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(71) Applicant *(for all designated States except US)*: **AIC** [BE/BE]; Drie Eikenstraat 661, B-2650 Edegem (BE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LAMBEIR, Anne-Marie** [BE/BE]; Sparrendreef 35, B-3001 Heverlee (BE). **BRANDT, Inger** [BE/BE]; Hogebrug 90, B-9280 Lebbeke (BE). **SCHARPÉ, Simon** [BE/BE]; Kerkhofstraat 7, B-9280 Wieze (BE). **DE MEESTER, Ingrid** [BE/BE]; Fort 7-straat 7, B-2610 Wilrijk (BE).

(74) Agent: **BRANTS, Johan, Philippe, Emile**; Edgard Gevaertdreef 10a, B-9830 Sint-Martens-latem (BE).

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(54) Title: BNP AGONISTS

(57) Abstract: The present invention relates to dipeptidyl peptidase resistant BNP agonists, their synthesis and therapeutic applications thereof. In particular the present invention relates to compounds of formula (I): R-Xaa1-Xaa2-Xaa3-Pep-OH ; wherein R is selected from the group comprising hydrogen, acetyl, formyl, or alkyl, wherein Xaa1 is an amino-acid selected from serine, alanine, threonine or asparagine, Xaa2 is a L-amino acid or a D-amino acid, different from L-proline, L-alanine, L-glycine or L-serine, optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Xaa3 is a L-amino acid or a D-amino acid, optionally substituted by at least one substituent selected from alkyl, acetyl, or aryl, wherein Xaa1, Xaa2, Xaa3 are linked to each other by peptide bonds or pseudo-peptide bonds, and wherein Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure said ring comprising between 12 and 20 amino acids, wherein the disulfide bond is formed by two cysteines.

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BNP agonists

Field of the invention

The present invention relates to methods for treatment and/or prevention of cardiac and cardiovascular diseases. More specifically, the methods and uses of the invention relate to the therapeutic use of synthetic BNP agonists.

Background of the invention

The natriuretic peptide family consists of four members which all have a characteristic 17 amino acid residue ring formed by an intramolecular disulfide bridge between two cysteine residues. Before they are released into the circulation they each exist as a high molecular weight pro-hormone [1]. The heart secretes two cardiac natriuretic peptides, atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which are involved in the regulation of extracellular fluid and blood pressure homeostasis by their potent natriuretic, diuretic, vasodilatory, and cell growth inhibitory activities. In addition, ANP and CNP promote bone proliferation and differentiation via the action of cGMP in a signal-transduction pathway mediated by specific receptors in osteoblast-like cells from rat calvariae and in cultured fetal mouse tibias (Hagiwara et al. (1996) Am. J. Physiol. 270,C1311-8, Yasoda et al. (1998) J. Biol. Chem. 273,11695-11700).

Cleavage of human pro-atrial natriuretic peptide, which contains 126 amino acids, by endoprotease releases a 98 amino acid aminoterminal fragment (NT-ANP) and the 28 amino acid active hormone into the circulation [2]. BNP prohormone, proBNP, consists of 108 amino acids and is produced mainly by the cardiac myocytes. The biologically active BNP-32 (starting with the amino acids Ser-Pro-Lys...) is separated from the N-terminal part of the prohormone, termed NT-proBNP. The ubiquitous endoprotease furin as well as the serine protease corin have been suggested to be involved in the maturation of proBNP [3]. The main stimulus for BNP peptide synthesis is cardiac wall stress. The physiological effects of BNP-32 are caused by binding to the cyclic GMP bound natriuretic peptide receptor type A. The biological effects thus produced include diuresis, vasodilatation, inhibition of renin and aldosterone production and of cardiac and vascular myocyte growth [4]. The clearance of natriuretic peptides involves two main pathways; the relative importance of these two mechanisms in the clearance is controversial. The first way of clearing the natriuretic peptides from the plasma is by binding to the natriuretic peptide receptor type C (NPR-C) (ANP>BNP). The ligand/NPR-C receptor is internalized and lysosomal hydrolysis of the ligand and subsequent recycling of the receptor to the cell surface occurs [5]. Another way in which natriuretic peptides are cleared is through

proteolysis by peptidases, the most closely studied being neutral endopeptidase (NEP, EC 3.4.24.11). This zinc metalloproteinase is widely distributed on the surface of endothelial cells, smooth-muscle cells, cardiac myocytes and fibroblasts and is particularly concentrated at the brush border membranes in the proximal tubule of the kidney. By cleaving the natriuretic peptides and thereby opening the ring structure, it inactivates the peptides [1]. Compared to ANP, BNP seems relatively resistant to NEP degradation [6].

Dipeptidyl peptidases (DPPs, EC 3.4.14) have been identified in various mammalian tissues and catalyze the sequential release of dipeptides from peptides. Among these enzymes, DPP II (EC 3.4.14.2, also called QPP or DPP7), DPP IV (EC 3.4.14.5), DPP8 and DPP9 preferentially release N-terminal dipeptide moieties (Xaa-Pro- or Xaa-Ala-) from some oligopeptides or proteins.

Fibroblast Activation Protease belongs to the same family of proteases (prolyloligopeptidase family) and has endopeptidase as well as dipeptidyl peptidase activity. Dipeptidyl peptidase IV and fibroblast activation protease are cell-surface proteases. Dipeptidyl peptidase IV is a highly specific exopeptidase with a serine type mechanism of peptidase activity, cleaving off dipeptides from the amino-terminus of peptides with proline or alanine at the penultimate position [reviewed in 8]. Cleavage after a penultimate Ser, Gly, Thr, Val and Leu has also been observed [9-12]. It plays, for example, an important role in glucose homeostasis through proteolytic inactivation of the incretins, glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). DPP IV is constitutively expressed on epithelial and endothelial cells of a variety of different tissues, and is also found in body fluids. In the hematopoietic system, DPP IV was identified as the leukocyte antigen CD26 (DPP IV/CD26; EC 3.4.14.5).

Several of the documented DPP IV substrates (incretins, growth factors, neuropeptides, chemokines) have multiple biological functions. For example GLP-1 has been shown to have an antihypertensive effect in a specific rat model [22], apart from its insulin releasing action and its function as a growth factor for pancreatic islet cells. Neuropeptide Y plays a role in the development of left ventricular hypertrophy in vivo [23]. The neuropeptide Y receptor types 1 and 2 play opposite roles in this process. DPP IV catalyzed truncation shifts the receptor specificity of neuropeptide Y [8]. Growth hormone and vasoactive intestinal peptide play a role in fluid homeostasis [24] and growth hormone releasing hormone improves cardiac dysfunction and cachexia and suppresses stress-related hormones and cardiomyocyte apoptosis in rats with heart failure [25]. Atrial fibrillation was shown to be associated with high titers of circulating SDF-1alpha. This chemokine plays a

role in the migration of hematopoeietic (and endothelial) progenitor cells to the site of damage after heart injury [26].

Determination of BNP concentrations in blood samples is used as a marker in cardiology. Patients with left ventricular dysfunction have elevated concentrations of BNP and proBNP fragments in their blood. Measurement thereof is used in diagnostic procedures. In addition natriuretic peptides, in particular BNP, are used therapeutically (eg as Nesiritide) in cardiac diseases.

Several patent applications relate to the use of natriuretic peptides. ANP and BNP are rapidly degraded and have a rapid clearance. Conjugates have been proposed to prevent proteolytic degradation and to increase the circulating half-life of the peptides. The use of DPP IV inhibitors to prevent N-terminal degradation has also been described. While this approach has met with success, it is costly, time consuming, and fraught with uncertainty in terms of pharmacokinetics and toxicity. There is therefore a need for natriuretic peptides resistant to degradation.

Summary of the invention:

The present invention provides dipeptidyl peptidase resistant natriuretic peptide agonists. In particular the present invention provides a compound having the structural formula I

R-Xaa1-Xaa2-Xaa3-Pep-OH
I

wherein R is selected from the group comprising hydrogen, acetyl, formyl, or alkyl,

wherein Xaa1 is an amino-acid selected from serine, alanine, threonine or asparagine, Xaa2 is a L-amino acid or a D-amino acid, different from L-proline, L-alanine, L-glycine or L-serine optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Xaa3 is a L-amino acid or a D-amino acid, optionally substituted by at least one substituent selected from alkyl, acyl, or aryl, wherein Xaa1, Xaa2, Xaa3 are linked to each other by peptide bonds or pseudo-peptide bonds,

and wherein Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure said ring comprising between 12 and 20 amino acids, wherein the disulfide bond is formed by two cysteines.

In a particular embodiment, the ring structure is of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acid. Preferably n is 15. In an embodiment, the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is

independently from each other an L or D amino acid. In another embodiment, the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid. In a further embodiment, the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid. Preferably the ring structure further comprises the sequence Asp-Arg-Ile. Preferably the ring structure is of formula Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys. In a particular embodiment, Pep comprises the 29 C-terminal amino acid sequence of BNP-32, mature BNP or a homolog thereof. Preferably, Pep comprises the sequence Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His wherein the two Cys form a disulfide bond, or a homologue thereof.

In a particular embodiment, Xaa2 is a D-amino acid. In another particular embodiment, Xaa1 is ser and Xaa2 is a D-Ala. According to an embodiment of the invention, Xaa3 is N-methyl-lysine. In another particular embodiment, R is acetyl, formyl or alkyl, preferably acetyl or methyl, more preferably acetyl.

According to an embodiment of the invention, Xaa1, Xaa2, Xaa3 are linked to each other by peptide bonds. According to another embodiment of the invention, Xaa1, Xaa2, Xaa3 are linked to each other by “pseudo-peptide” bonds. For example, Xaa2 and Xaa3 are linked to each other by a “pseudo-peptide” bond. In an embodiment said pseudo-peptide bond is selected from an N-methyl peptide bond, a ketomethylene group, a hydroxyethylene, an ethylene group, a carba group, an ether bond, a reduced amide bond, a urea bond or a retro-inverso dipeptide.

The present invention also provides a pharmaceutical composition comprising a compound according to the present invention and a pharmaceutically acceptable carrier.

The present invention also provides a compound according to the invention for use as a medicament. The present invention also encompasses the use of a compound according to the present invention, for the preparation of a medicament for preventing or treating at least one of the conditions selected from heart failure, nephrotic syndrome, cirrhosis of the liver, hypertension or kidney failure.

The present invention also concerns a method for treating or preventing at least one of the conditions selected from heart failure, nephrotic syndrome, cirrhosis of the liver, hypertension, kidney failure, in a subject, comprising administering to a subject a

therapeutically effective amount of a compound according to the invention, or a pharmaceutical composition according to the invention.

DPP resistant BNP agonists according to the invention are obtained by conserving the structural motifs involved in receptor binding and modifying the N-terminal amino acids of BNP or BNP-derived peptides in such a way that the resulting molecule has a reduced affinity for dipeptidyl peptidases. Such modifications include chemical modification of the terminal amine group and/or replacement of the N-terminal tripeptide by a peptidomimetic.

Brief description of the Figures

Figure 1 represents a graph plotting the time course of truncation of natriuretic peptides by DPP IV: Time course of truncation of 5 µM BNP-32 (—●—), 5 µM prepro ANF (26-55) (—■—) and 5 µM prepro ANF (104-123) (—▲—). Each peptide was incubated independently with 29 U/l DPP IV at 37°C.

Figure 2 represents a graph plotting the K_m and k_{cat} determination of BNP-32 truncation by DPP IV: Varying concentrations of BNP-32 were incubated with DPP IV at 37°C. The average rate of conversion was plotted versus the average substrate concentration of the chosen time interval. The results were directly fitted to the Michaelis-Menten equation. Inset: lineweaver-burk plot. The reported K_m and k_{cat} is the result of three independent measurements.

Figure 3 represents a graph plotting the inhibition of DPP IV-catalyzed truncation of BNP-32: Decay of BNP-32 with 29 U/L DPP IV in presence of increasing concentrations of Vildagliptin, (100 nM, 50 nM, 25 nM, 10 nM, 0 nM).

Figure 4 represents in 4a and 4b MS spectra showing for two patients the truncation of intact BNP (1-32) upon incubation (1 h, room temperature) of EDTA plasma with BNP in the absence (upper panels) or presence (lower panels) of 1 µmol/L Vildagliptin. Two individual patient samples are shown (Fig. 4a and Fig. 4b); the ions with m/z 693 and 496 belong to the intact BNP(1-32) and the ions with m/z 656 and 469 to the truncated BNP(3-32).

Detailed description

A "*BNP agonist*" as used herein refers to a molecule which imitates the function of BNP on at least one of its receptors.

A "*peptidomimetic*" as used herein refers to a molecule bearing identifiable resemblance to a peptide that can imitate the effect of a natural peptide.

An "*amino acid*" refers to a molecule that contains both amino and carboxylic acid functional groups. In an embodiment, an amino acid refers to alpha amino acids in which the amino and carboxylate functionalities are attached to the same carbon, the so-called α–carbon. The full name or the standard three letter codes for amino acids are used throughout this text. Unless specified otherwise, these represent the natural L-amino acids: alanine - Ala; arginine - Arg ; asparagine - Asn; aspartic acid - Asp; cysteine - Cys; glutamine - Gln; glutamic acid - Glu; glycine - Gly; histidine - His; isoleucine - Ile; leucine - Leu; lysine - Lys; methionine - Met; phenylalanine - Phe; proline - Pro; serine - Ser; threonine - Thr; tryptophan - Trp; tyrosine - Tyr; valine - Val. D-amino acids and N-methyl amino acids are notated as such, for e.g. D-alanine can be represented as D-Ala.

An "*amino acid residue*" refers to what is left of an amino acid once a molecule of water has been lost (an H+ from the nitrogenous side and an OH- from the carboxylic side) in the formation of a peptide bond.

A "*peptide bond*" as used herein refers to the C-N bond between two separate amino acids. The term 'peptide bond' implies the existence of the peptide group which is commonly written in text as -CONH- .

A "*pseudo-peptide bond*" as used herein refers to a peptide bond which has been replaced with an isostere or isoelectronic bond, including but not limited to N-methyl peptide bond, a ketomethylene group, a hydroxyethylene, an ethylene group, a carba group, an ether bond, a reduced amide bond, a urea bond or the insertion of a retro-inverso dipeptide.

The term "*peptide*" is used to refer to a compound of two or more amino acids joined through the main chain (as opposed to side chain) by a peptide amide bond (-CONH-). By convention, the amine end (N terminal) of an amino acid of a peptide is always on the left, while the acid end (C terminal) is on the right. The peptide alanine-alanine-alanine can be interchangeably written H-Ala-Ala-Ala-OH or H-Ala-Ala-Ala or Ala-Ala-Ala.

The term "*alkyl*" by itself or as part of another substituent refers to a hydrocarbyl radical of formula C_nH_{2n+1} wherein n is a number greater than or equal to 1. Generally, alkyl groups of this invention comprise from 1 to 20 carbon atoms, more preferably from 1 to 10 carbon atoms, still more preferably 1 to 8 carbon atoms, in particular 1 to 6 carbon atoms, preferably 1 to 4 carbon atoms. Alkyl groups may be linear or branched and may be substituted as indicated herein. When a subscript is used herein following a carbon atom, the subscript refers to the number of carbon atoms that the named group may contain. Thus, for example, C_{1-4}alkyl means an alkyl of one to four carbon atoms. Examples of

alkyl groups are methyl, ethyl, n-propyl, i-propyl, butyl and its isomers (e.g. n-butyl, i-butyl and t-butyl); pentyl and its isomers, hexyl and its isomers, heptyl and its isomers, octyl and its isomers, nonyl and its isomers; decyl and its isomers. C_1-C_6 alkyl includes all linear, branched or cyclic alkyl groups with between 1 and 6 carbon atoms, and thus includes methyl, ethyl, n-propyl, i-propyl, butyl and its isomers (e.g. n-butyl, i-butyl and t-butyl); pentyl and its isomers, hexyl and its isomers, cyclopentyl, 2-, 3- or 4-methylcyclopentyl, cyclopentylmethylene, and cyclohexyl.

The term "*aryl*" by itself or as part of another substituent refers to a polyunsaturated, aromatic hydrocarbyl group having a single ring (i.e. phenyl) or multiple aromatic rings fused together (e.g. naphthalene or anthracene) or linked covalently, typically containing 5 to 8 atoms; wherein at least one ring is aromatic. The aromatic ring may optionally include one to three additional rings (either cycloalkyl, heterocyclyl or heteroaryl) fused thereto. Aryl is also intended to include the partially hydrogenated derivatives of the carbocyclic systems enumerated herein. Non-limiting examples of aryl comprise phenyl, biphenylyl, biphenylenyl, 5- or 6-tetralinyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-azulenyl, 1- or 2-naphthyl, 1-, 2- or 3-indenyl, 1-, 2- or 9-anthryl, 1- 2-, 3-, 4- or 5-acenaphtylenyl, 3-, 4- or 5-acenaphtenyl, 1-, 2-, 3-, 4- or 10-phenanthryl, 1- or 2-pentalenyl, 1, 2-, 3- or 4-fluorenyl, 4- or 5-indanyl, 5-, 6-, 7- or 8-tetrahydronaphthyl, 1,2,3,4-tetrahydronaphthyl, 1,4-dihydronaphthyl, dibenzo[a,d]cylcoheptenyl, 1-, 2-, 3-, 4- or 5-pyrenyl.

The term "*acyl*" by itself or as part of another substituent refers to an alkanoyl group having 2 to 6 carbon atoms or a phenylalkanoyl group whose alkanoyl moiety has 1 to 4 carbon atoms, i.e. a carbonyl group linked to a radical such as, but not limited to, alkyl, aryl, more particularly, the group $-COR^1$, wherein R^1 can be selected from alkyl, aryl, substituted alkyl, or substituted aryl, as defined herein. The term acyl therefore encompasses the group alkylcarbonyl ($-COR^1$), wherein R^1 is alkyl. Said acyl can be exemplified by acetyl, propionyl, butyryl, valeryl and pivaloyl, benzoyl, phenylacetyl, phenylpropionyl and phenylbutytyl.

Whenever the term "*substituted*" is used in the present invention, it is meant to indicate that one or more hydrogens on the atom indicated in the expression using "substituted" is replaced with a selection from the indicated group, provided that the indicated atom's normal valency is not exceeded, and that the substitution results in a chemically stable compound, i.e. a compound that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into a therapeutic agent. Where groups may be optionally substituted, such groups may be substituted with once or more, and preferably once, twice or thrice.

A "*retro-inverso*" peptide is a linear peptide whose amino acid sequence is inversed and the alpha-center chirality is inversed for example comprising D-amino acids in which the amino acid residues are assembled in the opposite direction to the native peptide with respect to which it is retro-inverso modified.

The N-terminal "*tail*" of BNP consists of the 9 N-terminal amino acids that precede the disulfide-bonded ring structure that contains the receptor binding structures.

The "*specificity constant*" of an enzyme equals the ratio of the experimental parameters k_{cat}/K_m. The higher this value, the higher is the efficiency of the enzyme. This parameter also allows to predict which of two substrates will be preferentially converted by an enzyme when both are present at the same time.

By DPP IV "*resistant*" is meant that the K_m (or K_i in case the molecule or its cleavage products behave as an inhibitor of DPP IV activity) is at least 10 times and preferably 100 times larger than the K_m of BNP-32.

By "*DPP IV*" or "DPP" in this context is meant DPP IV/CD26 properly and all other proline specific dipeptidyl peptidases that are present at the site of BNP-32 production, the blood stream and the site where BNP binds to its receptors and exerts its biological activity.

The term "*homologue of a polypeptide*" as used herein refers to a polypeptide having an amino acid sequence that has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 97,5%, 98%, 98,5%, 99% or 99,5% identity with the amino acid sequence corresponding to BNP.

By a polypeptide with, for example, 95% "*identity*" to a reference amino acid sequence, it is intended that the amino acid sequence of said polypeptide is identical to the reference sequence except that the amino acid sequence may include up to five amino acid alterations per each 100 amino acids of the reference polypeptide amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence of at least 95% identity with a reference amino acid sequence, up to 5% of the amino acids in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acids in the reference sequence may be inserted into the reference sequence. As a practical matter, whether any particular polypeptide has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 97,5%, 98%, 98,5%, 99% or 99,5% identity to an amino acid sequence of the present invention can be determined using known algorithms. It should also be understood that instead of % "identity", also the corresponding % "similarity" can be used to define homologues according to the invention.

The recitation of numerical ranges by endpoints includes all integer numbers and, where appropriate, fractions subsumed within that range (*e.g.* 1 to 5 can include 1, 1.5, 2, 2.75, 3, 3.80, 4, and 5). For example, the term “n is an integer between 10 and 18” refers to “n is an integer selected from 10, 11, 12, 13, 14, 15, 16, 17 or 18”, for example, the term “t is an integer between 8 and 16” refers to “t is an integer selected from 8, 9, 10, 11, 12, 13, 14, 15 or 16”, for example, the term “s is an integer between 6 and 14” refers to “s is an integer selected from 6, 7, 8, 9, 10, 11, 12, 13 or 14”, and for example, the term “r is an integer between 4 and 12” refers to “r is an integer selected from 4, 5, 6, 7, 8, 9, 10, 11 or 12”.

As used in the specification and the appended claims, the singular forms "a", "an," and "the" include plural referents unless the context clearly dictates otherwise. By way of example, "a compound" means one compound or more than one compound.

The terms described above and others used in the specification are well understood to those in the art.

Preferred features of the compounds of this invention are now set forth.

The inventors have demonstrated the *in vitro* truncation of BNP-32 by dipeptidyl peptidase IV (DPP IV/CD26). The N-terminal truncation of BNP-32 by dipeptidyl peptidases affects the clearance rate or the susceptibility to proteolytic enzymes, in general. Nevertheless, the cleavage of BNP-32 by DPP IV has physiological implications and is critical for the biological activity of BNP-32.

The inventors have shown that BNP-32 is a very good substrate for DPP IV having a higher specificity constant than several known physiological substrates of the enzyme. An elevated concentration of BNP (equal or higher than 30 μM) – because it is a good substrate of DPP IV – interferes with the proteolytic action of DPP IV on other biologically active peptides, such as the incretins, growth factors, neuropeptides and chemokines. This is of special importance as the natriuretic peptides are used therapeutically (eg as Nesiritide) in cardiac diseases. During therapy the concentrations of natriuretic peptide are much higher than the normal physiological concentrations and their presence will result in a subtle modulation of the action of the other DPP IV peptide substrates. This will be even more pronounced when natriuretic peptides are administered together with a DPP IV inhibitor. The observation that BNP-32 is the major form of mature BNP found in the circulation and the conserved motif of the N-terminal “tail” in higher mammals (human, pig, sheep, dog, cat, cattle, but not in rodents) are additional arguments to keep this motif intact as much as possible in a therapeutic BNP agonist.

The present invention relates to a BNP agonist that resembles the intact BNP-32 peptide but is rendered DPP IV resistant by minimal modifications in the N-terminal amino acids. In addition these modifications render the molecule resistant to aminopeptidases that are able to further degrade BNP once the proline on position 2 has been removed.

The BNP agonists of the present invention have the following formula I:

R-Xaa1-Xaa2-Xaa3-Pep-OH
I

wherein R is H or a small N-terminal modification obtained by acetylation or formylation of the NH2- residue of the N-terminal amino-acid Xaa1 or by alkylation of the NH2- residue of the N-terminal amino-acid Xaa1 (for example a methylation giving a methyl amine or dimethyl-amine derivative of Xaa1),

Xaa1, Xaa2, Xaa3 as defined are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds as outlined below, wherein Xaa1 is selected from serine, alanine, threonine or asparagine, Xaa2 is a L- or D-amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, with the proviso that Xaa2 is not L-proline, L-alanine, L-glycine or L-serine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl, wherein Xaa3 is a L- or D-amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl,

and wherein Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure said ring comprising between 12 and 20 amino acids, wherein the disulfide bond is formed by two cysteines.

The present invention encompasses compounds of formula I or salt, hydrate, or solvate thereof.

Preferably, Pep is a peptide sequence comprising a ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer from 10 to 18 and each Xaa4 is independently an L or D amino acid.

In an embodiment, said ring structure comprises 17 amino acids, i.e. n is 15. In a preferred embodiment, in said ring structure the Cys most closely to the aminoterminus of the compound is followed by a -Phe-Gly sequence, i.e. the ring structure has a formula

Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently an L or D amino acid. In an embodiment, the Cys most closely to the carboxyterminus of the compound is preceded by Ser-Gly-Leu-Gly- i.e. the ring structure has a formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid. In another embodiment, the ring structure contains the sequence Asp-Arg-Ile. In another embodiment, the Cys most closely to the aminoterminus of the compound is followed by a -Phe-Gly sequence and the Cys most closely to the carboxyterminus of the compound is preceded by Ser-Gly-Leu-Gly-, i.e. the ring has a formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys, wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid. In another embodiment, the Cys most closely to the aminoterminus of the compound is followed by a -Phe-Gly sequence; the ring further contains the sequence Asp-Arg-Ile and the Cys most closely to the carboxyterminus of the compound is preceded by Ser-Gly-Leu-Gly-. In another embodiment the ring contains 17 amino acids wherein the Cys most closely to the aminoterminus of the compound is followed by a -Phe-Gly sequence; the ring further contains the sequence Asp-Arg-Ile and the Cys most closely to the carboxyterminus of the compound is preceded by Ser-Gly-Leu-Gly- . In a further embodiment, the ring structure comprises between the two cysteines: Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-.

Preferably, Pep comprises the sequence Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His wherein the two Cys form a disulfide bond, or a homologue thereof having an amino acid sequence that has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 97,5%, 98%, 98,5%, 99% or 99,5% identity with the amino acid sequence of said sequence.

In an embodiment, Pep comprises the 29 C-terminal amino acids of human BNP-32, or a homologous peptide containing conservative substitutions and deletions that do not affect the biological activity of BNP as defined by receptor binding studies and in vivo natriuretic effects. Pep can also be a homologous peptide containing an amino acid sequence of mature BNP from pig, cattle, sheep, dog, cat or another vertebrate or hybrids thereof.

In an embodiment, Xaa1 is serine.

In another embodiment, Xaa2 is a D-amino acid, for example, D-proline, D-alanine, D-glycine, D-serine, D-arginine; D-asparagine; D-aspartic acid; D-cysteine; D-glutamine; D-

glutamic acid; D-histidine; D-isoleucine; D-leucine; D-lysine; D-methionine; D-phenylalanine; D-threonine; D-tryptophan; D-tyrosine; D-valine, preferably D-alanine.

In a further embodiment, Pep comprises the 29 C-terminal amino acids of human BNP-32, or a homologous peptide thereof, containing conservative substitutions and deletions that do not affect the biological activity of BNP as defined by receptor binding studies and in vivo natriuretic effects. Pep can also be a homologous peptide containing an amino acid sequence of mature BNP from pig, cattle, sheep, dog, cat or another vertebrate or hybrids thereof. Such peptides are described in US pat. Nos 6,974,861 and 6,586,396; WO 03/081246; WO 98/17690; WO 2005/019819; WO 2005/116655; WO 2004/011498; WO 2005/072055; WO 2006/005140, all incorporated herein by reference.

In a certain embodiment of the invention, the proline on the second position of BNP-32 is replaced by a D-amino acid. In a preferred embodiment of the invention, the proline on the second position of BNP-32 is replaced by a D-alanine.

In another embodiment of the invention, the N-terminal amine group of the BNP agonist is acetylated. In a preferred embodiment of the invention the N-terminal amine group of the BNP agonist is acetylated and the second amino acid is a D-amino acid, preferably D-alanine.

In another embodiment of the invention the peptide bond between the first and second amino acid (not proline) of the BNP agonist is replaced by a “pseudo-peptide bond”. By “pseudo-peptide bond” is meant the replacement of the peptide bond by an N-methyl peptide bond, a ketomethylene group, a hydroxyethylene, an ethylene group, a carba group, an ether bond, a reduced amide bond, a urea bond or the insertion of a retro-inverso dipeptide. A preferred embodiment contains an N-methyl-D-alanine on the second position.

In another embodiment of the invention the peptide bond between the second and third amino acid of the BNP agonist is replaced by a “pseudo-peptide bond”. A preferred embodiment contains an N-methyl-D-alanine on the second position and an N-methyl-D-lysine on the third position.

In an embodiment, R is H or a small N-terminal modification obtained by acetylation or formylation, or a dimethyl-amine group, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or “pseudo-peptide” bonds, Xaa1 is an amino acid selected from the group comprising serine, alanine, threonine, and asparagine and Xaa2 is a D-amino acid.

In another embodiment, R is acetyl, formyl, or alkyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds, Xaa1 is serine, Xaa2 is D-alanine.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds, Xaa1 is serine and Xaa2 is D-alanine.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by "pseudo-peptide" bonds, Xaa1 is serine and Xaa2 is D-alanine.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond, Xaa1 is serine and Xaa2 is D-alanine.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond wherein Xaa3 is N-methyl-lysine, Xaa1 is serine and Xaa2 is D-alanine.

In another embodiment, R is acetyl, formyl or alkyl such as methyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds wherein Xaa1 is an amino acid selected from serine, alanine, threonine or asparagine, Xaa2 is a D-amino acid, Xaa3 is selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, formyl or alkyl such as methyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds wherein Xaa1 is an amino acid selected from serine, alanine, threonine or asparagine, Xaa2 is a D-amino acid and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His, and Xaa3 is selected from D or L

amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl.

In another embodiment, R is acetyl, formyl or alkyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds, Xaa1 is serine, Xaa2 is D-alanine, Xaa3 is selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, formyl or alkyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds or "pseudo-peptide" bonds, Xaa1 is serine, Xaa2 is D-alanine and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His, and Xaa3 is selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds, Xaa1 is serine, Xaa2 is D-alanine, Xaa3 is selected from selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence

comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by peptide bonds, Xaa1 is serine, Xaa2 is D-alanine and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His, and Xaa3 is selected from selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by "pseudo-peptide" bonds selected from N-methyl peptide bond, a ketomethylene group, a hydroxyethylene, an ethylene group, a carba group, an ether bond, a reduced amide bond, a urea bond or a retro-inverso dipeptide, Xaa1 is serine, Xaa2 is D-alanine, Xaa3 is selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, Xaa1, Xaa2, Xaa3 are amino acid residues, linked by "pseudo-peptide" bonds selected from N-methyl peptide bond, a ketomethylene group, a hydroxyethylene, an ethylene group, a carba group, an ether bond, a reduced amide bond, a urea bond or a retro-inverso dipeptide, Xaa1 is serine, Xaa2 is D-alanine and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His and Xaa3 is selected from D or L amino acid selected from proline, alanine, glycine, serine, arginine; asparagine; aspartic acid; cysteine; glutamine; glutamic acid; histidine; isoleucine; leucine; lysine; methionine; phenylalanine; threonine; tryptophan; tyrosine; valine, each amino acid being optionally substituted by at least one substituent selected from alkyl, aryl or acyl.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond, Xaa1 is serine, Xaa2 is D-alanine and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond, Xaa1 is serine, Xaa2 is D-alanine and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond, specifically Xaa3 is N-methyl-lysine, L-lysine, N-methyl(D)lysine or D-lysine, Xaa1 is serine, Xaa2 is D-alanine and Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acids; preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid; more preferably the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer

between 6 and 14 and each Xaa6 is independently an L or D amino acid, yet more preferably the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein r is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

In another embodiment, R is acetyl, Xaa2 and Xaa3 are amino acid residues, linked by a "pseudo-peptide" bond, specifically Xaa3 is N-methyl-lysine, L-lysine, N-methyl(D)lysine or D-lysine, Xaa1 is serine, Xaa2 is D-alanine and Pep is Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His.

The compounds of the invention may be in the form of pharmaceutically and/or veterinary acceptable salts, as generally described below. Some preferred, but non-limiting examples of suitable pharmaceutically acceptable organic and/or inorganic acids are as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, acetic acid and citric acid, as well as other pharmaceutically acceptable acids known *per se* (for which reference is made to the prior art referred to below).

When the compounds of the invention contain an acidic group as well as a basic group the compounds of the invention may also form internal salts, and such compounds are within the scope of the invention. When the compounds of the invention contain a hydrogen-donating heteroatom (e.g. NH), the invention also covers salts and/or isomers formed by transfer of said hydrogen atom to a basic group or atom within the molecule.

It will also be clear that when the desired compounds of the invention, and/or the starting materials, precursors and/or intermediates used in the preparation thereof, contain functional groups that are sensitive to the reaction conditions used in the preparation of the compounds of the invention (i.e. that would undergo undesired reactions under those conditions if they were not suitably protected) can be protected during said reaction with one or more suitable protective group, which protective group can then be suitably removed after either completion of said reaction and/or as a later or final step in the preparation of the compounds of the invention. Protected forms of the inventive compounds are included within the scope of the present invention. Suitable protective groups, as well as methods and conditions for inserting them and removing them, will be clear to the skilled person and are generally described in the standard handbooks of organic chemistry, such as Greene and Wuts, "Protective groups in organic synthesis", 3rd Edition, Wiley and Sons, 1999, which is incorporated herein by reference in its entirety. It will also be clear to the skilled person that compounds of the invention in which one or more functional groups have been protected with suitable functional groups can

find use as intermediates in the production and/or synthesis of the compounds of the invention, and as such form a further aspect of the invention.

The present invention further relates to compounds of formula I as defined herein for use as a medicament.

The present also concerns a pharmaceutical composition comprising a compound of Formula I, and a pharmaceutically acceptable carrier, excipient and/or diluent.

Preferably, the present invention relates to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a therapeutic effective amount of a compound according to the invention.

The term "therapeutically effective amount" as used herein means that amount of active compound or component or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease being treated.

For pharmaceutical use, the compounds of the invention may be used as a free acid or base, and/or in the form of a pharmaceutically acceptable acid-addition and/or base-addition salt (e.g. obtained with non-toxic organic or inorganic acid or base), in the form of a hydrate, solvate and/or complex, and/or in the form or a pro-drug or pre-drug, such as an ester. As used herein and unless otherwise stated, the term “solvate” includes any combination which may be formed by a compound of this invention with a suitable inorganic solvent (e.g. hydrates) or organic solvent, such as but not limited to alcohols, ketones, esters and the like. Such salts, hydrates, solvates, etc. and the preparation thereof will be clear to the skilled person; reference is for instance made to the salts, hydrates, solvates, etc. described in US-A-6,372,778, US-A-6,369,086, US-A-6,369,087 and US-A-6,372,733.

The pharmaceutically acceptable salts of the compounds according to the invention, i.e. in the form of water-, oil-soluble, or dispersible products, include the conventional non-toxic salts or the quaternary ammonium salts which are formed, e.g., from inorganic or organic acids or bases. Examples of such acid addition salts include acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalene-sulfonate, nicotinate, oxalate, pamoate, pectinate,

persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups may be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl–bromides and others. Other pharmaceutically acceptable salts include the sulfate salt ethanolate and sulfate salts.

The pharmaceutical composition can be prepared in a manner known per se to one of skill in the art. For this purpose, at least one compound of the present invention one or more solid or liquid pharmaceutical excipients and, if desired, in combination with other pharmaceutical active compounds, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human medicine or veterinary medicine.

Generally, for pharmaceutical use, the compounds of the inventions may be formulated as a pharmaceutical preparation comprising at least one compound of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers, diluents and excipients for use in the preparation thereof, will be clear to the skilled person; reference is again made to for instance US-A-6,372,778, US-A-6,369,086, US-A-6,369,087 and US-A-6,372,733, as well as to the standard handbooks, such as the latest edition of Remington's Pharmaceutical Sciences.

Some preferred, but non-limiting examples of such preparations include tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, ointments, creams, lotions, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders (which are usually reconstituted prior to

use) for administration as a bolus and/or for continuous administration, which may be formulated with carriers, excipients, and diluents that are suitable per se for such formulations, such as lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, polyethylene glycol, cellulose, (sterile) water, methylcellulose, methyl- and propylhydroxybenzoates, talc, magnesium stearate, edible oils, vegetable oils and mineral oils or suitable mixtures thereof. The formulations can optionally contain other pharmaceutically active substances (which may or may not lead to a synergistic effect with the compounds of the invention) and other substances that are commonly used in pharmaceutical formulations, such as lubricating agents, wetting agents, emulsifying and suspending agents, dispersing agents, desintegrants, bulking agents, fillers, preserving agents, sweetening agents, flavoring agents, flow regulators, release agents, and the like. The compositions may also be formulated so as to provide rapid, sustained or delayed release of the active compound(s) contained therein, for example using liposomes or hydrophilic polymeric matrices based on natural gels or synthetic polymers. In order to enhance the solubility and/or the stability of the compounds of a pharmaceutical composition according to the invention, it can be advantageous to employ α-, β- or γ-cyclodextrins or their derivatives. In addition, co-solvents such as alcohols may improve the solubility and/or the stability of the compounds. In the preparation of aqueous compositions, addition of salts of the compounds of the invention can be more suitable due to their increased water solubility.

The pharmaceutical preparations of the invention are preferably in a unit dosage form, and may be suitably packaged, for example in a box, blister, vial, bottle, sachet, ampoule or in any other suitable single-dose or multi-dose holder or container (which may be properly labeled); optionally with one or more leaflets containing product information and/or instructions for use. Generally, such unit dosages will contain between 1 and 1000 mg, and usually between 5 and 500 mg, of the at least one compound of the invention, e.g. about 10, 25, 50, 100, 200, 300 or 400 mg per unit dosage.

The compounds can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous, intravenous, intramuscular or intranasal routes, depending mainly on the specific preparation used and the condition to be treated or prevented, and with transdermal, subcutaneous and intravenous administration usually being preferred.

The compound of the invention will generally be administered in an effective amount, which, upon suitable administration, is sufficient to achieve the desired therapeutic or prophylactic effect in the individual to which it is administered. Usually, depending on the

condition to be prevented or treated and the route of administration, such an effective amount will usually be between 0.01 μg to 1000 mg per kilogram body weight, preferably between 0.01-1000 μg/kg per kilogram body weight, more often between 0.1 and 500 mg per kilogram body weight, such as between 0.1 and 250 mg, for example about 0.1, 1, 5, 10, 20, 50, 100, 150, 200 or 250 mg, per kilogram body weight of the patient per day, which may be administered as a single daily dose, divided over one or more daily doses, or essentially continuously, e.g. using a drip infusion. The amount(s) to be administered, the route of administration and the further treatment regimen may be determined by the treating clinician, depending on factors such as the age, gender and general condition of the patient and the nature and severity of the disease/symptoms to be treated. Reference is again made to US-A-6,372,778, US-A-6,369,086, US-A-6,369,087 and US-A-6,372,733 and the further prior art mentioned above, as well as to the standard handbooks, such as the latest edition of Remington's Pharmaceutical Sciences. It will be understood, however, that specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition.

The present invention further relates to the use of a BNP agonist in the preparation of a medicament for the treatment of cardiac diseases.

The present invention further relates to the use of a BNP agonist with a modified amino terminus in the preparation of a medicament for the treatment of cardiac diseases.

The present invention further relates to the use of a DPP resistant BNP agonist with a modified amino terminus as defined herein in the preparation of a medicament for the treatment of cardiac diseases. The present invention further relates to method for treating or preventing cardiac diseases in a subject, comprising administering to a subject in need thereof a therapeutically effective amount of a compound or a composition according to the invention.

A number of disease states are characterized by abnormal fluid retention, including congestive heart failure, cirrhosis of the liver, and nephrotic syndrome. These diseases are associated with excessive fluid accumulation on the venous side of circulation, and an underperfusion of the kidneys, leading to a fall in glomerular filtration rate. Hypertension is another result of an increase in extracellular fluid volume and is a major risk factor for cardiac disease.

BNP agonists according to the invention, and compositions containing them, can find use as therapeutic agents in the treatment of various edematous states such as, for example, congestive heart failure, nephrotic syndrome and hepatic cirrhosis, in addition to hypertension and renal failure due to ineffective renal perfusion or reduced glomerular filtration rate. The BNP agonists of the invention are particularly effective in the treatment of congestive heart failure.

The BNP agonists of the invention are particularly effective in treating or preventing at least one of the conditions selected from heart failure, nephrotic syndrome, cirrhosis of the liver, hypertension, kidney failure, in a subject.

The invention will now be illustrated by means of the following synthetic and biological examples, which do not limit the scope of the invention in any way.

Example 1: In vitro truncation of natriuretic peptides with purified human DPPIV

Soluble DPP IV was purified from human seminal fluid as described before [14]. Natriuretic peptides were synthesized using standard solid phase peptide synthesis (Bachem).

To study the decay curves of the natriuretic peptide substrates, BNP-32, prepro-atrial natriuretic factor (26-55) and prepro-atrial natriuretic factor (104-123), 70 µl of DPP IV was mixed with 70 µl of a 10 µM substrate solution and incubated at 37 °C in presence of 50 mM Tris/HCl buffer, pH 7.5, 1 mM EDTA. The final DPP IV concentration was 29 U/L. One unit of DPP IV activity is the amount of enzyme required to catalyze the conversion of 1 micromole of substrate per minute in presence of 0.5 mM Gly-Pro-*p*NA and 50 mM Tris buffer, pH 8.3 at 37 °C. The substrate concentration was 5 µM. At certain time intervals, 20 µl samples were withdrawn and quenched in 0.2% trifluoroacetic acid (TFA). C18 ZipTips (Millipore Corp., Bedford, MA) were used to desalt the samples. Elution was performed step-wise with 10 µl of 30 and 50 % acetonitrile in 0.1% acetic acid. The composition of the mixture was determined with an Esquire ESI Ion Trap mass spectrometer (Bruker, Bremen, Germany). The instrument was used in a normal range, normal resolution setting, optimized on an *m/z* value near the most abundant ion of the intact peptide. The spectra were deconvoluted and the concentrations of the intact and truncated peptides were calculated from their relative abundance. The concentration of intact peptide was plotted against time.

Concentrations of BNP-32 varying from 5 to 100 µM were incubated with DPP IV at 37°C for 2-8 minutes (10-50% conversion). The amount of converted substrate was calculated from the relative abundance of intact and cleaved forms. The average rate of conversion

was plotted versus the average substrate concentration of the chosen time interval [15]. The results were directly fitted to the Michaelis-Menten equation.

In Figure 1 the time course of degradation BNP-32, prepro-atrial natriuretic factor (ANF) (26-55) and prepro-ANF (104-123) by DPPIV is shown. The DPP IV activity used is similar to the normal level in human plasma. In addition to BNP-32, prepro-ANF (26-55) and prepro-ANF (104-123) were also incubated with DPP IV, since it was reported that other fragments of the pro-atrial natriuretic peptide also circulate in plasma. Several data suggest that these pro-ANF fragments may be even more important than ANP as a natriuretic hormone [1]. Prepro-ANF (26-55), like BNP-32, carries a proline in the penultimate position of its N-terminus and thereby is a possible candidate for DPP IV cleavage. This peptide is also known as long acting natriuretic peptide. It has potent vasodilatory properties and is present in higher concentration in patients with congestive heart failure [16, 17]. Prepro-ANF(104-123) is also known as kaliuretic peptide, because it has the strongest potassium excreting properties of all atrial natriuretic peptides in humans. In addition this peptide has blood pressure lowering and diuretic properties [18, 19]. In its N-terminal penultimate position it has a serine, which is not the preferred amino acid for DPP IV in this position. However since several known DPP IV substrates, such as glucagon, vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP), have a serine in this position [8], the possible cleavage of this substrate by DPP IV was also analyzed. With this particular enzyme preparation the degradation of BNP-32 was much faster than the one of prepro-ANF(26-55). Prepro-ANF(104-123) was not degraded by DPP IV during the incubation time and in the conditions used in this experiment.

Fitting the data on concentration dependency with the Michaelis-Menten equation (figure 2) yielded a K_m of 35 ± 4 µM and a k_{cat} of 13 s^{-1}. The resulting specificity constant k_{cat}/K_m of 0.37 $\mu M^{-1}.s^{-1}$ was compared with the ones from other DPP IV substrates, studied with DPP IV from the same source and in an identical experimental set-up. BNP-32 clearly is a very good substrate for DPP IV having a higher specificity constant than GLP-1, substance P, GIP and several chemokines. For these substrates the *in vivo* relevance has been proven [8].

Table 1: specificity constants for BNP in comparison with other DPP IV peptide substrates.

Peptide substrate	**k_{cat}/K_m $\mu M^{-1}.s^{-1}$**
NPY	0,76
GRP	1,8

Peptide substrate	k_{cat}/K_m $\mu M^{-1}.s^{-1}$
PACAP27	0,0042
GLP-1	0,2
GLP-2	0,02
PACAP38	0,024
GIP	0,22
Mig	0,4
IP-10	0,5
I-TAC	1,2
SDF-1alfa	5
LD78beta	0,003
RANTES	0,04
Eotaxin	0,08
MDC (1-69)	4
MDC (3-69)	0,5
BNP	0,37
SP(1-11)	0,134
SP(3-11)	0,107
GIP	0,0796

Example 2: Use of Vildagliptin as DPPIV inhibitor to prevent ex vivo cleavage of natriuretic peptides by DPPIV.

Vildagliptin was custom synthesized by GLSynthesis Inc. (Worcester, MA, USA). Soluble DPP IV was purified from human seminal fluid as described before [14].

The substrate (Gly-Pro-pNA) concentration was 5 µM and Vildagliptin concentrations varied between 10 and 100 µM.

The reaction between DPP IV and BNP-32, and its inhibition by Vildagliptin, was followed by determining the composition of the reaction mixture at several time points using mass spectrometry as described above.

There was an increase in the inhibition of the cleavage of BNP-32 by DPP IV by adding progressive amounts of the specific DPP IV inhibitor Vildagliptin (NVP-LAF237/(2S)-{[(3-hydroxyadamantan-1-yl)amino]acetyl}-pyrrolidine-2-carbonitrile) (Figure 3). This compound was developed by Novartis as a potential once daily therapeutic agent for type 2 diabetes. Phase III safety and efficacy clinical trials were started in the first quarter of 2004 [21]. The inhibitor concentrations used in our experiments were at the least 20 times lower than the C_{max} of the inhibitor in patients with type 2 diabetes treated with Vildagliptin for 28 days [21].

In order to study the truncation of BNP[1-32] in human EDTA-plasma, 5 µL of a 100 µmol/L solution of BNP[1-32] was added to 90 µL of EDTA-plasma (n=5) with or without 1 µmol/L of Vildagliptin (n=5). The samples were incubated at room temperature (22° C). After 1 hour 0.25% TFA was added and the samples were centrifuged twice for 5 minutes at 12 000 rpm (Microfuge®18 Centrifuge, Beckman Coulter). The resulting supernatants were desalted and analyzed as described above.

When BNP[1-32] was added exogenously to human EDTA-plasma at room temperature, more than 50% was degraded to *des*-SerPro-BNP during an incubation of one hour (Figure 4). This truncation was prevented by adding Vildagliptin, indicating that the endogenous DPP IV present in plasma is able to truncate BNP[1-32]. Under identical conditions, the incubation of BNP[1-32] with 20 U/L of purified DPP IV in PBS also resulted in a partial, although slightly faster degradation.

The experiments described in examples 1 and 2 demonstrate that BNP is degraded by DPP IV in the blood and that this truncation can be avoided by making a DPP IV resistant BNP agonist.

Example 3: Synthesis and properties of a DPP IV resistant BNP agonist.

The DPP resistant BNP agonists of this invention can be synthesized by standard Fmoc solid phase peptide synthesis or by chemically combining (using solution phase methods) the N-terminal peptidomimetic portion to a peptide produced by recombinant methods.

Introduction of pseudo-amide bonds between the first and second and/or second and third amino acid of the BNP agonists render them DPP resistant. Replacements for peptide bonds are well known to those skilled in the art and include N-methyl, ketomethylene, hydroxyethylene, (E)-ethylene, carba, ether, reduced amide, retro-inverso, phosphonoamide, phosphonate, phosphinate, and urea bonds. A good source of synthetic methods is "Houben-Weyl Methods of Organic Chemistry, 4th Edition, additional an supplementary volumes, Vol E22: Synthesis of Peptides and Peptidomimetcs, Thieme Chemistry". The peptidomimetic N-terminal tripeptide building block can be synthesized in solution and coupled to the rest of the molecule by solid phase chemistry.

The BNP agonist N-acetyl-Ser-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His, can be synthesized using standard Fmoc solid phase synthesis procedures using, for example, the Applied Biosystems ABI 433A peptide synthesizer. Protected D-alanine and N-methyl-lysine as well as the common L-amino acid building blocks are available from commercial suppliers. Amino terminal acetylation is achieved by reacting with acetic

anhydride/pyridine. The peptide is purified by reverse phase chromatography, the correct sequence, formation of the disulfide bridge and the purity is verified using a mass spectrometer such as the Bruker Esquire 4000 ESI ion trap.

To study the DPP resistance of the BNP agonist, 10 µl of DPP IV is mixed with 10 µl of a 10 µM BNP agonist solution and incubated at 37 °C in presence of 50 mM Tris/HCl buffer, pH 7.5, 1 mM EDTA. The final DPP IV concentration is 29 U/L. In a separate vessel the same mixture is made except for the addition of the DPP IV inhibitor Vildagliptin (10 µM final). In a separate vessel the BNP agonist is diluted in reaction buffer without DPP IV or Vildagliptin (control sample). After 1 hour of incubation the solutions are quenched in 0.2% trifluoroacetic acid (TFA). C18 ZipTips (Millipore Corp., Bedford, MA) are used to desalt the samples. Elution is performed step-wise with 10 µl of 30 and 50 % acetonitrile in 0.1% acetic acid. The composition of the mixture is determined with an Esquire ESI Ion Trap mass spectrometer (Bruker, Bremen, Germany). The instrument is used in a normal range, normal resolution setting, optimized on an *m/z* value near the most abundant ion of the intact molecule. Comparison of the mass spectra of the three samples allows to estimate the percentage of DPP IV catalyzed hydrolysis of the BNP agonist. A DPP resistant agonist is found to be converted for less than 20% compared to natural BNP subjected to an identical incubation with DPPIV.

To verify whether the BNP agonist has affinity for DPP IV (without being cleaved) the K_i is determined using six BNP agonist concentrations (ranging from 10 µM to 1 mM final) and six concentrations of the chromogenic substrate Gly-Pro-pNA (in presence of 2 U/L DPP IV). The assay buffer is 50 mM TRIS, pH 8.3. The initial rate is measured in a volume of 200 µl using a kinetic method on the SpectramaxPlus microtiterplate reader using untreated flat bottom 96 well microtiterplates (NUNC). The release of p-nitroanilide is measured spectrofotometrically at 405 nm during 10 min. Readings are performed in duplicate. The K_i is determined from the dependency of the initial rate on the substrate and inhibitor (= BNP agonist) concentration. A DPP resistant BNP agonist has a K_i > 300 µM.

Table 2 shows the results for compounds of Formula I. As used herein the term "ND" means "not determined". Disulfide Bridge: Cys10-Cys26 means that the BNP agonist sequence comprises a disulfide bond between the cysteine at position 10 and the cysteine at position 26.

Table 2

SEQ ID NO:	Sequence BNP agonist	K_i µM.
1	N-acetyl-Ser-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	> 300
2	N-acetyl-Ala-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
3	N-acetyl-Thr-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
4	N-acetyl-Asp-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
5	N-acetyl-Ser-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
6	N-acetyl-Ala-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
7	N-acetyl-Thr-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
8	N-acetyl-Asp-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
9	N-acetyl-Ser-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
10	N-acetyl-Ala-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
11	N-acetyl-Thr-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
12	N-acetyl-Asp-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
13	N-acetyl-Ser-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
14	N-acetyl-Ala-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
15	N-acetyl-Thr-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
16	N-acetyl-Asp-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
17	N(dimethyl)Ser-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
18	N(dimethyl)Ala-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
19	N(dimethyl)Thr-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
20	N(dimethyl)Asp-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
21	N(dimethyl)Ser-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
22	N(dimethyl)Ala-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
23	N(dimethyl)Thr-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
24	N(dimethyl)Asp-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
25	N(dimethyl)Ser-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His,	ND
26	N(dimethyl)Ala-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
27	N(dimethyl)Thr-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
28	N(dimethyl)Asp-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
29	N(dimethyl)Ser-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
30	N(dimethyl)Ala-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
31	N(dimethyl)Thr-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
32	N(dimethyl)Asp-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
33	N-acetyl-Ser-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	> 300
34	N-acetyl-Ala-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
35	N-acetyl-Thr-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
36	N-acetyl-Asp-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
37	N-acetyl-Ser-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
38	N-acetyl-Ala-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
39	N-acetyl-Thr-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
40	N-acetyl-Asp-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
41	N-acetyl-Ser-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
42	N-acetyl-Ala-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
43	N-acetyl-Thr-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
44	N-acetyl-Asp-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
45	N-acetyl-Ser-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
46	N-acetyl-Ala-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
47	N-acetyl-Thr-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
48	N-acetyl-Asp-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
49	N(dimethyl)Ser-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
50	N(dimethyl)Ala-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
51	N(dimethyl)Thr-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
52	N(dimethyl)Asp-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
53	N(dimethyl)Ser-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
54	N(dimethyl)Ala-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
55	N(dimethyl)Thr-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
56	N(dimethyl)Asp-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
57	N(dimethyl)Ser-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
58	N(dimethyl)Ala-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
59	N(dimethyl)Thr-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
60	N(dimethyl)Asp-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
61	N(dimethyl)Ser-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
62	N(dimethyl)Ala-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
63	N(dimethyl)Thr-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
64	N(dimethyl)Asp-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
65	H-Ser-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	> 300
66	H-Ala-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
67	H-Thr-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
68	H-Asp-D-Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
69	H-Ser-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
70	H-Ala-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
71	H-Thr-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
72	H-Asp-D-Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
73	H-Ser-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
74	H-Ala-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
75	H-Thr-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
76	H-Asp-N-methyl(D)Ala-N-methyl-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
77	H-Ser-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
78	H-Ala-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
79	H-Thr-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
80	H-Asp-N-methyl(D)Ala-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
81	H-Ser-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	> 300
82	H-Ala-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
83	H-Thr-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
84	H-Asp-D-Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile- Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
85	H-Ser-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
86	H-Ala-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
87	H-Thr-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
88	H-Asp-D-Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
89	H-Ser-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

SEQ ID NO:	Sequence BNP agonist	K_i µM.
90	H-Ala-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
91	H-Thr-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
92	H-Asp-N-methyl(D)Ala-N-methyl(D)Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
93	H-Ser-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
94	H-Ala-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
95	H-Thr-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND
96	H-Asp-N-methyl(D)Ala-D-Lys-Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His (Disulfide Bridge: Cys10-Cys26)	ND

The present invention encompasses the agonists of SEQ ID No: 1 to 96 or a salt, hydrate, or solvate thereof.

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Claims

1. A compound having the structural formula I

R-Xaa1-Xaa2-Xaa3-Pep-OH
I

wherein R is selected from the group comprising hydrogen, acetyl, formyl, or alkyl,

wherein Xaa1 is an amino-acid selected from serine, alanine, threonine or asparagine, Xaa2 is a L-amino acid or a D-amino acid, different from L-proline, L-alanine, L-glycine or L-serine, optionally substituted by at least one substituent selected from alkyl, aryl or acyl, and Xaa3 is a L-amino acid or a D-amino acid, optionally substituted by at least one substituent selected from alkyl, acetyl, or aryl, wherein Xaa1, Xaa2, Xaa3 are linked to each other by peptide bonds or pseudo-peptide bonds,

and wherein Pep is a peptide sequence comprising not more than 29 amino acid residues said sequence comprising a disulfide linked ring structure said ring comprising between 12 and 20 amino acids, wherein the disulfide bond is formed by two cysteines.

2. A compound according to claim 1, wherein the ring structure is of formula Cys-$(Xaa4)_n$-Cys, wherein n is an integer between 10 and 18 and each Xaa4 is independently from each other an L or a D amino acid.

3. A compound according to claim 1 or 2, wherein n is 15.

4. A compound according to any of claims 1 to 3, wherein the ring structure is of formula Cys-Phe-Gly-$(Xaa5)_t$-Cys, wherein t is an integer between 8 and 16 and each Xaa5 is independently from each other an L or D amino acid.

5. A compound according to any of claims 1 to 3, wherein the ring structure is of formula Cys-$(Xaa6)_s$-Ser-Gly-Leu-Gly-Cys wherein s is an integer between 6 and 14 and each Xaa6 is independently an L or D amino acid.

6. A compound according to any of claims 1 to 3, wherein the ring structure is of formula Cys-Phe-Gly-$(Xaa7)_r$-Ser-Gly-Leu-Gly-Cys wherein n is an integer between 4 and 12 and each Xaa7 is independently an L or D amino acid.

7. A compound according to any of claims 1 to 6, wherein the ring structure comprises the sequence Asp-Arg-Ile.

8. A compound according to any of claims 1 to 7, wherein the ring structure comprises between the two cysteines Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly.

9. A compound according to any of claims 1 to 8, wherein Pep comprises the 29 C-terminal amino acid sequence of BNP-32, mature BNP or a homolog thereof.

10. A compound according to any of claims 1 to 9, wherein Xaa2 is a D-amino acid.

11. A compound according to any of claims 1 to 10, wherein Xaa2 is D-alanine.

12. A compound according to any of claims 1 to 11, wherein Xaa1 is serine and Xaa2 is D-alanine.

13. A compound according to any of claims 1 to 12, wherein R is acetyl, formyl or alkyl.

14. A compound according to any of claims 1 to 13, wherein Xaa1, Xaa2, Xaa3 are linked to each other by peptide bonds.

15. A compound according to any of claims 1 to 13, wherein Xaa1, Xaa2, Xaa3 are linked to each other by "pseudo-peptide" bonds.

16. A compound according to any of claims 1 to 13, wherein Xaa2 and Xaa3 are linked to each other by a "pseudo-peptide" bond.

17. A compound according to claim 16, wherein Xaa3 is N-methyl-lysine.

18. A compound according to any of claims 1 to 17, wherein

Pep comprises the sequence Met-Val-Gln-Gly-Ser-Gly-Cys-Phe-Gly-Arg-Lys-Met-Asp-Arg-Ile-Ser-Ser-Ser-Ser-Gly-Leu-Gly-Cys-lys-Val-Leu-Arg-Arg-His wherein the two Cys form a disulfide bond, or a homologue thereof.

19. A pharmaceutical composition comprising a compound according to any of claims 1 to 18, and a pharmaceutically acceptable carrier.

20. A compound according to any of claims 1 to 18 for use as a medicament.

21. Use of a compound according to any of claims 1 to 18, for the preparation of a medicament for preventing or treating at least one of the conditions selected from heart failure, nephrotic syndrome, cirrhosis of the liver, hypertension, or kidney failure.

22. Use of a compound according to any of claims 1 to 18, for the preparation of a medicament for preventing or treating cardiac diseases.

23. A method for treating or preventing at least one of the conditions selected from heart failure, nephrotic syndrome, cirrhosis of the liver, hypertension, kidney failure, in a subject, comprising administering to a subject a therapeutically effective amount of the composition according to claim 19.

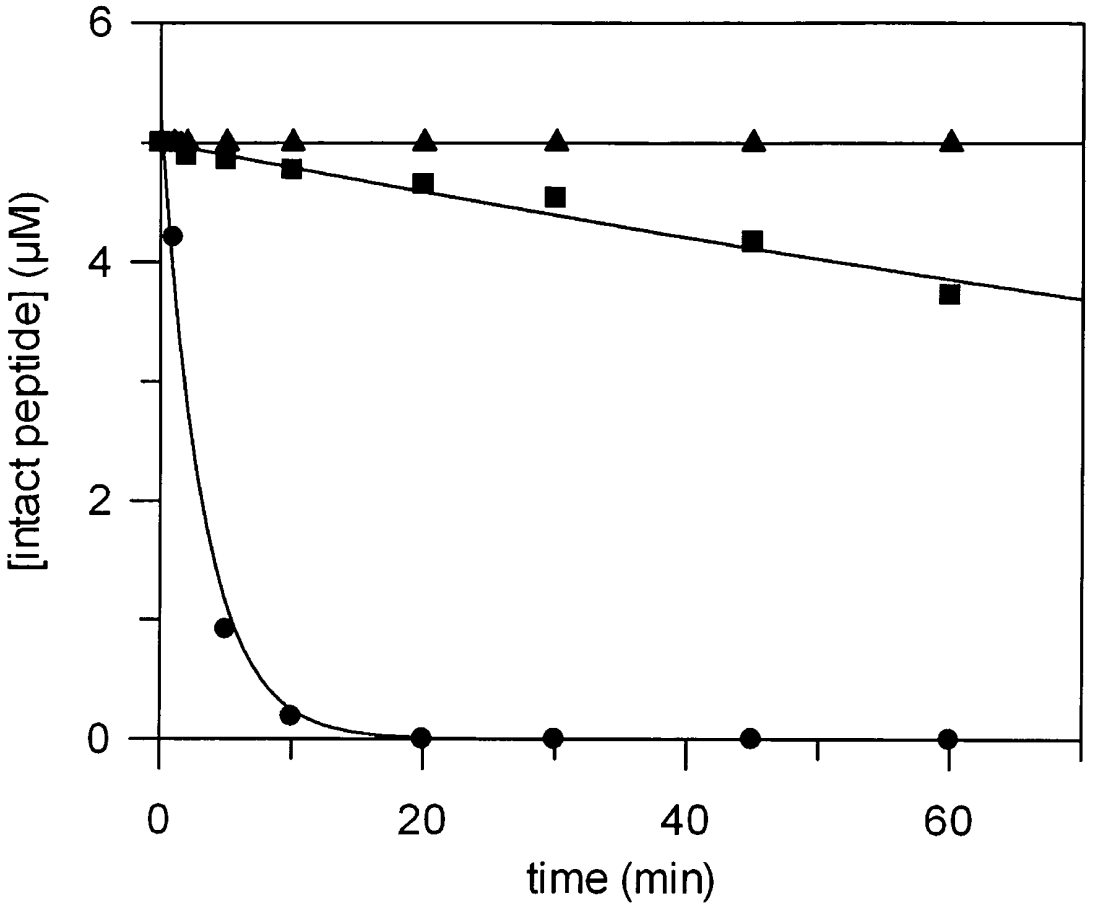


FIG. 1

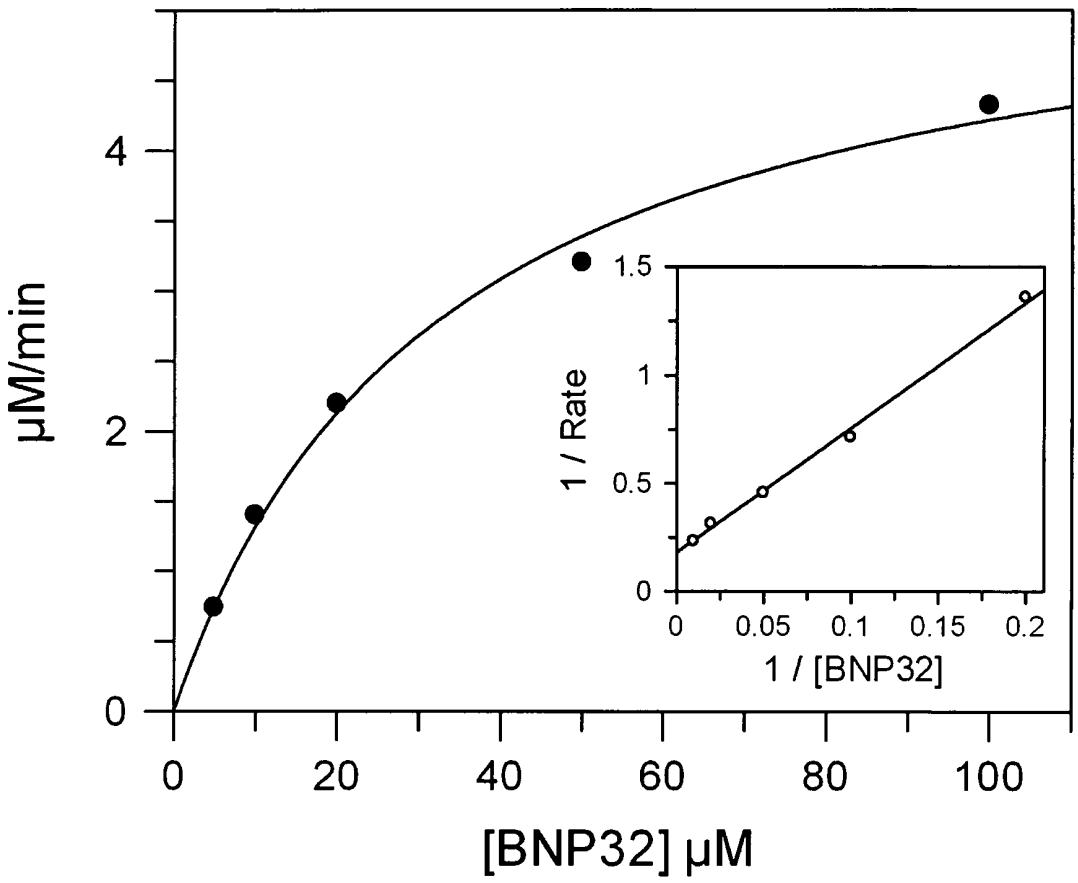

µM/min
0
2
4
0
20
40
60
80
100
[BNP32] µM
1 / Rate
0
0.5
1
1.5
0
0.05
0.1
0.15
0.2
1 / [BNP32]

FIG. 2

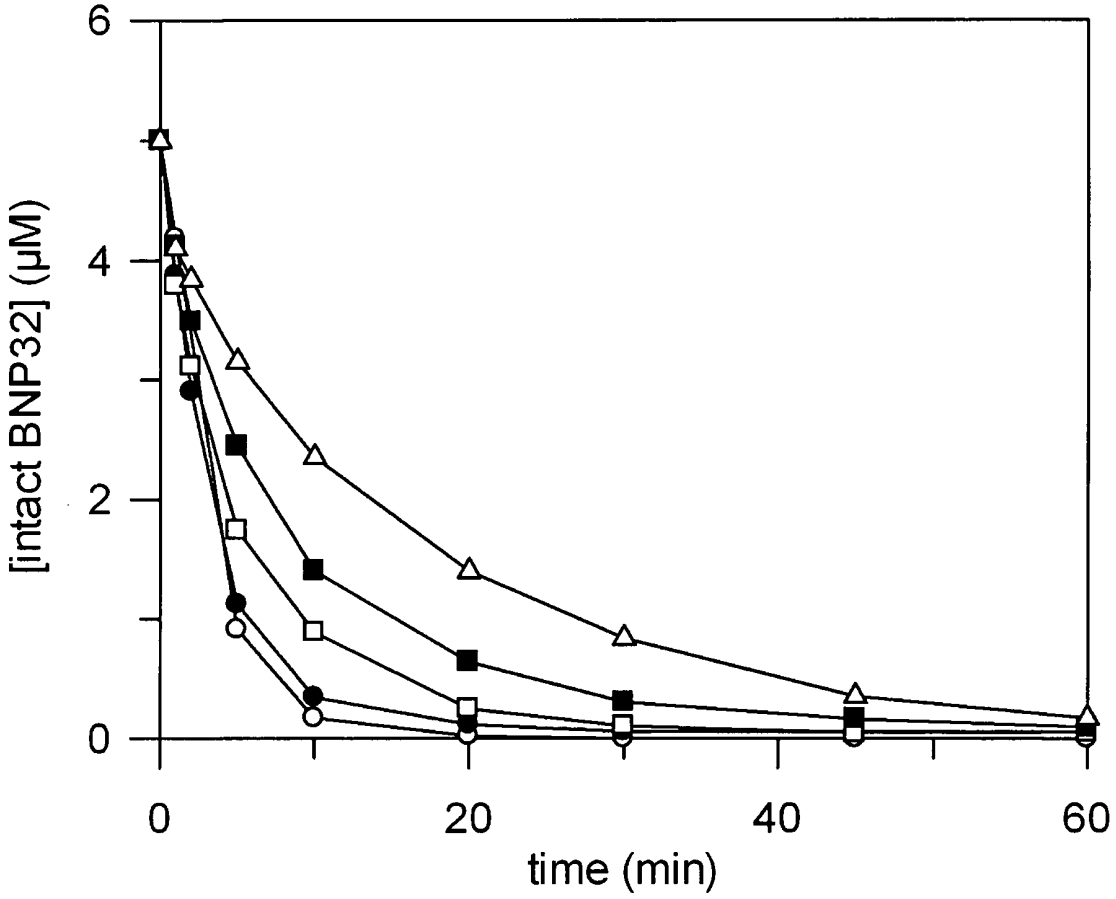
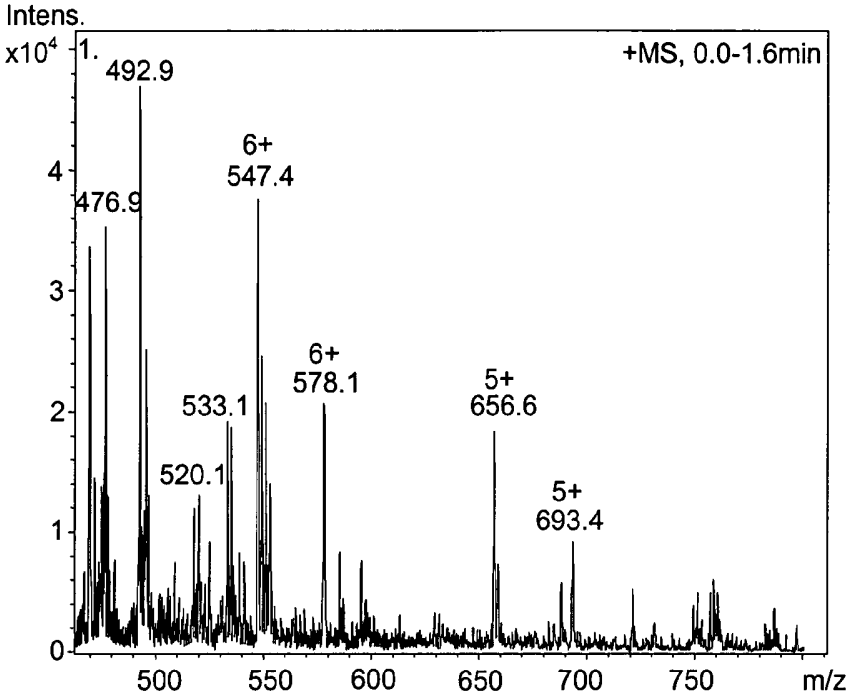


FIG. 3


Intens.
x10^4
1.
+MS, 0.0-1.6min
492.9
6+
547.4
476.9
6+
578.1
5+
656.6
533.1
520.1
5+
693.4
0
1
2
3
4
500
550
600
650
700
750
m/z

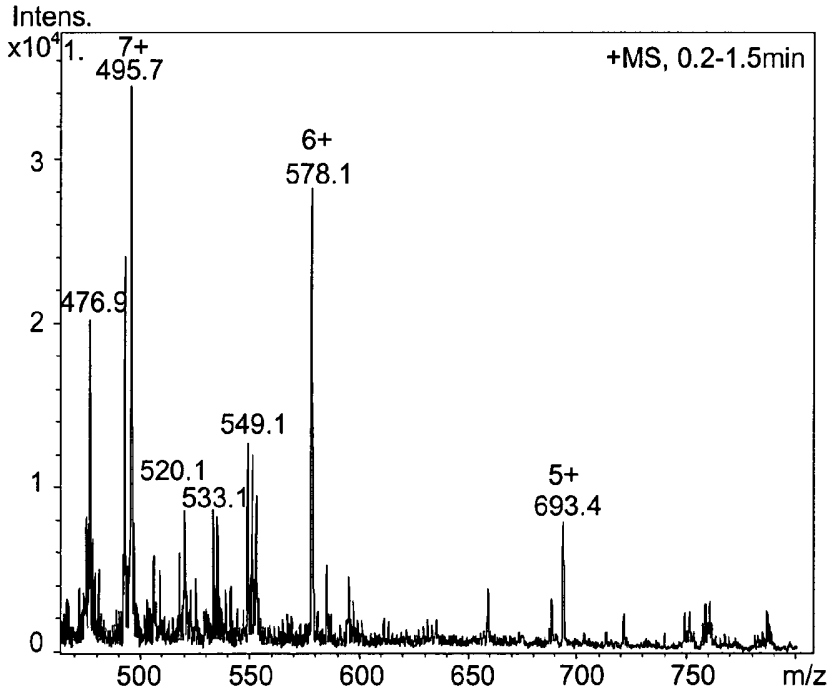
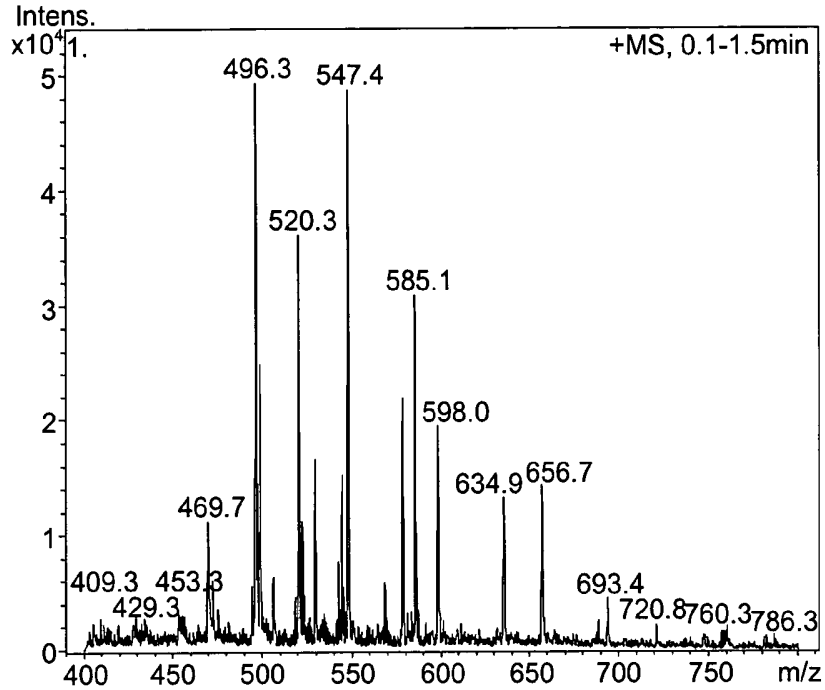

Intens.
x10^4
1.
7+
495.7
+MS, 0.2-1.5min
6+
578.1
476.9
549.1
520.1
533.1
5+
693.4
0
1
2
3
500
550
600
650
700
750
m/z

FIG. 4a


Intens.
x10^4
1.
+MS, 0.1-1.5min
496.3
547.4
520.3
585.1
598.0
634.9
656.7
469.7
409.3
453.3
429.3
693.4
720.8
760.3
786.3
m/z

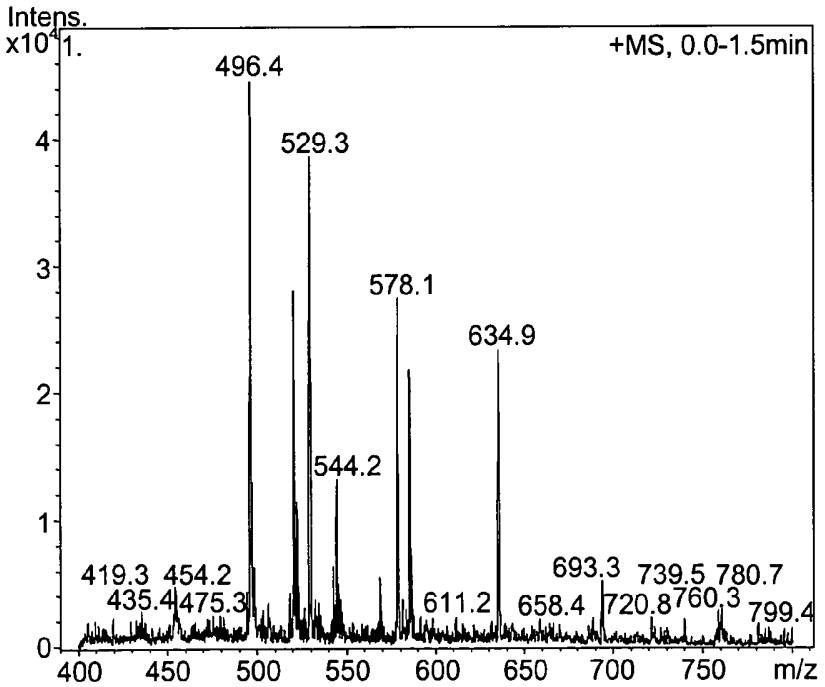

Intens.
x10^4
1.
+MS, 0.0-1.5min
496.4
529.3
578.1
634.9
544.2
419.3
454.2
435.4
475.3
611.2
658.4
693.3
720.8
739.5
760.3
780.7
799.4
m/z

FIG. 4b