a) anionic surface active agents such as cholesterol derivatives (e.g. bile acids), (b) cationic surface agents (e.g. acyl carnitines, phospholipids and the like), (c) non-ionic surface active agents, and (d) mixtures of anionic surface active agents and negative charge neutralizers, and combinations thereof. Negative charge neutralizers include but are not limited to acyl carnitines, cetyl pyridinum chloride, and the like.

[0151] In some embodiments, an acid soluble bile acid and a cationic surface active agent with be used together as absorption enhancers. Acyl carnitines (such as lauroyl carnitine),

phospholipids and bile acids may be particularly effective absorption enhancers in some embodiments.

[0152] While a variety of absorption enhancers are suitable for use in various embodiments, the following exemplary list is intended to illustrate some embodiments of the invention. Without limitation, some suitable absorption enhancers include: (a) salicylates such as sodium salicylate, 3-methoxysalicylate, 5-methoxysalicylate and homovanilate; (b) bile acids such as taurocholic, tauorodeoxycholic, deoxycholic, cholic, glycholic, lithocholate, chenodeoxycholic, ursodeoxycholic, ursocholic, dehydrocholic, fusidic, etc.; (c) non-ionic surfactants such as polyoxyethylene ethers (e.g. Brij 36T, Brij 52, Brij 56, Brij 76, Brij 96, Texaphor A6, Texaphor A14, Texaphor A60 etc.), p-t-octyl phenol polyoxyethylenes (Triton X-45, Triton X-100, Triton X-114, Triton X-305 etc.) nonylphenoxypoloxyethylenes (e.g. Igepal CO series), polyoxyethylene sorbitan esters (e.g. Tween-20, Tween-80 etc.); (d) anionic surfactants such as dioctyl sodium sulfosuccinate; (e) lyso-phospholipids such as lysolecithin and lysophosphatidylethanolamine; (f) acylcarnitines, acylcholines and acyl amino acids such as lauroylcarnitine, myristoylcarnitine, palmitoylcarnitine, lauroylcholine, myristoylcholine, palmitoylcholine, hexadecyllysine, N-acylphenylalanine, N-acylglycine etc.; g) water soluble phospholipids such as diheptanoylphosphatidylcholine, dioctylphosphatidylcholine etc.; (h) medium-chain glycerides which are mixtures of mono-, di- and triglycerides containing medium-chain-length fatty acids (caprylic, capric and lauric acids); (i) ethylene-diaminetetraacetic acid; (j) cationic surfactants such as cetylpyridinium chloride; (k) fatty acid derivatives of polyethylene glycol such as Labrasol, Labrafac, etc.; and (l) alkylsaccharides such as lauroyl maltoside, lauroyl sucrose, myristoyl sucrose, palmitoyl sucrose, etc.

[0153] In some embodiments, the absorption enhancer(s) will be present in a quantity measured as a percent by weight, relative to the overall weight of the pharmaceutical composition (typically exclusive of enteric coating). By way of additional non-limiting example, the quantity of absorption enhancer present in an embodiment may range from 0.1 to 20 percent by weight; from 0.5 to 20 percent by weight; from 1.0 to 20 percent by weight, from 2.0 to 20 percent by weight, from 3.0 to 20 percent by weight, from 4.0 to 20 percent by weight, from from 5.0 to 20 percent by weight, from 5.0 to 15 percent by weight, from 5.0 to 14 percent by weight, from 5.0 to 13 percent by weight, from 5.0 to 12 percent by weight, from 5.0 to 12 percent by weight, from 5.0 to 11 percent by weight, from 5.0 to 10 percent by weight, from 6.0

to 10 percent by weight, from 7.0 to 10 percent by weight, from 8.0 to 10 percent by weight, from 9.0 to 10 percent by weight, from 5.0 to 9.0 percent by weight, from 5.0 to 8.0 percent by weight, from 5.0 to 7.0 percent by weight, and from 5.0 to 6.0 percent by weight.

[0154] In some embodiments, the weight ratio of pH-lowering agent(s) to absorption enhancer(s) may be about 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1 or between any two of the foregoing exemplary ratios. The total weight of all pH-lowering agents and the total weight of all absorption enhancers in a given pharmaceutical composition is included in the foregoing exemplary ratios. For example, if a pharmaceutical composition includes two pH-lowering agents and three absorption enhancers, the foregoing ratios will be computed on the total combined weight of both pH-lowering agents and the total combined weight of all three absorption enhancers.

[0155] In some embodiments, the absorption enhancer(s) will be soluble at acid pH, such as less than pH 5.5, and in particular, between pH 3.0 and pH 5.0.

Protective Vehicles

[0156] As used herein, a protective vehicle refers to any protective component and/or structure, such as a carrier, a layer, a coating or other vehicle, that protects an active peptide (e.g., an angiotensin (1-7) peptide) from stomach proteases. Typically, a protective vehicle dissolves eventually so that the active and other ingredients in a particular dosage form may be released. A common form of protective vehicle is an enteric coating. In some embodiments, a suitable enteric costing may prevent breakdown of the pharmaceutical composition of the invention in 0.1N HCl for at least two hours, then capable of permitting complete release of all contents of the pharmaceutical composition within thirty minutes after pH is increased to 6.3 in a dissolution bath in which said composition is rotating at 100 revolutions per minute.

[0157] Many enteric coatings are known in the art and are useful in one or more embodiments. Non-limiting examples of enteric coatings include cellulose acetate phthalate, hydroxypropyl methylethylcellulose succinate, hydroxypropyl methylcellulose phthalate, carboxyl methylethylcellulose and methacrylic acid-methyl methacrylate copolymer. In some embodiments, an angiotensin (1-7) peptide, absorption enhancers such as solubility and/or uptake enhancer(s), and pH-lowering agent(s), are included in a sufficiently viscous protective syrup to permit protected passage of the components of the embodiment through the stomach.

[0158] Suitable enteric coatings may be applied, for example, to capsules after the active and other components of the invention have been loaded within the capsule. In other embodiments, enteric coating is coated on the outside of a tablet or coated on the outer surface of particles of active components which are then pressed into tablet form, or loaded into a capsule.

[0159] In some embodiments it may be desirable that all components of the invention be released from the carrier or vehicle, and solubilized in the intestinal environment as simultaneously as possible. It may also be preferred in some embodiments that the vehicle or carrier release the active components in the small intestine where uptake enhancers that increase transcellular or paracellular transport are less likely to cause undesirable side effects than if the same uptake enhancers were later released in the colon. It will be appreciated, however, that the present invention is believed effective in the colon as well as in the small intestine. Numerous vehicles or carriers, in addition to the ones discussed above, are known in the art.

[0160] In some embodiments, it may be desirable (especially in optimizing how simultaneously the components of the invention are released) to keep the amount of enteric coating low. In some embodiments, an enteric coating adds no more than 30% to the weight of the remainder of pharmaceutical composition such as a solid dosage form (the "remainder" being the pharmaceutical composition exclusive of enteric coating itself). In other embodiments, an enteric coating adds less than 20%, less than 19%, less than 18%, less than 17%, less than 16%, less than 15%, less than 14%, less than 13%, less than 12%, less than 11%, or less than 10%. In some embodiments, a protective vehicle such as an enteric coating constitutes an amount of or less than approximately 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5% of the total weight of a pharmaceutical composition (e.g., a solid dosage form).

Dosage Forms

[0161] As used herein, dosage forms refer to a mixture of active drug components and nondrug components. Various dosage forms may be used according to the invention, including but not limited to, liquid dosage forms, solid dosage forms and semisolid dosage forms. Common dosage forms include pill, tablet, capsule, drink or syrup. In some embodiments, solid dosage forms such as pill, tablet or capsule are used.

[0162] Typically, a particularly desirable dosage form provides simultaneous release of the angiotensin-(1-7) peptide, pH-lowering agent and absorption enhancers. This is highly desirable because the acid is best able to reduce undesirable proteolytic attack on the peptide when the acid is released in close time proximity to release of the peptide. Near simultaneous release is best achieved by administering all components of the invention as a single pill, tablet or capsule.

[0163] Various embodiments may optionally include common pharmaceutical excipients such as diluents, glycants, lubricants, gelatin capsules, preservatives, colorants and the like in their usual known sizes and amounts. Exemplary, non-limiting excipients include pro-salts, polyplastum and sodium stearyl fumerate. In some embodiments, another peptide (such as albumin, casein, soy protein, other animal or vegetable proteins and the like) is included to reduce non-specific adsorption (e.g., binding of angiotensin (1-7) peptide to the intestinal mucus barrier) thereby lowering the necessary concentration of the expensive peptide active agent. When added, the additional peptide is typically from 1.0 to 10.0 percent by weight relative to the weight of the overall pharmaceutical composition (excluding protective vehicle). Typically, this additional peptide is not physiologically active and is most preferably a food peptide such as soy bean peptide or the like. Without intending to be bound by theory, this second peptide may also increase bioavailability by acting as a protease scavenger that desirably competes with the peptide active agent for protease interaction. The second peptide may also aid the active compound's passage through the liver.

[0164] In some embodiments, the pH-lowering agent(s), the angiotensin (1-7) peptide, the absorption enhancer(s) and other excipients (whether single compounds or a plurality of compounds in each category) be uniformly dispersed in a dosage form. In other embodiments, a dosage form may comprise granules that include a pharmaceutical binder having the angiotensin (1-7) peptide, the pH-lowering agent and the absorption enhancer uniformly dispersed within said binder. In yet other embodiments, granules may also consist of an acid core, surrounded by a uniform layer of organic acid, a layer of enhancer and a layer of peptide that is surrounded by an outer layer of organic acid. Granules may be prepared from an aqueous mixture consisting of pharmaceutical binders such as polyvinyl pyrrolidone or hydroxypropyl methylcellulose, together with the pH-lowering agents, absorption enhancers and peptide active agents of the invention.

[0165] As described, various embodiments may have differing amounts of ingredients and differing ingredients as well. Regardless of the recipe of a particular embodiment, the total weight of all ingredients present in that embodiment may fall within a certain weight range, such as from about 500-1500 (e.g., from about 500-1200 mg, 500-1000 mg, 600-1500 mg, 600-1200 mg, 600-1000 mg, 700-1500 mg, 700-1200 mg, 700-1000 mg, 800-1500 mg, 800-1200 mg, 800-1000 mg). In some embodiments, a suitable solid dosage form has a total weight of or greater than about 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, or 1500 mg. In some embodiments, a suitable solid dosage form has a total weight of or less than about 2000 mg, 1900 mg, 1800 mg, 1700 mg, 1600 mg, 1500 mg, 1400 mg, 1300 mg, 1200 mg, 1100 mg, 1000 mg, 900 mg, 800 mg, 700 mg, 600 mg, or 500 mg.

EXAMPLES

Example 1. Oral Delivery of Angiotensin (1-7) Peptides

[0166] This example demonstrates that angiotensin (1-7) can be effectively delivered orally using an exemplary formulation according to the present invention. Specifically, the feasibility of orally delivering an angiotensin (1-7) peptide was demonstrated by administrating it in a liquid formulation to an anesthetized rat via intra-duodenal injection (ID). This model mimics the release and absorption expected from an orally delivered enteric coated solid dosage form such as capsule or tablet.

[0167] Initially, a baseline pharmacokinetic profile in female Sprague-Dawley rats was obtained by subcutaneous (SC) administration of angiotensin (1-7) in phosphate buffered saline (PBS). Blood samples (0.6 ml) was taken from a cannula implanted into the right carotid artery before and 5, 10, 20, 30, 60 and 90 minutes after the injection of the peptide and replaced with an equal volume of heparinized saline.

[0168] After extraction, the samples were then transferred to ice-cold tubes containing a protease inhibitor cocktail. The samples were kept on ice until they were centrifuged at 4°C to obtain plasma. The plasma supernatants were then frozen -70°C until analysis using an LC-MS assay.

[0169] Table 1 summarized exemplary individual baseline A(1-7) levels achieved at the designated time points and the non-compartmental PK values. Three rats were administered 0.3

mL of 10 mg/mL A(1-7) as a subcutaneous injection. The pharmacokinetics of A(1-7) were determined using a non-compartmental model, where individual pharmacokinetics were determined. The mean concentration for each time point was calculated and the PK values for these mean values were estimated. The Tmax was achieved approximately 10 to 90 minutes after administration. Half-lives are approximately 15 minutes for this treatment group. Total mean A(1-7) exposure over the observation period was 614 ng*min/mL with a range of 585 to 656 ng*min/mL.

Table 1:

	0.3 mL: A(1-7) Subcutaneous injection				
	Angiotensin (1-7) (ng/mL)				
Time Point (min)	Rat1	Rat 2	Rat 3	Mean PK Value	Average
0	0.121	10.2	1.41		4.50
5	0.120	7.47	4.37		3.99
10	18.6	0.94	1.09		6.88
20	6.25	15.7	5.71		9.22
40	15.30	6.38	4.47		8.72
60	1.03	2.65	7.46		3.71
90	1.14	6.59	17.4		8.38
Cmax (ng/mL)	18.6	15.7	17.4	17.2	9.2
Tmax (ng/mL)	10	20	90	40.0	20
Half-Life (min)	15	15.58		15.1	
AUC_{0-90} (ng*min/mL)	583	598.1	656.1	612.4	614

[0170] The oral delivery of an angiotensin (1-7) peptide (e.g., TXA127) was then evaluated in a rat model that mimics the release of the peptide into the intestine by enteric-coated capsule. Briefly, the duodenums of anesthetized rats were surgically exposed, and an

angiotensin (1-7) peptide was delivered through a 27 gauge needle into the duodenum. A baseline was obtained by ID administration of an angiotensin (1-7) in PBS. Samples of blood was removed from the carotid artery before and 5, 10, 20, 40, 60 and 90 minutes after peptide administration. Subsequently, an angiotensin (1-7) peptide was administered ID in 400 mM citrate buffer (pH 3.5) and lauroyl-L-carnitine (LLC) (10 mg/ml), a formulation that mimics the contents of the enteric-coated capsules. In order to maximize the stability of TXA127 in rat circulation, captopril (e.g., 0.5 mg/ml or 5 mg/ml) may be added to the formulations. Blood samples were taken at the same time points as the baseline study and handled as described above for analysis. Exemplary results were summarized in Tables 2-5.

[0171] Table 2 summarizes exemplary results from a total of 6 rats treated with 0.3 mL of 10 mg/mL Angiotensin (1-7) (A(1-7)) formulated in PBS. The pharmacokinetics of A(1-7) were determined using a non-compartmental model. The average concentration for each time point was calculated and the PK values for these mean values were estimated. These were compared with the mean PK values, which were calculated by taking the average of all individual PK parameters. The Tmax was achieved approximately 10 to 60 minutes after administration. Half-lives ranged from 7 to 140 minutes for this treatment group. Total mean A(1-7) exposure over the observation period was 403 ng*min/mL with a range of 123 to 881 ng*min/mL.

Table 2:

	0.3 ml A, ID [10 mg/ml Angiotensin(1-7) in PBS]							
	Angiotensin (1-7) (ng/mL)							
Time Point (min)	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Mean PK Values	Average
0	0.23	0.0	1.54	1.00	1.84	5.81		1.74
5	0.84	12.3	0.94	0.88	0.891	9.24		4.18
10	3.44	6.38	1.08	0.99	1.12	4.08		2.85

20	0.62	13.3	1.91	1.79	1.80	2.05		3.58
40	0.27	15.4	1.22	4.14	1.30	3.89		4.37
60	1.55	6.27	3.97	8.75	3.07	10.4		5.67
90	2.61	7.19	0.78	7.54	1.23	10.4		4.96
Cmax (ng/mL)	3.44	15	4	9	3	10.4	7.5	5.7
Tmax (ng/mL)	10	40	60	60	60		46.0	60
Half-Life (min)		51	13	140	23	7	38.9	155
AUC_{0-90} (ng*min/mL)	123	881	181	456	166	616	403	404

[0172] Table 3 summarizes exemplary A(1-7) concentration found in rats treated with 0.3 mL of 10 mg/mL A(1-7) in preparation containing 10 mg/mL LLC, 400 mM Citrate, 150 mM NaCl pH 3.5. The pharmacokinetics of A(1-7) was determined using a non-compartmental model. Additionally, the mean concentration for each time point was calculated and the PK values for these mean values were estimated. These mean concentration values were compared with the mean PK values, which is calculated by taking the average of all individual PK parameters. The Tmax was achieved approximately 5 to 10 minutes after administration. Half-lives ranged from 9.3 to 173.3 minutes for this treatment group. Total mean A(1-7) exposure over the observation period was 4,274 ng*min/mL with a range of 422 to 19,502 ng*min/mL.

Table 3:

	0.3 ml B, ID [10 mg/ml Angiotensin(1-7), 10mg/ml LLC, 400mM Citrate pH3.5,150 mM NaCl]							
	Angiotensin (1-7) (ng/mL)							
Time Point (min)	Rat 3**	Rat 4	Rat 3	Rat 4	Rat 3	Rat 4	Mean PK Values	Average
0	1.46	0.171	3.02	1.31	1.81	1.29		1.51
5	>1000	259	3.48	89.7	60.7	56.38		416.54

10	>1000	21	19.3	15.2	59.6	17.92		203.84
20	59.9	7.35	6.02	18.4	51.2	6.02		24.82
40	4.88	5.6	2.32	5.05	11.7	1.11		5.11
60	4.74	1.7	3.99	6.36	10.9	3.48		5.20
90	3.93	3.99	1.11	4.43	4.74	3.39		3.60
Cmax (ng/mL)	>1000	259.0	19.3	89.7	60.7	56.4	419.2	416.5
Tmax (ng/mL)	5.0	5.0	10.0	5.0	5.0	5.0	5.8	5
Half-Life (min)	9.3	173.3	23.8	25.2	21.6	25.0	46.4	13
AUC_{0-90} (ng*min/mL)	19,502.2	1,777.5	422.8	1,168.2	2,100.6	669.9	4,273.5	4274

** - data for this rat was not included in averages or analysis

[0173] Table 4 summarizes exemplary pharmacokinetics results from seven rats administered with 0.3 mL of 10 mg/mL A(1-7) in a preparation containing 0.5 mg/mL captopril, 10 mg/mL LLC, 400 mM Citrate, 150 mM NaCl at pH 3.5. The pharmacokinetics of A(1-7) were determined using a non-compartmental model. The average concentration for each time point was calculated and the PK values for these mean values were estimated. These mean concentration values were compared with the mean PK values, which is calculated by taking the average of all individual PK parameters. AUCs were determined using values >1,000 ng/mL to provide a perspective of the range of exposure. In addition mean concentration for each time point was calculated and the PK values for these mean values were estimated. The Tmax was achieved approximately 5 to 10 minutes after administration. Half-lives ranged from 11.9 to 29.1 minutes for this treatment group. Total mean A(1-7) exposure over the observation period was 7,152 ng*min/mL with a range of 1,969 to 9,257 ng*min/mL.

Table 4:

	0.3 ml C, ID [10 mg/ml Angiotensin(1-7), Captopril 0.5 mg/ml, 10mg/ml LLC, 400 mM Citrate pH 3.5, 150 mM NaCl]								
	Angiotensin (1-7) (ng/mL)								
Time Point (min)	Rat 5	Rat 6**	Rat 5	Rat 6	Rat 5	Rat 6		Mean PK Values	Average
0	1.24	1.13	1.54	0.51	1.29	0.98			1.1
5	879.00	>1000	468.00	57.20	735.00	325.00			628.31
10	407.00	349.00	313.00	83.00	248.00	145.67			233.21
20	88.70	496.00	141.00	64.10	16.30	70.14			129.13
40	4.24	19.60	17.30	7.68	8.42	22.85			13.87
60	12.30	10.10	4.96	6.16	12.30	37.30			14.45
90	5.61	5.83	7.84	4.93	15.80	7.36			7.74
Cmax (ng/mL)	879.0	>1000	468.0	83.0	735.0	83.0		609.3	628.3
Tmax (ng/mL)	5.0	5.0	5.0	10.0	5.0	10.0		7.1	5
Half-Life (min)	11.9	29.1	13.9	18.3	17.9	18.3		19.4	14
AUC0-90 (ng*min/mL)	9,257	20,442	7,394	2,252	6,495	2,252		7,152	7,587

** - data for this rat was not included in averages or analysis

[0174] Table 5 summarizes exemplary A(1-7) levels achieved in six rats given 0.3 mL of 10 mg/mL A(1-7) in a preparation containing 0.5 mg/mL captopril, 10 mg/mL LLC, 400 mM Citrate, 150 mM NaCl at pH 3.5. Again, the pharmacokinetic parameters of A(1-7) was determined using a non-compartmental model, where individual pharmacokinetics parameters were also determined. In this analysis, AUCs were determined using values >1,000 ng/mL to provide a perspective of the range of exposure. The mean concentration for each time point was

calculated and the PK values for these mean values were estimated. The Tmax was achieved approximately 5 to 10 minutes after administration. Half-lives ranged from 7.97 to 25.6 minutes for this treatment group. Total mean A(1-7) exposure over the observation period was 9,399 ng*min/mL with a range of 1,008 to 26,654 ng*min/mL.

Table 5:

	0.3 ml C, ID [10 mg/ml Angiotensin(1-7), Captopril 5 mg/ml, 10mg/ml LLC, 400 mM Citrate pH 3.5,150 mM NaCl]							
	Angiotensin (1-7) (ng/mL)							
Time Point (min)	Rat 7	Rat 8	Rat 7	Rat 8	Rat 7	Rat 8	Mean PK Value	Average
0	0.99	0.40	3.31	7.63	1.55	0.63		2.42
5	>1000	33.50	504.00	746.00	268.89	432.86		619.21
10	>1000	12.30	410.00	404.00	391.25	5.34		423.82
20	466.00	8.12	70.40	178.00	63.50	41.25		137.88
40	27.20	20.20	18.40	39.90	22.70	16.10		24.08
60	16.60	22.10	17.60	13.50	19.90	25.70		19.23
90	10.20	0.00	11.60	5.58	2.75	4.83		5.83
Cmax (ng/mL)	<1000	33.5	504	746.0	391.25	432.85	639.6	619.2
Tmax (ng/mL)	5.0	5.0	5.0	5.0	10	5.0	5.8	5
Half-Life (min)	10.55	7.97	15.45	11.94	12.86	25.62	14.1	13
AUC_{0-90} (ng*min/mL)	26,654	1,008	7,641	10,668	6,228	3,862	9,343.5	9,399

[0175] In addition, Figure 1 illustrates the AUC values compared between various administration routes and formulations.

[0176] As shown in Tables 1-5 and Figure 1, angiotensin (1-7) delivered in a formulation according to the present invention using a rat model mimicking oral delivery has significantly improved half-life and total exposure over the observation period as compared to the baseline profile of angiotensin (1-7) delivered in PBS. These results demonstrate that angiotensin (1-7) can be delivered orally according to the present invention and achieve therapeutically effective bioavailability in circulation.

EQUIVALENTS AND SCOPE

[0177] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

CLAIMS

We claim:

1. A solid dosage form for oral administration comprising

(a) an angiotensin (1-7) peptide;

(b) at least one pharmaceutically acceptable pH-lowering agent;

(c) at least one absorption enhancer effective to promote bioavailability of the angiotensin (1-7) peptide; and

(d) a protective vehicle.

2. The solid dosage form of claim 1, wherein the solid dosage form is a capsule or tablet.

3. The solid dosage form of claim 1 or 2, wherein the pH-lowering agent is citric acid.

4. The solid dosage form of claim 3, wherein the citric acid is present in an amount greater than 400 mg.

5. The solid dosage form of claim 3, wherein the citric acid is present in an amount greater than 500 mg.

6. The solid dosage form of claim 3, wherein the citric acid is present in an amount greater than 50% of the total weight of the solid dosage form.

7. The solid dosage form of claim 1 or 2, wherein the pH-lowering agent is tartaric acid.

8. The solid dosage form of any one of the preceding claims, wherein the absorption enhancer is an acylcarnitine.

9. The solid dosage form of claim 8, wherein the acylcarnitine is lauroyl carnitine.

10. The solid dosage form of claim 9, wherein the lauroyl carnitine is present in an mount ranging from 50-100 mg.

11. The solid dosage form of claim 9, wherein the lauroyl carnitine is present in an mount ranging from 5-10% of the total weight of the solid dosage form.

12. The solid dosage form of any one of the preceding claims, wherein the protective vehicle constitutes less than 20% of the total weight of the solid dosage form.

13. The solid dosage form of any one of the preceding claims, wherein the protective vehicle is an enteric coat.

14. The solid dosage form of any one of the preceding claims, wherein the solid dosage form further comprises one or more excipients selected from fillers such as PROSOLV®, disintegrants such as POLYPLASDONE™ crospovidone, glidants such as silicon dioxide or lubricants such as sodium stearyl fumarate.

15. The solid dosage form of any one of the preceding claims, wherein the solid dosage form further comprises captopril.

16. The solid dosage form of any one of the preceding claims, wherein the solid dosage form has a total weight ranging from 800-1200 mg.

17. The solid dosage form of any one of the preceding claims, wherein the angiotensin (1-7) peptide is present in an amount ranging from 10-1000 mg.

18. A solid dosage form for oral administration comprising

(a) an angiotensin (1-7) peptide;

(b) citric acid;

(c) lauroyl carnitine; and

(d) a protective vehicle.

19. The solid dosage form of claim 18, wherein the citric acid is present in an amount great than 500 mg and the lauroyl carnitine is present in an amount ranging from 50-100 mg.

20. The solid dosage form of claim 18 or 19, wherein the solid dosage form is a capsule or tablet.

21. The solid dosage form of any one of claims 18-20, wherein the protective vehicle is an enteric coat.

22. The solid dosage form of any one of the preceding claims, wherein the angiotensin (1-7) peptide comprises the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7} (SEQ ID NO:1).

23. The solid dosage form of any one of claims 1-21, wherein the angiotensin (1-7) peptide is a functional equivalent of SEQ ID NO:1.

24. The solid dosage form of claim 23, wherein the functional equivalent is a linear peptide.

25. The solid dosage form of claim 24, wherein the linear peptide comprises a sequence that includes at least four amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least four amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

26. The solid dosage form of claim 24, wherein the linear peptide comprises a sequence that includes at least five amino acids from the seven amino acids that appear in the naturally-occurring Angiotensin (1-7), wherein the at least five amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

27. The solid dosage form of claim 24, wherein the linear peptide comprises a sequence that includes at least six amino acids from the seven amino acids that appear in the naturally-

occurring Angiotensin (1-7), wherein the at least six amino acids maintain their relative positions as they appear in the naturally-occurring Angiotensin (1-7).

28. The solid dosage form of any one of claim 25-27, wherein the at least four, five or six amino acids, respectively, further maintain their relative spacing as they appear in the naturally-occurring Angiotensin (1-7).

29. The solid dosage form of any one of claims 24-28, wherein the linear peptide contains 4-25 amino acids.

30. The solid dosage form of any one of claims 24-29, wherein the linear peptide is a fragment of the naturally-occurring Angiotensin (1-7).

31. The solid dosage form of any one of claims 24-30, wherein the linear peptide contains amino acid substitutions, deletions and/or insertions in the naturally-occurring Angiotensin (1-7).

32. The solid dosage form of claim 31, wherein the linear peptide has an amino acid sequence of Asp^1-Arg^2-Nle^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:2).

33. The solid dosage form of claim 31, wherein the linear peptide has an amino acid sequence of Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7 (SEQ ID NO:6).

34. The solid dosage form of claim 23, wherein the functional equivalent is a cyclic peptide.

35. The solid dosage form of claim 34, wherein the cyclic peptide comprises a linkage between amino acids.

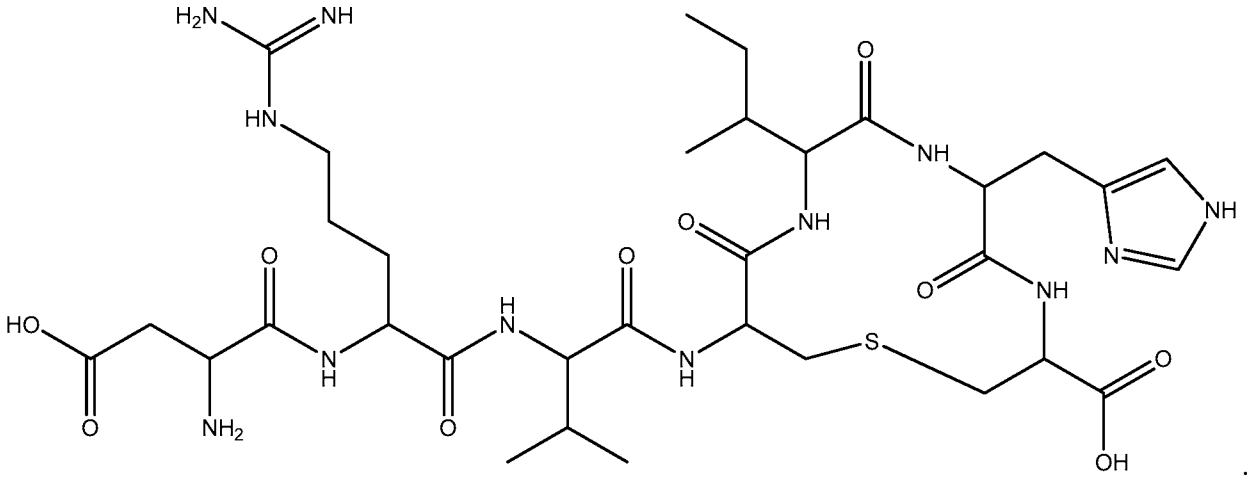
36. The solid dosage form of claim 35, wherein the linkage is located at residues corresponding to positions Tyr^4 and Pro^7 in naturally-occurring Angiotensin (1-7).

37. The solid dosage form of claim 35 or 36, wherein the linkage is a thioether bridge.

38. The solid dosage form of any one of claims 34-37, wherein the cyclic peptide comprises an amino acid sequence otherwise identical to the naturally-occurring Angiotensin (1-7) amino acid sequence of Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7 (SEQ ID NO:1).

39. The solid dosage form of any one of claims 34-38, wherein the cyclic peptide comprises a norleucine (Nle) replacing position Val^3 in naturally-occurring Angiotensin (1-7).

40. The solid dosage form of claim 36, wherein the cyclic peptide is a 4,7-cyclized angiotensin (1-7) with the following formula:



41. The solid dosage form of any one of claims 34-40, wherein the angiotensin (1-7) peptide comprises one or more chemical modifications to increase protease resistance, serum stability and/or bioavailability.

42. The solid dosage form of claim 41, wherein the one or more chemical modifications comprise pegylation.

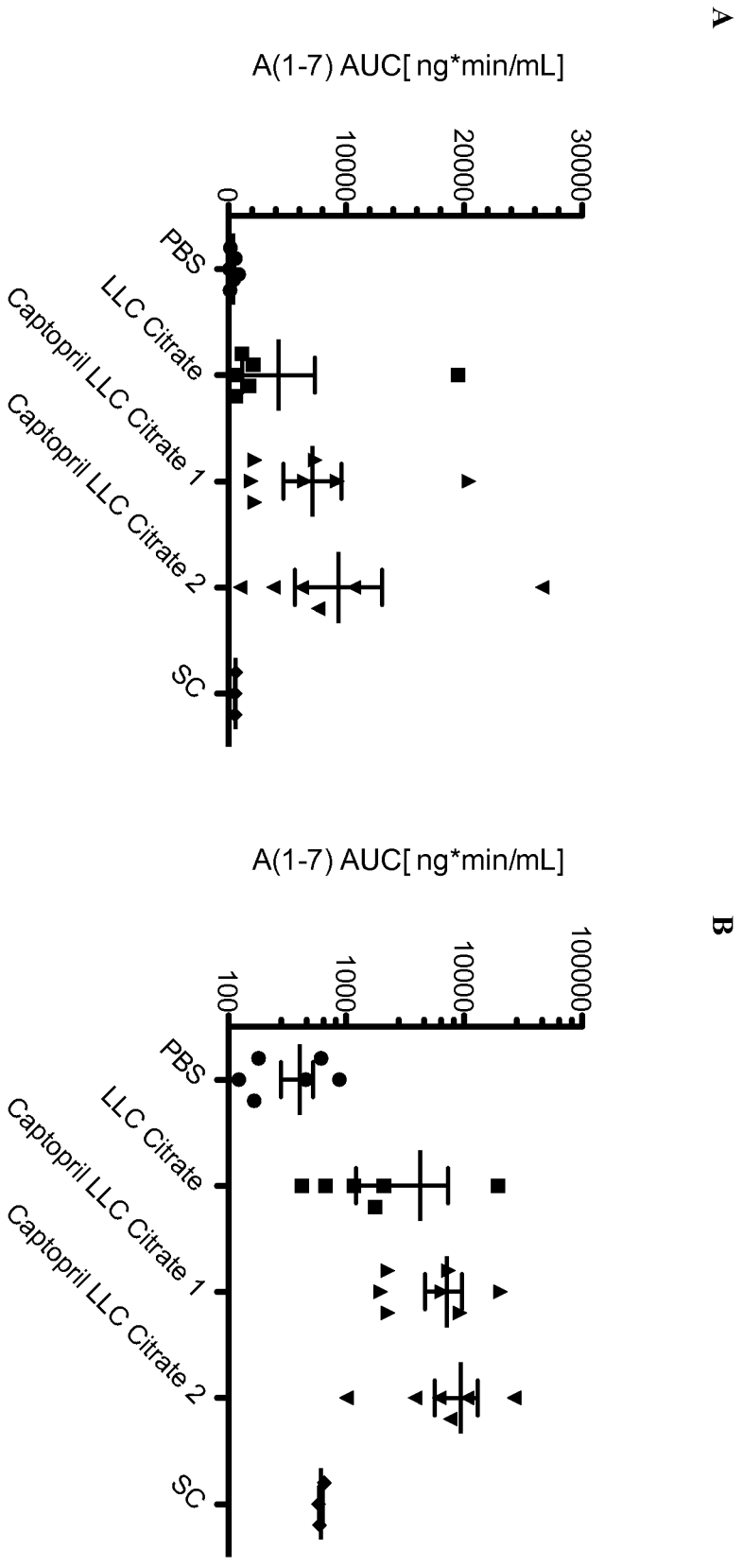

A
A(1-7) AUC[ng*min/mL]
0
10000
20000
30000
PBS
LLC Citrate
Captopril LLC Citrate 1
Captopril LLC Citrate 2
SC
B
A(1-7) AUC[ng*min/mL]
100
1000
10000
100000
PBS
LLC Citrate
Captopril LLC Citrate 1
Captopril LLC Citrate 2
SC

FIG. 1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2013/060139

A. CLASSIFICATION OF SUBJECT MATTER

A61K 9/20 *(2006.01)*
A61K 38/08 *(2006.01)*
A61K 47/12 *(2006.01)*
A61K 47/48 *(2006.01)*
A61P 43/00 *(2006.01)*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 9/20, 38/08, 47/12, 47/48, A61P 43/00

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

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

Espacenet, USPTO DB, PubMed, RUPAT, PatSearch (RUPTO internal)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2010/0055146 A1 (MARIJKE HAAS et al.) 04.03.2010, abstract, paragraphs [0008], [0024], [0035], [0036], [0041], [0043], [0073], [0077], [0088], [0092], [0093], [0095], [0098], [0099], claims 1, 3, 10, 11	1-42
Y	US 2010/0260858 A1 (ELAN PHARMA INTERNATIONAL LIMITED) 14.10.2010, abstract, paragraphs [0007], [0010], [0014], [0018]-[0020], [0060], [0063], [[0072], claims 1, 11, 12, 15, 16	1-42
Y	US 2003/0180352 A1 (MAHESH V. PATEL et al.) 25.09.2003, abstract, paragraphs [0003], [0009], [0100], [0144], [0191], [0204]	1-42
Y	US 2008/0167251 A1 (E. ANN TALLANT et al.) 10.07.2008, paragraphs [0034], [0040], [0102]-[0104], claim 1	13, 21-22, 24-31
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[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
27 December 2013 (27.12.2013)	27 February 2014 (27.02.2014)
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INTERNATIONAL SEARCH REPORT

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 8207233 B1 (UNIVERSITY OF SOUTHERN CALIFORNIA) 26.06.2012, abstract, claims 1-2	32, 38-39
Y	WO 2010/028845 A2 (CHARITE-UNIVERSITATSMEDIZIN BERLIN et al.) 18.03.2010, abstract, p. 3, paragraphs 3, 10-10, p. 4, paragraphs 1-2, p. 5, paragraph 8, p. 6, last paragraph, p. 7, paragraphs 1-3, claims 1, 5	33, 40
Y	VERONESE FM et al. The impact of PEGylation on biological therapies. BioDrugs, 2008, 22(5), p. 315-329 (abstract) [online] [retrieved on 13.01.2014] Retrieved from PubMed, PMID: 18778113	41-42

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权利要求书3页 说明书30页 附图1页

(54)发明名称

血管紧张素的口服制剂

(57)摘要

本发明提供了用于经口递送血管紧张素肽的各种制剂。

CN 104853749 A

1. 一种用于经口施用的固体剂型，其包含

(a) 血管紧张素 (1-7) 肽；

(b) 至少一种药学上可接受的降 pH 剂；

(c) 至少一种有效提高所述血管紧张素 (1-7) 肽的生物利用度的吸收促进剂；和

(d) 保护性媒介物。

2. 根据权利要求 1 所述的固体剂型，其中所述固体剂型是胶囊剂或片剂。

3. 根据权利要求 1 或 2 所述的固体剂型，其中所述降 pH 剂是柠檬酸。

4. 根据权利要求 3 所述的固体剂型，其中所述柠檬酸存在的量为大于 400mg。

5. 根据权利要求 3 所述的固体剂型，其中所述柠檬酸存在的量为大于 500mg。

6. 根据权利要求 3 所述的固体剂型，其中所述柠檬酸存在的量为大于所述固体剂型总重的 50％。

7. 根据权利要求 1 或 2 所述的固体剂型，其中所述降 pH 剂是酒石酸。

8. 根据前述权利要求中任一项所述的固体剂型，其中所述吸收促进剂是酰基肉碱。

9. 根据权利要求 8 所述的固体剂型，其中所述酰基肉碱是月桂酰肉碱。

10. 根据权利要求 9 所述的固体剂型，其中所述月桂酰肉碱存在的量的范围为 50-100mg。

11. 根据权利要求 9 所述的固体剂型，其中所述月桂酰肉碱存在的量的范围为所述固体剂型总重的 5-10％。

12. 根据前述权利要求中任一项所述的固体剂型，其中所述保护性媒介物构成了小于 20％的所述固体剂型总重。

13. 根据前述权利要求中任一项所述的固体剂型，其中所述保护性媒介物是肠溶包衣。

14. 根据前述权利要求中任一项所述的固体剂型，其中所述固体剂型还包含了一种或多种选自填充剂诸如 PROSOLV®、崩解剂诸如 POLYPLASDONE™ 交联聚维酮、助流剂诸如二氧化硅或润滑剂诸如硬脂富马酸钠的赋形剂。

15. 根据前述权利要求中任一项所述的固体剂型，其中所述固体剂型还包含了卡托普利。

16. 根据前述权利要求中任一项所述的固体剂型，其中所述固体剂型的总重范围为 800-1200mg。

17. 根据前述权利要求中任一项所述的固体剂型，其中所述血管紧张素 (1-7) 肽存在的量的范围为 10-1000mg。

18. 一种用于经口施用的固体剂型，其包含

(a) 血管紧张素 (1-7) 肽；

(b) 柠檬酸；

(c) 月桂酰肉碱；和

(d) 保护性媒介物。

19. 根据权利要求 18 所述的固体剂型，其中所述柠檬酸存在的量为大于 500mg 并且所述月桂酰肉碱存在的量的范围为 50-100mg。

20. 根据权利要求 18 或 19 所述的固体剂型，其中所述固体剂型是胶囊剂或片剂。

21. 根据权利要求 18-20 中任一项所述的固体剂型，其中所述保护性媒介物是肠溶包衣。

22. 根据前述权利要求中任一项所述的固体剂型，其中所述血管紧张素 (1-7) 肽包含天然存在的血管紧张素 (1-7) 氨基酸序列 Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}(SEQ ID NO:1)。

23. 根据权利要求 1-21 中任一项所述的固体剂型，其中所述血管紧张素 (1-7) 肽是 SEQ ID NO:1 的功能等同物。

24. 根据权利要求 23 所述的固体剂型，其中所述功能等同物是线性肽。

25. 根据权利要求 24 所述的固体剂型，其中所述线性肽包含包括来自所述天然存在的血管紧张素 (1-7) 中出现的七种氨基酸的至少四种氨基酸的序列，其中所述至少四种氨基酸如它们在所述天然存在的血管紧张素 (1-7) 中出现那样保持它们的相对位置。

26. 根据权利要求 24 所述的固体剂型，其中所述线性肽包含包括来自所述天然存在的血管紧张素 (1-7) 中出现的七种氨基酸的至少五种氨基酸的序列，其中所述至少五种氨基酸如它们在所述天然存在的血管紧张素 (1-7) 中出现那样保持它们的相对位置。

27. 根据权利要求 24 所述的固体剂型，其中所述线性肽包含包括来自所述天然存在的血管紧张素 (1-7) 中出现的七种氨基酸的至少六种氨基酸的序列，其中所述至少六种氨基酸如它们在所述天然存在的血管紧张素 (1-7) 中出现那样保持它们的相对位置。

28. 根据权利要求 25-27 中任一项所述的固体剂型，其中所述至少四种、五种或六种氨基酸如它们在所述天然存在的血管紧张素 (1-7) 中出现那样分别进一步保持它们的相对间距。

29. 根据权利要求 24-28 中任一项所述的固体剂型，其中所述线性肽含有 4-25 个氨基酸。

30. 根据权利要求 24-29 中任一项所述的固体剂型，其中所述线性肽是所述天然存在的血管紧张素 (1-7) 的片段。

31. 根据权利要求 24-30 中任一项所述的固体剂型，其中所述线性肽含有所述天然存在的血管紧张素 (1-7) 中的氨基酸取代、缺失和 / 或插入。

32. 根据权利要求 31 所述的固体剂型，其中所述线性肽具有氨基酸序列 Asp^{1}-Arg^{2}-Nle^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}(SEQ ID NO:2)。

33. 根据权利要求 31 所述的固体剂型，其中所述线性肽具有氨基酸序列 Asp^{1}-Arg^{2}-Val^{3}-Ser^{4}-Ile^{5}-His^{6}-Cys^{7}(SEQ ID NO:6)。

34. 根据权利要求 23 所述的固体剂型，其中所述功能等同物是环肽。

35. 根据权利要求 34 所述的固体剂型，其中所述环肽包含氨基酸之间的键。

36. 根据权利要求 35 所述的固体剂型，其中所述键位于对应于天然存在的血管紧张素 (1-7) 中位置 Tyr^{4} 和 Pro^{7} 的残基处。

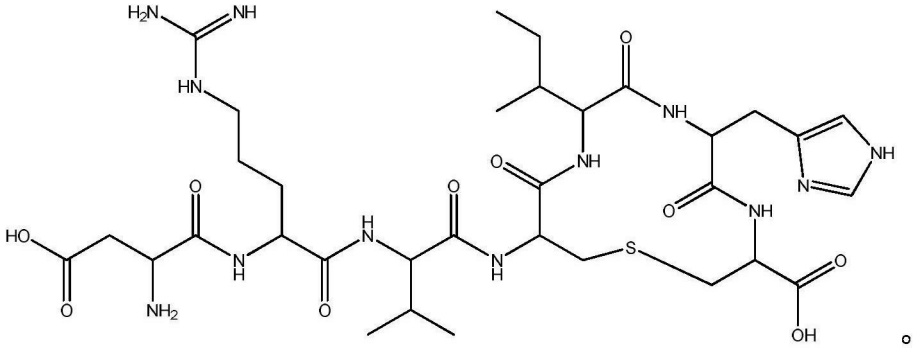
37. 根据权利要求 35 或 36 所述的固体剂型，其中所述键是硫醚桥。

38. 根据权利要求 34-37 中任一项所述的固体剂型，其中所述环肽包含另外与所述天然存在的血管紧张素 (1-7) 氨基酸序列 Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}(SEQ ID NO:1) 相同的氨基酸序列。

39. 根据权利要求 34-38 中任一项所述的固体剂型，其中所述环肽包含替代天然存在

的血管紧张素 (1-7) 中位置 Val^3 的正亮氨酸 (Nle)。

40. 根据权利要求 36 所述的固体剂型，其中所述环肽是具有下式的 4,7- 环化血管紧张素 (1-7)：



。

41. 根据权利要求 34-40 中任一项所述的固体剂型，其中所述血管紧张素 (1-7) 肽包含一个或多个化学修饰以增加蛋白酶耐受性、血清稳定性和 / 或生物利用度。

42. 根据权利要求 41 所述的固体剂型，其中所述一个或多个化学修饰包括聚乙二醇化。

血管紧张素的口服制剂

[0001] 相关申请的交叉引用

[0002] 本申请要求 2012 年 9 月 17 日提交的美国临时专利申请序列 No.61/701,972 的优先权，本公开的全部内容在此以其整体并入。

[0003] 背景

[0004] 经口递送通常是一种需要的施用途径，因为其与注射、经鼻施用和其它施用途径相比更便利且给患者带来更少的不舒服。然而一般很难经口施用肽，因为肽容易降解。经口施用短肽如血管紧张素倾向于甚至更有问题，因为短肽通常缺乏二级或三级结构并因此更易受胃和肠的蛋白水解酶的影响。这些酶可以快速降解短肽，从而使得其失活之后其可被吸收至血流中。

[0005] 发明概述

[0006] 本发明提供了用于有效经口递送血管紧张素肽的组合物和方法。特别地，本发明提供了保持血管紧张素肽的稳定性并增强其吸收至血流中的各种口服制剂。结果，根据本发明递送的血管紧张素肽可获得延长的半衰期和治疗有效的生物利用度。

[0007] 在一方面，本发明提供了经口施用的固体剂型，其包含 (a) 血管紧张素 (1-7) 肽，(b) 至少一种药学上可接受的降 pH 剂，(c) 至少一种有效促进血管紧张素 (1-7) 肽的生物利用度的吸收促进剂，和 (d) 保护性媒介物。

[0008] 在一些实施方案中，合适的固体剂型是胶囊剂或片剂。

[0009] 在一些实施方案中，合适的降 pH 剂是柠檬酸。在一些实施方案中，柠檬酸存在的量为大于约 200mg(如，大于约 250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg)。在一些实施方案中，柠檬酸存在的量为大于固体剂型总重的约 20％ (如，大于 25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％)。

[0010] 在一些实施方案中，合适的降 pH 剂是酒石酸。

[0011] 在一些实施方案中，合适的吸收促进剂是酰基肉碱。在一些实施方案中，酰基肉碱是月桂酰肉碱。在一些实施方案中，月桂酰肉碱存在的量的范围是约 20-200mg(如，范 围 为 20-150mg、20-100mg、20-90mg、20-80mg、50-200mg、50-150mg、50-100mg、50-90mg、50-80mg)。在一些实施方案中，月桂酰肉碱存在的量为约 20mg,30mg、40mg、50mg、60mg、70mg、80mg、90mg、100mg、110mg、120mg、130mg、140mg、150mg、160mg、170mg、180mg、190mg 或 200mg。在一些实施方案中，月桂酰肉碱存在的量范围为固体剂型总重的约 2-20％ (如，2-15％、2-10％、2-7.5％、5-20％、5-15％、5-10％、5-7.5％)。在一些实施方案中，月桂酰肉碱存在的量为或大于固体剂型总重的约 1％、2％、3％、4％、5％、6％、7％、8％、9％、10％、11％、12％、13％、14％、15％、16％、17％、18％、19％或 20％。在一些实施方案中，月桂酰肉碱存在的量为或小于固体剂型总重的约 25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％。

[0012] 在一些实施方案中，合适的保护性媒介物是肠溶包衣。在一些实施方案中，保护性媒介物组成量为或小于固体剂型总重的约 25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％。

[0013] 在一些实施方案中，根据本发明的固体剂型还包含一种或多种赋形剂。在具体的实施方案中，所述一种或多种赋形剂选自填充剂（诸如PROSOLV®）、崩解剂（诸如POLYPLASDONE™交联聚维酮）、助流剂（诸如二氧化硅）或润滑剂（诸如硬脂富马酸钠）。

[0014] 在一些实施方案中，根据本发明的固体剂型还包含卡托普利。

[0015] 在一些实施方案中，合适的固体剂型的总重范围为约500-1500（如，约500-1200mg、500-1000mg、600-1500mg、600-1200mg、600-1000mg、700-1500mg、700-1200mg、700-1000mg、800-1500mg、800-1200mg、800-1000mg）。在一些实施方案中，合适的固体剂型的总重范围为大于约500mg、600mg、700mg、800mg、900mg、1000mg、1100mg、1200mg、1300mg、1400mg或1500mg。在一些实施方案中，合适的固体剂型的总重为或小于约2000mg、1900mg、1800mg、1700mg、1600mg、1500mg、1400mg、1300mg、1200mg、1100mg、1000mg、900mg、800mg、700mg、600mg或500mg。

[0016] 在一些实施方案中，血管紧张素(1-7)肽存在的量范围为约10-1000mg（如，约10-900mg、10-800mg、10-700mg、10-600mg、10-500mg、100-1000mg、100-900mg、100-800mg、100-700mg、100-600mg、100-500mg、100-400mg、100-300mg、200-1000mg、200-900mg、200-800mg、200-700mg、200-600mg、200-500mg、200-400mg、300-1000mg、300-900mg、300-800mg、300-700mg、300-600mg、300-500mg）。在一些实施方案中，血管紧张素(1-7)肽存在的量为或大于约10mg、50mg、100mg、150mg、200mg、250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg。在一些实施方案中，血管紧张素(1-7)肽存在的量为或小于约1000mg、950mg、900mg、850mg、800mg、750mg、700mg、650mg、600mg、550mg、500mg、450mg、400mg、350mg、300mg、250mg、200mg、150mg或100mg。

[0017] 在具体的实施方案中，本发明提供了用于经口施用的固体剂型，其包含(a)血管紧张素(1-7)肽，(b)柠檬酸，(c)月桂酰肉碱，和(d)保护性媒介物。在某些实施方案中，柠檬酸存在的量为大于500mg并且月桂酰肉碱存在的量范围为50-100mg。

[0018] 在某些实施方案中，固体剂型是胶囊剂或片剂。在某些实施方案中，合适的保护性媒介物是肠溶包衣。

[0019] 在各种实施方案中，血管紧张素(1-7)肽包含天然存在的血管紧张素(1-7)氨基酸序列Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}(SEQ ID NO:1)。

[0020] 在各种实施方案中，血管紧张素(1-7)肽是SEQ ID NO:1的功能等同物。在一些实施方案中，功能等同物是线性肽。在一些实施方案中，线性肽包含了包括至少4种选自天然存在的血管紧张素(1-7)中出现的7种氨基酸的氨基酸的序列，其中至少4种氨基酸将它们的相对位置保持在与其天然存在的血管紧张素(1-7)中出现的位置处。在一些实施方案中，线性肽包含包括至少5种选自天然存在的血管紧张素(1-7)中出现的7种氨基酸的氨基酸的序列，其中至少5种氨基酸将它们的相对位置保持在与其天然存在的血管紧张素(1-7)中出现的位置处。在一些实施方案中，线性肽包含了包括至少6种选自天然存在的血管紧张素(1-7)中出现的7种氨基酸的氨基酸的序列，其中至少6种氨基酸将它们的相对位置保持在与其天然存在的血管紧张素(1-7)中出现的位置处。在一些实施方案中，至少4、5或6种氨基酸各自进一步将它们的相对间距保持在它们在天然存在的血管紧张素(1-7)中出现的位置处。

[0021] 在一些实施方案中，线性肽含有4-25个氨基酸（如，4-20、4-15、4-14、4-13、4-12、

4-11、4-10、4-9、4-8、4-7 个氨基酸）。在一些实施方案中，线性肽含有 4、5、6、7、8、9、10、11、12、13、14、16、17、18、19、20、21、22、23、24 或 25 个氨基酸。

[0022] 在一些实施方案中，线性肽是天然存在的血管紧张素 (1-7) 的片段。

[0023] 在一些实施方案中，线性肽含有天然存在的血管紧张素 (1-7) 中的氨基酸取代、缺失和 / 或插入。

[0024] 在具体的实施方案中，线性肽具有 Asp^1-Arg^2-Nle^3-Tyr^4-Ile^5-His^6-Pro^7的氨基酸序列 (SEQ ID NO:2)。

[0025] 在具体的实施方案中，线性肽具有 Asp^1-Arg^2-Val^3-Ser^4-Ile^5-His^6-Cys^7的氨基酸序列 (SEQ ID NO:6)。

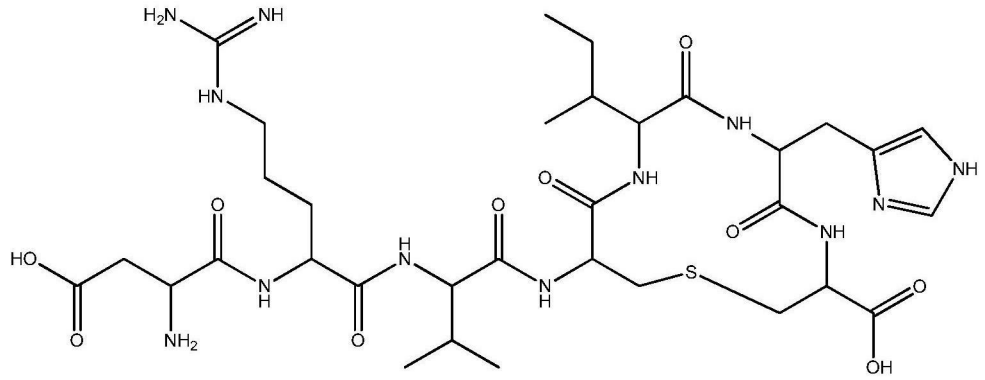
[0026] 在一些实施方案中，功能等同物是环肽。在一些实施方案中，环肽包含了氨基酸之间的键。在一些实施方案中，所述键位于对应于天然存在的血管紧张素 (1-7) 中的位置 Tyr^4 和 Pro^7残基处。在一些实施方案中，所述键是硫醚桥。

[0027] 在具体的实施方案中，环肽包含另外与天然存在的血管紧张素 (1-7) 氨基酸序列 Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7(SEQ ID NO:1) 相同的氨基酸序列。

[0028] 在某些实施方案中，环肽包含替代天然存在的血管紧张素 (1-7) 中的位置 Val^3的正亮氨酸 (Nle)。

[0029] 在某些实施方案中，环肽是具有下式的 4,7- 环化血管紧张素 (1-7)：

[0030]



[0031] 在各种实施方案中，血管紧张素 (1-7) 肽包含一个或多个化学修饰以增加蛋白酶耐受性、血清稳定性和 / 或生物利用度。在一些实施方案中，一个或多个化学修饰包括聚乙二醇化。

[0032] 本发明进一步提供了用于施用本文描述的口服制剂的方法。

[0033] 如本申请所用，术语“约 (about，approximately)”可用作等同物。本申请所用的任何具有或不具有约的数字意在涵盖相关技术领域的普通技术人员所理解的任何正常波动。

[0034] 本发明的其它特征、目标和优势以随后的详述变得显而易见。然而，应该理解，详述在说明本发明的实施方案的同时，仅通过举例说明的方式给出而非限制的方式给出。本发明范围内的各种变化和修改将通过详述而对本领域的技术人员变得显而易见。

[0035] 附图简述

[0036] 附图仅用于说明目的而非限制。

[0037] 图 1 描绘了示例性结果，说明了各种施用途径的由曲线下面积 (AUC) 表示的血管

紧张素 (1-7) 的总暴露的比较。

[0038] 定义

[0039] 为了本发明更容易被理解，首先如下定义了某些术语。对以下术语和其它术语的另外定义在本说明书全文示出。

[0040] 动物：如本文所用，术语“动物”是指任何数目的动物界。在一些实施方案中，“动物”是指任何发育阶段的人。在一些实施方案中，“动物”是指任何发育阶段的非人动物。在某些实施方案中，非人动物是哺乳动物（如，啮齿类动物、小鼠、大鼠、兔、猴、狗、猫、羊、牛、灵长类动物和 / 或猪）。在一些实施方案中，动物包括但不限于哺乳动物、鸟、爬行动物、两栖动物、鱼、昆虫、和 / 或虫。在一些实施方案中，动物可为转基因动物、基因工程动物和 / 或克隆。

[0041] 约 (approximately, about)：如本文所用，如应用于一个或多个目标值的术语“约 (approximately, about)”是指与指出的参考值类似的值。在某些实施方案中，术语“约 (approximately, about)”是指除非另外说明或另外从上下文中显而易见，否则落入 25％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％、1％或更小范围（在指明的参考值的任一方向（大于或小于））的值（除了当此类数字将超过可能值的 100％时）。

[0042] 生物利用度：如本文所用，术语“生物利用度”一般是指施用剂量到达受试者血流的百分比。

[0043] 生物活性：如本文所用，短语“生物活性”是指任何试剂在生物系统特别是有机体中具有活性的特征。例如，当向有机体施用制剂时，所述制剂对有机体具有生物作用，那么所述制剂被认为具有生物活性。在其中肽具有生物活性的具体实施方案中，此肽中与肽共有至少一种生物活性的部分通常被称为“生物活性”部分。在某些实施方案中，肽不具有固有生物活性但抑制一种或多种天然存在的血管紧张素化合物的作用被认为具有生物活性。

[0044] 载体或稀释剂：如本文所用，术语“载体”和“稀释剂”是指药学上可接受的（如，安全且无毒地施用给人）载体或稀释物质以用于制备药物制剂。示例性稀释剂包括无菌水、注射用抑菌水 (BWFI)、pH 缓冲溶液（如磷酸盐缓冲盐水）、无菌盐水溶液、林格氏溶液 (Ringer's solution) 或葡萄糖溶液。

[0045] 剂型：如本文所用，术语“剂型”和“单位剂型”是指用于待治疗患者的物理分散单位的治疗剂。每个单位含有预定量的活性物质，其经计算产生目标疗效。然而，应该理解，组合物的总剂量将由主治医生在可靠的医学判断范围内决定。

[0046] 给药方案：如本文所用，术语“给药方案”（或“治疗方案”）是一组通常在一段间隔的时期向受试者各自施用的单位剂量（通常多于一个）。在一些实施方案中，给定的治疗剂具有推荐的可涉及一个或多个剂量的给药方案。在一些实施方案中，给药方案包括多个剂量，所述剂量的每一个可与另一个以相同长度的时间分开；在一些实施方案中，给药方案包括多个剂量和分隔各个剂量的至少两个不同时间段。在一些实施方案中，将治疗剂在预定的使其内连续施用。在一些实施方案中，将治疗剂每天施用一次 (QD) 或每天施用两次 (BID) 施用。

[0047] 赋形剂：如本文所用，术语“赋形剂”是指任何添加至药物和 / 或制剂以改善所述药物和 / 或制剂质量（即一致性）、药代动力学特性（即生物利用度）、药效学特性及其组

合的惰性物质。

[0048] 功能等同物或功能衍生物：如本文所用，术语“功能等同物”或“功能衍生物”表示在氨基酸序列的功能衍生物的情况下，保留与原始序列的生物活性基本上类似的生物活性的分子。功能衍生物或等同物可为天然衍生物，或者重组或合成制备。示例性功能衍生物包括具有一个或多个氨基酸的取代、缺失或添加的氨基酸序列，前提条件是所述蛋白的生物活性是保守的。取代氨基酸具有所需的与经取代的氨基酸的化学－物理性质类似的化学－物理性质。所需的类似的化学－物理性质包括电荷、膨松性、疏水性、亲水性等方面的类似性。

[0049] 改善、增加或减少：如本文所用，术语“改善”、“增加”或“减少”或语法等同物表明了相对于基线测量（诸如在相同个体中在开始本文所述治疗之前的测量或在缺乏本文所述治疗时在对照个体（或多个对照个体）中的测量）的值。“对照个体”是罹患与正在治疗的个体相同形式疾病的个体，所述对照个体与正在治疗的个体处于大概相同的年纪（以确保经治疗的个体和对照个体中的疾病的阶段是可比较的）。

[0050] 体外：如本文所用，术语“体外”是指在人工环境如在测试管或反应容器、细胞培养物而非多细胞有机物中发生的事件。

[0051] 体内：如本文所用，术语“体内”是指在多细胞有机体诸如人和非人动物中发生的事件。在基于细胞的系统的上下文中，术语可用于指在活细胞中发生的事件（与之相反，例如，体外系统）。

[0052] 分离的：如本文所用，术语“分离的”是指物质和/或实体已经(1)与至少一些在所述物质和/或实体初始产生时相联的组分中分离（无论是在天然和/或实验设定中），和/或(2)人工产生、制备和/或制造。经分离的物质和/或实体可与与其初始相联的至少约10％、约20％、约30％、约40％、约50％、约60％、约70％、约80％、约90％、约95％、约98％、约99％、基本上100％或100％的其它组分分离。在一些实施方案中，分离的试剂为大于约80％、约85％、约90％、约91％、约92％、约93％、约94％、约95％、约96％、约97％、约98％、约99％、基本上100％或100％纯。如本文所用，如果物质基本上不含其它组分，则物质是“纯的”。如本文所用，术语“分离的细胞”是指多细胞有机物中不含的细胞。

[0053] 肽：如本文所用的术语“肽”是指经由肽键连在一起的氨基酸的顺序链(sequential链)。通常，术语用来指长度短的氨基酸链，但本领域的普通技术人员将理解所述术语不限于任何特定长度的链并且可指包含经由肽键连在一起的两个氨基酸的最小链。然而，通常，肽是指具有或小于50、45、40、35、30、25、20、15、10个氨基酸的氨基酸链。如本领域的技术人员所知，可加工和/或修饰肽。

[0054] 药学上可接受的：如本文所用，术语“药学上可接受的”是指当施用给受试者时不产生非所需的过敏反应或抗原反应的任何实体或组合物。

[0055] 蛋白：如本文所用的术语“蛋白”是指作为分散单位起作用的一种或多种多肽。如果单个多肽是分散功能单位且为了形成分散功能单位而不需要与其它多肽有永久性或临时性物理相关性，则术语“多肽”和“蛋白”可互换使用。如果分散功能单位由多于一种物理上彼此分散的多肽组成，则术语“蛋白”是指物理上偶联在一起并一起作为分散单位起作用的多种多肽。

[0056] 稳定性：如本文所用，术语“稳定的”是治疗剂长期保持其治疗效果（如，所有或

其大部分的期望生物活性和/或物理化学完整性）的能力。治疗剂的稳定性，和药物组合物保持此类治疗剂的稳定性的能力可长期评估（如，至少1、3、6、12、18、24、30、36个月或更久）。在某些实施方案中，已经配制了本文描述的药物组合物适，使得它们能够稳定或另外地减缓或预防与其一起配制的一种或多种治疗剂的降解。在制剂的情况下，稳定的制剂是一种其中的治疗剂在贮藏和处理过程中（诸如冷冻/解冻、机械混合和冻干）基本上保持其物理和/或化学完整性和生物活性。

[0057] 受试者：如本文所用，术语“受试者”是指人或任何非人动物（如，小鼠、大鼠、狗、猫、牛、猪、羊、马或灵长类动物）。人包括产前形式和产后形式。在许多实施方案中，受试者是人类。受试者可为患者，其是指呈现给医疗提供者以诊断或治疗疾病的人。术语“受试者”在本文中可与“个体”或“患者”互换使用。受试者可罹患或易感疾病或病症但可能或不可能呈现出所述疾病或病症的症状。

[0058] 基本上：如本文所用，术语“基本上”是指展现出目标特征或性质的完全或近乎完全程度或度的定质条件。生物领域的技术人员将理解生物和化学现象如果存在基本上不完全进行和/或进行至完全或者达到或避免绝对结果。术语“基本上”因此在本文中用于捕获许多生物和化学现象缺乏所固有的完整性的潜能。

[0059] 治疗有效量：如本文所用，术语“治疗有效量”的治疗剂意为当向患有或易患疾病、病症和/或疾患的受试者施用时足以治疗、诊断、预防所述疾病、病症和/或疾患和/或延缓其症状发作的量。本领域的普通技术人员将理解，通常经由包括至少一个单位剂量的给药方案施用治疗有效量。

[0060] 治疗/处理：如本文所用，术语“治疗/处理(treat，treatment，treating)”是指任何用于部分或完全减轻、改善、缓减、预防具体疾病、病症和/或疾患，延迟具体疾病、病症和/或疾患发作，降低具体疾病、病症和/或疾患的严重性和/或降低具体疾病、病症和/或疾患的一种或多种症状或特征的发病率。可将治疗施用给未展现出疾病迹象和/或仅展现出疾病早期迹象的受试者以用于降低患有与所述疾病相关的病状的风险。

[0061] 本发明的其它特征、目标和优势在以下详述中显而易见。然而，应该理解，详述在表明本发明的实施方案时，仅举例说明的方式给出而非限制的方式给出。本发明范围内的各种变化和修改将通过详述而对本领域的技术人员变得显而易见。

[0062] 某些实施方案的详述

[0063] 本发明尤其提供了适用于经口施用给受试者的血管紧张素(1-7)(Ang-(1-7))的制剂。此类施用可用于多种目的，包括治疗疾病、病症或疾患。

[0064] 在一些实施方案中，提供了用于经口施用的固体剂型，其包含(a)血管紧张素(1-7)肽，(b)至少一种药学上可接受的降pH剂，(c)至少一种有效促进血管紧张素(1-7)肽的生物利用度的吸收促进剂，和(d)保护性媒介物。

[0065] 在一些实施方案中，固体剂型是胶囊剂或片剂。各种制备口服制剂的方法和成分是本领域已知的，并且期待本领域的技术人员将能够确定这些方法中的哪种将与如本发明书所述的本发明相容。还可在本发明的范围内考虑了此类方法和成分。

[0066] 本发明的各个方面详述于以下章节中。使用章节并非意在限制本发明。每个章节可适用于本发明的任何方面。在本申请中，除非另外说明，否则使用“或”意为“和/或”。

[0067] 血管紧张素(1-7)肽

[0068] 如本文所用，术语“血管紧张素 (1-7) 肽”是指天然存在的血管紧张素 (1-7) 和天然存在的血管紧张素 (1-7) 的任何功能等同物、类似物或衍生物。如本文所用，“肽”和“多肽”是可互换的术语，并是指通过肽键结合在一起的两个或多个氨基酸。如本文所用，术语“肽”和“多肽”包括线状肽和环状肽。术语“血管紧张素 -(1-7)”、“血管紧张素 -(1-7)”和“Ang-(1-7)”可互换使用。

[0069] 天然存在的血管紧张素 (1-7)

[0070] 天然存在的血管紧张素 (1-7)(也被称为 Ang-(1-7)) 是如下所示的七氨基酸肽 ：

[0071] Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}(SEQ ID NO:1)

[0072] 其是肾素 - 血管紧张素系统的一部分，并且由也被称为血管紧张素原的前体转化而来，所述血管紧张素原是一种组成性产生且主要通过肝释放至循环的 α -2- 球蛋白。血管紧张素原是丝氨酸蛋白酶抑制蛋白家族的一个成员并且也被称为肾素底物。人血管紧张素原长为 452 个氨基酸，但另外的肿瘤具有各种大小的血管紧张素原。通常，开始的 12 个氨基酸对于血管紧张素活性来说是最重要的 ：

[0073] Asp^{1}-Arg^{2}-Val^{3}-Tyr^{4}-Ile^{5}-His^{6}-Pro^{7}-Phe^{8}-His^{9}-Leu^{10}-Val^{11}-Ile^{12}(SEQ ID NO:4)

[0074] 不同类型的血管紧张素可通过各种酶的作用形成。例如，血管紧张素 (1-7) 是通过血管紧张素 - 转化酶 2(ACE 2) 的作用产生的。

[0075] Ang-(1-7) 是 Mas 受体的内源性配体。Mas 受体是含有 7 个跨膜区的 G- 蛋白偶联的受体。如本文所用，术语“血管紧张素 -(1-7) 受体’涵盖了 G 蛋白偶联的 Mas 受体。

[0076] 如本文所用，术语“天然存在的血管紧张素 (1-7)”包括具有与天然存在的血管紧张素 (1-7) 的氨基酸序列相同的氨基酸序列的从天然来源纯化的和任何重组产生肽或化学合成肽的任何血管紧张素 (1-7) 肽。

[0077] Ang-(1-7) 的功能等同物、类似物或衍生物

[0078] 在一些实施方案中，适用于本发明的血管紧张素 (1-7) 肽是天然存在的 Ang-(1-7) 的功能等同物。如本文所用，天然存在的 Ang-(1-7) 的功能等同物是指与天然存在的 Ang-(1-7) 共有氨基酸序列同一性且基本上保留了与天然存在的 Ang-(1-7) 相同或类似活性的任何肽。例如，在一些实施方案中，本文描述的天然存在的 Ang-(1-7) 的功能等同物具有如使用本文所述或本领域已知的方法确定的促血管生成活性，或者具有活性诸如一氧化氮释放、血管舒张、改善的内皮功能、抑制尿分泌或本文讨论的积极影响血管生成的其它性质之一。在一些实施方案中，本文描述的天然存在的 Ang-(1-7) 的功能等同物可结合或活化如使用本文所述或本领域已知的各种测定确定的血管紧张素 -(1-7) 受体 (如，G 蛋白偶联的 Mas 受体)。在一些实施方案中，Ang-(1-7) 的功能等同物也被称为血管紧张素 (1-7) 类似物或衍生物或功能衍生物。

[0079] 通常，血管紧张素 (1-7) 的功能等同物与天然存在的 Ang-(1-7) 共有氨基酸序列类似性。在一些实施方案中，根据本发明的 Ang-(1-7) 的功能等同物含有包括来自天然存在的 Ang-(1-7) 中出现的其 7 种氨基酸的至少 3 种 (如，至少 4 种、至少 5 种、至少 6 种、至少 7 种) 氨基酸的序列，其中所述的至少 3 种 (如，至少 4 种、至少 5 种、至少 6 种或至少 7 种) 氨基酸如它们在天然存在的 Ang-(1-7) 出现的那样保持它们的位置。

[0080] 在一些实施方案中，Ang-(1-7) 的功能等同物还涵盖了含有与天然存在的 Ang-(1-7) 的氨基酸序列具有至少 50％ (如，至少 60％、70％、80％或 90％) 同一性的序

列的任何肽。氨基酸序列同一性的百分比可通过比对氨基酸序列来确定。氨基酸序列的比对可以本发明领域内的各种方式（例如使用可公开获得的计算机软件诸如 BLAST、ALIGN 或 M egalign(DNASTAR) 软件）实现。本领域技术人员可确定适当的参数以测量比对，包括任何需要获得全长比较序列的最大比对的算法。优选地，WU-BLAST-2 软件用于测定氨基酸序列同一性 (Altschul 等，Me thods in Enzymology 266,460-480(1996)；http://blast.wustl/edu/blas t/README.html)。WU-BLAST-2 使用多种搜索参数，大多数参数被设定为缺省值。可调整的参数按照以下值设定：重叠跨度＝ 1，重叠分数＝ 0.125，词阈值 (T) ＝ 11。HSP 得分 (S) 和 HSP S2 参数是动态值并且可由程序本身根据具体序列的组成建立，燃和最小值可被调节并如上指定的那样设定。

[0081] 在一些实施方案中，Ang-(1-7) 的功能等同物、类似物或衍生物是天然存在的 Ang-(1-7) 的片段。在一些实施方案中，Ang-(1-7) 的功能等同物、类似物或衍生物含有天然存在的 Ang-(1-7) 中的氨基酸取代、缺失和 / 或插入。Ang-(1-7) 功能等同物、类似物或衍生物可经由取代、添加和 / 或缺失通过改变氨基酸序列来制备。例如，天然存在的 Ang-(1-7) 的序列 (SEQ ID NO:1) 内的一个或多个氨基酸残基可被作为功能等同物起作用的类似极性的另一种氨基酸取代，从而导致沉默变化。序列内氨基酸的取代可选自所述氨基酸所属类别的其它成员。例如，带正电（碱性）氨基酸包括精氨酸、赖氨酸和组氨酸。非极性（疏水性）氨基酸包括亮氨酸、异亮氨酸、丙氨酸、苯丙氨酸、缬氨酸、脯氨酸、色氨酸和蛋氨酸。不带电的极性氨基酸包括丝氨酸、苏氨酸、半胱氨酸、酪氨酸、天冬酰胺和谷氨酰胺。带负电（酸性）氨基酸包括谷氨酸和天冬氨酸。氨基酸甘氨酸可被纳入非极性氨基酸家族或不带电（中性）极性氨基酸家族中。氨基酸家族内进行的取代一般被理解为保守取代。例如，肽抑制剂的氨基酸序列可被修饰或取代。

[0082] Ang-(1-7) 功能等同物、类似物和衍生物的实例描述于以下标题为“示例性血管紧张素 (1-7) 肽”的章节中。

[0083] 血管紧张素 -(1-7) 肽可具有任何长度。在一些实施方案中，根据本发明的血管紧张素 -(1-7) 肽可含有例如 4-25 个氨基酸（如，4-20、4-15、4-14、4-13、4-12、4-11、4-10、4-9、4-8、4-7 个氨基酸）。在一些实施方案中，线性肽含有 4 个、5 个、6 个、7 个、8 个、9 个、10 个、11 个、12 个、13 个、14 个、16 个、17 个、18 个、19 个、20 个、21 个、22 个、23 个、24 个或 25 个氨基酸。

[0084] 在一些实施方案中，血管紧张素 -(1-7) 肽含有增加蛋白酶耐受性、血清稳定性和 / 或生物利用度的一种或多种修饰。在一些实施方案中，合适的修饰选自肽的聚乙二醇化、乙酰化、糖基化、生物素化、用 D- 氨基酸和 / 或非天然氨基酸取代和 / 或肽的环化。

[0085] 如本文所用，术语“氨基酸”就其最广泛的含义来说是指任何可掺入多肽链的化合物和 / 或物质。在某些实施方案中，氨基酸具有一般结构 $H_2N-C(H)(R)-COOH$。在某些实施方案中，氨基酸是天然存在的氨基酸。在某些实施方案中，氨基酸是合成或非天然氨基酸（如，α，α - 二取代的氨基酸、N- 烷基氨基酸）；在一些实施方案中，氨基酸是 d- 氨基酸；在某些实施方案中，氨基酸是 l- 氨基酸。“标准氨基酸”是指天然存在的肽中通常存在的二十种氨基酸的任何一种，包括天然掺入肽中的 l- 和 d- 氨基酸。“不标准”或“非常规氨基酸”是指除了标准氨基酸之外的任何氨基酸，无论其是合成制备还是从天然来源获得的。如本文所用，“合成或非天然氨基酸”涵盖化学修饰的氨基酸，包括但不限于盐、氨基酸衍生

物（诸如酰胺）和/或取代。可将氨基酸，包括肽中的羧基端和/或氨基端的氨基酸，通过改变肽的循环半衰期而不不利影响其活性的甲基化、酰胺化、乙酰化和/或用其它化学基团取代来修饰。非常规或非天然氨基酸的实例包括但不限于瓜氨酸、鸟氨酸、正亮氨酸、正缬氨酸、4-(E)-丁烯基-4(R)-甲基-N-甲基苏氨酸(MeBmt)、N-甲基-亮氨酸(MeLeu)、氨基异丁酸、抑胃酶氨酸和N-甲基-丙氨酸(MeAla)。氨基酸可参与二硫键。术语“氨基酸”可与“氨基酸残基”互换使用，并且可以指游离氨基酸和/或肽的氨基酸残基。从使用术语的上下文中显而易见其时指游离氨基酸还是肽的残基。

[0086] 在某些实施方案中，血管紧张素-(1-7)肽含有一种或多种L-氨基酸、D-氨基酸和/或非天然氨基酸。

[0087] 除了仅含有天然存在的氨基酸的肽之外，本发明还涵盖了模拟肽或肽类似物。肽类似物常在制药业中用作具有与模板肽的那些性质类似的性质的非肽药物。非肽化合物被定义为“肽模拟物”或模拟肽(Fauchere等，Infect.Immun.54:283-287(1986)；Evans等，J.Med.Chem.30:1229-1239(1987))。与治疗有效肽结构相关的且可用来产生等同物或者增强的治疗或预防效果的肽模拟物。通常，模拟肽与示例多肽（即，具有生物或药理学活性的多肽）诸如天然存在的受体-结合多肽在结构上类似，但具有可被诸如 $-CH_2NH-$、$-CH_2S-$、$-CH_2-CH_2-$、$-CH=CH-$（顺式和反式）、$-CH_2SO-$、$-CH(OH)CH_2-$、$-COCH_2-$等的键通过本领域熟知的方法任选替换的一个或多个肽键(Spatola,Peptide Backbone Modifications,Vega Data,1(3):267(1983)；Spatola等Life Sci.38:1243-1249(1986)；Hudson等Int.J.Pept.Res.14:177-185(1979)；和Weinstein.B.,1983,Chemistry and Biochemistry,of Amino Acids,Peptides and Proteins,Weinstein编辑,Marcel Dekker,New-York,)。此类肽模拟物可较天然存在的多肽具有显著的优势，所述优势包括更经济的生产、更佳的化学稳定性、增强的药理学性质（如，半衰期、吸收、效能、效率等）、降低抗原性的及其它。

[0088] Ang-(1-7)肽还包括了含有另外的化学部分并非通常的肽的一部分的其它类型的肽衍生物，前提条件是衍生物保留了所述肽所需的功能。此类衍生物的实例包括(1)氨基端或另一氨基的N-酰基衍生物，其中酰基可为烷酰基（如，乙酰基、己酰基、辛酰基）、芳酰基（如，苯甲酰基）或阻断基团（诸如F-moc（芴基甲基-O-CO-））；(2)羧基端酯或另一种游离羧基酯或羟基酯；(3)通过与氨或合适的胺反应而产生羧基端酰胺或另一游离羧基酰胺；(4)磷酸化衍生物；(5)与抗体或其它生物配体缀合的衍生物和其它类型的衍生物；和(6)与聚乙二醇(PEG)链缀合的衍生物。

[0089] Ang-(1-7)肽可通过本领域技术人员已知的任何肽合成方法（如，专一性固体相合成、部分固体相合成、片段缩合、经典溶液合成、天然化学连接）和重组技术获得。例如，肽或肽衍生物可通过固体相肽合成获得，所述固体相肽合成简而言之由C末端氨基酸的羧基与树脂（如，二苯甲胺树脂、氯甲基化树脂、羟甲基树脂）偶联并连续添加N-α保护的氨基酸组成。保护基团可以是本领域已知的任何此类基团。在向生长链添加每个新氨基酸之前，之前向所述链添加的氨基酸的保护基团被去除。此类固体相合成已经公开在例如美国专利号4,305,872和4,316,891中的Merrifield,J.Am.Chem.Soc.85:2149(1964)；Vale等，Science 213:1394-1397(1981)，及Lubell等“Peptides”Science of Synthesis 21.11,Chemistry of Amides.Thieme,Stuttgart,713-809(2005)中综述的技术Bodonsky

等 Chem. Ind. (London), 38:1597(1966) ;与 Pietta 和 Marshall, Chem. Comm. 650(1970) 中。氨基酸与适当的树脂的偶联也是本领域所熟知的并且已经公开在美国专利号 4,244,946 中 (综述于 Houver-Weyl, Methods of Organic Chemistry. 第 E22a 卷 . Synthesis of Peptides and Peptidomimetics, Murray Goodman, 主编 , Thieme. Stuttgart. New York 2002 中)。

[0090] 除非另外定义,否则本文所用的科学术语和技术术语及命名法具有与本发明所属领域普通技术人员通常所理解的含义相同的含义。通常,细胞培养、感染、分子生物学方法等的程序是本领域使用的一般方法。此类标准技术可见于参考手册诸如 , 例如 Ausubel 等, Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001 ;和 Sambrook 等 , Molecular Cloning:A Laboratory Manual, 第 3 版 , Cold Spring Harbor Laboratory Press, N. Y., 2001 中。

[0091] 在制备 Ang-(1-7) 肽的任何过程中,需要保护考虑的任何分子上的敏感反应基团。这可通过常规保护基团的方式实现,诸如 Protective Groups In Organic Synthesis by T. W. Greene&P. G. M. Wuts, 1991, John Wiley and Sons, New-York ; 和 Peptides:chemistry and Biology by Sewald 和 Jakubke, 2002, Wiley-VCH, Wheinheim 第 142 页中描述的那些方式。例如, α 氨基保护基团包括酰基型保护基团 (如,三氟乙酰基、甲酰基、乙酰基)、脂肪族氨基甲酸乙酯保护基团 (如,叔丁基氧基羰基 (BOC)、环己基氧基羰基)、芳香族氨基甲酸乙酯型保护基团 (如,芴基 -9- 甲氧基 - 羰基 (Fmoc)、苄氧基羰基 (Cbz)、Cbz 衍生物) 和烷基型保护基团 (如,三苯基甲基、苄基)。氨基酸侧链保护基团包括苄基 (用于 Thr 和 Ser)、Cbz(Tyr、Thr、Ser、Arg、Lys)、甲基乙基、环己基 (Asp、His)、Boc(Arg、His、Cys) 等。保护基团可在方便的随后阶段使用本领域已知的方法去除。

[0092] 此外, Ang-(1-7) 肽可根据 FMOC 方案在有机相中用保护基团合成。期望地,可将肽以 70％的产率用高压液相色谱 (HPLC) 在 C18 色谱柱上纯化并用 10-60％的乙腈梯度洗脱。肽的分子量可通过质谱验证 (综述于 Fields, G. B. “Solid-Phase Peptide Synthesis” Methods in Enzymology. 第 289 卷 , Academic Press, 1997 中)。

[0093] 可替代地, Ang-(1-7) 肽可在重组系统中使用例如编码多肽的多核苷酸序列来制备。应理解,多肽可含有相同多肽中多于一种的所述修饰。

[0094] 当肽可有效地引发体外生物活性时,它们的体内效果可由于蛋白酶的存在而减少。血清蛋白酶具有特定的底物要求。所述底物必需具有用于清除的 L- 氨基酸和肽键。此外,代表了血清终蛋白酶活性的最主要组分的外肽酶通常作用于所述肽的第一个肽键上,并且需要游离的 N- 末端 (Powell 等, Pharm. Res. 10:1268-1273(1993))。鉴于此,使用修饰型的肽通常是有利的。经修饰的肽保持了赋予 Ang-(1-7) 生物活性但有利地不容易受到蛋白酶和 / 或外肽酶裂解影响的原始 L- 氨基酸肽的结构特征。

[0095] 用相同类型的 D- 氨基酸系统性取代共有序列的一种或多种氨基酸 (如,D- 赖氨酸替代 L- 赖氨酸) 可用于产生更稳定的肽。因此,本发明的肽衍生物或拟肽物可以都是 L、都是 D 或者以正向或反向混合的 D、L 肽。N 末端或 C 末端 D- 氨基酸的存在增加了肽的体内稳定性因为肽酶不能使用 D- 氨基酸作为底物 (Powell 等, Pharm. Res. 10:1268-1273(1993))。反向 -D 肽是以相对于含有 L- 氨基酸的肽以相反序列排列的含有 D- 氨基酸的肽。因此, L- 氨基酸肽的 C 末端残基变成 D- 氨基酸肽的 N 末端等。反向 D- 肽保留了与 L- 氨基酸肽相

同的二级构象并因此具有类似的活性，但是对体外和体内酶降解更耐受，并因此可具有比原始肽更佳的治疗效果 (Brady 和 Dodson, Nature368:692-693(1994) ;Jameson 等 , Nature 368:744-746(1994))。类似地，反向 -L 肽可使用标准方法产生，其中母体肽的 C 末端替代了反向 -L 肽的 N 末端。应考虑到不具有显著二级结构的 L- 氨基酸肽的反向 L- 肽（如，短肽）保留了 L- 氨基酸肽侧链的相同的间距和构象并因此通常具有与原始 L- 氨基酸肽类似的活性。此外，反向肽可含有 L- 和 D- 氨基酸的组合。可维持氨基酸之间的间距和侧链的构象，从而产生与原始 L- 氨基酸肽类似的活性。

[0096] 另一种有效赋予作用于肽的 N 末端或 C 末端残基的肽酶耐受性的方式是在肽末端添加化学基团，是的经修饰的肽不再是肽酶的底物。一种此类化学修饰是在任一端或两端对肽进行糖基化。某些化学修饰，特别是 N 末端糖基化，已经显示可增加人血清中肽的稳定性 (Powell 等 , Pharm. Res. 10:1268-1273(1993))。其它增强血清稳定性的化学修饰包括但不限于添加由 1 至 20 个碳的低级烷基组成的 N 末端烷基（诸如乙酰基），和 / 或添加 C 末端酰胺或取代的酰胺基团。特别地，本发明包括经修饰的肽，所述肽由带有 N 末端乙酰基和 / 或 C 末端酰胺基的肽组成。

[0097] 在肽的子序列中用非天然存在的氨基酸替代天然氨基酸还可赋予对蛋白水解的耐受性。此类取代可例如赋予对作用于 N 末端的外肽酶蛋白水解的耐受性而不影响生物活性。非天然存在的氨基酸的实例包括 α , α - 二取代氨基酸、N- 烷基氨基酸、C- α - 甲基氨基酸、β - 氨基酸和 β - 甲基氨基酸。用于本发明的氨基酸类似物可包括但不限于 β - 丙氨酸、正缬氨酸、正亮氨酸、4- 氨基丁酸、鸟氨酸、羟基脯氨酸、肌氨酸、瓜氨酸、磺基丙氨酸、环己基丙氨酸、2- 氨基异丁酸、6- 氨基己酸、叔丁基甘氨酸、苯基甘氨酸、o- 磷酸丝氨酸、N- 乙酰基丝氨酸、N- 甲酰基蛋氨酸、3- 甲基组氨酸和其它非常规氨基酸。此外，非天然存在的氨基酸合成肽在本领域是常规做法。

[0098] 此外，包含共有序列或基本上相同的共有序列变体的限制性肽可通过本领域熟知的方法产生 (Rizo 和 Gierasch, Ann. Rev. Biochem. 61:387-418(1992))。例如，限制性肽可通过添加能够形成二硫桥从而产生环肽的半胱氨酸残基来产生。环肽可被限定为不具有游离的 N 末端或 C 末端。因此，它们不易受外肽酶蛋白水解的影响，尽管它们易受不在肽末端理解的内肽酶的影响。具有 N 末端或 C 末端 D- 氨基酸的肽的氨基酸序列与环肽的氨基酸序列通常与它们所对应的肽的序列相同，除了在分别存在 N 末端或 C 末端 D- 氨基酸残基或它们的环状结构的情况下。

[0099] 环肽

[0100] 在一些实施方案中，天然存在的 Ang-(1-7) 的功能等同物、类似物或衍生物是环肽。如本文所用，环肽具有两个不相邻的残基之间的分子内的共价键。分子内的键可为主链与主链之间的键、侧链与主链之间的键或侧链与侧链之间的键（即，线性肽的末端官能团和 / 或末端或内部残基的侧链官能团可连接以实现环化）。典型的分子内的键包括二硫键、酰胺键和硫醚键。多种环化多肽的方式是本领域所熟知的，可对此类肽进行的许多其它修饰也是如此。对于一般讨论，参见国际专利公布号 WO 01/53331 和 WO 98/02452，其内容通过引用并入本文。此类环键和其它修饰还可应用于本发明的环肽和衍生物化合物。

[0101] 如本文所述的环肽可包含 L- 氨基酸、D- 氨基酸的残基或其任何组合。氨基酸可来自于天然或非天然来源，前提条件是至少一个氨基和至少一个羧基存在于所述分子中；

α-和 β-氨基酸通常是优选的。环肽还可含有一种或多种罕见氨基酸(诸如4-羟基脯氨酸或羟基赖氨酸)、有机酸或酰胺和/或常见氨基酸的衍生物,诸如C末端羧酸根酯化(如,苄基酯、甲基酯或乙基酯)或酰胺化的氨基酸和/或N末端氨基修饰的氨基酸(如,乙酰化或烷氧基羰基化)、具有或不具有各种侧链修饰和/或取代的任何一种的氨基酸(如,甲基化、苄基化、叔丁基化、甲苯磺酰化、烷氧基羰基化等)。合适的衍生物包括具有N-乙酰基的氨基酸(使得代表环化前线性N末端的肽氨基被乙酰基化)和/或C末端酰胺基(即,环化前线性肽的羧基端酰胺化)。除了可与环肽一同呈现的常见氨基酸之外的残基包括但不限于青霉胺、β,β-环戊烷半胱氨酸、β,β-环戊烷半胱氨酸、β-巯基丙酸、β,β-环戊烷-β-巯基丙酸、2-巯基苯、2-巯基苯胺、2-巯基脯氨酸、鸟氨酸、二氨基丁酸、α-氨基己二酸、间氨甲基苯甲酸和 α,β-二氨基丙酸。

[0102] 在合成具有或不具有N-乙酰化和/或C-酰胺化的线性肽之后,环化可以本领域熟知的多种技术的任何一种来实现。在一个实施方案中,可在反应性氨基酸侧链之间产生键。例如,二硫桥可通过使用多种方法的任何一种氧化所述肽从而从包含两个含硫醇残基的线性肽形成。在一种此类方法中,硫醇的空气氧化可使用碱性或中性水性介质经数天的时间产生二硫键。所述肽以高稀释度使用以将聚集和分子内副反应最小化。可替代地,强氧化剂诸如 I_2和 $K_3Fe(CN)_6$可用于形成二硫键。本领域的普通技术人员将认识到必需小心不能氧化Met、Tyr、Trp或His的敏感侧链。在另外的实施方案中,环化可通过酰胺键形成来实现。例如,肽键可在末端官能团之间形成(即,环化前线性肽的氨基端和羧基端)。在另一个此类实施方案中,线性肽包括D-氨基酸。可替代地,环化可通过连接一个末端与残基侧链或者使用两条具有或不具有N末端乙酰基和/或C末端酰胺的侧链来实现。能够形成内酰胺键的残基包括赖氨酸、鸟氨酸(Orn)、α-氨基己二酸、间氨基甲基苯甲酸、α,β-二氨基丙酸、谷氨酸或天冬氨酸。用于形成酰胺键的方法一般为本领域所熟知的。在一个此类方法中,碳二亚胺介导的内酰胺形成羧酸与DCC、DIC、ED AC或DCCI反应,从而形成可与游离氨基立即反应完成环化的O-酰基脲来实现。可替代地,环化可使用叠氮化物法进行,其中反应性叠氮化物中间体是从烷基酯经由酰肼形成的。可替代地,环化可使用活化的酯来实现。在酯的烷氧基碳上存在吸电子取代基增加了它们对氨解的易感性。对硝基酚、N-羟基化合物和多卤化酚的酯的高反应性已经使得这些"活性酯"用于合成酰胺键。在另外的实施方案中,硫醚键可在含硫醇残基的侧链和适当衍化的 α-氨基酸之间形成。举例来说,赖氨酸侧链可与溴乙酸通过碳二亚胺偶联方法(DCC、EDAC)偶联,然后与以上提及的含硫醇残基的任何一个的侧链反应形成硫醚键。为了形成二硫醚,任何两条含硫醇侧链可与二溴乙烷和二异丙胺于DMF中反应。

[0103] 示例性血管紧张素-(1-7)肽

[0104] 在某些方面,本发明提供了线性血管紧张素-(1-7)肽。如上所讨论,天然存在的Ang-(1-7)的结构如下:

[0105] Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7(SEQ ID NO:1)

[0106] 本发明的肽和肽类似物可一般由以下序列表示:

[0107] Xaa^1-Xaa^2-Xaa^3-Xaa^4-Xaa^5-Xaa^6-Xaa^7(SEQ ID NO:5),

[0108] 或其药学上可接受的盐。

[0109] Xaa^1是任何氨基酸或二羧酸。在某些实施方案中,Xaa^1是Asp、Glu、Asn、Acpc(1-氨

基环戊烷羧酸）、Ala、Me_2Gly(N,N- 二甲基甘氨酸）、Pro、Bet（甜菜碱、1- 羧基 -N,N,N- 三甲基甲铵氢氧化物）、Glu、Gly、Asp、Sar（肌氨酸）或 Suc（琥珀酸）。在某些此类实施方案中，Xaa^1是带负电的氨基酸，诸如 Asp 或 Glu，通常是 Asp。

[0110] Xaa^2是 Arg、Lys、Ala、Cit（瓜氨酸）、Orn（鸟氨酸）、乙酰基化 Ser、Sar、D-Arg 和 D-Lys。在某些实施方案中，Xaa^2是带正电的氨基酸，诸如 Arg 或 Lys，通常是 Arg。

[0111] Xaa^3是 Val、Ala、Leu、Nle（正亮氨酸）、Ile、Gly、Lys、Pro、羟基 Pro（羟基脯氨酸）、Aib(2- 氨基异丁酸）、Acpc 或 Tyr。在某些实施方案中，Xaa^3是脂肪族氨基酸诸如 Val、Leu、Ile 或 Nle，通常是 Val 或 Nle。

[0112] Xaa^4是 Tyr、Tyr(PO_3)、Thr、Ser、homoSer（高丝氨酸）、azaTyr（氮杂 - α 1- 高 -L- 酪氨酸）或 Ala。在某些实施方案中，Xaa^4是羟基取代的氨基酸，诸如 Tyr、Ser 或 Thr，通常是 Tyr。

[0113] Xaa^5是 Ile、Ala、Leu、norLeu、Val 或 Gly。在某些实施方案中，Xaa^5是脂肪族氨基酸，诸如 Val，Leu，Ile 或 Nle，通常是 Ile。

[0114] Xaa^6是 His、Arg 或 6-NH_2-Phe(6- 氨基苯基丙氨酸）。在某些实施方案中，Xaa^6是完全或部分带正电的氨基酸诸如 Arg 或 His。

[0115] Xaa^7是 Cys、Pro 或 Ala。

[0116] 在某些实施方案中，Xaa^1-Xaa^7的一个或多个与天然存在的 Ang-(1-7) 中相应的氨基酸相同。在某些此类实施方案中，所有而非 Xaa^1-Xaa^7的一个或两个与天然存在的 Ang-(1-7) 中相应的氨基酸相同。在其它实施方案中，所有 Xaa^1-Xaa^6与天然存在的 Ang-(1-7) 中相应的氨基酸相同。

[0117] 在某些实施方案中，Xaa^3是 Nle。当 Xaa^3是 Nle 时，Xaa^1-Xaa^2和 Xaa^{4-7}的一个或多个可任选地与天然存在的 Ang-(1-7) 中相应的氨基酸相同。在某些此类实施方案中，所有而非 Xaa^1-Xaa^2和 Xaa^{4-7}的一个或两个与天然存在的 Ang-(1-7) 中相应的氨基酸相同。在其它实施方案中，所有 Xaa^1-Xaa^2和 Xaa^{4-7}与天然存在的 Ang-(1-7) 中相应的氨基酸相同，从而产生氨基酸序列 :Asp-Arg-Nle-Tyr-Ile-His-Pro(SEQ ID NO:2)。

[0118] 在某些实施方案中，所述肽具有氨基酸序列 Asp-Arg-Nle-Tyr-Ile-His-Pro(SEQ ID NO:2)。

[0119] 在某些实施方案中，所述肽具有氨基酸序列 Asp-Arg-Val-Ser-Ile-His-Cys(SEQ ID NO:6) 或 Asp-Arg-Val-ser-Ile-His-Cys(SEQ ID NO:3)。

[0120] 在一些实施方案中，如本文所用的线性血管紧张素 (1-7) 肽是具有 Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-Phe^8-His^9(SEQ ID NO:23) 序列的肽，所述序列与 Ang(1-9) 的序列相同。在一些实施方案中，血管紧张素 (1-7) 肽是 Ang(1-9) 的衍生物。对于示例性 Ang(1-9) 肽，包括 Ang(1-9) 衍生物，参见美国专利公布 2012/0172301 中，其公开内容在此通过引用并入。

[0121] 在一些实施方案中，线性血管紧张素 (1-7) 肽是具有氨基酸序列 of Ala^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7(SEQ ID NO:24) 的肽。另外来自于 SEQ ID NO:24 的序列可参见欧洲专利申请 2,264,048，其公开的内容在此通过引用并入。

[0122] 另外考虑了本文所述的线性肽的变型，其中所述变型保持了比较肽的一种或多种功能性质。变型可与本文所述的示例性线性肽具有至少 70％、至少 75％、至少 80％、至少

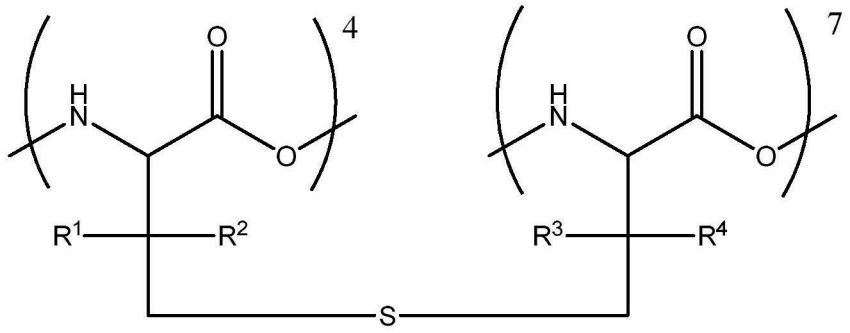
85％、至少 90％、至少 95％、至少 98％或至少 99％的序列同一性。

[0123] 示例性环状血管紧张素 (1-7) 肽

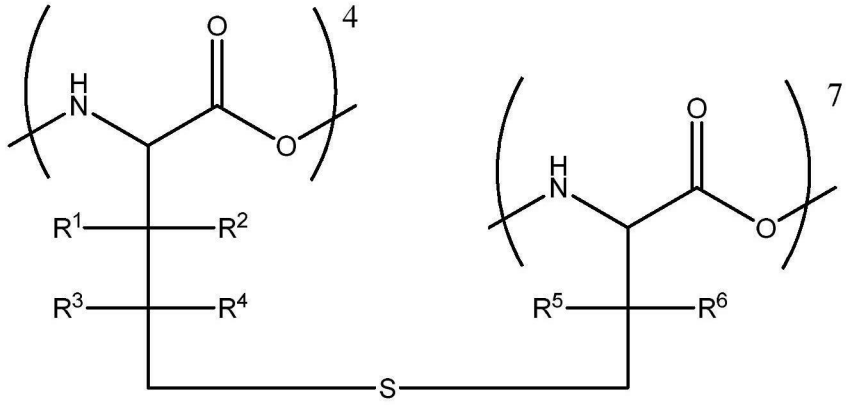
[0124] 在某些方面，本发明提供了环状血管紧张素 -(1-7)（包含键（诸如对应于 Ang 中的位置 Tyr^4 和 Pro^7 的氨基酸侧链之间的键）的 Ang-(1-7)）肽类似物。这些肽类似物通常包含了 7 种氨基酸残基，但还可包含可裂解的序列。如下详细讨论的那样，本发明包括了其中一种或多种氨基酸被另一种氨基酸（包括片段）取代的片段和类似物。

[0125] 尽管以下章节根据连接 4- 和 7- 位置处的残基的硫醚键描述了本发明的各方面，但应理解其它键（如上所述）可替代硫醚桥兵器其它残基可被环化。硫醚桥也被称为一硫化物桥或在 Ala-S-Ala 的情况下被称为羊毛硫氨酸。含肽硫醚桥可通过两种具有以下各式之一的氨基酸形成：

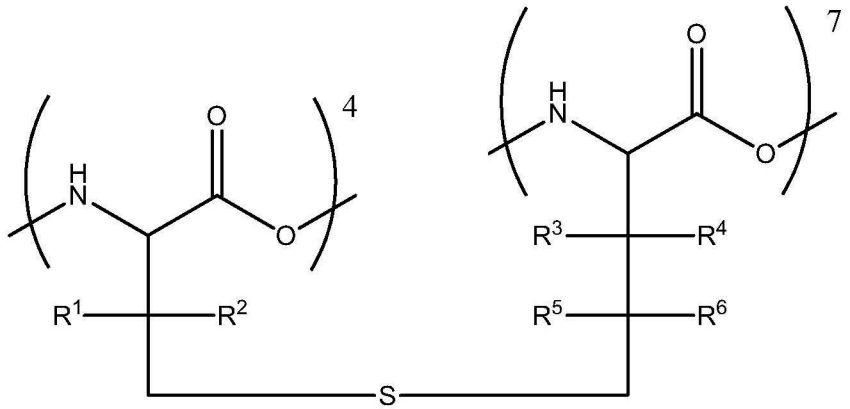
[0126]



式(I)



式(II)



式(III)

[0127] 在这些式中，R^1、R^2、R^3、R^4、R^5和R^6独立为-H、烷基（如，C_1-C_6烷基、C_1-C_4烷基）或芳烷基，其中烷基和芳烷基被一个或多个卤素、-OH或-NRR'基团任选地取代（其中R和R'独立为-H或C_1-C_4烷基）。在某些实施方案中，R^1、R^2、R^3、R^4、R^5和R^6各自独立为-H或$-CH_3$，其中所有都为-H的基团。

[0128] 在某些实施方案中，本发明提供了根据式(I)的包含硫醚桥的Ang类似物或衍生物。通常，R^1、R^2、R^3和R^4独立选自-H和$-CH_3$。根据式(I)的包含硫醚桥的肽可通过例如羊毛硫氨酸抗生素酶(lantibiotic enzyme)或通过二硫化物的硫挤出产生。在一个实例中，

挤出硫的二硫化物可通过位置4的D-半胱氨酸和位置7的L-半胱氨酸或者位置4的D-半胱氨酸和位置7的L-青霉胺形成(参见,如,Galande,Trent和Spatola(2003)Biopolymers 71,534-551)。

[0129] 在其它实施方案中,两个氨基酸的键可为式(II)或式(III)中所述的桥。根据式(II)的包含硫醚桥的肽可例如通过经由D-高半胱氨酸在位置4和L-半胱氨酸在位置7形成的二硫化物的硫挤出制备。类似地,根据式(III)的包含硫醚桥的肽可通过例如经由D-半胱氨酸在位置4和L-高半胱氨酸在位置7形成的二硫化物的硫挤出制备。

[0130] 如上所讨论,本发明的Ang类似物和衍生物的长度和氨基酸组成有差异。本发明的Ang类似物和衍生物优选地具有生物活性或为可蛋白水解活化的非活性前体分子(诸如具有10个氨基酸的血管紧张素(I)如何通过2个氨基酸的切割转化为活性片段)。Ang类似物或衍生物的大小可变化,但通常为约5至10个氨基酸,只要涵盖了包含3-7个Nle-硫醚-环结构的"核心"五聚物片段。本发明的类似物或衍生物的氨基酸序列可变化,通常只要其具有生物活性或可被蛋白水解活化。类似物或衍生物的生物活性可使用本领域已知的方法确定,所述方法包括放射性配体结合研究、体外细胞活化测定和体内实验。参见,例如,Godeny和Sayeski,(2006)Am.J.Physiol.Cell.Physiol.291:C1297-1307;Sarr等,Cardiovasc.Res.(2006)71:794-802;和Koziarz等,(1933)Gen.Pharmacol.24:705-713。

[0131] 其中仅肽的长度变化的Ang类似物和衍生物包括以下:

[0132] 命名的4,7-环化类似物[Cyc^{4-7}]Ang-(1-7),其源自天然Ang-(1-7)(Asp^{1}-Arg^{2}-Val^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7},SEQ ID NO:7)。

[0133] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(1-10),其源自天然血管紧张素I(Ang-(1-10))(Asp^{1}-Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8}-His^{9}-Leu^{10},SEQ ID NO:8);

[0134] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(1-8),其源自天然血管紧张素II(Ang-(1-8))(Asp^{1}-Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8},SEQ ID NO:9);

[0135] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(2-8),其源自天然血管紧张素III(Ang-(2-8))(Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8},SEQ ID NO:10);

[0136] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(3-8),其源自天然血管紧张素IV(Ang-(3-8))(Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8},SEQ ID NO:11);

[0137] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(1-7),其源自天然Ang-(1-7)(Asp^{1}-Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7},SEQ ID NO:12);和

[0138] 命名的4,7-环化类似物[Nle^{3},Cyc^{4-7}]Ang-(1-9),其源自天然Ang-(1-9)(Asp^{1}-Arg^{2}-Nle^{3}-Cyc^{4}-Ile^{5}-His^{6}-Cyc^{7}-Phe^{8}-His^{9},SEQ ID NO:13)。

[0139] 这些类似物可具有式(I)-(III)所示的硫醚桥的一种作为Cyc^{4-7}部分,例如其中Cyc^{4}和Cyc^{7}由式(I)表示,诸如其中R^{1}-R^{4}各自为-H或$-CH_3$,通常为-H。

[0140] 与天然血管紧张素肽的氨基酸序列相比,Cyc^{4-7}类似物的位置4和7处的氨基酸被修饰以使得能够引入上示的硫醚环。除了Ang类似物的长度之外,除了位置3、4和7处之外的氨基酸可与天然存在的肽相同或不同,一般只要类似物保留生物功能。对于非活性前体的类似物,如[Cyc^{4-7}]Ang-(1-10),生物功能是指一种或两种类似物对可将其裂解为生物活性片段(如Ang-(1-8)或Ang-(1-7))的血管紧张素转化酶的易感性,或者其片段的生物

活性。在某些实施方案中，本发明的 Ang 类似物或衍生物不具有固有功能但抑制一种或多种天然存在的血管紧张素化合物的作用。

[0141] 在某些实施方案中，本发明的 Ang 类似物由式 (IV) 表示：

[0142] Xaa^1-Xaa^2-Xaa^3-Cyc^4-Xaa^5-Xaa^6-Cyc^7(IV,SEQ ID NO:14)

[0143] Xaa^1是任何氨基酸，但通常为带负电的氨基酸，诸如 Glu 或 Asp，更通常为 Asp。

[0144] Xaa^2是带正电的氨基酸，诸如 Arg 或 Lys，通常为 Arg。

[0145] Xaa^3是脂肪族氨基酸，诸如 Leu、Ile 或 Val，通常为 Val。

[0146] Cyc^4连同 Cyc^7形成了硫醚桥。Cyc^4可为 D- 立体异构体和 / 或 L- 立体异构体，通常为 D- 立体异构体。Cyc^4(连同 Cyc^7) 的实例显示于式 (I)、(II) 和 (III) 中。通常，式 (I)、(II) 和 (III) 中的 R 基团为 -H 或 $-CH_3$，特别为 -H。

[0147] Xaa^5是脂肪族氨基酸，诸如 Leu、Ile 或 Val，通常为 Ile。

[0148] Xaa^6是 His。

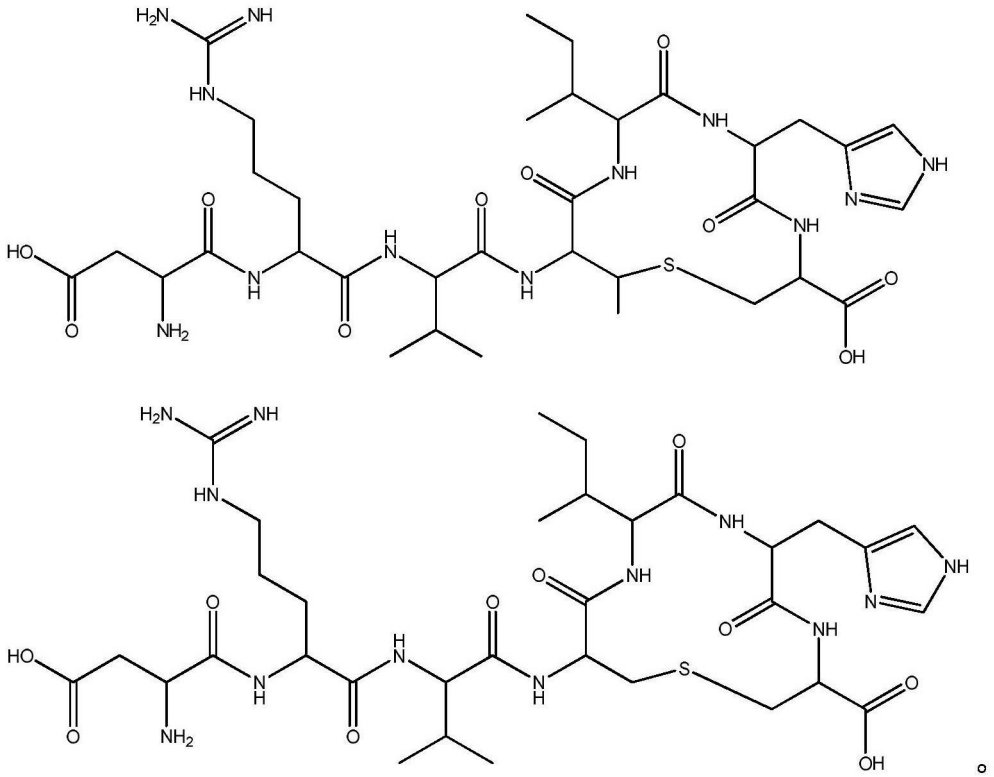
[0149] Cyc^7连同 Cyc^4诸如在式 (I)、(II) 或 (III) 中形成硫醚桥。Cyc^7可为 D- 立体异构体和 / 或 L- 立体异构体，通常为 L- 立体异构体。Cyc^7(连同 Cyc^4) 的实例显示于式 (I)、(II)、(III) 和 (IVIII) 中。通常，式 (I)、(II),) 和 (III) 及 (IV) 中的 R 基团为 -H 或 $-CH_3$，特别为 -H。

[0150] 在某些实施方案中，Xaa^1-Xaa^6(排除 Cyc^4和 Cyc^7) 的一个或多个与天然存在的 Ang-(1-7) 中的相应的氨基酸相同。在某些此类实施方案中，Xaa^1-Xaa^6的所有并非一个或两个与天然存在的 Ang-(1-7) 中相应的氨基酸相同。在其它实施方案中，所有 Xaa^1-Xaa^6与天然存在的 Ang-(1-7) 中相应的氨基酸相同。

[0151] 在某些实施方案中，Cyc^4和 Cyc^7独立选自 Abu(2- 氨基丁酸) 和 Ala(丙氨酸)，其中 Ala 存在于至少一个位置处。因此，环状类似物可具有通过 -Ala^4-S-Ala^7- 形成的硫醚键 (式 (I)，其中 R^1-R^4各自为 -H)；-Ala^4-S-Abu^7-(式 (I)：R^1-R^3为 -H 且 R^4为 $-CH_3$) 或 -Abu^4-S-Ala^7-(式 (I)：R^1、R^3和 R^4为 -H 且 R^2为 $-CH_3$)。环状类似物的特定实例包括 -Abu^4-S-Ala^7- 或 -Ala^4-S-Ala^7- 键。

[0152] 在某些实施方案中，本发明提供了在位置 4 和位置 7 之间具有硫醚桥的 Ang-(1-7) 类似物，其具有氨基酸序列 Asp-Arg-Val-Abu-Ile-His-Ala(SEQ ID NO:15) 或氨基酸序列 Asp-Arg-Val-Ala-Ile-His-Ala(SEQ ID NO:16)，其由以下结构图表示：

[0153]



[0154] 在某些实施方案中，本发明的 Ang 类似物或衍生物由式 (IV) 表示：

[0155] Xaa^1-Xaa^2-Nle^3-Cyc^4-Xaa^5-Xaa^6-Cyc^7-Xaa^8-Xaa^9-Xaa^{10}(IV，SEQ ID NO:17)

[0156] 如上所讨论，Xaa^1、Xaa^2、Xaa^8、Xaa^9和 Xaa^{10}的一个或多个在某些实施方案中不存在。例如，(1)Xaa^{10}不存在，(2)Xaa^9和 Xaa^{10}不存在，(3)Xaa^8、Xaa^9和 Xaa^{10}不存在，(4)Xaa^1不存在，(5)Xaa^1和 Xaa^{10}不存在，(6)Xaa^1、Xaa^9和 Xaa^{10}不存在，(7)Xaa^1、Xaa^8、Xaa^9和 Xaa^{10}不存在，(8)Xaa^1和 Xaa^2不存在，(9)Xaa^1、Xaa^2和 Xaa^{10}不存在，(10)Xaa^1、Xaa^2、Xaa^9和 Xaa^{10}不存在，或 (11)Xaa^1、Xaa^2、Xaa^8、Xaa^9和 Xaa^{10}不存在。对于这些实施方案的每一个，剩下的氨基酸具有下述值。

[0157] Xaa^1当存在时是任何氨基酸，但通常是带负电的氨基酸，诸如 Glu 或 Asp，更通常为 Asp。

[0158] Xaa^2当存在时是带正电的氨基酸，诸如 Arg 或 Lys，通常是 Arg。

[0159] Nle^3是正亮氨酸。

[0160] Cyc^4连同 Cyc^7形成硫醚桥。Cyc^4可为 D- 立体异构体和 / 或 L- 立体异构体，通常为 D- 立体异构体。Cyc^4(连同 Cyc^7) 的实例示于式 (I)、(II) 和 (III) 中。通常，式 (I)、(II) 和 (III) 中的 R 基团是 -H 或 $-CH_3$，特别是 -H。

[0161] Xaa^5是脂肪族氨基酸，诸如 Leu、Nle、Ile 或 Val，通常为 Ile。

[0162] Xaa^6是 His。

[0163] Cyc^7连同 Cyc^4在式 (I)、(II) 或 (III) 中形成了硫醚桥。Cyc^7可为 D- 立体异构体和 / 或 L- 立体异构体，通常为 L- 立体异构体。Cyc^7(连同 Cyc^4) 的实例示于式 (I)、(II) 和 (III) 中。通常，式 (I)、(II) 和 (III) 中的 R 基团为 -H 或 $-CH_3$，特别为 -H。

[0164] Xaa^8当存在时为除了 Pro 之外的氨基酸，通常是 Phe 或 Ile。在某些实施方案中，Ile 产生了 Ang(1-8) 的抑制剂。在某些实施方案中，Phe 保持了 Ang(1-8) 或 Ang(1-10) 的

生物活性。

[0165] Xaa^{9}当存在时为 His。

[0166] Xaa^{10}当存在时为脂肪族残基，例如 Ile、Val 或 Leu，通常为 Leu。

[0167] 在某些实施方案中，Xaa^{1}-Xaa^{10}的一个或多个（排除了 Nle^{3}、Cyc^{4}和 Cyc^{7}）与天然存在的 Ang 中（包括 Ang-(1-7)、Ang(1-8)、Ang(1-9)、Ang(1-10)、Ang(2-7)、Ang(2-8)、Ang(2-9)、Ang(2-10)、Ang(3-8)、Ang(3-9) 和 Ang(3-10) 相应的氨基酸相同。在某些此类实施方案中，Xaa^{1}-Xaa^{10}的所有而非一个或两个（对于存在的那些）与天然存在的 Ang 中相应的氨基酸相同。在其它实施方案中，所有的 Xaa^{1}-Xaa^{10}（对于存在的那些）与天然存在的 Ang 中相应的氨基酸相同。

[0168] 在某些实施方案中，Cyc^{4}和 Cyc^{7}独立选自 Abu(2- 氨基丁酸）和 Ala（丙氨酸），其中 Ala 存在于至少一个位置处。因此，涵盖了包含由 -Ala^{4}-S-Ala^{7}- 形成的硫醚键的环状类似物（式 (I)，其中 R^{1}-R^{4}各自为 -H）；由 -Ala^{4}-S-Abu^{7}- 形成的硫醚键的环状类似物（式 (I)：R^{1}-R^{3}为 -H 且 R^{4}为 $-CH_3$）或由 -Abu^{4}-S-Ala^{7}- 形成的硫醚键的环状类似物（式 (I)：R^{1}、R^{3}和 R^{4}为 -H 且 R^{2}为 $-CH_3$）。特定的环状类似物包含 -Abu^{4}-S-Ala^{7}- 或 -Ala^{4}-S-Ala^{7}- 键。

[0169] 特别地，本方面提供了在位置 4 和位置 7 之间具有硫醚桥的 An g-(1-7) 类似物或衍生物，其具有氨基酸序列 Asp-Arg-Nle-Abu-Ile-His-Ala(SEQ ID NO:18) 或氨基酸序列 Asp-Arg-Nle-Ala-Ile-His-Ala(SE Q ID NO:19)。

[0170] 在另一方面，本发明提供了具有 Ang-(1-8) 拮抗活性的在位置 4 和位置 7 之间具有硫醚桥的 Ang-(1-8) 类似物或衍生物，特别是具有氨基酸序列 Asp-Arg-Nle-Abu-Ile-His-Ala-Ile(SEQ ID NO:20)、氨基酸序列 Asp-Arg-Nle-Ala-Ile-His-Ala-Ile(SEQ ID NO:21) 或氨基酸序列 Asp-Arg-Nle-Abu-Ile-His-Ala-Ile(SEQ ID NO:22) 的 Ang(1-8) 类似物或衍生物。

[0171] 烷基是完全饱和的直链或支链非芳族烃。通常，直链或支链烷基具有 1 至约 20 个碳原子，优选 1 至约 10 个。直链和支链烷基的实例包括甲基、乙基、正丙基、异丙基、正丁基、仲丁基、叔丁基、戊基、己基、戊基和辛基。C1-C4 直链或支链烷基也被称为″低级烷基″基团。

[0172] 芳烷基是被芳基取代的烷基。芳族（芳基）基团包括碳环芳族基团（诸如苯基、萘基和蒽基）和杂芳基（诸如噻唑基、噻吩基、呋喃基、吡啶基、嘧啶基、吡喃基、吡唑基、吡咯基、吡嗪基、噻唑基、噁唑基和四唑基）。芳族基团还包括其中碳环芳族环或杂芳基环与一种或多种其他芳基环稠合的稠合多环芳族环系统。实例包括苯丙噻吩基、苯丙呋喃基、吲哚基、喹啉基、苯丙噻唑、苯并噁唑、苯并咪唑、喹啉基、异喹啉基和异吲哚基。

[0173] 血管紧张素 (1-7) 肽（包括衍生物和类似物）可以各种量存在于各种实施方案中。例如，血管紧张素 (1-7) 肽存在的量范围为约 10-1000mg（如，约 20mg－1,000mg、30mg－1,000mg、40mg－1,000mg、50mg－1,000mg、60mg－1,000mg、70mg－1,000mg、80mg－1,000mg、90mg－1,000mg、 约 10-900mg、10-800mg、10-700mg、10-600mg、10-500mg、100-1000mg、100-900mg、100-800mg、100-700mg、100-600mg、100-500mg、100-400mg、100-300mg、200-1000mg、200-900mg、200-800mg、200-700mg、200-600mg、200-500mg、200-400mg、300-1000mg、300-900mg、300-800mg, 300-700mg、300-600mg、300-500mg、400mg－1,000mg、500mg－1,000mg、100mg-900mg、200mg－800mg、300mg－700mg、400mg－

700mg 和 500mg - 600mg)。在一些实施方案中，血管紧张素 (1-7) 肽存在的量为或大于约 10mg、50mg、100mg、150mg、200mg、250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg。在一些实施方案中，血管紧张素 (1-7) 肽存在的量为或小于约 1000mg、950mg、900mg、850mg、800mg、750mg、700mg、650mg、600mg、550mg、500mg、450mg、400mg、350mg、300mg、250mg、200mg、150mg 或 100mg。

[0174] 在一些实施方案中，环状血管紧张素 (1-7) 肽是环化 Ang(1-9) 肽或包含 SEQ ID NO:24 的环化肽。

[0175] 另外还考虑了本文描述的环肽的变型，其中所述变型保持了比较肽的一种或多种功能性质。环化变型的序列可与本文描述的示例性环肽的任何序列具有至少 70％、至少 75％、至少 80％、至少 85％、至少 90％、至少 95％、至少 98％或至少 99％序列同一性。

[0176] 降 pH 剂

[0177] 考虑了适用于本发明的降 pH 剂包括任何药学上可接受的降 pH 剂或降 pH 剂的组合，它们 a) 对胃肠道无毒性，b) 能够递送氢离子或能够从环境中诱导更高含量的氢离子，和 / 或 c) 能够以足以降低局部肠道 pH 低于对于那里发现的蛋白酶是最佳的 pH 的量经口施用。各种测试可用于确定降 pH 剂是否适用于本发明和多少量是适当的。例如，降 pH 剂或降 pH 剂的组合适用于本发明，如果当特定量添加至 10 毫升 0.1M 碳酸氢钠溶液时其将溶液的 pH 降低至不超过 5.5、4.7 或 3.5。在一些实施方案中，可添加一定量的降 pH 剂或试剂以降低于 10 毫升 0.1M 碳酸氢钠溶液中的 pH 至不超过 3.4、3.2、3.0 或 2.8。

[0178] 在一些实施方案中，合适的降 pH 剂或药剂包括至少一种 pKa 不超过 4.2(如，不超过 4.0、3.8、3.6、3.4、3.2、3.0 或 2.8) 的降 pH 剂。适用于本发明的示例性降 pH 剂包括但不限于羧酸，诸如乙酰水杨酸、乙酸、抗坏血酸、柠檬酸、富马酸、葡萄糖醛酸、戊二酸、甘油酸、甘氨胆酸 (glycocolic)、乙醛酸、异柠檬酸、异戊酸、乳酸、顺丁烯二酸、草酰乙酸、草酰琥珀酸、丙酸、丙酮酸、琥珀酸、酒石酸和缬草酸 ;氯化铝 ;氯化锌 ;氨基酸 (或其衍生物) 的酸性盐，包括乙酰基谷氨酸、丙氨酸、精氨酸、天冬酰胺、天冬氨酸、甜菜碱、肉毒碱、肌肽、瓜氨酸、肌酸、谷氨酸、甘氨酸、组氨酸、羟基赖氨酸、羟基脯氨酸、亚牛磺酸、异亮氨酸、亮氨酸、赖氨酸、甲基组氨酸、正亮氨酸、鸟氨酸、苯丙氨酸、脯氨酸、肌氨酸、丝氨酸、牛磺酸、苏氨酸、色氨酸、酪氨酸和缬氨酸的酸性盐 ;某些磷酸盐，包括果糖 1,6 二磷酸盐和葡萄糖 1,6 二磷酸盐，在某些实施方案中也可为适当的降 pH 剂。在具体的实施方案中，柠檬酸或酒石酸可用作降 pH 剂。

[0179] 任何特定降 pH 剂或降 pH 剂的组合所需的量可变化。通常，合适的量可使用本领域已知和本文描述的各种测试测定 (例如，于上述的 10 毫升 0.1M 碳酸氢钠的溶液中使用 pH 降低测试)。作为非限制性实例，根据本发明的制剂中所用的降 pH 剂的合适的量可为或大于 约 100mg、200mg、250mg、300mg、350mg、400mg、425mg、450mg、475mg、500mg、525mg、550mg、575mg、600mg、625mg、650mg、675, mg、700mg、725mg、750mg、775mg、800mg、825mg、850mg、875mg、900mg、925mg、950mg、975mg 或 1,000mg 的量。在其它实施方案中，所用的柠檬酸的量可超过 1,000mg。

[0180] 在一些实施方案中，所用的降 pH 剂 (如，柠檬酸或酒石酸) 的合适的量可测量为特定剂型的总重的百分比。作为非限制性实例，降 pH 剂的合适的量可为或大于固体剂型总重的约 10％ (如，或大于 15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、

70％、75％、80％、85％、90％或 95％）的量。

[0181] 吸收促进剂

[0182] 在各种实施方案中，本发明的制剂具有一种或多种吸收促进剂。如本文所用，吸收促进剂是指增加其它组分在被释放其中的水性或亲脂性环境中的溶解度和 / 或增强活性肽（如，血管紧张素 (1-7) 肽）经肠壁吸收的试剂。在一些实施方案中，吸收促进剂被称为增溶剂和 / 或吸收增强剂。

[0183] 在一些实施方案中，可能具有吸收促进剂的混合物，其中一些提供增强的溶解度，一些提供增强的吸收及一些提供两者。在给定的环境中可能具有个各种数量的吸收促进剂，包括但不限于一种、两种、三种、四种、五种、六种、七种、八种、九种或十种吸收促进剂。

[0184] 表面活性剂是具有增溶剂和吸收增强剂性质的有用的吸收促进剂的实例。在一些实施方案中，当表面活性剂用作吸收促进剂时，它们可为用于在制造过程中促进混合和装载胶囊的游离流动的粉末。在其它实施方案中，当表面活性剂用于增加血管紧张素 (1-7) 肽的生物利用度时，表面活性剂可选自 (a) 阴离子表面活性剂，诸如胆固醇衍生物（如胆酸），(b) 阳离子表面剂（如酰基肉毒碱、磷脂等），(c) 非离子表面活性剂，和 (d) 阴离子表面活性剂和带负电的中和剂的混合物，及其组合。带负电的中和剂包括但不限于酰基肉毒碱、氯化十六烷吡啶等。

[0185] 在一些实施方案中，酸可溶性胆酸和阳离子表面活性剂将一起用作吸收促进剂。酰基肉毒碱（诸如月桂酰肉碱）、磷脂和胆酸在一些实施方案中可为特别有效的吸收促进剂。

[0186] 当各种吸收促进剂适用于各种实施方案中时，以下示例性列表意在说明本发明的一些实施发难。在不受限制的情况下，一些合适的吸收促进剂包括 :(a) 水杨酸盐，诸如水杨酸钠、3- 甲氧基水杨酸盐、5- 甲氧基水杨酸盐和高香草酸盐 ;(b) 胆酸，诸如牛磺胆酸、牛去氧胆酸、去氧胆酸、胆酸、甘氨胆酸 (glycholic)、石胆酸、鹅去氧胆酸、熊去氧胆酸、熊胆酸、去氢胆酸、夫西地酸等 ;(c) 非离子表面活性剂，诸如聚氧乙烯醚（如 Brij 36T、Brij 52、Brij 56、Brij 76、Brij 96、Texaphor A6、Texaphor A14、Texaphor A60 等）、p-t- 辛基酚聚氧乙烯醚 (Triton X-45、Triton X-100、Triton X-114、Triton X-305 等）壬基酚氧基聚氧乙烯（如 Igepal CO 系列）、聚氧乙烯脱水山梨糖醇酯（如 Tween-20、Tween-80 等）；(d) 阴离子表面活性剂，诸如磺基琥珀酸二辛酯钠 ;(e) 溶血磷脂，诸如溶血卵磷脂和溶血磷脂酰乙醇胺 ;(f) 酰基肉碱、乙酰胆碱和酰基氨基酸，诸如月桂酰基肉毒碱、肉豆蔻酰肉毒碱、棕榈酰肉毒碱、月桂酰基胆碱、肉豆蔻酰胆碱、棕榈酰胆碱、十六烷基赖氨酸、N- 酰基苯丙氨酸、N- 酰基甘氨酸等 ;g) 水溶性磷脂，诸如二庚酰磷脂酰胆碱、二辛基磷脂酰胆碱等 ;(h) 中链甘油酯，其为甘油一酯、甘油二脂和甘油三酯的混合物，含有中链长的脂肪酸（辛酸、癸酸和月桂酸）;(i) 乙二胺四乙酸 ;(j) 阳离子表面活性剂，诸如氯化十六烷基吡啶 ;(k) 聚乙二醇的脂肪酸衍生物，诸如 Labrasol、Labrafac 等 ;和 (l) 烷基糖，诸如月桂酰基麦芽糖苷、月桂酰基蔗糖、肉豆蔻酰蔗糖、棕榈酰蔗糖等。

[0187] 在一些实施方案中，一种或多种吸收促进剂将以如以按重量计相对于药物组合物总重（通常不包括肠溶包衣）的百分比测量的量存在。举另外非限制实例的来说，环境中存在的吸收促进剂的量的范围可为 0.1 至 20 重量％ ;0.5 至 20 重量％ ;1.0 至 20 重量％、2.0 至 20 重量％、3.0 至 20 重量％、4.0 至 20 重量％、5.0 至 20 重量％、5.0 至 15 重量％、

5.0 至 14 重量%、5.0 至 13 重量%、5.0 至 12 重量%、5.0 至 12 重量%、5.0 至 11 重量%、5.0 至 10 重量%、6.0 至 10 重量%、7.0 至 10 重量%、8.0 至 10 重量%、9.0 至 10 重量%、5.0 至 9.0 重量%、5.0 至 8.0 重量%、5.0 至 7.0 重量%和 5.0 至 6.0 重量%。

[0188] 在一些实施方案中，一种或多种降 pH 剂与一种或多种吸收促进剂的比率可为约 3:1、4:1、5:1、6:1、7:1、8:1、9:1、10:1、11:1、12:1、13:1、14:1、15:1、16:1、17:1、18:1、19:1、20:1 或前述示例性比率的任何两者之间。给定药物组合物中的所有降 pH 剂的总重和所有吸收促进剂的总重被包括在前述的示例性比率中。例如，如果药物组合物包括两种降 pH 剂和三种吸收促进剂，则前述比率将按照两种降 pH 剂的总组合重量和所有三种吸收促进剂的总组合重量计算。

[0189] 在一些实施方案中，一种或多种吸收促进剂将在酸性 pH（诸如小于 pH 5.5）下并且特别在 pH 3.0 与 pH 5.0 之间可溶。

[0190] 保护性媒介物

[0191] 如本文所用，保护性媒介物是指任何保护性组分和 / 或结构，诸如载体、层、包衣或保护活性肽（如，血管紧张素 (1-7) 肽）免受胃蛋白酶的其它媒介物。通常，保护性媒介物最终溶解，使得特定剂型中的活性和其它成分可释放。常见形式的保护性媒介物是肠溶包衣。在一些实施方案中，合适的肠溶包衣可预防本发明的药物组合物在 0.1N HCl 中分解至少两小时，然后能够使得药物组合物的所有组分在溶解浴中的 pH 增加至 6.3 之后在三十分钟内完全释放，其中所述组合物以每分钟 100 转旋转。

[0192] 许多肠溶包衣是本领域已知的，并且在一个或多个实施方案中使用。肠溶包衣的非限制性实例包括邻苯二甲酸乙酸纤维素、羟丙基甲基乙基纤维素丁二酸酯、羟丙基甲基纤维素邻苯二甲酸酯、羧甲基乙基纤维素和甲基丙烯酸 - 甲基甲基丙烯酸酯共聚物。在一些实施方案中，将血管紧张素 (1-7) 肽、吸收促进剂（诸如增溶剂和 / 或一种或多种吸收增强剂及一种或多种降 pH 剂）包含在足够粘的保护性浆料中使得本实施方案的组分的保护的通道通过胃。

[0193] 在将本发明的活性和其它组分装载在胶囊中之后，可将合适的肠溶包衣例如应用给胶囊。在其它实施方案中，将肠溶包衣涂覆在片剂的外部或涂覆在活性组分的颗粒的外表面，然后将其压制为片剂形式或装载在胶囊中。

[0194] 在一些实施方案中，可能希望本发明的所有组分可从载体或媒介物中释放，并尽可能同时溶解于肠道环境中。在一些实施方案中，媒介物或载体还优选在小肠中释放活性组分，其中相较于相同的吸收增强剂随后在结肠中释放增加跨细胞或细胞旁路运输的吸收增强剂更不可能引起不希望的副作用。然而，应该理解，本发明据信在结肠以及在小肠中都有效。除了以上讨论的那些媒介物或载体之外的多种媒介物或载体为本领域已知的。

[0195] 在一些实施方案中，可能希望（特别是在优化本发明的组分如何同时释放时）保持较少量的肠溶包衣。在一些实施方案中，肠溶包衣增加了不超过 30％的药物组合物剩余物的重量（诸如固体剂型）(″剩余物″为除了肠溶包衣本身之外的药物组合物）。在其它实施方案中，肠溶包衣增加了小于 20％、小于 19％、小于 18％、小于 17％、小于 16％、小于 15％、小于 14％、小于 13％、小于 12％、小于 11％或小于 10％。在一些实施方案中，保护性媒介物诸如肠溶包衣的组成量为或小于药物组合物（如，固体剂型）总重的约 25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、

8%、7%、6%、5%。

[0196] 剂型

[0197] 如本文所用，剂型是指活性药物组分和非药物组分的混合物。各种剂型可根据本发明使用，包括但不限于液体剂型、固体剂型和半固体剂型。常见剂型包括丸剂、片剂、胶囊剂、饮剂或糖浆剂。在一些实施方案中，使用诸如丸剂、片剂或胶囊剂的固体剂型。

[0198] 通常，特别需要的剂型提供了同时释放血管紧张素-(1-7)肽、降pH剂和吸收促进剂。这是极其需要的，因为当酸在与释放肽相近的时间释放时，所述酸最能够降低不需要的对肽的蛋白水解攻击。通过施用为单一丸剂、片剂或胶囊剂的本发明的所有组分可最容易实现近乎同时释放。

[0199] 各种实施方案可任选地包括具有常见已知尺寸和量的常见的药物赋形剂，诸如稀释剂、聚糖(glycant)、润滑剂、明胶胶囊剂、防腐剂、着色剂等。示例性非限制性赋形剂包括pro-盐、polyplastum和硬脂富马酸钠。在一些实施方案中，包含另一种肽（诸如白蛋白、酪蛋白、大豆蛋白、其它动物或植物蛋白等）以减少非特异性吸附（如，血管紧张素(1-7)肽与肠粘膜屏障的结合），从而降低昂贵肽活性剂的必需浓度。当添加时，另外的肽通常为相对于总药物组合物（排除保护性媒介物）的重量1.0至10.0重量%。通常，这种另外的肽并不具有生理活性，并且最优选为食物肽诸如大豆肽等。不希望被理论所束缚，该第二肽还可通过作为期望与用于蛋白酶相互作用的肽活性剂竞争的蛋白酶清除剂来增加生物利用度。第二肽还有助于通过肺的活性化合物通道。

[0200] 在一些实施方案中，一种或多种降pH剂、血管紧张素(1-7)肽、一种或多种吸收促进剂及其它赋形剂（在每个类别中无论是单一化合物还是多种化合物）应该均匀地分散于剂型中。在其它实施方案中，剂型可包括颗粒，所述颗粒包含了均匀分散于所述粘合剂中的血管紧张素(1-7)肽、降pH剂和吸收促进剂的药物粘合剂。在其它实施方案中，颗粒还可由有机酸的均一层、增强剂层和肽层围绕的酸性核心组成，所述肽层被有机酸的外层所围绕。颗粒可从由本发明的药物粘合剂诸如聚乙烯吡咯烷酮或羟丙基甲基纤维素连同降pH剂、吸收促进剂和肽活性剂组成的水性混合物制备。

[0201] 如所述，各种实施方案可具有不同量的成分以及不同成分。不考虑具体实施方案的配方，此实施方案中存在的所有成分的总重可落入某些重量范围中，诸如约500-1500(如，约500-1200mg、500-1000mg、600-1500mg、600-1200mg、600-1000mg、700-1500mg、700-1200mg、700-1000mg、800-1500mg、800-1200mg、800-1000mg)。在一些实施方案中，合适的固体剂型的总重为或大于约500mg、600mg、700mg、800mg、900mg、1000mg、1100mg、1200mg、1300mg、1400mg或1500mg。在一些实施方案中，合适的固体剂型的总重为或小于约2000mg、1900mg、1800mg、1700mg、1600mg、1500mg、1400mg、1300mg、1200mg、1100mg、1000mg、900mg、800mg、700mg、600mg或500mg。

实施例

[0202] 实施例1.血管紧张素(1-7)肽的经口递送

[0203] 该实施例说明了血管紧张素(1-7)可使用根据本发明的示例性制剂有效经口递送。特别地，经口递送血管紧张素(1-7)肽的可行性通过将其在液体制剂中经由十二指肠内注射(ID)施用给麻醉大鼠得以说明。该模型模拟了从经口递送的肠溶包衣的固体剂型

（诸如胶囊剂或片剂）期望的释放和吸收。

[0204] 首先，雌性 Sprague-Dawley 大鼠中的基线药代动力学曲线通过皮下 (SC) 施用于磷酸盐缓存盐水 (PBS) 中的血管紧张素 (1-7) 来获得。血样 (0.6ml) 从植入右颈动脉的套管在注射肽之前和之后 5、10、20、30、60 和 90 分钟获得，并用等体积的肝素化盐水替代。

[0205] 提取后，然后将样品转移至含有蛋白酶抑制剂混合物的冰冷却的管中。将样品保持在冰上直至将它们在 4℃下离心以获得血浆。然后将血浆上清液在 -70℃下冷冻直至使用 LC-MS 测定分析。

[0206] 表 1 总结了在指定的时间点和非隔室 (non-compartmental)PK 值下获得的示例性各基线 A(1-7) 水平。向三只大鼠施用 0.3mL 的 10mg/mL A(1-7) 皮下注射剂。A(1-7) 的药代动力学使用非隔室模型确定，其中确定各自的药代动力学。计算每个时间点的平均浓度，并且估计这些平均值的 PK 值。在施用后约 10 至 90 分钟达到 Tmax。对于这一治疗组来说，半衰期为约 15 分钟。观察期间的总平均 A(1-7) 暴露为 614ng*min/mL，范围为 585 至 656ng*min/mL。

[0207] 表 1：

[0208]

	0.3 mL：A(1-7)皮下注射				
	血管紧张素(1-7) (ng/mL)				
时间点(min)	大鼠 1	大鼠 2	大鼠 3	平均 PK 值	均值
0	0.121	10.2	1.41		4.50
5	0.120	7.47	4.37		3.99
10	18.6	0.94	1.09		6.88
20	6.25	15.7	5.71		9.22
40	15.30	6.38	4.47		8.72
60	1.03	2.65	7.46		3.71
90	1.14	6.59	17.4		8.38
Cmax (ng/mL)	18.6	15.7	17.4	17.2	9.2
Tmax (ng/mL)	10	20	90	40.0	20
半衰期 (min)	15	15.58		15.1	
AUC_{0-90} (ng*min/mL)	583	598.1	656.1	612.4	614

[0209] 然后在模拟肽通过肠溶胶囊释放至肠的大鼠模型中评估血管紧张素 (1-7) 肽

(如,TXA127)的经口递送。简而言之,可通过手术暴露麻醉大鼠的十二指肠,并且血管紧张素(1-7)肽通过27规格针头递送至十二指肠内。通过ID施用于PBS中的血管紧张素(1-7)来获得基线。将血样在肽施用后的5、10、20、40、60和90分钟从颈动脉去除。随后,将血管紧张素(1-7)肽于400mM柠檬酸盐缓冲液(pH 3.5)和月桂酰基-L-肉毒碱(LLC)(10mg/ml)(一种模拟肠溶胶囊内含物的制剂)中ID施用。为了将大鼠循环中的TXA127的稳定性最大化,可将卡托普利(如,0.5mg/ml或5mg/ml)添加至制剂。在与基线研究相同的时间点取血样,并入上所述处理以用于分析。示例性结果总结于表2-5中。

[0210] 表2总结了用于PBS中配制的0.3mL的10mg/mL血管紧张素(1-7)(A(1-7))处理的总共6只大鼠的示例性结果。使用非隔室模型测定A(1-7)的药代动力学。计算每个时间点的平均浓度并估计这些平均值的PK值。将这些值与从各PK参数的均值计算而来的平均PK值相比较。在施用之后的约10至60分钟达到。对于该治疗组来说,半衰期的范围为7至140分钟。观察期间的总平均A(1-7)暴露为403ng*min/mL,其范围为123至881ng*min/mL。

[0211] 表2:

[0212]

	0.3 ml A, ID [于 PBS 中的 10 mg/ml 血管紧张素(1-7)]							
	血管紧张素(1-7) (ng/mL)							
时间点(min)	大鼠 1	大鼠 2	大鼠 1	大鼠 2	大鼠 1	大鼠 2	平均 PK 值	均值
0	0.23	0.0	1.54	1.00	1.84	5.81		1.74
5	0.84	12.3	0.94	0.88	0.891	9.24		4.18
10	3.44	6.38	1.08	0.99	1.12	4.08		2.85
20	0.62	13.3	1.91	1.79	1.80	2.05		3.58
40	0.27	15.4	1.22	4.14	1.30	3.89		4.37
60	1.55	6.27	3.97	8.75	3.07	10.4		5.67
90	2.61	7.19	0.78	7.54	1.23	10.4		4.96
Cmax (ng/mL)	3.44	15	4	9	3	10.4	7.5	5.7
Tmax (ng/mL)	10	40	60	60	60		46.0	60
半 衰 期 (min)		51	13	140	23	7	38.9	155
AUC_{0-90} (ng*min/m	123	881	181	456	166	616	403	404

[0213]

L)								

[0214] 表 3 总结了用于含有 10mg/mL LLC、400mM 柠檬酸盐、150mM NaCl pH 3.5 的制剂中的 0.3mL 的 10mg/mL A(1-7) 处理的大鼠中出现的示例性 A(1-7) 浓度。使用非隔室模型测定 A(1-7) 的药代动力学。此外，计算每个时间点的平均浓度并估计这些平均值的 PK 值。将这些平均浓度值与从所有各个 PK 参数的均值计算而来的平均 PK 值相比较。在施用之后约 5 至 10 分钟达到 Tmax。对于该处理组来说，半衰期的范围为 9.3 至 173.3 分钟。观察期间的总平均 A(1-7) 暴露为 4,274ng*min/mL，其范围为 422 至 19,502ng*min/mL。

[0215] 表 3：

[0216]

	0.3 ml B, ID [10 mg/ml 血管紧张素(1-7)、10mg/ml LLC、400mM 柠檬酸盐，pH3.5，150 mM NaCl]							
	血管紧张素(1-7) (ng/mL)							
时间点 (min)	大鼠 3**	大鼠 4	大鼠 3	大鼠 4	大鼠 3	大鼠 4	平均 PK 值	均值
0	1.46	0.171	3.02	1.31	1.81	1.29		1.51
5	>1000	259	3.48	89.7	60.7	56.38		416.54
10	>1000	21	19.3	15.2	59.6	17.92		203.84
20	59.9	7.35	6.02	18.4	51.2	6.02		24.82
40	4.88	5.6	2.32	5.05	11.7	1.11		5.11
60	4.74	1.7	3.99	6.36	10.9	3.48		5.20
90	3.93	3.99	1.11	4.43	4.74	3.39		3.60
Cmax (ng/mL)	>1000	259.0	19.3	89.7	60.7	56.4	419.2	416.5
Tmax (ng/mL)	5.0	5.0	10.0	5.0	5.0	5.0	5.8	5

[0217]

半衰期 (min)	9.3	173.3	23.8	25.2	21.6	25.0	46.4	13
AUC_{0-90} (ng*min /mL)	19,502.2	1,777.5	422.8	1,168.2	2,100.6	669.9	4,273.5	4274

[0218] **- 该大鼠的数据未包括在均值或分析中

[0219] 表 4 总结了用于含有 0.5mg/mL 卡托普利、10mg/mL LLC、400mM 柠檬酸盐、150mM NaCl(pH 3.5) 中的 0.3mL 的 10mg/mL A(1-7) 施用的七只大鼠的示例性药代动力学结果。使用非隔式模型测定 A(1-7) 的药代动力学。计算每个时间点的平均浓度并估计这些平均值的 PK 值。将这些平均浓度值与从所有各个 PK 参数的均值计算而来的平均 PK 值相比较。使用值 >1,000ng/mL 测定 AUC 以提供就暴露范围的考量。此外，计算每个时间点的平均浓度之外并估计这些平均值的 PK 值。在施用之后约 5 至 10 分钟达到 Tmax。对于该处理组来说，半衰期的范围为 11.9 至 29.1 分钟。观察期间的总平均 A(1-7) 暴露为 7,152ng*min/mL，其范围为 1,969 至 9,257ng*min/mL。

[0220] 表 4：

[0221]

	0.3 ml C，ID [10 mg/ml 血管紧张素(1-7)，卡托普利 0.5 mg/ml, 10mg/ml LLC，400 mM 柠檬酸盐 pH 3.5，150 mM NaCl]								
	血管紧张素(1-7) (ng/mL)								
时间点 (min)	大鼠 5	大鼠 6**	大鼠 5	大鼠 6	大鼠 5	大鼠 6		平均 PK 值	均值
0	1.24	1.13	1.54	0.51	1.29	0.98			1.1
5	879.00	>1000	468.00	57.20	735.00	325.00			628.31
10	407.00	349.00	313.00	83.00	248.00	145.67			233.21

[0222]

20	88.70	496.00	141.00	64.10	16.30	70.14			129.13
40	4.24	19.60	17.30	7.68	8.42	22.85			13.87
60	12.30	10.10	4.96	6.16	12.30	37.30			14.45
90	5.61	5.83	7.84	4.93	15.80	7.36			7.74
Cmax (ng/mL)	879.0	>1000	468.0	83.0	735.0	83.0		609.3	628.3
Tmax (ng/mL)	5.0	5.0	5.0	10.0	5.0	10.0		7.1	5
半衰期 (min)	11.9	29.1	13.9	18.3	17.9	18.3		19.4	14
AUC0-90 (ng*min/mL)	9,257	20,442	7,394	2,252	6,495	2,252		7,152	7,587

[0223] **- 该大鼠的数据未包括在均值或分析中

[0224] 表 5 总结了在给予 0.3mL 于含有 0.5mg/mL 卡托普利、10mg/mL LLC、400mM 柠檬酸

盐、150mM NaCl(pH 3.5)的制剂中的10mg/mL A(1-7)的六只大鼠中获得的示例性A(1-7)水平。再次,使用非隔室模型测定A(1-7)的药代动力学参数,其中还测定了各代动力学参数。在该分析中,使用>1,000ng/mL的值测定AUC,以提供就暴露范围的考量。计算每个时间点的平均浓度并估计这些平均值的PK值。在施用之后约5至10分钟达到Tmax。对于该处理组来说,半衰期的范围为7.97至25.6分钟。观察期间的总平均A(1-7)暴露为9,399ng*min/mL,其范围为1,008至26,654ng*min/mL。

[0225] 表5:

[0226]

	0.3 ml C，ID [10 mg/ml 血管紧张素(1-7)，卡托普利 5 mg/ml，10mg/ml LLC，400 mM 柠檬酸盐 pH 3.5，150 mM NaCl]							
	血管紧张素(1-7) (ng/mL)							
时间点 (min)	大鼠7	大鼠8	大鼠7	大鼠8	大鼠7	大鼠8	平均PK值	均值
0	0.99	0.40	3.31	7.63	1.55	0.63		2.42
5	>1000	33.50	504.00	746.00	268.89	432.86		619.21
10	>1000	12.30	410.00	404.00	391.25	5.34		423.82
20	466.00	8.12	70.40	178.00	63.50	41.25		137.88
40	27.20	20.20	18.40	39.90	22.70	16.10		24.08
60	16.60	22.10	17.60	13.50	19.90	25.70		19.23
90	10.20	0.00	11.60	5.58	2.75	4.83		5.83
Cmax (ng/mL)	<1000	33.5	504	746.0	391.25	432.85	639.6	619.2
Tmax (ng/mL)	5.0	5.0	5.0	5.0	10	5.0	5.8	5
半衰期 (min)	10.55	7.97	15.45	11.94	12.86	25.62	14.1	13
AUC_{0-90} (ng*min/mL)	26,654	1,008	7,641	10,668	6,228	3,862	9,343.5	9,399

[0227] 此外，图 1 说明了各种施用途径和制剂间 AUC 值的比较。

[0228] 如表 1-5 和图 1 中所示，与于 PBS 中递送的血管紧张素 (1-7) 的基线特征相比，根据本发明的制剂中递送（使用大鼠模型模拟经口递送）的血管紧张素 (1-7) 在观察期内具有显著改善的半衰期和总暴露。这些结果说明血管紧张素 (1-7) 可根据本发明经口递送并在循环中获得治疗有效的生物利用度。

[0229] 等同形式和范围

[0230] 本领域技术人员将认识到或能够确定仅使用常规实验、对本文描述的本发明的特定实施方案特异的许多等同物。本发明的范围并非意在限于以上描述，而是如以下权利要求所示出：

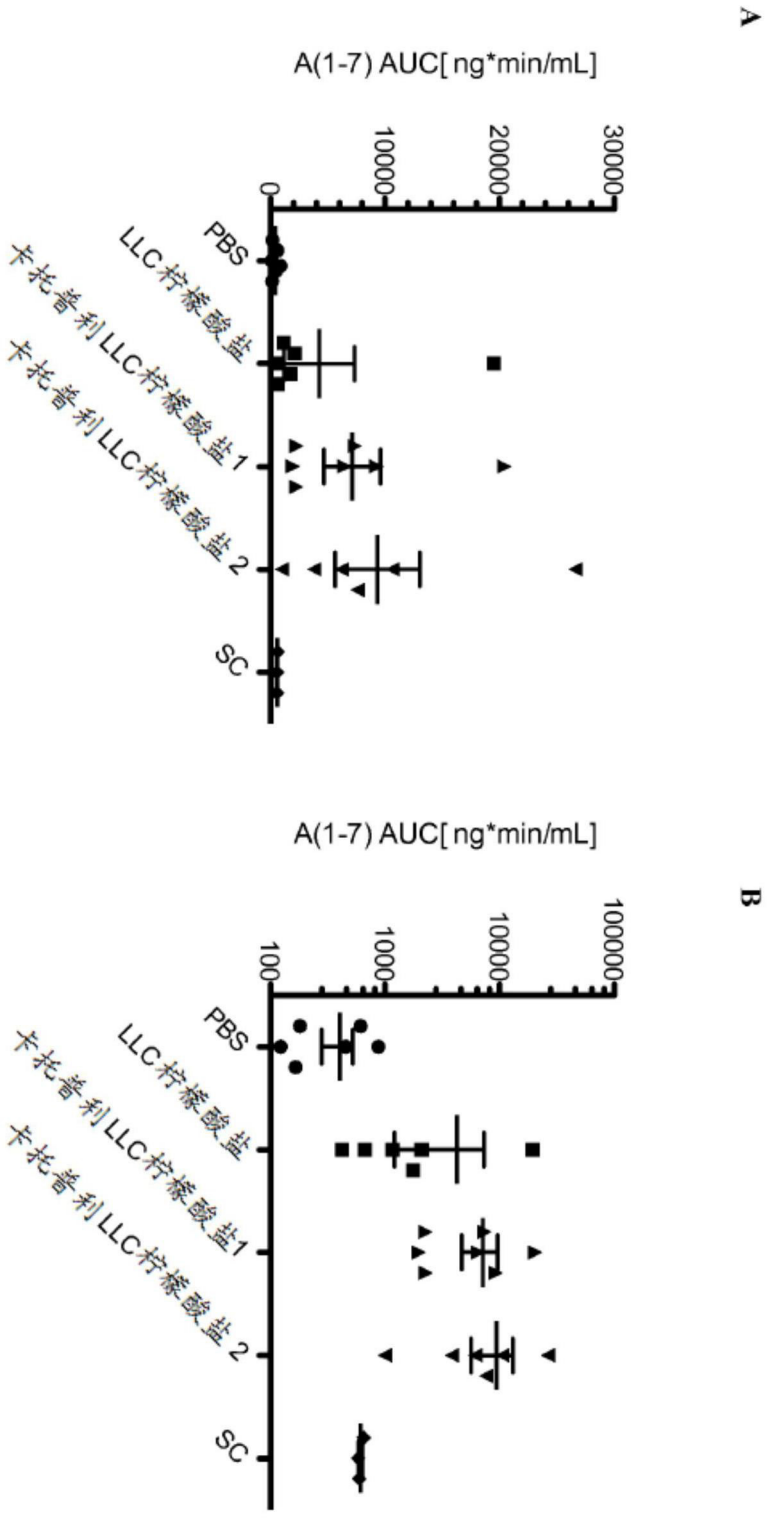

A
A(1-7) AUC[ng*min/mL]
0
10000
20000
30000
PBS
LLC柠檬酸盐
卡托普利LLC柠檬酸盐1
卡托普利LLC柠檬酸盐2
SC
B
A(1-7) AUC[ng*min/mL]
100
1000
10000
100000
PBS
LLC柠檬酸盐
卡托普利LLC柠檬酸盐1
卡托普利LLC柠檬酸盐2
SC

图 1

Abstract

The present invention provides various formulations for oral delivery of angiotensin peptides.