



- (51) **International Patent Classification:**  
*A61K 38/00* (2006.01) *C07K 7/08* (2006.01)
- (21) **International Application Number:**  
PCT/US2014/047232
- (22) **International Filing Date:**  
18 July 2014 (18.07.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**  
61/847,909 18 July 2013 (18.07.2013) US
- (71) **Applicant:** ANCHOR THERAPEUTICS, INC.  
[US/US]; One Kendall Square, Building 200, Suite 2203,  
Cambridge, MA 02139 (US).
- (72) **Inventors:** LOOBY, Richard, J.; 32 William Road, Read-  
ing, MA 01867 (US). GROSS, Brett; 176 Summer Street,  
#2, Somerville, MA 02143 (US).
- (74) **Agents:** ABELLEIRA, Susan, M. et al.; Hamilton, Brook,  
Smith & Reynolds, P.C., 530 Virginia Rd, P.O. Box 9133,  
Concord, MA 01742-9133 (US).
- (81) **Designated States** (*unless otherwise indicated, for every  
kind of national protection available*): AE, AG, AL, AM,  
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,

BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,  
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,  
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,  
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,  
OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA,  
SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM,  
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM,  
ZW.

- (84) **Designated States** (*unless otherwise indicated, for every  
kind of regional protection available*): ARIPO (BW, GH,  
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,  
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,  
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,  
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,  
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,  
TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,  
KM, ML, MR, NE, SN, TD, TG).

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments (Rule 48.2(h))*



WO 2015/010045 A1

(54) **Title:** APJ RECEPTOR COMPOUNDS

(57) **Abstract:** The invention relates generally to compounds which are allosteric modulators (e.g., negative and positive allosteric modulators, allosteric agonists, and ago-allosteric modulators) of the G protein coupled receptor apelin, also known as the APJ receptor. The APJ receptor compounds are derived from the il intracellular loop and domain of the APJ receptor. The invention also relates to the use of these APJ receptor compounds and pharmaceutical compositions comprising the APJ receptor compounds in the treatment of diseases and conditions associated with APJ receptor modulation, such as cardiovascular diseases, (e.g., hypertension and heart failure, such as congestive heart failure), cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection.

## APJ RECEPTOR COMPOUNDS

## RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/847,909, filed on July 18, 2013. The entire teachings of the above application is incorporated herein by reference.

## BACKGROUND OF THE INVENTION

[0002] G protein coupled receptors (GPCRs) constitute one of the largest families of genes in the human genome. GPCRs are integral membrane signaling proteins. Hydrophobicity mapping of the amino acid sequences of G-protein coupled receptors has led to a model of the typical G-protein-coupled receptor as containing seven hydrophobic membrane-spanning regions with the amino terminal on the extracellular side of the membrane and the carboxyl terminal on the intracellular side of the membrane.

[0003] GPCRs mediate the transmission of intracellular signals (“signal transduction”) by activating guanine nucleotide-binding proteins (G proteins) to which the receptor is coupled. GPCRs are activated by a wide range of endogenous stimuli, including peptides, amino acids, hormones, light, and metal ions. The following reviews are incorporated by reference: Hill, *British J. Pharm* 147: s27 (2006); Palczeski, *Ann Rev Biochemistry* 75: 743-767 (2006); Dorsham & Gutkind, *Nature Reviews* 7: 79-94 (2007); Kobilka & Schertler, *Trends Pharmacol Sci.* 2: 79-83 (2008).

[0004] GPCRs are important targets for drug discovery as they are involved in a wide range of cellular signaling pathways and are implicated in many pathological conditions (e.g., cardiovascular and mental disorders, cancer, AIDS). In fact, GPCRs are targeted by 40-50% of approved drugs, illustrating the critical importance of this class of pharmaceutical targets. Interestingly, this number represents only about 30 GPCRs, a small fraction of the total number of GPCRs thought to be relevant to human disease. Over 1000 GPCRs are known in the human genome, and GPCRs remain challenging targets from a research and development perspective in part because these amembrane bound receptors with complex pharmacology.

- 2 -

[0005] There remains a need for the development of new pharmaceuticals that are allosteric modulators of GPCRs (e.g., negative and positive allosteric modulators, allosteric agonists, and ago-allosteric modulators).

#### SUMMARY OF THE INVENTION

[0006] The invention relates generally to compounds which are allosteric modulators (e.g., negative and positive allosteric modulators, allosteric agonists, and ago-allosteric modulators) of the G protein coupled receptor apelin, also known as the APJ receptor. The APJ receptor compounds are derived from the intracellular loops and domains of the the APJ receptor. The invention also relates to the use of these APJ receptor compounds and pharmaceutical compositions comprising the APJ receptor compounds in the treatment of diseases and conditions associated with APJ receptor modulation, such as cardiovascular diseases, (e.g., hypertension and heart failure, such as congestive heart failure), cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection. In another embodiment, the compounds of the invention are combined with a second therapeutic agent. The second therapeutic is an agent useful in the treatment or prevention of a disease or condition selected from hypertension and heart failure, in particular, congestive heart failure and hypertrophic cardiomyopathy. In addition, the second therapeutic would be useful in the treatment or prevention of coronary artery disease, atherosclerosis, stable and unstable angina pectoris, restenosis, acute myocardial infarction, pulmonary hypertension, diseases related to cardiac ischemia, and sudden heart death.

[0007] More specifically, the compounds of the invention are represented by Formula A:

T-L-X<sub>1</sub>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-X<sub>17</sub>-X<sub>18</sub>-R<sub>1</sub>;

[0008] or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and

- 3 -

heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>, wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>) aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub> is absent, a proline residue or a D-proline residue;

X<sub>2</sub> is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>3</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>4</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>5</sub> is N-methylarginine, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 4 -

- X<sub>7</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, a D-proline residue, an Aib residue, or a glycine residue;
- X<sub>8</sub> is a serine residue, a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, or a tyrosine residue;
- X<sub>9</sub> is D-ornithine, is a glutamic acid residue, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or an arginine residue, or a tyrosine residue;
- X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>15</sub> is an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 5 -

X<sub>16</sub> an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>17</sub> a phenylalanine residue, an isoleucine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>18</sub> is absent or an isoleucine residue, a proline residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue;

wherein seventeen contiguous amino acids are present and optionally 1- 5 amino acid residues are present in the D configuration.

**[0009]** In another aspect of the invention the compounds are represented by Formula B:

T-L-X<sub>1</sub>'-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub> X<sub>17</sub>-X<sub>18</sub> -R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>' and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>' at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

- 6 -

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3 \text{ alkyl})$ ,  $-C(O)(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3 \text{ alkyl})_2$ ,  $-NHC(O)O(C_1-C_3 \text{ alkyl})$ ,  $-C(O)OH$ ,  $-C(O)O(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)N(C_1-C_3 \text{ alkyl})_2$ , or  $-SO_2NR_2$ ;

$X_1'$  is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue, a proline residue or a D-proline residue;

$X_2$  is a threonine residue, a valine residue, a proline residue, histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

$X_3$  is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

$X_4$  is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, an arginine residue, or a proline residue;

$X_5$  is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, a serine residue, or a glycine residue;

$X_6$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$X_7$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an arginine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$X_8$  is a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a serine residue, a glycine residue, or a tyrosine residue;

- 7 -

- X<sub>9</sub> is D-ornithine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a proline residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, an arginine residue, or a tyrosine residue;
- X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, a glutamic acid residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>15</sub> is an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>16</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>17</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;



- 8 -

X<sub>18</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally 1- 5 amino acid residues are present in the D configuration.

**[0010]** In another embodiment, the compound is selected from the group consisting of: Compound 1, 2, 3 or 5, or pharmaceutically acceptable salt thereof.

**[0011]** More specifically, the compounds of the invention are represented by Formula C:

T-L-X<sub>1</sub>''-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>'' and is selected from: C(O), C(S), S(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>'' at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

- 9 -

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub>' is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is absent, is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is absent, is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is D-ornithine;

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 10 -

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

**[0012]** More specifically, the compounds of the invention are represented by Formula D:

T-L-X<sub>1</sub>''-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>'' and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>'' at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

- 11 -

n is 1-20

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3 \text{ alkyl})$ ,  $-C(O)(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3 \text{ alkyl})_2$ ,  $-NHC(O)O-(C_1-C_3 \text{ alkyl})$ ,  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3 \text{ alkyl})$ ,  $-NHC(O)N(C_1-C_3 \text{ alkyl})_2$ , or  $-SO_2NR_2$ ;

$X_1'''$  is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

$X_2$  is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

$X_3$  is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

$X_4$  is N-methylarginine, is an asparagine residue, an arginine residue, a histidine residue, or a glutamine residue;

$X_5$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$X_6$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$X_7$  is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$X_8$  is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;

$X_9$  is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 12 -

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an asparagine residue; isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein at least one of X<sub>4</sub> or X<sub>14</sub> is an asparagine residue,

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

**[0013]** In another aspect of the invention the compounds are represented by Formula E:

T-L-X<sub>1</sub><sup>''''</sup>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub><sup>''''</sup> and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub><sup>''''</sup> at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl,

- 13 -

aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub>''' is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is N-methylarginine;

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 14 -

X<sub>8</sub> is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

[0014] In another aspect of the invention the compounds are represented by Formula I:

T-L-P,

or pharmaceutically acceptable salts thereof, wherein:

P is a peptide comprising SEQ ID Nos. 2-37, wherein

- 15 -

L is a linking moiety bonded to P at an N-terminal nitrogen of an N-terminal amino-acid residue selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to P at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; and T is a lipophilic tether moiety bonded to L, wherein the C-terminal amino acid residue of P is functionalized by replacement of the acid moiety with C(O)NR<sub>3</sub><sup>1</sup>R<sub>4</sub><sup>1</sup>; R<sub>3</sub><sup>1</sup> is selected from hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>; R<sub>4</sub><sup>1</sup> is selected from (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>;

n is 1-20

or -NR<sub>3</sub><sup>1</sup>R<sub>4</sub><sup>1</sup> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>.

**[0015]** In one embodiment, the compounds T-L-P of the invention exhibit inhibition of cAMP production at the hAPJ receptor with an EC<sub>50</sub> ≤ 50 nM (hAPJ) according to the HTRF cAMP Assay described under Functional Assays and the data of Table 6. Compounds of the invention with EC<sub>50</sub> ≤ 50 nM (hAPJ) are Compound Nos. 1-36.

**[0016]** In a particular embodiment, the compounds exhibit EC<sub>50</sub> from 25- 50 nM (hAPJ), %IA from 50 - 79% and either % histamine ≤ 35% or formal (+) charge ≤ 1 according to the according to the HTRF cAMP Assay and Histamine Assay described under Functional Assays



- 16 -

and the data of Table 6. Compounds of the invention with EC<sub>50</sub> from 25- 50 nM (hAPJ), %IA from 50 - 79% and either % histamine  $\leq$  35% or formal (+) charge  $\leq$  1 are Compound Nos. 6, 7, 19, 20, 33, 34, 35, and 16.

**[0017]** In a particular embodiment, the compounds exhibit EC<sub>50</sub>  $\leq$  25 nM (hAPJ), %IA  $\geq$  80% and either % histamine  $>$  35% or formal (+) charge  $>$  1 according to the according to the HTRF cAMP Assay and Histamine Assay described under Functional Assays and the data of Table 6. Compounds of the invention with EC<sub>50</sub>  $\leq$  25 nM (hAPJ), %IA  $\geq$  80% and either % histamine  $>$  35% or formal (+) charge  $>$  1 are Compound Nos. 3, 14, 18, 21, 24, 25, 26, 27, 28, 29, and 32.

**[0018]** In a particular embodiment, the compounds exhibit EC<sub>50</sub>  $\leq$  25 nM (hAPJ), %IA  $\geq$  80% and either % histamine  $\leq$  35% or formal (+) charge  $\leq$  1 according to the according to the HTRF cAMP Assay and Histamine Assay described under Functional Assays and the data of Table 6. Compounds of the invention with EC<sub>50</sub>  $\leq$  25 nM (hAPJ), %IA  $\geq$  80% and either % histamine  $\leq$  35% or formal (+) charge  $\leq$  1 are Compound Nos. 8, 12, 13, 15, 17, 30, and 36.

**[0019]** The invention also relates to pharmaceutical compositions comprising one or more compounds of the invention and a carrier, and the use of the disclosed compounds and compositions in methods of treating diseases and conditions responsive to modulation (inhibition or activation) of the APJ receptor.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0020]** The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

**[0021]** FIGS. 1A-1D show the concentration response curves illustrating inhibition of forskolin analog NKH477-induced cAMP generation in HEK cells stably expressing the human APJ receptor (hAPJ) for selected APJ Compounds 8, 12, 13, 15, 17, 30, and 36. Compounds 8, 12, 13, 15, 17, 30, and 36 inhibited the forskolin analog NKH477-stimulated increase in cAMP in HEK cells stably expressing the Gi-coupled receptor APJ in a dose dependent manner.

- 17 -

[0022] FIGS. 2A-2E show concentration response curves for selected APJ Compounds 14, 18, 21, 24, 25, 26, 27, 29, and 32. Compounds 14, 18, 21, 24, 25, 26, 27, 29, and 32 inhibited the forskolin analog NKH477-stimulated increase in cAMP in HEK cells stably expressing the Gi-coupled receptor APJ in a dose dependent manner.

[0023] FIGS. 3A-3B show concentration response curves illustrating  $\beta$ -arrestin recruitment for selected APJ compounds: Endogenous ligand apelin-13, Compound 3, Compound 25, Compound 26, and Compound 27. The endogenous ligand apelin-13 robustly recruits  $\beta$ -arrestin as measured by a dose dependent increase in chemiluminescence.  $\beta$ -arrestin is weakly engaged for Compounds 3, 25, 26, and 27 in a dose dependent manner.

[0024] FIGS. 4A-4B illustrate compound structures for selected APJ receptor compounds.

[0025] FIGS. 5A-5D illustrate compound structures for selected APJ receptor compounds.

[0026] FIGS. 6A-6C show representative data showing lack of dose-responsive activity at the CXCR4 receptor, another Gi-coupled GPCR for Compounds 12, 13, 14, 18, and 28. These data demonstrate that the compounds of the invention exhibit receptor selectivity and are capable of selectively activating the hAPJ receptor but not CXCR4, another GPCR that couples to the same Gi protein as the APJ receptor. Because CXCR4 is also Gi coupled and signals through cAMP, it therefore provides a desirable measure of selectivity.

## DETAILED DESCRIPTION OF THE INVENTION

[0027] A description of example embodiments of the invention follows.

### G PROTEIN COUPLED RECEPTORS (GPCRS)

[0028] G protein coupled receptors (GPCRs) constitute one of the largest superfamilies of genes in the human genome; these transmembrane proteins enable the cell to respond to its environment by sensing extracellular stimuli and initiating intracellular signal transduction cascades. GPCRs mediate signal transduction through the binding and activation of guanine nucleotide-binding proteins (G proteins) to which the receptor is coupled. Wide arrays of ligands bind to these receptors, which in turn orchestrate signaling networks integral to many cellular functions. Diverse GPCR ligands include small proteins, peptides, amino acids, biogenic amines, lipids, ions, odorants and even photons of light. The following reviews are incorporated

by reference: Hill, *British J. Pharm* 147: s27 (2006); Dorsham & Gutkind, *Nature Reviews* 7: 79-94 (2007).

[0029] In addition to modulating a diverse array of homeostatic processes, GPCR signaling pathways are integral components of many pathological conditions (e.g., cardiovascular and mental disorders, cancer, AIDS). In fact, GPCRs are targeted by 40-50% of approved drugs illustrating the critical importance of this class of pharmaceutical targets. Interestingly, this number represents only about 30 GPCRs, a small fraction of the total number of GPCRs thought to be relevant to human disease. GPCRs are membrane bound receptors that exhibit complex pharmacological properties and remain challenging targets from a research and development perspective. Given their importance in human health combined with their prevalence (over 1000 known GPCRs in the human genome) GPCRs represent an important target receptor class for drug discovery and design.

[0030] GPCRs are integral membrane proteins that mediate diverse signaling cascades through an evolutionarily conserved structural motif. All GPCRs are thought to consist of seven hydrophobic transmembrane spanning  $\alpha$ -helices with the amino terminus on the extracellular side of the membrane and the carboxyl terminus on the intracellular side of the membrane. The transmembrane helices are linked together sequentially by extracellular (e1, e2, e3) and intracellular (cytoplasmic) loops (i1, i2, i3). The intracellular loops or domains are intimately involved in the coupling and turnover of G proteins and include: i1, which connects TM1-TM2; i2, connecting TM3-TM4; i3, connecting TM5-TM6; and a portion of the C-terminal cytoplasmic tail (domain 4). Due in part to the topological homology of the 7TM domains and the recent high resolution crystal structures of several GPCRs (Palczewski et al., *Science* 289, 739-45 (2000), Rasmussen, S.G. et al., *Nature* 450, 383-7 (2007)) skilled modelers are now able to predict the general boundaries of GPCR loop domains through the alignment of several related receptors. These predictions are aided in part by a number of programs used by computational biologists, including EMBOSS, ClustalW2, Kalign, and MAFFT (Multiple Alignment using Fast Fourier Transform). Importantly, many of these programs are publically available (see, for example, The European Bioinformatics Institute (EMBL-EBI) web site <http://www.ebi.ac.uk/Tools/>) and most have web-based interfaces.

[0031] GPCR mediated signal transduction is initiated by the binding of a ligand to its cognate receptor. In many instances GPCR ligand binding is believed to take place in a

hydrophilic pocket generated by a cluster of helices near the extracellular domain. However, other ligands, such as large peptides, are thought to bind to the extracellular region of protein and hydrophobic ligands are postulated to intercalate into a receptor binding pocket through the membrane between gaps in the helices. The process of ligand binding induces conformational changes within the receptor. These changes involve the outward movement of helix 6, which in turn alters the conformations of the intracellular loops and ultimately results in a receptor form that is able to bind and activate a heterotrimeric G protein (Farrens, D., et al. *Science* 274, 768-770 (1996), Gether, U. and Kobilka, B., *J. Biol. Chem.* 273, 17979-17982 (1998)). Upon binding the receptor catalyzes the exchange of GTP for GDP in the alpha subunit of the heterotrimeric G protein, which results in a separation of the G protein from the receptor as well a dissociation of the alpha and beta/gamma subunits of the G protein itself. Notably, this process is catalytic and results in signal amplification in that activation of one receptor may elicit the activation and turnover of numerous G proteins, which in turn may regulate multiple second messenger systems. Signaling diversity is further achieved through the existence of numerous G protein types as well as differing isoforms of alpha, beta and gamma subunits. Typically, GPCRs interact with G proteins to regulate the synthesis or inhibition of intracellular second messengers such as cyclic AMP, inositol phosphates, diacylglycerol and calcium ions, thereby triggering a cascade of intracellular events that eventually leads to a biological response.

**[0032]** GPCR signaling may be modulated and attenuated through cellular machinery as well as pharmacological intervention. Signal transduction may be 'switched off' with relatively fast kinetics (seconds to minutes) by a process called rapid desensitization. For GPCRs, this is caused by a functional uncoupling of receptors from heterotrimeric G proteins, without a detectable change in the total number of receptors present in cells or tissues. This process involves the phosphorylation of the receptor C terminus, which enables the protein Arrestin to bind to the receptor and occlude further G protein coupling. Once bound by Arrestin the receptor may be internalized into the cell and either recycled back to the cell surface or degraded. The alpha subunit of the G protein possesses intrinsic GTPase activity, which attenuates signaling and promotes re-association with the beta/gamma subunits and a return to the basal state. GPCR signaling may also be modulated pharmacologically. Agonist drugs act directly to activate the receptors, whereas antagonist drugs act indirectly to block receptor signaling by preventing agonist activity through their associating with the receptor.

- 20 -

[0033] GPCR binding and signaling can also be modified through allosteric modulation, that is by ligands that bind not at the orthosteric binding site but through binding at an allosteric site elsewhere in the receptors. Allosteric modulators can include both positive and negative modulators of orthosteric ligand mediated activity, allosteric agonists (that act in the absence of the orthosteric ligand), and ago-allosteric modulators (ligands that have agonist activity on their own but that can also modulate the activity of the orthosteric ligand).

[0034] The large superfamily of GPCRs may be divided into subclasses based on structural and functional similarities. GPCR families include Class A Rhodopsin like, Class B Secretin like, Class C Metabotropic glutamate / pheromone, Class D Fungal pheromone, Class E cAMP receptors (*Dictyostelium*), the Frizzled/Smoothed family, and various orphan GPCRs. In addition, putative families include Ocular albinism proteins, Insect odorant receptors, Plant Mlo receptors, Nematode chemoreceptors, Vomeronasal receptors (VIR & V3R) and taste receptors.

[0035] Class A GPCRs, also called family A or rhodopsin-like, are the largest class of receptors and characteristically have relatively small extracellular loops that form the basis for selectivity vs. endogenous agonists and small-molecule drugs. In addition, Class A receptors also have relatively small intracellular loops. Class A receptors include amine family members such as dopamine and serotonin, peptide members such as chemokine and opioid, the visual opsins, odorant receptors and an array of hormone receptors.

[0036] The apelin receptor (APJ) is a Class A receptor that has been implicated in conditions such as cardiovascular diseases, such as heart diseases (e.g., hypertension and heart failure, such as congestive heart failure), cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection.

## PEPTIDES

[0037] As defined herein, P is a peptide comprising Sequences 2-37. It is understood that the linking moiety is bonded to the N-terminal nitrogen of the N-terminal amino acid residue or it can be an amino acid residue distinct from the terminal amino acid residue.

[0038] Intracellular i1 loop as used herein refers to the loop which connects TM1 to TM2 and the corresponding transmembrane junctional residues.

Table 1:

Intracellular Loop Number	APJ Receptor Intercellular Loop
i1	TVFRSSREKRRSADIFI (SEQ ID NO: 1)

SEQ ID NOS: 2-37 are listed in Table 2a below:

Table 2a. APJ Sequence IDs

SEQ ID No.	Length (# AAs)	Sequence
2	18	TVPFRSSEPkRRSADIFI
3	18	TPVFRSSPEkRRSADIFI
4	18	PTVFRSPSEkRRSADIFI
5	11	PEkRRSADIFP
6	18	TVDRSSREkRRRESADIFI
7	17	TVDRSSQEkRRSADIFI
8	17	TVDRSSKEkRRSADIFI
9	16	TVFRSSE(d-Orn)RRSADIFI
10	20	PK(PkRRSADIFP)RRSADIFP
11	16	TVFRSSEaRRSADIFI
12	16	TVFRSSEkRRSAD(Bip)FI
13	16	DVFQSSEkRRSADQFI
14	16	DVFRSSEkRRSADQFI
15	15	TVFRSEkRRSADQFI
16	15	TVFQSSEkRRSADFI
17	14	TVFQSEkRRSADFI
18	15	DVFQSEkRRSADQFI
19	16	TVFRSSEkRRSADNFI
20	16	DVFNSEkRRSADNFI
21	16	DVFRSSEkRRSADNFI
22	16	TVKRSSEkRRSADIFI
23	17	TVFRSPSEkRRPSADIF
24	17	TVFRSSPEkRRSPADIF
25	17	TVFRSSEkRRPSADIFP
26	17	TVFRSSPEkRRSADIFI
27	17	TVFRSSPEkRRSADIFP
28	17	TVFRSSEPkRRSADIFI
29	17	TVFRSSEPkRRSADIFP

- 22 -

30	17	pTVFRSpSEkRRSADIF
31	11	PkRRSADIFIP
32	16	TVF(N-methyl-Arg)SSEkRRSADIFI
33	16	TVD(N-methylArg)SSEkRRSADIFI
34	16	TVDRSSEkRRSAD(4-fluoro-Phe)FI
35	16	TVDRSSEkRRSAD(p-aminomethyl-Phe)FI
36	16	DVHRSSEkRRSADIFI
37	16	DVHRSSEkRRSADQFI

[0039] In another even more specific embodiment, P is selected from the group consisting of SEQ ID NOS: 9, 13, 14, 16, 18, 31, and 37 as listed in Table 2b below:

Table 2b:

APJ i-loop	SEQ ID No.	Sequence
il	9	TVFRSSE(d-Orn)RRSADIFI
il	13	DVFQSSEkRRSADQFI
il	14	DVFRSSEkRRSADQFI
il	16	TVFQSSEkRRSADFI
il	18	DVFQSEkRRSADQFI
il	31	PkRRSADIFIP
il	37	DVHRSSEkRRSADQFI

[0040] In another even more specific embodiment, P is selected from the group consisting of SEQ ID NOS: 4, 15, 19, 22, 25, 26, 27, 28, 29, 30 and 33 as listed in Table 2c below:

Table 2c:

APJ i-loop	SEQ ID No.	Sequence
il	4	PTVFRSPSEkRRSADIFI
il	15	TVFRSEkRRSADQFI
il	19	TVFRSSEkRRSADNFI
il	22	TVKRSSEkRRSADIFI
il	25	TVFRSSEkRRPSADIFP
il	26	TVFRSSPEkRRSADIFI
il	27	TVFRSSPEkRRSADIFP

- 23 -

i1	28	TVFRSSEPkRRSADIFI
i1	29	TVFRSSEPkRRSADIFP
i1	30	pTVFRSpSEkRRSADIF
i1	33	TVD(N-Me Arg)SSEkRRSADIFI

It is understood that the i1 loop sequences, including those sequences presented in Tables 2a - 2c, can be optionally functionalized at the C-terminus by  $-R_1$  of the Formulas described herein wherein  $R_1$  is  $-OR_2$  or  $-NR_3R_4$ , each  $R_2$  is independently hydrogen or  $(C_1-C_{10})$ alkyl; and

$R_3$  and  $R_4$  are each independently selected from hydrogen,  $(C_1-C_{10})$  alkyl,  $(C_1-C_{10})$ aralkyl,  $[CH_2CH_2O]_nCH_2CH_2C(O)OR_2$  or  $[CH_2CH_2O]_nCH_2CH_2C(O)NR_2$ ;

n is 1-20

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3$  alkyl),  $-C(O)(C_1-C_3$  alkyl),  $-NHC(O)(C_1-C_3$  alkyl),  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3$  alkyl) $_2$ ,  $-NHC(O)O-(C_1-C_3$  alkyl),  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3$  alkyl),  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3$  alkyl),  $-NHC(O)N(C_1-C_3$  alkyl) $_2$ , or  $-SO_2NR_2$ ;

In another specific embodiment, P of Formula I is selected from the group consisting of SEQ ID NO: 2-37, for example those as listed in Tables 2a-2c, and the functionalization at the C-terminus is  $C(O)NR_3^1R_4^1$  (i.e., instead of the C-terminus ending with  $-C(O)OH$ , it ends with  $C(O)NR_3^1R_4^1$ );  $R_3^1$  and  $R_4^1$  are each independently selected from  $(C_1-C_{10})$ aralkyl,  $[CH_2CH_2O]_nCH_2CH_2C(O)OR_2$  or  $[CH_2CH_2O]_nCH_2CH_2C(O)NR_2$ ;

n is 1-20



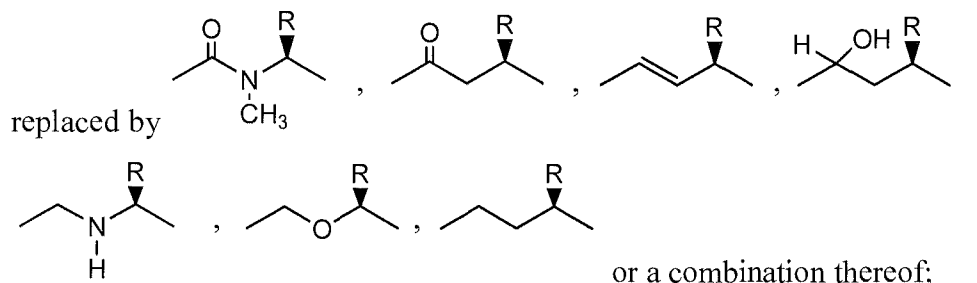
- 24 -

or  $-\text{NR}_3^1\text{R}_4^1$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $\text{R}_5$ ;

each  $\text{R}_5$  is independently halogen,  $-\text{OH}$ ,  $\text{C}_1\text{-C}_3$  alkyl,  $\text{C}_1\text{-C}_3$  haloalkyl,  $-\text{NO}_2$ ,  $-\text{C}_1\text{-C}_3$  alkoxy,  $-\text{C}_1\text{-C}_3$  haloalkoxy,  $-\text{CN}$ ,  $-\text{NH}_2$ ,  $-\text{C}_1\text{-C}_3$  alkylamino,  $-\text{C}_1\text{-C}_3$  dialkylamino,  $-\text{C}(\text{O})\text{NH}_2$ ,  $-\text{C}(\text{O})\text{NH}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{C}(\text{O})(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{H}$ ,  $-\text{C}(\text{O})\text{N}(\text{C}_1\text{-C}_3 \text{ alkyl})_2$ ,  $-\text{NHC}(\text{O})\text{O}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{C}(\text{O})\text{OH}$ ,  $-\text{C}(\text{O})\text{O}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{NH}_2$ ,  $-\text{NHC}(\text{O})\text{NH}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{N}(\text{C}_1\text{-C}_3 \text{ alkyl})_2$ , or  $-\text{SO}_2\text{NR}_2$ .

**[0041]** Furthermore, it is understood that Sequences 2-37 may be modified with peptide backbone modifications such as, but not limited to, retro-inverso peptide linkages; despsipeptide linkages; conformational restrictions; or a combination thereof. These modifications include:

- one or more unnatural amino acid residues;
- one or more peptide backbone modifications;
- one or more retro-inverso peptide linkages;
- a peptide sequence according to (b) wherein one or more peptide bonds are



**[0042]** It is noted that P of Formula I can be optionally functionalized at the C-terminus. Functionalized at the C-terminus means that the acid moiety present at the C-terminus is replaced by some other functional group as described herein.

**[0043]** Peptidomimetic as used herein refers to a compound comprising non-peptidic structural elements in place of a peptide sequence.

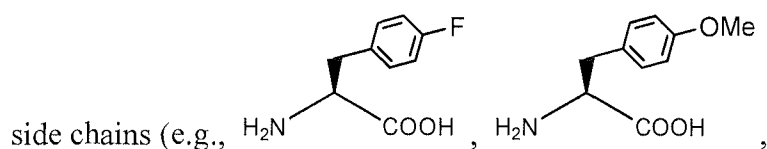
**[0044]** As used herein, the term “amino acid” includes both a naturally occurring amino acid and a non-natural amino acid.

[0045] As used herein, the term “naturally occurring amino acid” means a compound represented by the formula  $\text{NH}_2\text{-CHR-COOH}$ , wherein R is the side chain of a naturally occurring amino acids such as lysine, arginine, serine, tyrosine etc. as shown in the Table below.

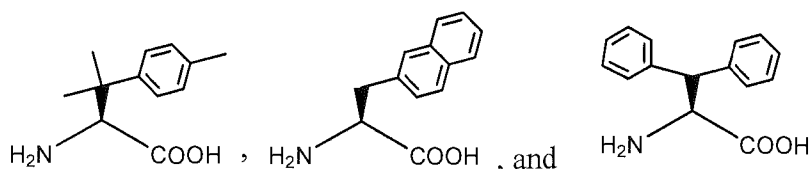
**Table of Common Naturally Occurring Amino Acids**

	Amino acid	Three letter code	One letter code
Non-polar; neutral at pH 7.4	alanine	Ala	A
	isoleucine	Ile	I
	leucine	Leu	L
	methionine	Met	M
	phenylalanine	Phe	F
	proline	Pro	P
	tryptophan	Trp	W
	valine	Val	V
Polar, uncharged at pH 7.0	asparagine	Asn	N
	cysteine	Cys	C
	glycine	Gly	G
	glutamine	Gln	Q
	serine	Ser	S
	threonine	Thr	T
	tyrosine	Tyr	Y
	Polar; charged at pH 7	glutamic acid	Glu
arginine		Arg	R
aspartic acid		Asp	D
histidine		His	H
lysine		Lys	K

"Non-natural amino acid" means an amino acid for which there is no nucleic acid codon. Examples of non-natural amino acids include, for example, the D-isomers of the natural  $\alpha$ -amino acids such as D-proline (D-P, D-Pro) as indicated above; natural  $\alpha$ -amino acids with non-natural



- 26 -



related to phenylalanine); Aib

(aminobutyric acid), bAib (3-aminoisobutyric acid), Nva (norvaline),  $\beta$ -Ala, Aad (2-aminoadipic acid), bAad (3-aminoadipic acid), Abu (2-aminobutyric acid), Gaba ( $\gamma$ -aminobutyric acid), Acp (6-aminocaproic acid), Dbu (2,4-diaminobutyric acid),  $\alpha$ -aminopimelic acid, TMSA (trimethylsilyl-Ala), alle (allo-isoleucine), Nle (norleucine), tert-Leu, Cit (citrulline), Orn (ornithine, O), Dpm (2,2'-diaminopimelic acid), Dpr (2,3-diaminopropionic acid),  $\alpha$  or  $\beta$ -Nal, Cha (cyclohexyl-Ala), hydroxyproline, Sar (sarcosine), Dap (2,3-diaminopropionic acid) and the like.

**[0046]** Unnatural amino acids also include cyclic amino acids; and amino acid analogs, for example,  $N^{\alpha}$ -alkylated amino acids such as MeGly ( $N^{\alpha}$ -methylglycine), EtGly ( $N^{\alpha}$ -ethylglycine) and EtAsn ( $N^{\alpha}$ -ethylasparagine); and amino acids in which the  $\alpha$ -carbon bears two side-chain substituents. As with the natural amino acids, the residues of the unnatural amino acids are what are left behind when the unnatural amino acid becomes part of a peptide sequence as described herein.

**[0047]** Amino acid residues are amino acid structures as described above that lack a hydrogen atom of the amino group or the hydroxyl moiety of the carboxyl group or both resulting in the units of a peptide chain being amino-acid residues.

**[0048]** The D-isomers of the natural amino acids are designated herein with a lower case letter of the corresponding naturally occurring amino acid. For example, d-proline is designated "p" rather than "P" as is used for naturally occurring proline.

## LINKERS (L)

**[0049]** The linker "L" of the invention connects the lipophilic tether moiety, T, to the N-terminal nitrogen of the N-terminal amino acid residue of P in the case of Formula I and to  $X_1$ ,  $X_1'$ ,  $X_1''$ ,  $X_1'''$ ,  $X_1''''$  or the next present amino acid if  $X_1$  is absent at the atom of L as marked herein with an asterisk in the other Formulas described herein. The linker can be linear or branched and optionally substituted. The linker can in some instances be used to vary the

- 27 -

distance between T and P or the amino acid of Formulas described herein to which it is attached providing a more desirable interaction of P with its cognate GPCR. In other instances, the linker can confer improvements on the physicochemical and pharmacological properties of the APJ receptor compound as compared with compounds lacking a linker. For example, the introduction of the linker can alter one or more of lipophilicity, solubility, partition coefficient, stability, and biological half life.

[0050] In one embodiment, wherein L is a linking moiety bonded to the N terminal Threonine nitrogen of sequences 2, 3, 6, 7, 8, 9, 11, 12, 15, 16, 17, 19, 22, 23, 24, 25, 26, 27, 28, 29, 32, 33, 34, and 35, the N-terminal proline of sequences 4, 5, 10, 30 or 31, or the N-terminal aspartic acid nitrogen of sequences 13, 14, 18, 20, 21, 36, and 37 and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to the terminal nitrogen at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted .

[0051] In another specific embodiment, R<sup>3</sup> is H or D.

[0052] In a further specific embodiment, L is selected from the group: C\*(O), S\*(O)<sub>2</sub>, NHC\*(O) and NHC\*(S).

[0053] Linkers can be attached to the N-terminal nitrogen of the N-terminal amino acid residue of P using chemistries that are compatible with covalent linkage to nitrogen, including, but not limited to, alkylation, amide bond, urea, thiourea, carbamate, and sulfonamide formation.

#### TETHERS (T)

[0054] T of the Formulas described herein is a lipophilic tether moiety which imparts lipophilicity to the APJ receptor compounds of the invention. The lipophilicity which T imparts, can promote penetration of the APJ receptor compounds into the cell membrane and tethering of the APJ receptor compounds to the cell membrane. As such, the lipophilicity imparted by T can facilitate interaction between the APJ receptor compounds of the invention and the cognate receptor.

**[0055]** The relative lipophilicity of compounds suitable for use as the lipophilic tether moiety of the Formulas described herein can be quantified by measuring the amount of the compound that partitions into an organic solvent layer (membrane-like) vs. an aqueous solvent layer (analogous to the extracellular or cytoplasmic environment). The partition coefficient in a mixed solvent composition, such as octanol/water or octanol/PBS, is the ratio of compound found at equilibrium in the octanol vs. the aqueous solvent (Partition coeff  $P = \frac{[\text{compound}]_{\text{octanol}}}{[\text{compound}]_{\text{aqueous}}}$ ). Frequently, the partition coefficient is expressed in logarithmic form, as the  $\log P$ . Compounds with greater lipophilicity have a more positive  $\log P$  than more hydrophilic compounds and tend to interact more strongly with membrane bilayers.

**[0056]** Computational programs are also available for calculating the partition coefficient for compounds suitable for use as the lipophilic tether moiety (T). In situations where the chemical structure is being varied in a systematic manner, for example by adding additional methylene units (-CH<sub>2</sub>-) onto to an existing alkyl group, the trend in  $\log P$  can be calculated using, for example, ChemDraw (CambridgeSoft, Inc).

**[0057]** In one embodiment, T is an optionally substituted (C<sub>6</sub>-C<sub>30</sub>)alkyl, (C<sub>6</sub>-C<sub>30</sub>)alkenyl, (C<sub>6</sub>-C<sub>30</sub>)alkynyl wherein 0-3 carbon atoms are replaced with oxygen, sulfur, nitrogen or a combination thereof.

**[0058]** In a specific embodiment, the (C<sub>6</sub>-C<sub>30</sub>)alkyl, (C<sub>6</sub>-C<sub>30</sub>)alkenyl, (C<sub>6</sub>-C<sub>30</sub>)alkynyl are substituted at one or more substitutable carbon atoms with halogen, -CN, -OH, -NH<sub>2</sub>, NO<sub>2</sub>, -NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>1</sub>-C<sub>6</sub>)haloalkyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>1</sub>-C<sub>6</sub>)haloalkoxy, aryloxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, -CONH<sub>2</sub>, -OCONH<sub>2</sub>, -NHCONH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylCONH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHCON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylCON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -NHC(S)NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHC(S)NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHC(S)N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -CONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl -CON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -C(S)(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>NH<sub>2</sub>, -S(O)<sub>p</sub>NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -CO(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OCO(C<sub>1</sub>-C<sub>6</sub>)alkyl, -C(O)O(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OC(O)O(C<sub>1</sub>-C<sub>6</sub>)alkyl, -C(O)H or -CO<sub>2</sub>H; and p is 1 or 2.

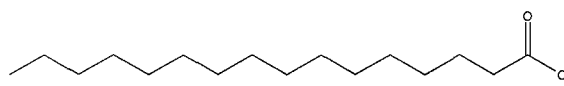
**[0059]** In a specific embodiment, T is selected from the group consisting of: CH<sub>3</sub>(CH<sub>2</sub>)<sub>9</sub>OPh-, CH<sub>3</sub>(CH<sub>2</sub>)<sub>6</sub>C=C(CH<sub>2</sub>)<sub>6</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>11</sub>O(CH<sub>2</sub>)<sub>3</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>9</sub>O(CH<sub>2</sub>)<sub>2</sub> and CH<sub>3</sub>(CH<sub>2</sub>)<sub>13</sub>.

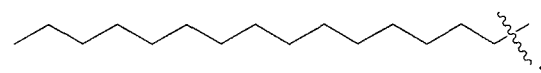
- 29 -

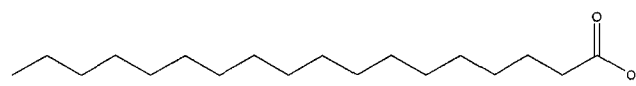
**[0060]** In a specific embodiment, T is selected from the group consisting of:  $\text{CH}_3(\text{CH}_2)_{16}$ ,  $\text{CH}_3(\text{CH}_2)_{15}$ ,  $\text{CH}_3(\text{CH}_2)_{14}$ ,  $\text{CH}_3(\text{CH}_2)_{13}$ ,  $\text{CH}_3(\text{CH}_2)_{12}$ ,  $\text{CH}_3(\text{CH}_2)_{11}$ ,  $\text{CH}_3(\text{CH}_2)_{10}$ ,  $\text{CH}_3(\text{CH}_2)_{9}$ ,  $\text{CH}_3(\text{CH}_2)_{8}$ ,  $\text{CH}_3(\text{CH}_2)_9\text{OPh-}$ ,  $\text{CH}_3(\text{CH}_2)_6\text{C}=\text{C}(\text{CH}_2)_6$ ,  $\text{CH}_3(\text{CH}_2)_{11}\text{O}(\text{CH}_2)_3$ , and  $\text{CH}_3(\text{CH}_2)_9\text{O}(\text{CH}_2)_2$  and  $\text{CH}_3(\text{CH}_2)_{13}$ .

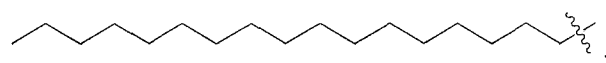
**[0061]** It is understood that the lipophilic moiety (T) of the Formulas described herein can be derived from precursor lipophilic compounds (e.g., fatty acids and bile acids). As used herein, "derived from" with regard to T, means that T is derived from a precursor lipophilic compound and that reaction of the precursor lipophilic compound in preparing the APJ receptor compounds of the Formulas described herein, results in a lipophilic tether moiety represented by T in the Formulas described herein that is structurally modified in comparison to the precursor lipophilic compound.

**[0062]** For example, the lipophilic tether moiety, T of the Formulas described herein can be derived from a fatty acid or a bile acid. It is understood that in accordance with the Formulas described herein when T is derived from a fatty acid (i.e., a fatty acid derivative), it is attached to L-P at the carbon atom alpha to the carbonyl carbon of the acid functional group in the fatty acid from which it is derived. For example, when T is derived from palmitic acid,

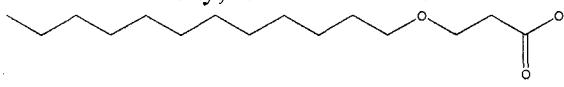
 , T of the Formulas described herein has the

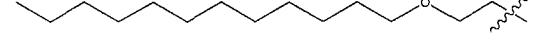
following structure:  . Similarly, when T is derived

from stearic acid,  , T of the Formulas

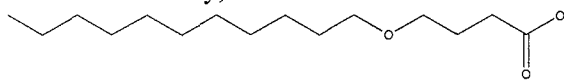
described herein has the following structure:  .

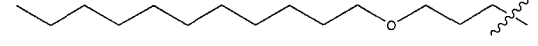
Similarly, when T is derived from 3-(dodecyloxy)propanoic acid,

 , T of the Formulas described herein has the

following structure:  .

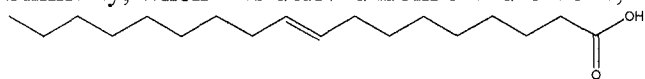
Similarly, when T is derived from 4-(undecyloxy)butanoic acid,

 , T of the Formulas described herein has the

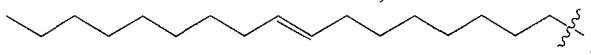
following structure:  .

- 30 -

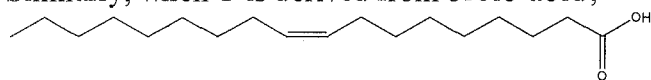
Similarly, when T is derived from elaidic acid,



, T of the Formulas described herein has the following structure:



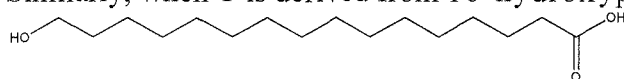
Similarly, when T is derived from oleic acid,



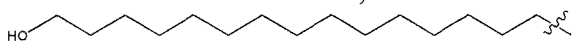
, T of the Formulas described herein has the following structure:



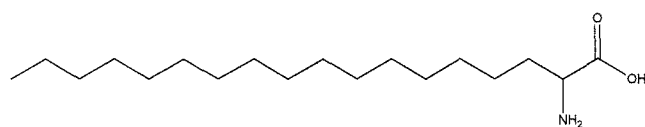
Similarly, when T is derived from 16-hydroxypalmitic acid,



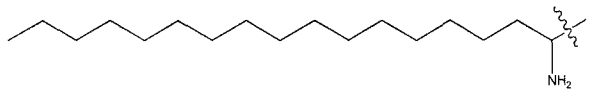
, T of the Formulas described herein has the following structure:



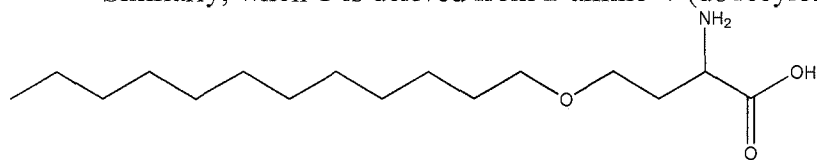
Similarly, when T is derived from 2-aminooctadecanoic acid



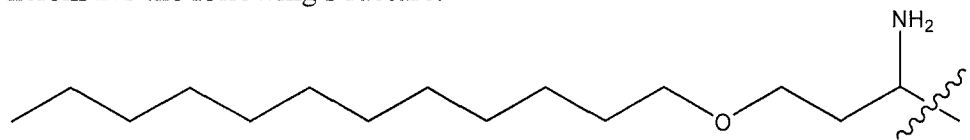
, T of the Formulas described herein has the following structure:



Similarly, when T is derived from 2-amino-4-(dodecyloxy)butanoic acid,



herein has the following structure:

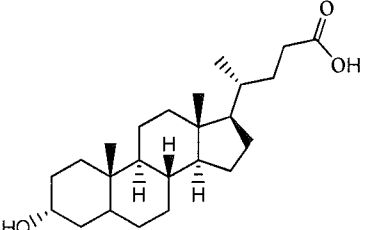


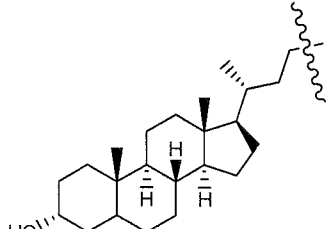
**[0063]** In a further embodiment, T is derived from a fatty acid. In a specific embodiment, T is derived from a fatty acid selected from the group consisting of: butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid.

- 31 -

[0064] In another specific embodiment, T is derived from a fatty acid selected from the group consisting of: myristoleic acid, palmitoleic acid, oleic acid, linoleic acid,  $\alpha$ -linolenic acid, arachidonic acid, eicosapentaenoic acid, erucic acid, docosahexaenoic acid

[0065] In another embodiment, T of the Formulas described herein can be derived from a bile acid. Similar to the embodiment where T is a fatty acid derivative, it is understood that in accordance with the Formulas described herein, when T is derived from a bile acid (i.e., a bile acid derivative) it is attached to L-P at the carbon atom alpha to the carbonyl carbon of the acid functional group in the bile acid from which it is derived. For example, when T is derived from

lithocholic acid, , T of the Formulas described herein has the

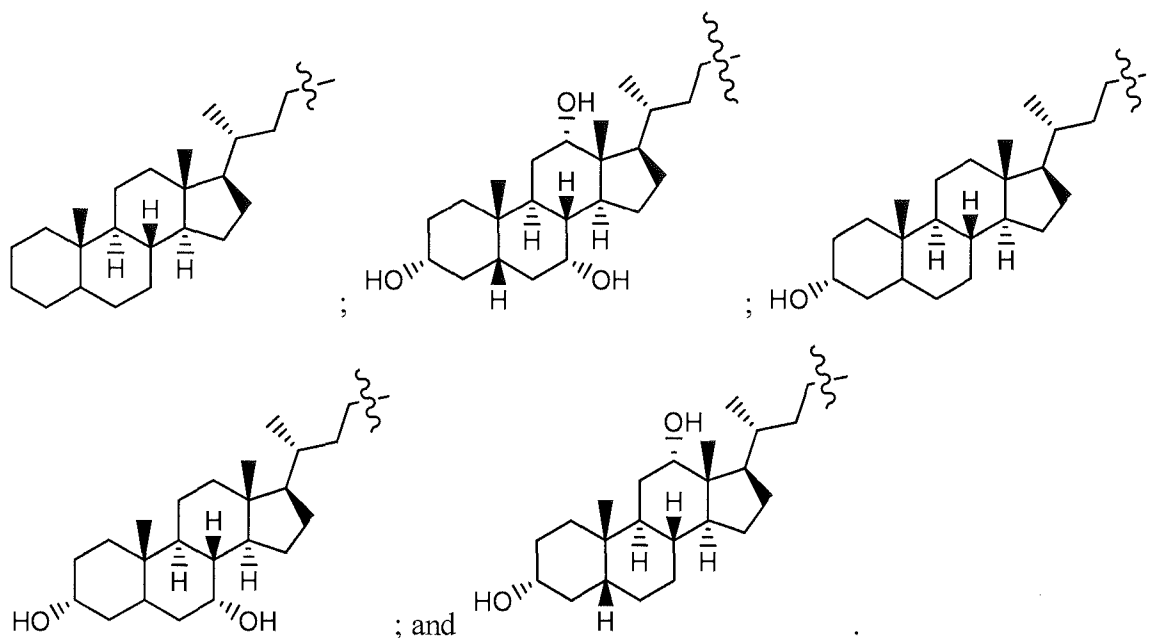
following structure: 

In a further embodiment, T is derived from a bile acid. In a specific embodiment, T is derived from a bile acid selected from the group consisting of: lithocholic acid, chenodeoxycholic acid, deoxycholic acid, cholanic acid, cholic acid, ursocholic acid, ursodeoxycholic acid, isoursodeoxycholic acid, lagodeoxycholic acid, dehydrocholic acid, hyocholic acid, hyodeoxycholic acid and the like.

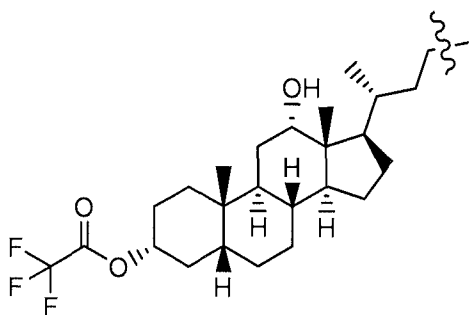
[0066] For example, T is selected from:



- 32 -



In another further embodiment, T is derived from a bile acid described above that has been modified at other than the acid functional group. For example, T can be derived from any of the bile acids described above, where the hydroxy position has been modified to form an ester or a halo ester. For example, T can be:



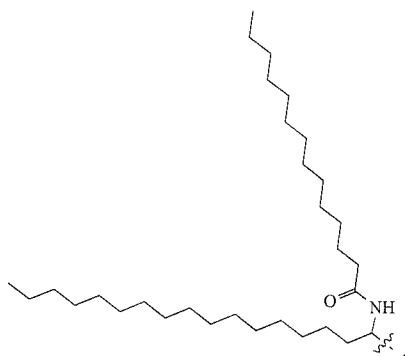
Other lipophilic moieties suitable for use as the lipophilic membrane tether, T, of the Formulas described herein, include but are not limited to steroids. Suitable steroids include, but are not limited to, sterols; progestagens; glucocorticoids; mineralcorticoids; androgens; and estrogens. Generally any steroid capable of attachment or which can be modified for incorporation into the Formulas described herein can be used. It is understood that the lipophilic membrane tether, T, may be slightly modified from the precursor lipophilic compound as a result of incorporation into the Formulas described herein.

- 33 -

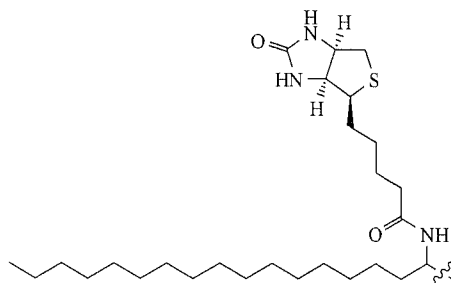
[0067] Suitable sterols for use in the invention at T, include but are not limited to: cholestanol, coprostanol, cholesterol, epicholesterol, ergosterol, ergocalciferol, and the like. Preferred sterols are those that provide a balance of lipophilicity with water solubility.

[0068] Suitable progestagens include, but are not limited to progesterone. Suitable glucocorticoids include, but are not limited to cortisol. Suitable mineralcorticoids include, but are not limited to aldosterone. Suitable androgens include, but are not limited to testosterone and androstenedione. Suitable estrogens include, but are not limited to estrone and estradiol.

[0069] In another specific embodiment, T can be derived from 2-tetradecanamideoctadecanoic acid. Similar to the embodiment where T is a fatty acid derivative, it is understood that in accordance with the Formulas described herein, when T is derived from 2-tetradecanamideoctadecanoic acid it is attached to L-P at the carbon atom alpha to the carbonyl carbon of the acid functional group in the bile acid from which it is derived. For example, when T is derived from 2-tetradecanamideoctadecanoic acid, the tether is:

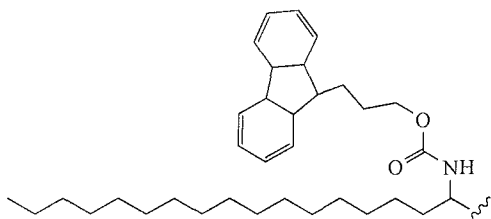


In another embodiment, T of the Formulas described herein can be derived from 2-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)octadecanoic acid. For example, when T is derived from 2-(5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamido)octadecanoic acid, the tether is:



- 34 -

In yet another embodiment, T of the Formulas described herein can be:



It is understood, that the compounds can contain one or more tether moieties.

In certain aspects, the tether moieties are the same. In other embodiments, the tether moieties are different.

#### COMPOUNDS (T-L-P)

[0070] In a first aspect of the invention, the APJ compounds of the invention are represented by Formula A:

T T-L-X<sub>1</sub>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-X<sub>17</sub>-X<sub>18</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>, wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

- 35 -

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub> is absent, a proline residue or a D-proline residue;

X<sub>2</sub> is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>3</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>4</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>5</sub> is N-methylarginine, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, a D-proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is a serine residue, a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, or a tyrosine residue;

X<sub>9</sub> is D-ornithine, is a glutamic acid residue, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline

- 36 -

residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;

X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or an arginine residue, or a tyrosine residue;

X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>17</sub> a phenylalanine residue, an isoleucine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>18</sub> is absent or an isoleucine residue, a proline residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue;

- 37 -

wherein seventeen contiguous amino acids are present and optionally 1- 5 amino acid residues are present in the D configuration.

[0071] In another aspect of the invention the compounds are represented by Formula B:

$$T-L-X_1'-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17}-X_{18}-R_1;$$

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of  $X_1'$  and is selected from:  $C^*(O)$ ,  $C^*(S)$ ,  $S^*(O)_2$ ,  $N(R^{13})S^*(O)$ ,  $N(R^{13})S^*(O)_2$ ,  $N(R^{13})C^*(O)$ ,  $N(R^{13})C^*(S)$ ,  $OC^*(O)$ ,  $OC^*(S)$ ,  $SC^*(O)$ ,  $SC^*(S)$ ,  $C^*(=NH)$ , and  $N(R^{13})C^*(=NH)$ ; wherein L is bonded to  $X_1'$  at the atom marked with an asterisk (\*) and  $R^{13}$  is selected from: H, D,  $(C_1-C_6)$ alkyl,  $(C_2-C_6)$ alkenyl,  $(C_2-C_6)$ alkynyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_9)$ cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L;  $R_1$  is  $-OR_2$ , - or  $NR_3R_4$ , wherein

each  $R_2$  is hydrogen or a  $(C_1-C_{10})$  alkyl group,

$R_3$  and  $R_4$  are each independently selected from hydrogen,  $(C_1-C_{10})$  alkyl,  $(C_1-C_{10})$ aralkyl,  $[CH_2CH_2O]_nCH_2CH_2C(O)OR_2$  or  $[CH_2CH_2O]_nCH_2CH_2C(O)NR_2$ ;

n is 1-20

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3$  alkyl),  $-C(O)(C_1-C_3$  alkyl),  $-NHC(O)(C_1-C_3$  alkyl),  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3$  alkyl) $_2$ ,  $-NHC(O)O-(C_1-C_3$  alkyl),  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3$  alkyl),  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3$  alkyl),  $-NHC(O)N(C_1-C_3$  alkyl) $_2$ , or  $-SO_2NR_2$ ;

- 38 -

- X<sub>1</sub>' is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue, a proline residue or a D-proline residue;
- X<sub>2</sub> is a threonine residue, a valine residue, a proline residue, histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;
- X<sub>3</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;
- X<sub>4</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, an arginine residue, or a proline residue;
- X<sub>5</sub> is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, a serine residue, or a glycine residue;
- X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>7</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an arginine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>8</sub> is a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a serine residue, a glycine residue, or a tyrosine residue;
- X<sub>9</sub> is D-ornithine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a proline residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, an arginine residue, or a tyrosine residue;

- 39 -

X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, a glutamic acid residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>17</sub> a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>18</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally 1- 5 amino acid residues are present in the D configuration.

**[0072]** More specifically, the compounds of the invention are represented by Formula C:

T-L-X<sub>1</sub>"-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub> -R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>" and is selected from: C(O), C(S), S(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>" at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H,



- 40 -

D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>, wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub>" is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is absent, is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;

- 41 -

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is absent, is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is D-ornithine;

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

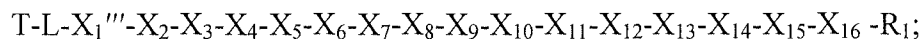
X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

- 42 -

[0073] More specifically, the compounds of the invention are represented by Formula D:



or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of  $X_1'''$  and is selected from:  $C^*(O)$ ,  $C^*(S)$ ,  $S^*(O)_2$ ,  $N(R^{13})S^*(O)$ ,  $N(R^{13})S^*(O)_2$ ,  $N(R^{13})C^*(O)$ ,  $N(R^{13})C^*(S)$ ,  $OC^*(O)$ ,  $OC^*(S)$ ,  $SC^*(O)$ ,  $SC^*(S)$ ,  $C(=NH)$ , and  $N(R^{13})C^*(=NH)$ ; wherein L is bonded to  $X_1'''$  at the atom marked with an asterisk (\*) and  $R^{13}$  is selected from: H, D,  $(C_1-C_6)$ alkyl,  $(C_2-C_6)$ alkenyl,  $(C_2-C_6)$ alkynyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_9)$ cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L;  $R_1$  is  $-OR_2$ , - or  $NR_3R_4$ , wherein

each  $R_2$  is hydrogen or a  $(C_1-C_{10})$  alkyl group,

$R_3$  and  $R_4$  are each independently selected from hydrogen,  $(C_1-C_{10})$  alkyl,  $(C_1-C_{10})$ aralkyl,  $[CH_2CH_2O]_nCH_2CH_2C(O)OR_2$  or  $[CH_2CH_2O]_nCH_2CH_2C(O)NR_2$ ;

n is 1-20

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3$  alkyl),  $-C(O)(C_1-C_3$  alkyl),  $-NHC(O)(C_1-C_3$  alkyl),  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3$  alkyl) $_2$ ,  $-NHC(O)O-(C_1-C_3$  alkyl),  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3$  alkyl),  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3$  alkyl),  $-NHC(O)N(C_1-C_3$  alkyl) $_2$ , or  $-SO_2NR_2$ ;

$X_1'''$  is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

- 43 -

- X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;
- X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;
- X<sub>4</sub> is N-methylarginine, is an asparagine residue, an arginine residue, a histidine residue, or a glutamine residue;
- X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>8</sub> is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;
- X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>14</sub> is an asparagine residue; isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 44 -

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein at least one of X<sub>4</sub> or X<sub>14</sub> is an asparagine residue,

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

**[0074]** In another aspect of the invention the compounds are represented by Formula E:

T-L-X<sub>1</sub><sup>''''</sup>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub><sup>''''</sup> and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub><sup>''''</sup> at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

- 45 -

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub>''' is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is N-methylarginine;

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 46 -

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

**[0075]** In one embodiment, the compounds T-L-P of the invention exhibit inhibition of cAMP production at the hAPJ receptor with an  $EC_{50} \leq 50$  nM (hAPJ) according to the HTRF cAMP Assay described under Functional Assays and the data of Table 6. Compounds of the invention with  $EC_{50} \leq 50$  nM (hAPJ) are Compound Nos. 1-36.

**[0076]** In a particular embodiment, the compounds exhibit  $EC_{50}$  from 25- 50 nM (hAPJ), %IA from 50 - 79% and either % histamine  $\leq 35\%$  or formal (+) charge  $\leq 1$  according to the according to the HTRF cAMP Assay and Histamine Assay described under Functional Assays and the data of Table 6. Compounds of the invention with  $EC_{50}$  from 25- 50 nM (hAPJ), %IA from 50 - 79% and either % histamine  $\leq 35\%$  or formal (+) charge  $\leq 1$  are Compound Nos. 6, 7, 19, 20, 33, 34, 35, and 16.

**[0077]** In a particular embodiment, the compounds exhibit  $EC_{50} \leq 25$  nM (hAPJ), %IA  $\geq 80\%$  and either % histamine  $> 35\%$  or formal (+) charge  $> 1$  according to the according to the HTRF cAMP Assay and Histamine Assay described under Functional Assays and the data of Table 6. Compounds of the invention with  $EC_{50} \leq 25$  nM (hAPJ), %IA  $\geq 80\%$  and either % histamine  $> 35\%$  or formal (+) charge  $> 1$  are Compound Nos. 3, 14, 18, 21, 24, 25, 26, 27, 28, 29, and 32.

**[0078]** In a particular embodiment, the compounds exhibit  $EC_{50} \leq 25$  nM (hAPJ), %IA  $\geq 80\%$  and either % histamine  $\leq 35\%$  or formal (+) charge  $\leq 1$  according to the according to the

HTRF cAMP Assay and Histamine Assay described under Functional Assays and the data of Table 6. Compounds of the invention with  $EC_{50} \leq 25$  nM (hAPJ),  $\%IA \geq 80\%$  and either  $\%$  histamine  $\leq 35\%$  or formal (+) charge  $\leq 1$  are Compound Nos. 8, 12, 13, 15, 17, 30, and 36.

[0079] In yet another embodiment, a GPCR compound of the invention is selected from one of the following compounds in Table 3a or 3b or shown in FIGS. 4 and 5 or a pharmaceutically acceptable salt thereof:

**Table 3a:**

Cmpd ID No.	Tether	Linker	Sequence	C-terminus
8	Pal	-C(O)-	TVFRSSE(d-Orn)RRSADIFI (SEQ ID NO: 9)	NH <sub>2</sub>
12	Pal	-C(O)-	DVFQSSEkRRSADQFI (SEQ ID NO: 13)	NH <sub>2</sub>
13	Pal	-C(O)-	DVFRSSEkRRSADQFI (SEQ ID NO: 14)	NH <sub>2</sub>
15	Pal	-C(O)-	TVFQSSEkRRSADFI (SEQ ID NO: 16)	NH <sub>2</sub>
17	Pal	-C(O)-	DVFQSEkRRSADQFI (SEQ ID NO: 18)	NH <sub>2</sub>
30	Pal	-C(O)-	PkRRSADIFIP (SEQ ID NO: 31)	NH <sub>2</sub>
36	Pal	-C(O)-	DVHRSSEkRRSADQFI (SEQ ID NO: 37)	NH <sub>2</sub>

**Table 3b:**

Cmpd ID No.	Tether	Linker	Sequence	C-terminus
3	Pal	-C(O)-	PTVFRSPSEkRRSADIFI (SEQ ID NO: 4)	NH <sub>2</sub>
14	Pal	-C(O)-	TVFRSEkRRSADQFI (SEQ ID NO: 15)	NH <sub>2</sub>
18	Pal	-C(O)-	TVFRSSEkRRSADNF (SEQ ID NO: 19)	NH <sub>2</sub>
21	Pal	-C(O)-	TVKRSSEkRRSADIFI (SEQ ID NO: 22)	NH <sub>2</sub>
24	Pal	-C(O)-	TVFRSSEkRRPSADIFP (SEQ ID NO: 25)	NH <sub>2</sub>
25	Pal	-C(O)-	TVFRSSPEkRRSADIFI (SEQ ID NO: 6)	NH <sub>2</sub>
26	Pal	-C(O)-	TVFRSSPEkRRSADIFP (SEQ ID NO: 27)	NH <sub>2</sub>



27	Pal	-C(O)-	TVFRSSEPkRRSADIFI (SEQ ID NO: 28)	NH <sub>2</sub>
28	Pal	-C(O)-	TVFRSSEPkRRSADIFP (SEQ ID NO: 29)	NH <sub>2</sub>
29	Pal	-C(O)-	pTVFRSpSEkRRSADIF (SEQ ID NO: 30)	NH <sub>2</sub>
32	Pal	-C(O)-	TVD(N-MeR)SSEkRRSADIFI (SEQ ID NO: 33)	NH <sub>2</sub>

Herein, “Cycloalkyl” used alone or as part of a larger moiety such as “cycloalkylalkyl” refers to a monocyclic or polycyclic, non-aromatic ring system of 3 to 20 carbon atoms, 3 to 12 carbon atoms, or 3 to 9 carbon atoms, which may be saturated or unsaturated. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cyclohexa-1,3-dienyl, cyclooctyl, cycloheptanyl, norbornyl, adamantyl, and the like.

[0080] “Heterocycloalkyl” refers to a saturated or unsaturated, non-aromatic, monocyclic or polycyclic ring system of 3 to 20 atoms, 3 to 12 atoms, or 3 to 8 atoms, containing one to four ring heteroatoms chosen from O, N and S. Examples of heterocycloalkyl groups include pyrrolidine, piperidine, tetrahydrofuran, tetrahydropyran, tetrahydrothiophene, tetrahydrothiopyran, isoxazolidine, 1,3-dioxolane, 1,3-dithiolane, 1,3-dioxane, 1,4-dioxane, 1,3-dithiane, 1,4-dithiane, morpholine, thiomorpholine, thiomorpholine-1,1-dioxide, tetrahydro-2H-1,2-thiazine-1,1-dioxide, isothiazolidine-1,1-dioxide, pyrrolidin-2-one, piperidin-2-one, piperazin-2-one, and morpholin-2-one, and the like.

[0081] “Halogen” and “halo” refer to fluoro, chloro, bromo or iodo.

[0082] “Haloalkyl” refers to an alkyl group substituted with one or more halogen atoms. By analogy, “haloalkenyl”, “haloalkynyl”, etc., refers to the group (for example alkenyl or alkynyl) substituted by one or more halogen atoms.

[0083] “Cyano” refers to the group –CN.

[0084] “Oxo” refers to a divalent =O group.

[0085] “Thioxo” refers to a divalent =S group.

[0086] “Ph” refers to a phenyl group.

[0087] “Carbonyl” refers to a divalent –C(O)- group.

[0088] “Alkyl” used alone or as part of a larger moiety such as “hydroxyalkyl”, “alkoxyalkyl”, “alkylamine” refers to a straight or branched, saturated aliphatic group having the

specified number of carbons, typically having 1 to 12 carbon atoms. More particularly, the aliphatic group may have 1 to 10, 1 to 8, 1 to 6, or 1 to 4 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, *tert*-butyl, n-hexyl, and the like.

[0089] “Alkenyl” refers to a straight or branched aliphatic group with at least one double bond. Typically, alkenyl groups have from 2 to 12 carbon atoms, from 2 to 8, from 2 to 6, or from 2 to 4 carbon atoms. Examples of alkenyl groups include ethenyl ( $-\text{CH}=\text{CH}_2$ ), n-2-propenyl (allyl,  $-\text{CH}_2\text{CH}=\text{CH}_2$ ), pentenyl, hexenyl, and the like.

[0090] “Alkynyl” refers to a straight or branched aliphatic group having at least 1 site of alkynyl unsaturation. Typically, alkynyl groups contain 2 to 12, 2 to 8, 2 to 6 or 2 to 4 carbon atoms. Examples of alkynyl groups include ethynyl ( $-\text{C}\equiv\text{CH}$ ), propargyl ( $-\text{CH}_2\text{C}\equiv\text{CH}$ ), pentynyl, hexynyl, and the like.

[0091] “Alkylene” refers to a bivalent saturated straight-chained hydrocarbon, e.g.,  $\text{C}_1\text{-C}_6$  alkylene includes  $-(\text{CH}_2)_6-$ ,  $-\text{CH}_2\text{-CH}-(\text{CH}_2)_3\text{CH}_3$ , and the like. “Bivalent means that the alkylene group is attached to the remainder of the molecule through two different carbon atoms.

[0092] “Alkenylene” refers to an alkylene group with in which one carbon-carbon single bond is replaced with a double bond.

[0093] “Alkynylene” refers to an alkylene group with in which one carbon-carbon single bond is replaced with a triple bond.

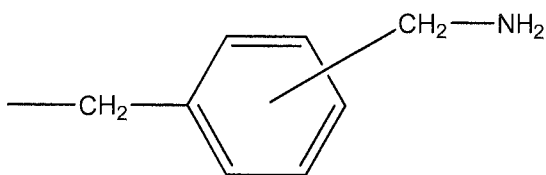
[0094] “Aryl” used alone or as part of a larger moiety as in “aralkyl” refers to an aromatic carbocyclic group of from 6 to 14 carbon atoms having a single ring or multiple condensed rings. The term “aryl” also includes aromatic carbocycle(s) fused to cycloalkyl or heterocycloalkyl groups. Examples of aryl groups include phenyl, benzo[*d*][1,3]dioxole, naphthyl, phenantrenyl, and the like.

[0095] “Aryloxy” refers to an  $-\text{OAr}$  group, wherein O is an oxygen atom and Ar is an aryl group as defined above.

[0096] An “aralkyl group” is an alkyl group substituted with an aryl group. Examples of aralkyl groups include  $-\text{CH}_2$ -phenyl, wherein the phenyl group is optionally substituted on a substitutable ring carbon. The term “ring atom” is an atom such as C, N, O or S that is in the ring of an aromatic group, cycloalkyl group or non-aromatic heterocyclic ring.

- 50 -

[0097] A “substitutable ring atom” in an aromatic group is a ring carbon or nitrogen atom bonded to a hydrogen atom. The hydrogen can be optionally replaced with a suitable substituent group. Thus, the term “substitutable ring atom” does not include ring nitrogen or carbon atoms which are shared when two rings are fused. In addition, “substitutable ring atom” does not include ring carbon or nitrogen atoms when the structure depicts that they are already attached to a moiety other than hydrogen. Examples of suitable substituents on a substitutable ring carbon atom of an aryl group include optionally substituted C<sub>1</sub>-C<sub>6</sub> alkyl. In a specific embodiment the



aryl group is

para to the methylene.

, more specifically the alkyl amine is

[0098] A “nitrogen-containing non-aromatic heterocyclic group” is a non-aromatic heterocyclic group with at least one nitrogen ring atom, and can be monocyclic, or polycyclic, for example, fused bicyclic or bridged bicyclic. Nitrogen-containing non-aromatic heterocyclic groups typically having three to fourteen members, preferably five to ten, in which one or more ring carbons, can each be replaced by a heteroatom such as N, O, or S, which can be saturated or unsaturated.

[0099] Examples of nitrogen-containing non-aromatic heterocyclic groups include pyrrolidinyl, piperazinyl, piperidinyl, morpholinyl

“Alkyl cycloalkyl” refers to an alkyl having at least one alkyl hydrogen atom replaced with a cycloalkyl moiety, such as -CH<sub>2</sub>-cyclohexyl, -CH<sub>2</sub>-cyclohexenyl, and the like.

[00100] “Heteroaryl” used alone or a part of a larger moiety as in “heteroaralkyl” refers to a 5 to 14 membered monocyclic, bicyclic or tricyclic heteroaromatic ring system, containing one to four ring heteroatoms independently selected from nitrogen, oxygen and sulfur. The term “heteroaryl” also includes heteroaromatic ring(s) fused to cycloalkyl or heterocycloalkyl groups. Particular examples of heteroaryl groups include optionally substituted pyridyl, pyrrolyl, pyrimidinyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, pyrazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl, 1,3,4-oxadiazolyl, 1,3,4-triazinyl, 1,2,3-triazinyl, benzofuryl, [2,3-dihydro]benzofuryl, isobenzofuryl,

benzothienyl, benzotriazolyl, isobenzothienyl, indolyl, isoindolyl, 3H-indolyl, benzimidazolyl, imidazo[1,2-a]pyridyl, benzothiazolyl, benzoxa-zolyl, quinoliziny, quinazoliny, phthalaziny, quinoxaliny, cinnoliny, naphthyridiny, pyrido[3,4-b]pyridyl, pyrido[3,2-b]pyridyl, pyrido[4,3-b]pyridyl, quinolyl, isoquinolyl, tetrazolyl, 1,2,3,4-tetrahydroquinolyl, 1,2,3,4-tetrahydroisoquinolyl, puriny, pteridiny, carbazolyl, xanthenyl, benzoquinolyl, and the like.

**[00101]** “Heteroaryloxy” refers to an –OHet group, wherein O is an oxygen atom and Het is a heteroaryl group as defined above.

**[00102]** “Heteroaralkyl” refers to an alkyl having at least one alkyl hydrogen atom replaced with a heteroaryl moiety, such as –CH<sub>2</sub>-pyridiny, -CH<sub>2</sub>-pyrimidiny, and the like.

**[00103]** “Alkoxy” refers to the group –O-R where R is “alkyl”, “cycloalkyl”, “alkenyl”, or “alkynyl”. Examples of alkoxy groups include for example, methoxy, ethoxy, ethenoxy, and the like.

**[00104]** “Alkyl heterocycloalkyl” refers to an alkyl having at least one alkyl hydrogen atom replaced with a heterocycloalkyl moiety, such as -CH<sub>2</sub>-morpholino, -CH<sub>2</sub>-piperidyl and the like.

**[00105]** “Alkoxy carbonyl” refers to the group –C(O)OR where R is “alkyl”, “alkenyl”, “alkynyl”, “cycloalkyl”, “heterocycloalkyl”, “aryl”, or “heteroaryl”.

**[00106]** “Hydroxyalkyl” and “alkoxyalkyl” are alky groups substituted with hydroxyl and alkoxy, respectively.

**[00107]** “Amino” means –NH<sub>2</sub>; “alkylamine” and “dialkylamine” mean –NHR and -NR<sub>2</sub>, respectively, wherein R is an alkyl group. “Cycloalkylamine” and “dicycloalkylamine” mean –NHR and -NR<sub>2</sub>, respectively, wherein R is a cycloalkyl group. “Cycloalkylalkylamine” means –NHR wherein R is a cycloalkylalkyl group. “[Cycloalkylalkyl][alkyl]amine” means –N(R)<sub>2</sub> wherein one R is cycloalkylalkyl and the other R is alkyl.

**[00108]** Haloalkyl and halocycloalkyl include mono, poly, and perhaloalkyl groups where the halogens are independently selected from fluorine, chlorine, bromine and iodine.

**[00109]** Suitable substituents for “alkyl”, “alkenyl”, “alkynyl”, “cycloalkyl”, “heterocycloalkyl”, “aryl”, or “heteroaryl”, etc., are those which will form a stable compound of the invention. Examples of suitable substituents are those selected from the group consisting of halogen, -CN, -OH, -NH<sub>2</sub>, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)haloalkyl, aryl, heteroaryl, (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl, (5-7 membered) heterocycloalkyl, -NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>1</sub>-C<sub>6</sub>)alkoxycarbonyl, -CONH<sub>2</sub>, -OCONH<sub>2</sub>, -NHCONH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylCONH<sub>2</sub>, -N(C<sub>1</sub>-

- 52 -

C<sub>6</sub>)alkylCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHCON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylCON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -NHC(S)NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)NH<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHC(S)NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -NHC(S)N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -N(C<sub>1</sub>-C<sub>6</sub>)alkylC(S)N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -CONH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OCONH(C<sub>1</sub>-C<sub>6</sub>)alkyl -CON((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -C(S)(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>NH<sub>2</sub>, -S(O)<sub>p</sub>NH(C<sub>1</sub>-C<sub>6</sub>)alkyl, -S(O)<sub>p</sub>N((C<sub>1</sub>-C<sub>6</sub>)alkyl)<sub>2</sub>, -CO(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OCO(C<sub>1</sub>-C<sub>6</sub>)alkyl, -C(O)O(C<sub>1</sub>-C<sub>6</sub>)alkyl, -OC(O)O(C<sub>1</sub>-C<sub>6</sub>)alkyl, -C(O)H or -CO<sub>2</sub>H. More particularly, the substituents are selected from halogen, -CN, -OH, -NH<sub>2</sub>, (C<sub>1</sub>-C<sub>4</sub>)alkyl, (C<sub>1</sub>-C<sub>4</sub>)haloalkyl, (C<sub>1</sub>-C<sub>4</sub>)alkoxy, phenyl, and (C<sub>3</sub>-C<sub>7</sub>)cycloalkyl. Within the framework of this invention, said "substitution" is also meant to encompass situations where a hydrogen atom is replaced with a deuterium atom. p is an integer with a value of 1 or 2.

**[00110]** Pharmaceutically acceptable salts of the compounds disclosed herein are included in the present invention. For example, an acid salt of a compound containing an amine or other basic group can be obtained by reacting the compound with a suitable organic or inorganic acid, resulting in pharmaceutically acceptable anionic salt forms. Examples of anionic salts include the acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glyceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, pamoate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teclate, tosylate, and triethiodide salts.

**[00111]** Salts of the compounds containing an acidic functional group can be prepared by reacting with a suitable base. Such a pharmaceutically acceptable salt can be made with a base which affords a pharmaceutically acceptable cation, which includes alkali metal salts (especially sodium and potassium), alkaline earth metal salts (especially calcium and magnesium), aluminum salts and ammonium salts, as well as salts made from physiologically acceptable organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine, collidine, quinine, quinoline, and basic amino acids such as lysine and arginine.

## PHARMACEUTICAL COMPOSITIONS

[00112] The invention also provides pharmaceutical compositions comprising an effective amount of a compound of the Formulas described herein (e.g., including any of the formulae herein), or a pharmaceutically acceptable salt of said compound; and a pharmaceutically acceptable carrier. The carrier(s) are “pharmaceutically acceptable” in that they are not deleterious to the recipient thereof in an amount used in the medicament.

[00113] Pharmaceutically acceptable carriers, adjuvants and vehicles that may be used in the pharmaceutical compositions of this invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

[00114] If required, the solubility and bioavailability of the compounds of the present invention in pharmaceutical compositions may be enhanced by methods well-known in the art. One method includes the use of lipid excipients in the formulation. See “Oral Lipid-Based Formulations: Enhancing the Bioavailability of Poorly Water-Soluble Drugs (Drugs and the Pharmaceutical Sciences),” David J. Hauss, ed. Informa Healthcare, 2007; and “Role of Lipid Excipients in Modifying Oral and Parenteral Drug Delivery: Basic Principles and Biological Examples,” Kishor M. Wasan, ed. Wiley-Interscience, 2006.

[00115] Another known method of enhancing bioavailability is the use of an amorphous form of a compound of this invention optionally formulated with a poloxamer, such as LUTROL™ and PLURONIC™ (BASF Corporation), or block copolymers of ethylene oxide and propylene oxide. See United States patent 7,014,866; and United States patent publications 20060094744 and 20060079502.

[00116] The pharmaceutical compositions of the invention include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), pulmonary, vaginal or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. In certain

embodiments, the compound of the formulae herein is administered transdermally (e.g., using a transdermal patch or iontophoretic techniques). Other formulations may conveniently be presented in unit dosage form, e.g., tablets, sustained release capsules, and in liposomes, and may be prepared by any methods well known in the art of pharmacy. See, for example, Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA (17th ed. 1985).

[00117] Such preparative methods include the step of bringing into association with the molecule to be administered ingredients such as the carrier that constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers, liposomes or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[00118] In certain embodiments, the compound is administered orally. Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets, or tablets each containing a predetermined amount of the active ingredient; a powder or granules; a solution or a suspension in an aqueous liquid or a non-aqueous liquid; an oil-in-water liquid emulsion; a water-in-oil liquid emulsion; packed in liposomes; or as a bolus, etc. Soft gelatin capsules can be useful for containing such suspensions, which may beneficially increase the rate of compound absorption.

[00119] In the case of tablets for oral use, carriers that are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

[00120] Compositions suitable for oral administration include lozenges comprising the ingredients in a flavored basis, usually sucrose and acacia or tragacanth; and pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia.

[00121] Compositions suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

- 55 -

The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets.

**[00122]** Such injection solutions may be in the form, for example, of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are mannitol, water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant.

**[00123]** The pharmaceutical compositions of this invention may be administered in the form of suppositories for rectal administration. These compositions can be prepared by mixing a compound of this invention with a suitable non-irritating excipient which is solid at room temperature but liquid at the rectal temperature and therefore will melt in the rectum to release the active components. Such materials include, but are not limited to, cocoa butter, beeswax and polyethylene glycols.

**[00124]** The pharmaceutical compositions of this invention may be administered by nasal aerosol or inhalation. Such compositions are prepared according to techniques well-known in the art of pharmaceutical formulation and may be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art. See, e.g.: Rabinowitz JD and Zaffaroni AC, US Patent 6,803,031, assigned to Alexza Molecular Delivery Corporation.



- 56 -

**[00125]** Topical administration of the pharmaceutical compositions of this invention is especially useful when the desired treatment involves areas or organs readily accessible by topical application. For topical application topically to the skin, the pharmaceutical composition should be formulated with a suitable ointment containing the active components suspended or dissolved in a carrier. Carriers for topical administration of the compounds of this invention include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax, and water. Alternatively, the pharmaceutical composition can be formulated with a suitable lotion or cream containing the active compound suspended or dissolved in a carrier. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, and water. The pharmaceutical compositions of this invention may also be topically applied to the lower intestinal tract by rectal suppository formulation or in a suitable enema formulation. Topically-transdermal patches and iontophoretic administration are also included in this invention.

**[00126]** Application of the patient therapeutics may be local, so as to be administered at the site of interest. Various techniques can be used for providing the patient compositions at the site of interest, such as injection, use of catheters, trocars, projectiles, pluronic gel, stents, sustained drug release polymers or other device which provides for internal access.

**[00127]** Thus, according to yet another embodiment, the compounds of this invention may be incorporated into compositions for coating an implantable medical device, such as prostheses, artificial valves, vascular grafts, stents, or catheters. Suitable coatings and the general preparation of coated implantable devices are known in the art and are exemplified in US Patents 6,099,562; 5,886,026; and 5,304,121. The coatings are typically biocompatible polymeric materials such as a hydrogel polymer, polymethylsiloxane, polycaprolactone, polyethylene glycol, polylactic acid, ethylene vinyl acetate, and mixtures thereof. The coatings may optionally be further covered by a suitable topcoat of fluorosilicone, polysaccharides, polyethylene glycol, phospholipids or combinations thereof to impart controlled release characteristics in the composition. Coatings for invasive devices are to be included within the definition of pharmaceutically acceptable carrier, adjuvant or vehicle, as those terms are used herein.

[00128] According to another embodiment, the invention provides a method of coating an implantable medical device comprising the step of contacting said device with the coating composition described above. It will be obvious to those skilled in the art that the coating of the device will occur prior to implantation into a mammal.

[00129] According to another embodiment, the invention provides a method of impregnating an implantable drug release device comprising the step of contacting said drug release device with a compound or composition of this invention. Implantable drug release devices include, but are not limited to, biodegradable polymer capsules or bullets, non-degradable, diffusible polymer capsules and biodegradable polymer wafers.

[00130] According to another embodiment, the invention provides an implantable medical device coated with a compound or a composition comprising a compound of this invention, such that said compound is therapeutically active.

[00131] According to another embodiment, the invention provides an implantable drug release device impregnated with or containing a compound or a composition comprising a compound of this invention, such that said compound is released from said device and is therapeutically active.

[00132] Where an organ or tissue is accessible because of removal from the patient, such organ or tissue may be bathed in a medium containing a composition of this invention, a composition of this invention may be painted onto the organ, or a composition of this invention may be applied in any other convenient way.

[00133] In another embodiment, a composition of this invention further comprises a second therapeutic agent. In one embodiment, the second therapeutic agent is one or more additional compounds of the invention.

[00134] In another embodiment, the second therapeutic agent may be selected from any compound or therapeutic agent known to have or that demonstrates advantageous properties when administered with a compound having the same mechanism of action as the APJ receptor compound of Formula I.

[00135] In a particular embodiment, the second therapeutic is an agent useful in the treatment or prevention of a disease or condition selected from cardiovascular diseases, (e.g., hypertension and heart failure, such as congestive heart failure), cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection. In another embodiment, the second therapeutic is an agent useful in the treatment or prevention

of a disease or condition selected from hypertension and heart failure, in particular, congestive heart failure.

[00136] For example, when the disease or condition is congestive heart failure, the second therapeutic agent can be selected from: ACE inhibitors, beta blockers, vasodilator, calcium channel blockers, loop diuretics, aldosterone antagonists, and angiotensin receptor blockers.

[00137] When the disease or condition being treated is hypertension, the second therapeutic agent can be selected from:  $\alpha$ -blockers,  $\beta$ -blockers, calcium channel blockers, diuretics, natriuretics, saluretics, centrally acting antihypertensives, angiotensin converting enzyme (ACE) inhibitors, dual ACE and neutral endopeptidase (NEP) inhibitors, angiotensin-receptor blockers (ARBs), aldosterone synthase inhibitor, aldosterone-receptor antagonists, or endothelin receptor antagonist.

[00138]  $\alpha$ -Blockers include doxazosin, prazosin, tamsulosin, and terazosin.

[00139]  $\beta$ -Blockers for combination therapy are selected from atenolol, bisoprol, metoprolol, acetutolol, esmolol, celiprolol, taliprolol, acebutolol, oxprenolol, pindolol, propranolol, bupranolol, penbutolol, mepindolol, carteolol, nadolol, carvedilol, and their pharmaceutically acceptable salts.

[00140] Calcium channel blockers include dihydropyridines (DHPs) and non-DHPs. The preferred DHPs are selected from the group consisting of amlodipine, felodipine, ryosidine, isradipine, lacidipine, nicardipine, nifedipine, nigulpidine, niludipine, nimodiphine, nisoldipine, nitrendipine, and nivaldipine and their pharmaceutically acceptable salts. Non-DHPs are selected from flunarizine, prenylamine, diltiazem, fendiline, gallopamil, mibefradil, anipamil, tiapamil, and verampimil and their pharmaceutically acceptable salts.

[00141] A diuretic is, for example, a thiazide derivative selected from amiloride, chlorothiazide, hydrochlorothiazide, methylchlorothiazide, and chlorothalidon.

[00142] Centrally acting antihypertensives include clonidine, guanabenz, guanfacine and methyldopa.

[00143] ACE inhibitors include alacepril, benazepril, benazaprilat, captopril, ceronapril, cilazapril, delapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipiril, moveltopril, perindopril, quinapril, quinaprilat, ramipril, ramiprilat, spirapril, temocapril, trandolapril, and zofenopril. Preferred ACE inhibitors are benazepril, enalpril, lisinopril, and ramipril.

[00144] Dual ACE/NEP inhibitors are, for example, omapatrilat, fasidotril, and fasidotrilat.

[00145] Preferred ARBs include candesartan, eprosartan, irbesartan, losartan, olmesartan, tasosartan, telmisartan, and valsartan.

[00146] Preferred aldosterone synthase inhibitors are anastrozole, fadrozole, and exemestane.

[00147] Preferred aldosterone-receptor antagonists are spironolactone and eplerenone.

[00148] A preferred endothelin antagonist is, for example, bosentan, enrasentan, atrasentan, darusentan, sitaxentan, and tezosentan and their pharmaceutically acceptable salts.

[00149] In one embodiment, the invention provides separate dosage forms of a compound of this invention and one or more of any of the above-described second therapeutic agents, wherein the compound and second therapeutic agent are associated with one another. The term “associated with one another” as used herein means that the separate dosage forms are packaged together or otherwise attached to one another such that it is readily apparent that the separate dosage forms are intended to be sold and administered together (within less than 24 hours of one another, consecutively or simultaneously).

[00150] In the pharmaceutical compositions of the invention, the compound of the present invention is present in an effective amount. As used herein, the term “effective amount” refers to an amount which, when administered in a proper dosing regimen, is sufficient to treat (therapeutically or prophylactically) the target disorder. For example, an effective amount is sufficient to reduce or ameliorate the severity, duration or progression of the disorder being treated, prevent the advancement of the disorder being treated, cause the regression of the disorder being treated, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy. Preferably, the compound is present in the composition in an amount of from 0.1 to 50wt.%, more preferably from 1 to 30 wt.%, most preferably from 5 to 20wt.%.

[00151] The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described in Freireich et al., (1966) *Cancer Chemother. Rep* 50: 219. Body surface area may be approximately determined from height and weight of the patient. See, e.g., *Scientific Tables*, Geigy Pharmaceuticals, Ardsley, N.Y., 1970, 537.

[00152] For pharmaceutical compositions that comprise a second therapeutic agent, an effective amount of the second therapeutic agent is between about 20% and 100% of the dosage normally utilized in a monotherapy regime using just that agent. Preferably, an effective amount is between about 70% and 100% of the normal monotherapeutic dose. The normal monotherapeutic dosages of these second therapeutic agents are well known in the art. See, e.g.,

Wells et al., eds., *Pharmacotherapy Handbook*, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); *PDR Pharmacopoeia*, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000), each of which references are incorporated herein by reference in their entirety.

**[00153]** The compounds for use in the method of the invention can be formulated in unit dosage form. The term “unit dosage form” refers to physically discrete units suitable as unitary dosage for subjects undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form can be for a single daily treatment dose or one of multiple daily treatment doses (e.g., about 1 to 4 or more times per day). When multiple daily treatment doses are used, the unit dosage form can be the same or different for each dose.

#### METHODS OF TREATMENT

**[00154]** As used herein the term “subject” and “patient” typically means a human, but can also be an animal in need of treatment, e.g., companion animals (dogs, cats, and the like), farm animals (cows, pigs, horses, sheep, goats, and the like) and laboratory animals (rats, mice, guinea pigs, and the like).

**[00155]** The terms “treat” and “treating” are used interchangeably and include both therapeutic treatment and prophylactic treatment (reducing the likelihood of development). Both terms mean decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease (e.g., a disease or disorder delineated herein), lessen the severity of the disease or improve the symptoms associated with the disease.

**[00156]** “Disease” means any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ.

**[00157]** As used herein, the term “effective amount” refers to an amount which, when administered in a proper dosing regimen, is sufficient to treat (therapeutically or prophylactically) the target disorder. For example, an effective amount is sufficient to reduce or ameliorate the severity, duration or progression of the disorder being treated, prevent the advancement of the disorder being treated, cause the regression of the disorder being treated, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

- 61 -

[00158] The invention also includes methods of treating diseases, disorders or pathological conditions which benefit from modulation of the APJ receptor comprising administering an effective amount of an APJ receptor compound of the invention to a subject in need thereof. Diseases and conditions which can benefit from modulation (inhibition or activation) of the APJ receptor include, but are not limited to cardiovascular diseases, (e.g., hypertension and heart failure, such as congestive heart failure), cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection. In another embodiment, the second therapeutic is an agent useful in the treatment or prevention of a disease or condition selected from hypertension and heart failure, in particular, congestive heart failure and hypertrophic cardiomyopathy. In addition, the second therapeutic would be useful in the treatment or prevention of coronary artery disease, atherosclerosis, stable and unstable angina pectoris, restenosis, acute myocardial infarction, pulmonary hypertension, diseases related to cardiac ischemia, sudden heart death and for identifying therapeutics that modulate angiogenesis.

[00159] In one embodiment, APJ receptor compounds of the invention are useful as inotropic agents for use in patients with heart failure.

[00160] In another embodiment, the APJ receptor compounds of the invention can be administered for treatment of the hypertension.

[00161] In another embodiment, the APJ receptor compounds of the invention can be administered for treatment of HIV infection.

[00162] In an additional aspect, the APJ receptor compounds of the invention can be administered for treatment of tumor metastases.

[00163] In one embodiment, an effective amount of a compound of this invention can range from about .005 mg to about 5000 mg per treatment. In more specific embodiments, the range is from about .05 mg to about 1000 mg, or from about 0.5 mg to about 500 mg, or from about 5 mg to about 50 mg. Treatment can be administered one or more times per day (for example, once per day, twice per day, three times per day, four times per day, five times per day, etc.). When multiple treatments are used, the amount can be the same or different.

It is understood that a treatment can be administered every day, every other day, every 2 days, every 3 days, every 4 days, every 5 days, etc. For example, with every other day administration, a treatment dose can be initiated on Monday with a first subsequent treatment administered on

- 62 -

Wednesday, a second subsequent treatment administered on Friday, etc. Treatment is typically administered from one to two times daily. Effective doses will also vary, as recognized by those skilled in the art, depending on the diseases treated, the severity of the disease, the route of administration, the sex, age and general health condition of the patient, excipient usage, the possibility of co-usage with other therapeutic treatments such as use of other agents and the judgment of the treating physician.

[00164] Alternatively, the effective amount of a compound of the invention is from about 0.01 mg/kg/day to about 1000 mg/kg/day, from about 0.1 mg/kg/day to about 100 mg/kg/day, from about 0.5 mg/kg/day to about 50 mg/kg/day, or from about 1 mg/kg/day to 10 mg/kg/day.

[00165] In another embodiment, any of the above methods of treatment comprises the further step of co-administering to said patient one or more second therapeutic agents. The choice of second therapeutic agent may be made from any second therapeutic agent known to be useful for co-administration with a compound that modulates the APJ receptor. The choice of second therapeutic agent is also dependent upon the particular disease or condition to be treated. Examples of second therapeutic agents that may be employed in the methods of this invention are those set forth above for use in combination compositions comprising a compound of this invention and a second therapeutic agent.

[00166] The term "co-administered" as used herein means that the second therapeutic agent may be administered together with a compound of this invention as part of a single dosage form (such as a composition of this invention comprising a compound of the invention and an second therapeutic agent as described above) or as separate, multiple dosage forms. Alternatively, the additional agent may be administered prior to, consecutively with, or following the administration of a compound of this invention. In such combination therapy treatment, both the compounds of this invention and the second therapeutic agent(s) are administered by conventional methods. The administration of a composition of this invention, comprising both a compound of the invention and a second therapeutic agent, to a subject does not preclude the separate administration of that same therapeutic agent, any other second therapeutic agent or any compound of this invention to said subject at another time during a course of treatment.

[00167] In one embodiment of the invention, where a second therapeutic agent is administered to a subject, the effective amount of the compound of this invention is less than its effective amount would be where the second therapeutic agent is not administered. In another

embodiment, the effective amount of the second therapeutic agent is less than its effective amount would be where the compound of this invention is not administered. In this way, undesired side effects associated with high doses of either agent may be minimized. Other potential advantages (including without limitation improved dosing regimens and/or reduced drug cost) will be apparent to those of skill in the art.

#### KITS

**[00168]** The present invention also provides kits for use to treat the target disease, disorder or condition. These kits comprise (a) a pharmaceutical composition comprising a compound of Formula I, or a salt thereof, wherein said pharmaceutical composition is in a container; and (b) instructions describing a method of using the pharmaceutical composition to treat the target disease, disorder or condition.

**[00169]** The container may be any vessel or other sealed or sealable apparatus that can hold said pharmaceutical composition. Examples include bottles, ampules, divided or multi-chambered holders bottles, wherein each division or chamber comprises a single dose of said composition, a divided foil packet wherein each division comprises a single dose of said composition, or a dispenser that dispenses single doses of said composition. The container can be in any conventional shape or form as known in the art which is made of a pharmaceutically acceptable material, for example a paper or cardboard box, a glass or plastic bottle or jar, a re-sealable bag (for example, to hold a "refill" of tablets for placement into a different container), or a blister pack with individual doses for pressing out of the pack according to a therapeutic schedule. The container employed can depend on the exact dosage form involved, for example a conventional cardboard box would not generally be used to hold a liquid suspension. It is feasible that more than one container can be used together in a single package to market a single dosage form. For example, tablets may be contained in a bottle, which is in turn contained within a box. In one embodiment, the container is a blister pack.

**[00170]** The kits of this invention may also comprise a device to administer or to measure out a unit dose of the pharmaceutical composition. Such device may include an inhaler if said composition is an inhalable composition; a syringe and needle if said composition is an injectable composition; a syringe, spoon, pump, or a vessel with or without volume markings if



- 64 -

said composition is an oral liquid composition; or any other measuring or delivery device appropriate to the dosage formulation of the composition present in the kit.

[00171] In certain embodiment, the kits of this invention may comprise in a separate vessel of container a pharmaceutical composition comprising a second therapeutic agent, such as one of those listed above for use for co-administration with a compound of this invention.

## GENERAL METHODS FOR PREPARING APJ RECEPTOR COMPOUNDS

### Synthesis of Peptides

[00172] The peptide component (P) of the compounds of the invention can be synthesized by incorporating orthogonally protected amino acids in a step-wise fashion. Any suitable synthetic methods can be used. Traditional Fmoc or Boc chemistry can be easily adapted to provide the desired peptide component (P) of the compounds of the invention. Fmoc is generally preferred, because the cleavage of the Fmoc protecting group is milder than the acid deprotection required for Boc cleavage, which requires repetitive acidic deprotections that lead to alteration of sensitive residues, and increase acid catalyzed side reactions (Fields, G.B. *et al.* in *Int. J. Pept. Protein*, 1990, 35, 161).

[00173] The peptides can be assembled linearly via Solid Phase Peptide Synthesis (SPPS), can be assembled in solution using modular condensations of protected or unprotected peptide components or a combination of both.

### Solid Phase Peptide Synthesis

[00174] For SPPS, an appropriate resin is chosen that will afford the desired moiety on the C-terminus upon cleavage. For example upon cleavage of the linear peptide, a Rink amide resin will provide a primary amide on the C-terminus, whereas a Rink acid resin will provide an acid. Rink acid resins are more labile than Rink amide resins and the protected peptide could also be cleaved and subsequently the free acid activated to react with amines or other nucleophiles. Alternatively, other resins could provide attachment of other moieties prior to acylation, leading to cleavage of an alkylated secondary amide, ester or other desired C-terminal modification. A review of commonly used resins and the functional moiety that results after cleavage can be found in manufacturer literature such as NovaBiochem or Advanced Chemtech catalogues.

- 65 -

[00175] Typically a resin is chosen such that after cleavage the C-terminus is an amide bond. Rink amide resin is a resin that results in a C-terminal amide during cleavage. The orthogonally protected Fmoc amino acids are added stepwise using methods well known in literature (Bodansky M., Principles of Peptide Synthesis (1993) 318p; Peptide Chemistry, a Practical Textbook (1993); Springer-Verlag). These procedures could be done manually or by using automated peptide synthesizers.

[00176] The process involves activating the acid moiety of a protected amino acid, using activating agents such as HCTU, HBTU, HATU, PyBop or simple carbodiimides. Often an additive is used to decrease racemization during coupling such as HOBT or HOAt (Schnölzer, M. *et al.*, *Int. J. Pept. Protein Res.*, 1992, 40, 180). Manually, the coupling efficiency can be determined photometrically using a ninhydrin assay. If the coupling efficiency is below 98%, a second coupling may be desired. After the second coupling a capping step may be employed to prevent long deletion sequences to form, simplifying the purification of the desired final compound. With automation, second couplings are not commonly required, unless a residue is known to be problematic such as arginine.

[00177] Deprotection of the Fmoc is most commonly accomplished using piperidine (20%) in dimethylformamide (DMF). Alternatively other secondary amines may also be used such as morpholine, diethylamine or piperazine. This reaction is facile and normally is accomplished within 20 minutes using piperidine. After deprotection the resin is washed several times with DMF and DCM prior to coupling with the next residue. This process is repeated, assembling the peptide linearly until the sequence is complete. The final Fmoc is removed, which allows for coupling with the tether moiety.

[00178] In a preferred synthesis, the peptide is formed by SPPS accomplished manually or in an automated fashion using a commercially available synthesizer such as the CEM Microwave peptide synthesizer, Rainin Symphony synthesizer, or ABI 433 flow-through synthesizer or for parallel synthesis an Intavis MultiPep RS. Commercially available Rink Amide resin is used for synthesizing the C-terminal amide peptides (Rink, H., *Tetrahedron Lett*, 28, 4645, 1967).

Although the NovaSyn TGR resin is preferred for the Intavis instrument and will also provide the C-terminal amide. Peptide synthesis reagents (coupling, deprotection agents) are commercially available and include HOBT, HBTU, HCTU (Novabiochem) as well as DMF, DCM, Piperidine, NMM, NMP, and DIEA (Sigma-Aldrich). Suitably protected amino acids for

- 66 -

use in solid phase peptide synthesis are commercially available from many sources, including Sigma-Aldrich and CEM Corporation.

[00179] For example, a convenient preparation of peptides can be done in parallel at 5  $\mu$ M scale using the INTAVIS ResPep RS with NovaSyn TGR resin with 0.25mM loading using about 5 fold excess reagents, amino acids and coupling reagent (HCTU). Deprotection of Fmoc can be accomplished with 20% piperidine in DMF.

[00180] In another preferred synthesis, peptides can be synthesized using a microwave instrument using 10 eq of reagents. Deprotection of Fmoc can be accomplished with 20% piperidine in DMF followed by washing with DMF and DCM.

[00181] In both cases (*i.e.*, Rink acid and Rink amide resins), final Fmoc deprotection of the N-terminus would leave a free amine after cleavage from the resin unless it is modified prior to cleavage. Compounds of the invention could be modified with tether moieties via couplings resulting in an amide, thioamide, sulfonamide, urea, thiourea, carbamate, thiocarbamate, carbamodithioate, imine, imidamide, or guanidine bonds. The amino terminus could be capped with a lipid such as palmitic acid.

#### Amino acid reagents

[00182] The following commercially available orthogonally protected amino acids used can be used in the synthesis of compounds of the invention: Fmoc-Tyr(tBu)-OH, Fmoc-Ala-OH\*H<sub>2</sub>O, Fmoc-Arg(Pbf)-OH, Fmoc, Asn(Trt)-OH, Fmoc-Asp(tBu), Fmoc-Cys(tBu)-OH, Fmoc-Glu(tBu)-OH, Fmoc-Glx(Pbf)-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Leu-OH, Fmoc-Ile-OH, Fmoc, Lys(tBu)-OH, Fmoc-Met-OH, Fmoc-Phe-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Typ-OH, and Fmoc-Val-OH. Additional amino acids suitable for incorporation into the compounds of the invention (*e.g.*, D- amino acids, substituted amino acids and other protecting group variations) are also commercially available or synthesized by methods known in the art.

#### Synthesis:

[00183] In the general operation of the Intavis ResPep SL (or MultiPep RS), 96 well plates having a small pore at the bottom of each well are used. Without resin in the well, the activated solutions would not have a very long residence time in the well plate. Using a resin with good swelling properties blocks the pore under normal gravity conditions allowing the activated amino

- 67 -

acids to react with the resin for a prescribed time. Afterwards the instrument will activate a vacuum removing the solution from the resin and directing it to waste. Likewise the resin is washed and Fmoc deprotection continues the cycle. The cycle repeats itself for each amino acid coupling until the elongation is complete. N-terminal lipidation is accomplished using the same protocol as an amino acid in the sequence. Final deprotection and cleavage is done by hand external to the instrument.

#### Preferred Protocol

**[00184]** NovaSyn TGR resin is the preferred resin for use on the Intavis instrument. With its lower loading of 0.25 mmol/g (as compared to .6 for rink amide resin), it has better capability to swell up and hold the active chemicals during synthesis. The APJ compounds are synthesized in a 96-well format. To initiate synthesis, the resin has to be placed into each well of the 96 well plate. The recommended loading of each well is at a 5  $\mu$ M scale. The total plate would require 0.48 mmol of resin which is ca. 1.92 g of resin ( $.25/(96 * .005\text{mM})$ ). About 10 mL of N-Methylpyrrolidinone (NMP) is added to create a "slurry" of the resin which is transferred to the 96 wells with a multichannel pipettor, (100uL to each well). The NMP is removed under vacuum filtration in the instrument.

**[00185]** The test sequences of compounds are uploaded to the Intavis using an excel spreadsheet (or alternatively could be entered by hand). The instrument program calculates the amounts of amino acids, coupling reagent (HCTU) needed with volumes and they are prepared using this printout. These quantities are loaded on the instrument at 0.5M dissolved in DMF using 5-10% excess. The program also calculates the base N-methylmorpholine (NMM) and piperidine (20% in DMF). If the sequences contain DG combinations 0.1M HOBT is added to the piperidine solution to minimize side reaction (Asp rearrangement).

**[00186]** A deprotection protocol begins the synthesis. The Fmoc deprotection step is set up to repeat three times (a triple Fmoc deprotection) using 150 $\mu$ L (10 min/cycle) throughout the entire synthesis. After the third cycles, the plate is rinsed with 1800uL DMF, and the waste is extracted through the plate to the waste container. Likewise the amino acids are coupled three times (20min/cycle) by adding 42.5 $\mu$ L (1eq) of activator, 13 $\mu$ L (2eq) base, 2 $\mu$ L NMP (for solvent), and 44 $\mu$ L (1.05eq) of amino acid at 5 fold excess-to the loading of the resin in each well for each coupling. During the final cycle we Fmoc deprotect 4 times and the plate is washed

- 68 -

again with DMF(1800  $\mu$ l). Once the peptide sequence is complete, palmitic acid is coupled to the N-term of our sequences (4 cycles) using the same reagents and concentrations for each amino acid coupling. The palmitic acid is added from a solution of palmitic acid at 0.2M concentration due to solubility-.

#### Cleavage:

[00187] After completion of the synthesis, the plates are washed with NMP. The plate is removed and placed into a collection apparatus external to the instrument. Added to each well is the deprotection cocktail: 400  $\mu$ l TFA/70% MSA/TIS/DDT9:1:1:1. The plate is covered and kept at room temperature for 18-24 hr. The wells are evacuated under positive pressure (via a pipette) and the TFA solution is collected into a 96 well collection plate. The solution is transferred to a 2 mL HPLC vial using a disposable pipette. The vials are capped, labeled and loaded onto an agilent 1100 LC/MS equipped with mass based fraction collection using the Purification gradient below).

[00188] Fractions are collected in 96 well plates. Fractions containing the desired MW are combined and lyophilized. Purity analysis is performed on an Agilent 1100 HPLC using area under curve at 220nm.

#### Purification:

[00189] The aqueous HPLC mobile phase used for purification is 10mM ammonium acetate buffer and acetonitrile is the organic modifier. To purify our crude compounds, an analytical Phenomenex column (10micron C5 Luna column, 250mm x 4.6 mm) is used with a flow rate of 1.2mL/min. The gradient runs 21 minutes including re-equilibration.

#### Analytical Methods

[00190] The compounds of the invention are analyzed for purity by HPLC using the methods listed below. Purification is achieved by preparative HPLC.

- 69 -

Fast LC/MS Method

Column: Phenomenex Luna C-5 20x 30mm  
Flow: 1.0 ml/min  
Solvent A: 0.1 % TFA in Type I water  
Solvent B: 0.1% TFA in Acetonitrile  
UV: 220 nm  
Injection: 20 ul  
Gradient: 5-95%B (7 minutes); 95-5%B (1 minute); 5% B (4 minutes)

Analytical Purity Method

Column: Phenomenex Luna C-5 20x 30mm  
Flow: 1.0 ml/min  
Solvent A: 0.1 % TFA in Type I water  
Solvent B: 0.1% TFA in Acetonitrile  
UV: 220 nm  
Injection: 20 ul  
Gradient: 2-95%B (10 minutes); 95-2%B (2 minutes); 2% B (2 minutes)

Preparative LC/MS Method (CEM)

Column: Phenomenex Luna C-5 250mmx 150 mm  
Flow: 5 ml/min  
Solvent A: 0.1% TFA in Type I water  
Solvent B: 0.1% TFA in Acetonitrile  
UV: 220 nm  
Injection: 900 ul  
Gradient: 35%B (5 minutes); 35-85%B (13 minutes); 85-35% B (0.5 minutes); 35%B (1.5 minutes)

Preparative LC/MS Method (Intavis)

Column: Phenomenex Luna C-5 250mmx 4.6mm  
Flow: 1.2 ml/min

- 70 -

Solvent A: 10 mM Ammonium Acetate in Type I water  
 Solvent B: Acetonitrile  
 UV: 220 nm  
 Injection: 400 ul  
 Gradient: 35%B (5 minutes); 35-85%B (13 minutes); 85-35% B (0.5 minutes); 35%B (1.5 minutes)

## SYNTHESIS OF SELECTED APJ COMPOUNDS

[00191] The compounds listed below in Tables 4a and 4b and pharmaceutically acceptable salts thereof were prepared according to the methods described herein. The lower case letters in the sequence represent the D-isomer of that amino acid. When the C-terminus is denoted as NH<sub>2</sub> this means that the -COOH group of the C-terminus is present as an amide (-CONH<sub>2</sub>).

Table 4a APJ i1 loop compounds

Cmpd ID No.	Tether	Linker	Sequence	C-terminus	Instrument
8	Pal	-C(O)-	TVFRSSE(d-Orn)RRSADIFI (SEQ ID NO: 9)	NH <sub>2</sub>	Intavis
12	Pal	-C(O)-	DVFQSSEkRRSADQFI (SEQ ID NO: 13)	NH <sub>2</sub>	Intavis
13	Pal	-C(O)-	DVFRSSEkRRSADQFI (SEQ ID NO: 14)	NH <sub>2</sub>	Intavis
15	Pal	-C(O)-	TVFQSSEkRRSADFI (SEQ ID NO: 16)	NH <sub>2</sub>	Intavis
17	Pal	-C(O)-	DVFQSEkRRSADQFI (SEQ ID NO: 18)	NH <sub>2</sub>	Intavis
30	Pal	-C(O)-	PkRRSADIFIP (SEQ ID NO: 31)	NH <sub>2</sub>	Intavis
36	Pal	-C(O)-	DVHRSSSEkRRSADQFI (SEQ ID NO: 37)	NH <sub>2</sub>	Intavis

Table 4b APJ i1 loop compounds

Cmpd ID No.	Tether	Linker	Sequence	C-terminus	Instrument
3	Pal	-C(O)-	PTVFRSPSEkRRSADIFI (SEQ ID NO: 4)	NH <sub>2</sub>	Intavis

14	Pal	-C(O)-	TVFRSEkRRSADQFI (SEQ ID NO: 15)	NH <sub>2</sub>	Intavis
18	Pal	-C(O)-	TVFRSSEkRRSADNF (SEQ ID NO: 19)	NH <sub>2</sub>	Intavis
21	Pal	-C(O)-	TVKRSSEkRRSADIFI (SEQ ID NO: 22)	NH <sub>2</sub>	Intavis
24	Pal	-C(O)-	TVFRSSEkRRPSADIFP (SEQ ID NO: 25)	NH <sub>2</sub>	Intavis
25	Pal	-C(O)-	TVFRSSPEkRRSADIFI (SEQ ID NO: 6)	NH <sub>2</sub>	Intavis
26	Pal	-C(O)-	TVFRSSPEkRRSADIFP (SEQ ID NO: 27)	NH <sub>2</sub>	Intavis
27	Pal	-C(O)-	TVFRSSEPkRRSADIFI (SEQ ID NO: 28)	NH <sub>2</sub>	Intavis
28	Pal	-C(O)-	TVFRSSEPkRRSADIFP (SEQ ID NO: 29)	NH <sub>2</sub>	Intavis
29	Pal	-C(O)-	pTVFRSpSEkRRSADIF (SEQ ID NO: 30)	NH <sub>2</sub>	Intavis
32	Pal	-C(O)-	TVD(N-MeR)SSEkRRSADIFI (SEQ ID NO: 33)	NH <sub>2</sub>	Intavis

Compound 8 (Pal- TVFRSSE(d-Orn)RRSADIFI -amide) (SEQ ID NO: 9)

**[00192]** Compound 8 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

**[00193]** Following deprotection of the Fmoc group on the N-terminal residue serine, the N-terminal amine was capped with Palmitic acid (2.5 eq x 2 cycles.). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400  $\mu$ L) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 2.2 mg of Compound 8 (Purity 92.4 %).

Compound 12 - (Pal- DVFQSSEkRRSADQFI -amide) (SEQ ID NO: 13)

**[00194]** Compound 12 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.



- 72 -

[00195] Following deprotection of the Fmoc group on the N-terminal residue Serine. the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 3 mg of Compound 12 (Purity 83.2%).

Compound 13 - (Pal- DVFRSSEkRRSADQFI -amide) ( SEQ ID NO: 14)

[00196] Compound 13 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

[00197] Following deprotection of the Fmoc group on the N-terminal residue Serine. the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 3.8 mg of Compound 13 (purity 83.8%).

Compound 15- (Pal- TVFQSSEkRRSADFI -amide) ( SEQ ID NO: 16)

[00198] Compound 15 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

- 73 -

**[00199]** Following deprotection of the Fmoc group on the N-terminal residue Serine. the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 2 mg of Compound 15, Purity (70%).

Compound No. 17- (Pal- DVFQSEkRRSADQFI -amide) ( SEQ ID NO: 18)

**[00200]** Compound 17 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

**[00201]** Following deprotection of the Fmoc group on the N-terminal residue Serine. the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 2.7 mg of Compound 17, Purity (80.7%).

Compound No. 30- (Pal- PkRRSADIFIP -amide) ( SEQ ID NO: 31)

**[00202]** Compound 30 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

**[00203]** Following deprotection of the Fmoc group on the N-terminal residue Serine. the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA MSA, TIS, DDT, and

- 74 -

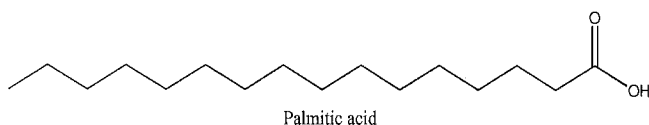
water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 2 mg of Compound 30, Purity (70.5%).

Compound No. 36- (Pal- DVHRSSEkRRSADQFI -amide) ( SEQ ID NO: 37)

**[00204]** Compound 36 was synthesized as described above using the Intavis MultiPep RS using Rink amide resin at 5  $\mu$ mol scale. Amino acids were coupled sequentially as described above following the sequence from C-term to N-term.

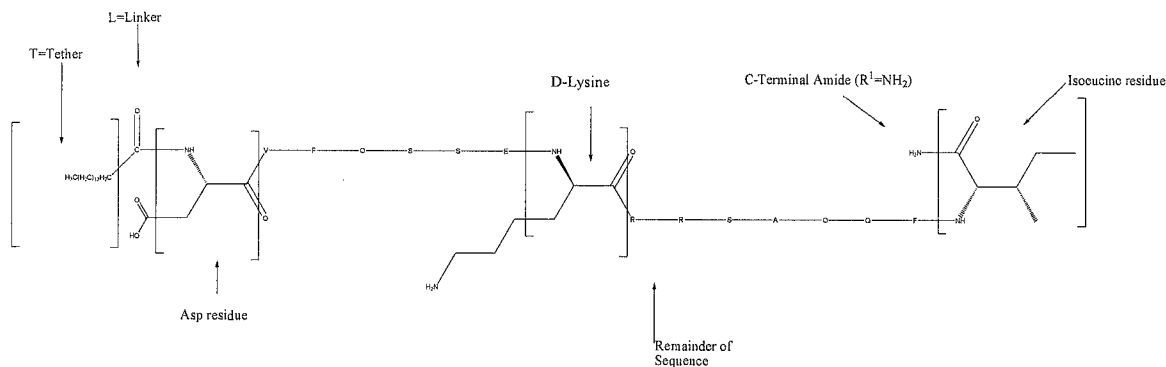
**[00205]** Following deprotection of the Fmoc group on the N-terminal residue Serine, the N-terminal amine was capped with Palmitic acid (2.5 eq x 4 cycles). The compound was manually cleaved from the resin by exposure to cleavage cocktail containing TFA/MSA, TIS, DDT, and water (82: 4.5:4.5:4.5:4.5; 400 uL) for 12 hours. The cocktail was filtered through the 96-well plate into a collection plate and each well was transferred to a 2 mL RPHPLC vial for direct injection onto HPLC/MS system utilizing mass based fraction collection. Fractions with correct MW were pooled and lyophilized and finally analyzed for purity using Method A to yield 2 mg of Compound 36, Purity (70%).

**[00206]** In Table 3a and 3b, specific compounds of the invention are described. For each of the compounds in Table 2, the N-terminal amino acid residue is bonded to  $-C(O)-$  (the Linker "L" of Formula I) and the  $-C(O)-$  is in turn bonded to the Tether (T). In the case of the compounds in Table 3a, the Tether (T) is  $CH_3(CH_2)_{14}$  derived from palmitic acid:



For example, the second compound listed in Table 3a shows the sequence as Pal-DVFQSSEkRRSADQFI -NH<sub>2</sub> (SEQ ID NO 13), the Tether as Pal, the linker as  $-C(O)-$  and the C-terminal carbon as an amide. This means that this compound has the following structure

when drawn out long hand, with the understanding that the flanked sequence of amino acids is in the form of a typical peptide:



Additional compounds that were synthesized following the above-described method are listed in Table 5.

TABLE 5

Cmpd ID No.	SEQ ID No.	Length	N term <sup>1</sup>	Sequence	C-term	MW	MS Theor	MS Obsd <sup>6</sup>	Purity <sup>7</sup>
1	2	18	Pal	TVPFRSSEPkRRSADIFI	NH <sub>2</sub>	2344	782.3	782.1	74
2	3	18	Pal	TPVFRSSPEkRRSADIFI	NH <sub>2</sub>	2344	782.3	782.1	82
3	4	18	Pal	PTVFRSPSEkRRSADIFI	NH <sub>2</sub>	2344	782.3	782.2	84.9
4	5	11	Pal	PEkRRSADIFP	NH <sub>2</sub>	1554	519.0		93.4
5	6	18	Pal	TVDRSSREkRRSADIFI	NH <sub>2</sub>	2403	801.9	801.8	80
6	7	17	Pal	TVDRSSQEkRRSADIFI	NH <sub>2</sub>	2246	1123.8	1123.7	85.4
7	8	17	Pal	TVDRSSKEkRRSADIFI	NH <sub>2</sub>	2247	749.9	749.3	70
8	9	16	Pal	TVFRSSE(d-Orn)RRSADIFI	NH <sub>2</sub>	2136	712.9	712	92
9	10	20	Pal	PK(PkRRSADIFP)RRSADIFP	NH <sub>2</sub>	2634	879.1	879.9	97
10	11	16	Pal	TVFRSSEaRRSADIFI	NH <sub>2</sub>	2092	698.5	698.3	89
11	12	16	Pal	TVFRSSEkRRSAD(Bip)FI	NH <sub>2</sub>	2260	754.2	754	94
12	13	16	Pal	DVFQSSEkRRSADQFI	NH <sub>2</sub>	2150	717.8	717.7	72
13	14	16	Pal	DVFRSSEkRRSADQFI	NH <sub>2</sub>	2179	727.2	726.9	70
14	15	15	Pal	TVFRSEkRRSADQFI	NH <sub>2</sub>	2077	693.5	693.3	70
15	16	15	Pal	TVFQSSEkRRSADFI	NH <sub>2</sub>	2008	670.5	670.3	70
16	17	14	Pal	TVFQSEkRRSADFI	NH <sub>2</sub>	1921	641.4	641.3	77
17	18	15	Pal	DVFQSEkRRSADQFI	NH <sub>2</sub>	2063	688.8	688.6	71
18	19	16	Pal	TVFRSSEkRRSADNFI	NH <sub>2</sub>	2151	717.8	717.6	84
19	20	16	Pal	DVFNSSSEkRRSADNFI	NH <sub>2</sub>	2122	708.5	708.3	87

- 76 -

20	21	16	Pal	DVFRSSEkRRSADNFI	NH <sub>2</sub>	2165	722.5	722.3	82
21	22	16	Pal	TVKRSSEkRRSADIFI	NH <sub>2</sub>	2131	533.7	533.5	72
22	23	17	Pal	TVFRSPSEkRRPSADIF	NH <sub>2</sub>	2231	558.7	558.5	84
23	24	17	Pal	TVFRSSPEkRRSPADIF	NH <sub>2</sub>	2231	558.7	558.5	84
24	25	17	Pal	TVFRSSEkRRPSADIFP	NH <sub>2</sub>	2231	558.7	558.6	75
25	26	17	Pal	TVFRSSPEkRRSADIFI	NH <sub>2</sub>	2247	562.7	562.6	82
26	27	17	Pal	TVFRSSPEkRRSADIFP	NH <sub>2</sub>	2231	558.7	558.5	89
27	28	17	Pal	TVFRSSEPkRRSADIFI	NH <sub>2</sub>	2247	562.7	562.6	77
28	29	17	Pal	TVFRSSEPkRRSADIFP	NH <sub>2</sub>	2231	558.7	558.6	75
29	30	17	Pal	pTVFRSpSEkRRSADIF	NH <sub>2</sub>	2231	744.6	744.4	88
30	31	11	Pal	PkRRSADIFIP	NH <sub>2</sub>	1537	769.5	769.5	71
31	32	16	Pal	TVF(N-Me-Arg)SSEkRRSADIFI	NH <sub>2</sub>	2164	722.2	723.8	70
32	33	16	Pal	TVD(N-Me-Arg)SSEkRRSADIFI	NH <sub>2</sub>	2132	711.5	713	71
33	34	16	Pal	TVDRSSEkRRSAD(p-fluoro-Phe)FI	NH <sub>2</sub>	2170	724.2	724	78
34	35	16	Pal	TVDRSSEkRRSAD(p-CH <sub>2</sub> NH <sub>2</sub> -Phe)FI	NH <sub>2</sub>	2181	727.9	727.7	92
35	36	16	Pal	DVHRSSEkRRSADIFI	NH <sub>2</sub>	2154	718.8	718.6	88
36	37	16	Pal	DVHRSSEkRRSADQFI	NH <sub>2</sub>	2169	723.8	723.6	96

## METHODS OF SCREENING

### FUNCTIONAL ASSAYS

[00207] Functional assays suitable for use in detecting and characterizing GPCR signaling include Gene Reporter Assays and Calcium Flux assays, cAMP and kinase activation assays. Several suitable assays are described in detail below.

#### Gene Reporter Assays

[00208] Cells expressing the APJ receptor can be transiently or stably transfected with a reporter gene plasmid construct containing an enhancer element which responds to activation of a second messenger signaling pathway or pathways, thereby controlling transcription of a cDNA encoding a detectable reporter protein. APJ expression can be the result of endogenous expression on a cell line or cell type or the result of stable or transient transfection of DNA encoding the receptor of interest into a cell line by means commonly used in the art. Immortalized cell lines or primary cell cultures can be used.

- 77 -

[00209] If the activated pathway is stimulatory (e.g., Gs or Gq), agonist activity results in activation of transcription factors, in turn causing an increase in reporter gene transcription, detectable by an increase in reporter activity. To test for agonist or inverse agonist activity, cells expressing the APJ receptor and the reporter gene construct can be challenged by the test compound for a predetermined period of time (e.g., 2-12 hours, typically 4 hours). Cells can then be assessed for levels of reporter gene product. Inverse agonists will suppress levels of reporter to below basal levels in a dose dependent manner. To test for antagonist or inhibitory activity through a stimulatory pathway, cells expressing both the APJ receptor and the reporter gene construct can be activated by a receptor agonist to increase gene reporter product levels. Treatment with antagonists will counter the effect of agonist stimulation in a dose- and receptor-dependent manner.

[00210] To test for agonist activity on receptor signaling through an inhibitory pathway (eg, Gi, which couples to APJ), cells can be treated with a systematic activator (e.g., forskolin) to increase levels of reporter gene product. Activation of Gi by treatment with receptor agonist will inhibit this expression by inhibiting adenylyl cyclase. To screen for antagonist activity, test compounds can be assessed for the ability to counter agonist inhibition of adenylyl cyclase, resulting in increase reporter transcription.

[00211] Alternatively, a plasmid construct expressing the promiscuous G-protein Ga16 can be used to obtain a positive signal from a GPCR which normally couples to an inhibitory G-protein. Co-expression of the chimeric G-protein Gaq/Gai5 (Coward et al. *Analytical Biochemistry* **270**, 242–248 (1999)) allows coupling to Gi-coupled receptors and conversion of second messenger signaling from the inhibitory Gi pathway to the stimulatory Gq pathway. Agonist and antagonist assessment in these systems is the same as the stimulatory pathways. Well-to-well variation caused by such factors as transfection efficiency, unequal plating of cells, and cell survival rates can be normalized in transient transfection assays by co-transfecting a constitutively expressing reporter gene with a non-interfering signal independent of the regulated reporter.

#### Calcium Flux Assay

[00212] Calcium Flux Assay is one of the most popular cell-based GPCR functional assays. It most often uses calcium sensing fluorescent dyes such as fura2 AM, fluo-4 and Calcium-4 to measure changes in intracellular calcium concentration. It is used mainly to detect GPCR

- 78 -

signaling via Gαq subunit. Activation of these Gq-coupled GPCRs leads to activation of phospholipase C, which subsequently leads to increase in inositol phosphate production. IP3 receptors on endoplasmic reticulum sense the change then release calcium into cytoplasm. Intracellular calcium binding to the fluorescent dyes can be detected by instruments that quantify fluorescent intensities, such as FLIPR Tetra, Flexstation (MDS) and FDSS (Hamamatsu). In addition to assess Gq-couple receptor signaling, calcium flux assay can also be used to study Gs and Gi couple receptors by co-expressing CNG (cyclic nucleotide gated calcium channel) or chimeric G-proteins (Gqi5, Gsi5 for example). Activation of some Gi-coupled receptors can also be detected by calcium flux assay via Gβγ mediated phospholipase C activation.

#### APJ Testing

**[00213]** An example of the use of the calcium flux assay can be assessing Apelin activation of APJ receptors in Molt3 human cell lines or in Rat RBL cells stably transfected with APJ. Cells can be seeded into 96-well black plates with clear bottom at 200K/well in Hank's balanced salt solution with 20mM HEPES, 0.1% BSA. After dye loaded by incubating in Calcium-4 dye at room temperature for 1 hour, cell plates can be placed in Flexstation 3. The addition of test compound or reference antagonists can be done either by manual pipetting or by liquid handling on Flexstation. The latter allows the assessment of agonist activity of the test compound. After incubation of 15 minutes at 37°C, Apelin can be added on Flexstation and receptor activation can be assessed by measuring changes in fluorescent intensity. This mode of assay also allows the detection of agonists and agonistic modulators of APJ activity.

#### HTRF cAMP Assay and IP-One Assay (Cisbio)

**[00214]** HTRF (homogeneous time resolved fluorescence) is a technology developed by Cisbio Bioassays based on TR-FRET (time-resolved fluorescence resonance energy transfer). Cisbio Bioassays has developed a wide selection of HTRF-based assays compatible with whole cells, thereby enabling functional assays to run under more physiological conditions. The IP-One assays are competitive immunoassays using cryptate-labeled anti-IP1 monoclonal antibody and d2-labeled IP1. IP1 is a relatively stable downstream metabolite of IP3, and accumulates in cells following Gq receptor activation.

**[00215]** cAMP kits based on a competitive immunoassay using cryptate-labeled anti-cAMP antibody and d2-labeled cAMP were used to assay the effects of APJ compounds of the present

- 79 -

invention. This assay measures the increase in intracellular cAMP upon Gs-coupled receptor activation as well as decrease in forskolin (or a more soluble version of forskolin – NKH477) stimulated increase in cAMP upon Gi-coupled receptor activation. For example, treatment of HEK cells stably expressing the Gi-coupled receptor APJ with its endogenous ligand Apelin inhibited NKH477 stimulated increase in cAMP with an EC<sub>50</sub> of 5 e-10 M. A second example of the use of the cAMP assay can be assessing Apelin activation of APJ receptor in Human TRex cells, which express APJ upon induction of doxycycline. Cells are treated with 1nG/mL doxycycline and seeded in all white 96 well plates @ 40k/well overnight. When Human TRex cells express APJ, Apelin inhibited NKH477 stimulated increase in cAMP with an EC<sub>50</sub> of 2 e-10 M. When Human TRex cells are not induced and therefore do not express the APJ receptor, Apelin does not inhibit NKH477 stimulated cAMP. This cell lines allows the assessment of activity (potency, as defined by EC<sub>50</sub>, and Intrinsic activity (%IA), which is a measure of efficacy) of the compounds with and without the APJ receptor.

#### Histamine Assay

[00216] More desirable pharmacological tolerability can include a decrease in an undesirable side effect (e.g., histamine release, an indicator of mast cell degranulation) without a significant loss in activity of the compound.

[00217] Screening of the variant G protein receptor compounds against a control compound for histamine release can be done following standard assays. The standard assay can include comparison to a control compound known to elicit a histamine response (e.g., 48/80 having CAS No. 94724-12-6). Such an assay using rat peritoneal cells is described herein for the APJ compounds..

[00218] Test compounds were assayed in the *ex vivo* histamine release assay using a 3-point dose response. Concentrations of total histamine contained in the cells is used to correct for variability in the numbers of mast cells in the lavage per well.

[00219] Resident peritoneal cells were harvested from naïve Sprague Dawley rats (male 200-300 gr, Charles River). Rats were euthanized by CO<sub>2</sub> asphyxiation and a skin incision was made along the middle-line of the abdomen. Thirty milliliters of HBSS (HyClone, cat# SH 30268.01) containing 20mM HEPES and 0.2% BSA (heretofore Assay Buffer) were injected intraperitoneally using a 30 mL syringe with the attached 18 gauge needle. The abdomen was



- 80 -

gently lavaged for 1-2 min and 7-10 milliliters of the peritoneal lavage were carefully aspirated. Cell count in the peritoneal lavage was determined using Cellometer Auto T4 (Nexcelom Bioscience). The peritoneal cell count was adjusted to  $2.75 \times 10^5$  cells/mL in Assay Buffer. Rat peritoneal cells were kept at room temperature before and during the assay.

**[00220]** For the 10-point assay, 3-fold serial dilutions were prepared for each test compound in 100% DMSO in 96-well plates (Greiner Bio One, Ref# 651261)- from 100  $\mu$ M down to 0.05  $\mu$ M. For the 3-point assay, 10-fold serial dilutions were prepared in 100% DMSO at 100  $\mu$ M, 10  $\mu$ M and 1  $\mu$ M concentrations. From this plate, 5  $\mu$ L of compound were transferred to a new plate containing 45  $\mu$ L Assay Buffer to generate a compound plate with 10X final concentrations in 10% DMSO with Assay Buffer. In the final plates, column 11 contained 1% DMSO for the low-histamine-releasing control.

**[00221]** Suspensions of the rat peritoneal cells (90  $\mu$ L of  $2.75 \times 10^5$  cells/mL;  $\rightarrow$  25,000 cells per well) were introduced into each well of a new 96-well microtiter plate (Greiner Bio One, Ref# 651261) and 10  $\mu$ L aliquots of the serially diluted compounds were transferred to the cells in each well. The microtiter plate was incubated for 30 min at room temperature. Following incubation cells were spun down (500 rpm, 7 min) and supernatants were collected for further analysis. Supernatants were diluted 6 times with the assay buffer (15 $\mu$ L to 75 $\mu$ L, for 90 $\mu$ L total) and released histamine content was determined using HTRF® Histamine assay kit (CisBio, cat# 62HTMPEB). To measure the total histamine in each well, cells were lysed by addition of 15  $\mu$ L 5% TritonX-100 to each well followed by incubation for 15 minutes. The plates were then spun at 3000 rpm for 5 minutes, and 10  $\mu$ L of supernatant were diluted 9-fold in assay buffer (10  $\mu$ L to 80  $\mu$ L for 90  $\mu$ L total) to make the "total" histamine plate. Histamine content was measured as described above for the "released" histamine values.

**[00222]** The standard positive control was Compound 48/80 (Enzo Life Sciences) , a oligomeric polymer mixture produced by the condensation of N-methyl-p-methoxyphenethylamine with formaldehyde. The trimer is the most abundant in the mixture Compound 48/80 is used to promote mast cell degranulation and activates G proteins by a mechanism analogous to that of mastoparan. Also Compound 48/80 inhibits calmodulin and human platelet PLC and exhibits a concentration dependent biphasic modulation of human platelet PLA. See references: T. Higashijima *et al.*, *J. Biol., Chem.*, **265** 14176 (1990);

M.Mousli *et al.*, *FEBS Lett.*, **259** 260 (1990); K.Gietzen *et al.*, *Biochim. Biophys. Acta*, **736** 109 (1983); K.Gietzen, *Biochem.J.*, 1983 **216** 611 and C.Bronner *et al.*, *Biochim. Biophys. Acta*, **920** 301(1987).

[00223] Representative testing of compounds was conducted and the results are set forth in Table 6. For the data in Table 6

- \*\*\*\*\* = EC<sub>50</sub> ≤ 25 nM (hAPJ), %IA ≥ 80% and either % histamine ≤ 35% or formal (+) charge ≤ 1  
 \*\*\*\* = EC<sub>50</sub> ≤ 25 nM (hAPJ), %IA ≥ 80% and either % histamine > 35% or formal (+) charge > 1  
 \*\*\* = EC<sub>50</sub> 25 - 50 nM (hAPJ), %IA 50 - 79% and either % histamine ≤ 35% or formal (+) charge ≤ 1  
 \*\* = EC<sub>50</sub> 25 - 50 nM (hAPJ), %IA 50 - 79% and either % histamine > 35% or formal (+) charge > 1  
 \* = EC<sub>50</sub> ≤ 50 nM (hAPJ), %IA < 50%

Formal positive charge is assigned by adding the number of basic residues (e.g., Arg, Lys) and subtracting the number of acidic residues (e.g., Asp, Glu).

Table 6

Cmpd ID No.	SEQ ID No.	Ranking	Sequence	#AAs	Formal (+) charge	EC <sub>50</sub> (cAMP inhibition, hAPJ HEK)	%IA (cAMP inhibition, hAPJ HEK)	Histamine (% vs. 48/80)
1	2	**	TVFPRSSEPkRRSADIFI	18	2	4	57	53
2	3	**	TPVFRSSPEkRRSADIFI	18	2	4	60	49
3	4	*****	PTVFRSPSEkRRSADIFI	18	2	13	90	64
4	5	*	PEkRRSADIFP	11	1	9	48	-1
5	6	*	TVDRSSREkRRSADIFI	18	2	40	41	
6	7	***	TVDRSSQEkRRSADIFI	17	1	19	77	
7	8	***	TVDRSSKEkRRSADIFI	17	1	9	59	
8	9	*****	TVFRSSE(d-orn)RRSADIFI	16	2	14	99	9
9	10	**	PK(PkRRSADIFP)RRSADIFP	20	4	44	84	65
10	11	**	TVFRSSEaRRSADIFI	16	1	39	106	3
11	12	**	TVFRSSEkRRSAD(Bip)FI	16	2	34	76	8
12	13	*****	DVFQSSEkRRSADQFI	16	0	12	97	9
13	14	*****	DVFRSSEkRRSADQFI	16	1	6	96	17
14	15	****	TVFRSEkRRSADQFI	15	2	8	87	
15	16	*****	TVFQSSEkRRSADFI	15	1	12	108	30
16	17	***	TVFQSEkRRSADFI	14	1	10	78	21
17	18	*****	DVFQSEkRRSADQFI	15	0	24	111	1
18	19	****	TVFRSSEkRRSADNFI	16	2	15	117	
19	20	***	DVFNSEkRRSADNFI	16	0	5	67	
20	21	***	DVFRSSEkRRSADNFI	16	1	32	98	
21	22	****	TVKRSSEkRRSADIFI	16	2	9	85	

22	23	*	TVFRSPSEKRRPSADIF	17	2	17	47	60
23	24	**	TVFRSSPEkRRSPADIF	17	2	44	63	67
24	25	****	TVFRSSEkRRPSADIFP	17	2	13	88	54
25	26	****	TVFRSSPEkRRSADIFI	17	2	15	138	51
26	27	****	TVFRSSPEkRRSADIFP	17	2	11	105	73
27	28	****	TVFRSSEPkRRSADIFI	17	2	7	105	67
28	29	****	TVFRSSEPkRRSADIFP	17	2	17	99	
29	30	****	pTVFRSpSEkRRSADIF	17	2	20	80	47
30	31	*****	PkRRSADIFIP	11	2	16	86	34
31	32	**	TVF(N-Me-Arg)SSEkRRSADIFI	16	2	34	84	
32	33	****	TVD(N-Me-Arg)SSEkRRSADIFI	16	2	24	97	
33	34	***	TVDRSSEkRRSAD(4-fluoro-Phe)FI	16	1	30	80	
34	35	***	TVDRSSEkRRSAD(4-CH <sub>2</sub> NH <sub>2</sub> -Phe)FI	16	1	30	104	
35	36	***	DVHRSSEkRRSADIFI	16	1	32	90	
36	37	*****	DVHRSSEkRRSADQFI	16	1	2	101	

### Receptor kinase activation (ERK and p38)

**[00224]** Experiments were performed to test activation of the ERK and p38 pathways using the Cellul'erk and the Phospho-p38 MAPK (Thr180/Tyr182) assay kits (Cisbio). APJ-TREX cells were optimized for signaling (pre-serum starved overnight with low FBS serum (2% FBS media) with 1 ng/ml doxycyclin (for induction of APJ receptor expression), 50,000 cells, 5 minutes agonist activation for ERK activity). Cells were pretreated with 3 or 0.3  $\mu$ M pepducin compound for 5 minutes and the reaction was stopped by lysis buffer. Lysates are then transferred to the assay plate for the detection of phosphorylated ERK or p38 by HTRF reagents. Using this assay system, apelin did not activate the p38 pathway through the APJ receptor but did activate ERK. Compound agonist activity was calculated using %Activity = 100% x (RLU of test sample - mean RLU of vehicle control)/(mean maximum control ligand (pyr-apelin-13 at 100 nM) - mean RLU of vehicle control).

The ERK agonist activity of Compounds 12, 13, 14, 25, and 28 are shown in Table 7. These compounds induced ERK activation at a level of 25 – 51% that of pyr-apelin-13.

**Table 7. APJ pepducin ERK agonist activity as assessed in Cellul'erk ERK Phosphorylation Assay. Max pyr-apelin-13 activity assessed at 100 nM.**

Cmpd ID No.	Average % Activity
12	51.8
13	44.0
14	58.2
25	25.5
28	34.7

$\beta$ -Arrestin signaling assays.

[00225] Activation of the  $\beta$ -Arrestin signaling pathway was monitored using the commercially available DiscoverX PathHunter<sup>®</sup> assay. This assay employs a homogenous, non-imaging assay format called Enzyme Fragment Complementation (EFC) using  $\beta$ -galactosidase ( $\beta$ -Gal) as the functional reporter. The enzyme is split into two inactive complementary portions (EA for Enzyme Acceptor and ED for Enzyme Donor) expressed as fusion proteins in the cell. EA is fused to  $\beta$ -Arrestin and ED is fused to the GPCR of interest. When the GPCR is activated and  $\beta$ -Arrestin is recruited to the receptor, ED and EA complementation occurs, restoring  $\beta$ Gal activity which is measured using chemiluminescent PathHunter<sup>®</sup> Detection Reagent. Using this assay format, the endogenous ligand apelin-13 robustly recruits  $\beta$ -arrestin as measured by an increase in chemiluminescence.

[00226] Representative  $\beta$ -arrestin data are illustrated in Table 8. The compounds listed in Table 8 efficiently promote activation of Gi as exemplified by the potent inhibition of NKH477-stimulated cAMP production (EC50's < 150 nM). In contrast,  $\beta$ -arrestin is weakly engaged for compounds 3, 25, 26 and 27 and therefore the signaling is biased towards the Gi pathway. This bias has potential therapeutic advantage for the treatment of cardiovascular disease, including ischemia-reperfusion injury and heart failure. (ref: Scimia et al., Nature. 2012 Aug 16; 488(7411):394-8)

Table 8.

Cmpd #	Sequences	Gi coupling (cAMP inhibition) <sup>1</sup>	β-arrestin Signaling	
		EC <sub>50</sub> (nM)	EC <sub>50</sub> (uM)	Max Response <sup>2</sup>
Apelin-13	N/A	1	0.0017	101.7
3	PTVFRSPSEKRRSADIFI (SEQ ID NO: 4)	13	0.0082	11.0
25	TVFRSSPEKRRSADIFI (SEQ ID NO: 26)	15	0.0031	12.6
26	TVFRSSPEKRRSADIFP (SEQ ID NO: 27)	11	0.0596	46.0
27	TVFRSSEPKRRSADIFI (SEQ ID NO: 28)	7	0.0081	23.7

<sup>1</sup>Inhibition of NKH477 stimulated cAMP production in hAPJ HEK 293 cells

<sup>2</sup>Relative to apelin-13

### IN VIVO ASSAYS

[00227] The *in vivo* efficacy of selected lead candidates is evaluated using animal models of cardiovascular function, heart failure, lipid metabolism, glucose homeostasis and body weight gain. Examples of such models are described below. However, it is noted that; the list of models described is not all encompassing, additional models are available and that these additional models may be used to exemplify the activity of our compounds.

#### Cardiovascular Function

[00228] This model is commonly conducted in various wild type and genetically modified rodents, however, it is noted that such studies may also be conducted in higher species such as dog, pig and non-human primate. The most commonly used rodent species are rat and mice. In addition to normal wild type rodents various genetic or environmental modifications have been used to create animals that better mimic various aspects of human disease. For example, the spontaneously hypertensive rat (commonly described as SHR rat) or rats fed on a high salt diet have elevated blood pressure akin to human hypertension.

[00229] It is noted that the use of large animals (typically dog or pig) allow for the fine placement of multiple measuring devices, which in turn allows for the measurement of multiple complex cardiovascular parameters. For example, placement of a balloon catheter into the left ventricle allows the measurement of both cardiac stroke volume and cardiac output. However, due to the small size of rats and mice such fine placement of measuring devices cannot be easily

done. Consequently, in the rodent protocols described below we focus only on the measurement of crude cardiovascular parameters such as blood and heart rate.

[00230] It is also noted that measurement of cardiovascular function in rodents can be conducted in both anaesthetized and conscious animals. In conscious animals measurement devices are implanted surgically under anesthesia. Once the animal has regained consciousness these measurement devices send cardiovascular data from the animals to electronic recording stations. Such animals are commonly described as telemetered. Although this approach yields viable data its use can be hampered by data artifacts induced by handling of the animals, environmental factors and technical difficulties associated with the surgical implantation of the measuring devices. Consequently, the most common approach to measuring cardiovascular parameters in rodents is the use of anaesthetized animals. This approach is described in detail below.

[00231] Typically for anaesthetized models of cardiovascular function Wistar or CD rats, and C57Bl6 mice are used. However, it is noted that the procedure described below can be easily adapted for different strains of rat and mouse.

[00232] On the day of experimentation the animals are first anaesthetized. Common anesthetics used for rodents include Inactin or ketamine with xylazine. Once a level of full surgical anesthesia is confirmed the carotid or femoral artery is exposed and cannulated. This cannulae is typically filled with heparinized saline, to prevent blood clotting, and connected to a commercial pressure transducer such as those available from AD Instruments (adstruments.com), to allow for the measurement of blood pressure. Typically, systolic and diastolic blood pressure will be recorded, and from this mean blood pressure and heart rate will be interpolated. To facilitate the intravenous administration of test substances the jugular vein may also be cannulated.

[00233] Using this model the effects of lead compounds on cardiovascular function can be evaluated. Typically the lead compound will be administered through; sub-cutaneous, oral, intra-peritoneal or intra-venous routes. To determine the effectiveness of our lead candidates the effects on blood pressure and heart rate in lead candidate treated animals will be compared to those observed in vehicle control treated animals.

*Heart Failure*

[00234] A number of animal models of heart failure and myocardial injury have been reported in the literature. Such models include the use of large animals such as pig and dog, however, due to their small size and lower cost rodents are the most common species used. A useful review of rodent models of heart failure and myocardial injury can be found in the journal of Circulation - Heart Failure, 2009, Volume 2; pages 138 to 144. Such models require either; ligation of cardiac blood vessels or genetic manipulation (such as cardiomyocyte specific overexpression of TNF $\alpha$ ).

[00235] The spontaneous hypertensive rat (SHR) is an especially useful acute rat models of chronic ventricular pressure overload whereas the mean arterial pressure in male wild type Wistar or SHR rats can be accessed via cannulation of the femoral or carotid (Regul. Pept. 2001:99:87). In addition, isolated rat heart preps have been used to demonstrate the inotropic effects of apelin and therefore could be used to evaluate APJ agonists, antagonists, or modulators (Circ. Res 2002; 91(5):434-40) For a more chronic model of heart failure, a useful exemplary model is the aortic banding methodology in the mouse (Circ Res.2007:101:e32-e42).

[00236] Mouse and rat Langendorff heart preparations were used to characterize the direct cardiac effects apelin and APJ compounds have on the target tissue (Szokodi, Circ. Res 2002:91 434-440). Hearts were perfused with (in mM) 118 NaCl, 4.7 KCl, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 1.5 CaCl<sub>2</sub>, 1.2 MgCl<sub>2</sub>, 23 NaHCO<sub>3</sub>, and 10.0 dextrose, gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> and adjusted to a pH of 7.4. A pressure-sensing balloon catheter was inserted in the LV cavity to record changes in the developed pressure. Heart function was assessed by measuring standard parameters such as heart rate, mean peak systolic pressure, mean end diastolic pressure, developed pressure, dP/dt max, and dP/dt min. An increase in developed pressure is consistent with the known inotropic effect of the endogenous ligand for APJ, apelin.

#### *Lipolysis and Body Weight*

[00237] This model is most commonly conducted in a variety of mouse strains, however, it is noted that such studies may also be conducted in rats and in higher species such as dog and non-human primate. For mice, the most commonly used strain is the C57Bl6. For these C57Bl6 mice, the ability of compounds to modify lipid metabolism (lipolysis) may be evaluated in animals fed a standard laboratory diet, or they may be evaluated in mice fed a modified high calorie diet. Mice fed a high calorie diet are commonly described as DIO (diet induced obesity)

because such a diet induces a disease state that shares a number of characteristics with human cardiovascular and metabolic disease. Such DIO mice have elevated blood lipid levels, similar to makers of human heart disease, cardiovascular disease, and atherosclerosis.

**[00238]** When mice fed on standard laboratory diet are used, the animals are typically 8 to 16 weeks of age when used for experimentation. When mice fed on a high calorie diet are used, the animals are typically 13 to 16 weeks of age (equating to 7 weeks feeding with standard laboratory diet, followed by 6 to 9 weeks feeding with a high calorie diet).

**[00239]** The activity of test compounds will be evaluated in mice where lipid metabolism is first elevated by either overnight fasting or by pretreatment with a pharmacological stimulator of lipid metabolism (typically isoproterenol). Most typically overnight fasting will be used to elevate lipid metabolism prior to testing our lead candidates, consequently, this is the protocol described in detail below.

**[00240]** One day prior to the day of experimentation the mice are fasted overnight (minimum of 12 hours and maximum of 18 hours) to elevate lipid metabolism. During this period the mice are allowed free access to drinking water, but the food is removed.

**[00241]** On the day of experimentation the test compound will be administered through; sub-cutaneous, oral, intra-peritoneal or intra-venous routes. After a predefined period (typically 5 to 360 minutes dependent on the pharmacokinetic properties of the lead candidate) a blood sample will be obtained, typically through terminal cardiac puncture. Blood will be transferred into a tube with a suitable anti-coagulant (typically heparin or EDTA), and centrifuged to separate blood cells from plasma. Plasma will then be harvested. The concentration of blood lipids (typically glycerol and non-esterified free fatty acid) will then be determined in these plasma samples using commercial assay kits such as those available from Zen Bio ([www.zen-bio.com](http://www.zen-bio.com)).

**[00242]** To determine the effectiveness of test compound the profile of blood lipids in mice receiving test compound will be compared to that observed in vehicle control mice.

**[00243]** The protocol described above assesses the activity of test compounds after a single acute dose. This protocol can be adapted to determine the activity of test compounds after chronic administration. In this situation the compound will be administered once or multiple times during a 24 hour period, and for periods extending up to 3 months. During the course of compound dosing, or at the end of a predefined period of compound dosing, the animal's response to fasting-induced lipolysis will be determined using the protocol laid out in the above



section. In addition, throughout the course of chronic compound dosing the body weight of the animals will also be recorded. Comparison of the change in body weight over time for compound treated vs. vehicle treated animals will elucidate any compound-mediated effect on body weight.

#### *Glucose Tolerance and Body Weight*

[00244] This model is most commonly conducted in a variety of mouse strains, however, it is noted that such studies may also be conducted in rats and in higher species such as dog and non-human primate. For mice, the most commonly used strain is the C57Bl6. For these C57Bl6 mice, the ability of test compounds to modify glucose homeostasis may be evaluated in animals fed a standard laboratory diet, or they may be evaluated in mice fed a modified high calorie diet. Mice fed a high calorie diet are commonly described as DIO (diet induced obesity) because such a diet induces a disease state that shares a number of characteristics with human metabolic and cardiovascular disease. Such DIO mice have a compromised ability to regulate blood glucose, similar to human type II diabetes.

[00245] When mice fed on standard laboratory diet are used, the animals are typically 8 to 16 weeks of age when used for experimentation. When mice fed on a high calorie diet are used, the animals are typically 13 to 16 weeks of age (equating to 7 weeks feeding with standard laboratory diet, followed by 6 to 9 weeks feeding with a high calorie diet).

[00246] One day prior to the day of experimentation the mice are fasted overnight (minimum of 12 hours and maximum of 18 hours). During this period the mice are allowed free access to drinking water, but the food is removed.

[00247] On the day of experimentation a small drop of blood (5 to 10  $\mu$ l) will be obtained from the animal's tail and applied to a calibrated commercial glucometer to determine basal blood glucose concentration. Subsequently, the test compound will be administered through; sub-cutaneous, oral, intra-peritoneal or intra-venous routes. After a predefined period (typically 5 to 360 minutes dependent on the pharmacokinetic properties of the lead candidate) a further blood sample will be obtained from the tail and blood glucose concentration determined. Immediately afterwards the mice will be given a single glucose challenge. Typically, this glucose challenge will be in the form of a solution of D-glucose administered via oral, intra-peritoneal or intra-venous routes. Subsequent to this glucose challenge, blood glucose will be measured, at various

- 89 -

time intervals using the same tail bleed/glucometer method. Typically, blood glucose will be measured at 30, 60 and 120 minutes after the glucose challenge.

[00248] To determine the effectiveness of test compounds, the profile of blood glucose concentration over time observed in mice receiving test compound will be compared to that observed in vehicle control mice.

[00249] The protocol described above assesses the activity of test compounds after a single acute dose. We may also adapt this protocol to determine the activity of test compounds after chronic administration. In this situation the compound will be administered once or multiple times during a 24 hour period, and for periods extending up to 3 months. During the course of compound dosing, or at the end of a predefined period of compound dosing, the animal's response to a glucose challenge will be determined using the protocol laid out in the above section. In addition, throughout the course of chronic compound dosing the body weight of the animals will also be recorded. Comparison of the change in body weight over time for compound treated vs. vehicle treated animals will elucidate any compound-mediated effect on body weight.

[00250] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

[00251] While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

- 90 -

## CLAIMS

What is claimed is:

1. The compound represented by Formula A:

T-L-X<sub>1</sub>-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-X<sub>17</sub>-X<sub>18</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub> or X<sub>2</sub> if X<sub>1</sub> is absent at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>, wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

- 91 -

- X<sub>1</sub> is absent, a proline residue or a D-proline residue;
- X<sub>2</sub> is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;
- X<sub>3</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;
- X<sub>4</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;
- X<sub>5</sub> is N-methylarginine, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;
- X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>7</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, a D-proline residue, an Aib residue, or a glycine residue;
- X<sub>8</sub> is a serine residue, a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, or a tyrosine residue;
- X<sub>9</sub> is D-ornithine, is a glutamic acid residue, an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or an arginine residue, or a tyrosine residue;
- X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 92 -

X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>17</sub> is a phenylalanine residue, an isoleucine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>18</sub> is absent or an isoleucine residue, a proline residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue;

wherein seventeen contiguous amino acids are present and optionally 1- 5 amino acid residues are present in the D configuration.

2. The compound of Claim 1, wherein

X<sub>1</sub> is absent, a proline residue or a D-proline residue;

X<sub>2</sub> is a threonine residue, or an aspartic acid residue;

X<sub>3</sub> is a valine residue;

X<sub>4</sub> is a phenylalanine residue, a histidine residue, or an aspartic acid residue;

X<sub>5</sub> is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or an Aib residue;

- 93 -

- X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>7</sub> is a serine residue, a glutamine residue, an aspartic acid residue, a proline residue, or a D-proline residue;
- X<sub>8</sub> is a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or an Aib residue;;
- X<sub>9</sub> is an arginine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a glutamic acid, a histidine residue, a glutamine residue, an aspartic acid residue, or an Aib residue;
- X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>13</sub> is a serine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue
- X<sub>15</sub> an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>16</sub> an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>17</sub> a phenylalanine residue, an isoleucine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or a proline residue;
- X<sub>18</sub> is absent or an isoleucine residue, a proline residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, or an Aib residue.

3. The compound of Claim 1 or Claim 2, wherein X<sub>1</sub> is absent.

4. The compound of any one of Claims 1-3, wherein X<sub>9</sub> or X<sub>10</sub> is D-lysine.
5. The compound of any one of Claims 1-4, wherein L is C\*(O).
6. The compound of any one of Claims 1-5, wherein the lipophilic moiety is a palmitic acid derivative.
7. The compound represented by Formula B:

T-L-X<sub>1</sub>'-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub> X<sub>17</sub>-X<sub>18</sub> -R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>' and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>' at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>, wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub>

- 95 -

alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>,  
-NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

- X<sub>1</sub>' is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue, a proline residue or a D-proline residue;
- X<sub>2</sub> is a threonine residue, a valine residue, a proline residue, histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;
- X<sub>3</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;
- X<sub>4</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, an arginine residue, or a proline residue;
- X<sub>5</sub> is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, a serine residue, or a glycine residue;
- X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>7</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an arginine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;
- X<sub>8</sub> is a lysine, a phenylalanine residue, a proline residue, a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a serine residue, a glycine residue, or a tyrosine residue;
- X<sub>9</sub> is D-ornithine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a glutamic acid residue, a proline residue, an Aib residue, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, or a glycine residue;
- X<sub>10</sub> is a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue, a phenylalanine residue, a proline residue, a



- 96 -

glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, or a glycine residue, an arginine residue, or a tyrosine residue;

X<sub>11</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, a glutamic acid residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an alanine residue, a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> an aspartic acid residue, an alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>17</sub> a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>18</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally 1- 5 amino acid residues are present in the D configuration.

8. The compound of Claim 7, wherein X<sub>9</sub> or X<sub>10</sub> is D-lysine.

- 97 -

9. The compound of Claim 7, wherein the compound is selected from the group consisting of: Compounds 1, 2, 3 or 5, or a pharmaceutically acceptable salt thereof.

10. The compound of Claim 7 or Claim 8, wherein L is C\*(O).

11. The compound of any one of Claims 7, 8 or 10, wherein the lipophilic moiety is a palmitic acid derivative.

12. The compound represented by Formula C:

T-L-X<sub>1</sub>"-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub>-R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>" and is selected from: C(O), C(S), S(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>" at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

each R<sub>2</sub> is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

R<sub>3</sub> and R<sub>4</sub> are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>,

- 98 -

$-\text{C}(\text{O})\text{NH}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{C}(\text{O})(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{H}$ ,  $-\text{C}(\text{O})\text{N}(\text{C}_1\text{-C}_3 \text{ alkyl})_2$ ,  $-\text{NHC}(\text{O})\text{O}-(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{C}(\text{O})\text{OH}$ ,  $-\text{C}(\text{O})\text{O}-(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{NH}_2$ ,  $-\text{NHC}(\text{O})\text{NH}(\text{C}_1\text{-C}_3 \text{ alkyl})$ ,  $-\text{NHC}(\text{O})\text{N}(\text{C}_1\text{-C}_3 \text{ alkyl})_2$ , or  $-\text{SO}_2\text{NR}_2$ ;

$\text{X}_1''$  is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

$\text{X}_2$  is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

$\text{X}_3$  is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

$\text{X}_4$  is absent, is N-methylarginine, is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a proline residue, or a glycine residue;

$\text{X}_5$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_6$  is absent, is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_7$  is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_8$  is D-ornithine;

$\text{X}_9$  is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_{10}$  is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_{11}$  is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

$\text{X}_{12}$  is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

- 99 -

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

13. The compound of Claim 12, wherein L is C\*(O).
14. The compound of any one of Claims 12-13, wherein the lipophilic moiety is a palmitic acid derivative.
15. The compound represented by Formula D:

T-L-X<sub>1</sub>'''-X<sub>2</sub>-X<sub>3</sub>-X<sub>4</sub>-X<sub>5</sub>-X<sub>6</sub>-X<sub>7</sub>-X<sub>8</sub>-X<sub>9</sub>-X<sub>10</sub>-X<sub>11</sub>-X<sub>12</sub>-X<sub>13</sub>-X<sub>14</sub>-X<sub>15</sub>-X<sub>16</sub> -R<sub>1</sub>;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of X<sub>1</sub>''' and is selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to X<sub>1</sub>''' at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L; R<sub>1</sub> is -OR<sub>2</sub>, - or NR<sub>3</sub>R<sub>4</sub>; wherein

- 100 -

each  $R_2$  is hydrogen or a (C<sub>1</sub>-C<sub>10</sub>) alkyl group,

$R_3$  and  $R_4$  are each independently selected from hydrogen, (C<sub>1</sub>-C<sub>10</sub>) alkyl, (C<sub>1</sub>-C<sub>10</sub>) aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub> ;

n is 1-20

or -NR<sub>3</sub>R<sub>4</sub> is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono- or di-substituted at one or more substitutable carbon atoms with an R<sub>5</sub>;

each R<sub>5</sub> is independently halogen, -OH, C<sub>1</sub>-C<sub>3</sub> alkyl, C<sub>1</sub>-C<sub>3</sub> haloalkyl, -NO<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkoxy, -C<sub>1</sub>-C<sub>3</sub> haloalkoxy, -CN, -NH<sub>2</sub>, -C<sub>1</sub>-C<sub>3</sub> alkylamino, -C<sub>1</sub>-C<sub>3</sub> dialkylamino, -C(O)NH<sub>2</sub>, -C(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)H, -C(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, -NHC(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -C(O)OH, -C(O)O-(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)NH<sub>2</sub>, -NHC(O)NH(C<sub>1</sub>-C<sub>3</sub> alkyl), -NHC(O)N(C<sub>1</sub>-C<sub>3</sub> alkyl)<sub>2</sub>, or -SO<sub>2</sub>NR<sub>2</sub>;

X<sub>1</sub><sup>'''</sup> is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

X<sub>2</sub> is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is N-methylarginine, is an asparagine residue, an arginine residue, a histidine residue, or a glutamine residue;

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, or a D-2,3-diaminopropionic acid residue;

- 101 -

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is an asparagine residue; isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

wherein at least one of X<sub>4</sub> or X<sub>14</sub> is an asparagine residue,

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

16. The compound of Claim 15, wherein X<sub>8</sub> is D-ornithine.
17. The compound of any one of Claims 15-16, wherein L is C\*(O).
18. The compound of any one of Claims 15-17, wherein the lipophilic moiety is a palmitic acid derivative.

19. The compound represented by Formula E:

$T-L-X_1^{''''}-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-R_1$ ;

or a pharmaceutically acceptable salt thereof, wherein L is a linking moiety bonded to the N terminal nitrogen of  $X_1^{''''}$  and is selected from:  $C^*(O)$ ,  $C^*(S)$ ,  $S^*(O)_2$ ,  $N(R^{13})S^*(O)$ ,  $N(R^{13})S^*(O)_2$ ,  $N(R^{13})C^*(O)$ ,  $N(R^{13})C^*(S)$ ,  $OC^*(O)$ ,  $OC^*(S)$ ,  $SC^*(O)$ ,  $SC^*(S)$ ,  $C(=NH)$ , and  $N(R^{13})C^*(=NH)$ ; wherein L is bonded to  $X_1^{''''}$  at the atom marked with an asterisk (\*) and  $R^{13}$  is selected from: H, D,  $(C_1-C_6)$ alkyl,  $(C_2-C_6)$ alkenyl,  $(C_2-C_6)$ alkynyl,  $(C_1-C_6)$ alkoxy,  $(C_3-C_9)$ cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; T is a lipophilic moiety bonded to L;  $R_1$  is  $-OR_2$ , - or  $NR_3R_4$ , wherein

each  $R_2$  is hydrogen or a  $(C_1-C_{10})$  alkyl group,

$R_3$  and  $R_4$  are each independently selected from hydrogen,  $(C_1-C_{10})$  alkyl,  $(C_1-C_{10})$ aralkyl,  $[CH_2CH_2O]_nCH_2CH_2C(O)OR_2$  or  $[CH_2CH_2O]_nCH_2CH_2C(O)NR_2$  ;

n is 1-20

or  $-NR_3R_4$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono- or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3$  alkyl),  $-C(O)(C_1-C_3$  alkyl),  $-NHC(O)(C_1-C_3$  alkyl),  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3$  alkyl) $_2$ ,  $-NHC(O)O-(C_1-C_3$  alkyl),  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3$  alkyl),  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3$  alkyl),  $-NHC(O)N(C_1-C_3$  alkyl) $_2$ , or  $-SO_2NR_2$ ;

$X_1^{''''}$  is a threonine residue, a histidine residue, a glutamine residue, an aspartic acid residue, an Aib residue, a phenylalanine residue, or a glycine residue;

$X_2$  is a valine residue, a phenylalanine residue, a histidine residue, an Aib residue, a glutamine residue, an aspartic acid residue, a proline residue, or a glycine residue;

- 103 -

X<sub>3</sub> is a phenylalanine residue, a histidine residue, an aspartic acid residue, a glycine residue, or a proline residue;

X<sub>4</sub> is N-methylarginine;

X<sub>5</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>6</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>7</sub> is a glutamic acid, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>8</sub> is a lysine, a D-lysine residue, a D-ornithine residue, a D-2,4-diaminobutyric acid residue, a D-2,3-diaminopropionic acid residue;

X<sub>9</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>10</sub> is an arginine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>11</sub> is a serine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>12</sub> is alanine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>13</sub> is an aspartic acid residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>14</sub> is isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>15</sub> is a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;

X<sub>16</sub> is a phenylalanine residue, an isoleucine residue, a phenylalanine residue, a histidine residue, a glutamine residue, an aspartic acid residue, a proline residue, an Aib residue, or a glycine residue;



- 104 -

wherein optionally a total of 1- 5 amino acid residues are present in the D configuration.

20. The compound of Claim 19, wherein the compounds are selected from any one of Compound Nos 31 and 32 or a pharmaceutically acceptable salt thereof.
21. The compound of Claim 19, wherein L is C\*(O).
22. The compound of Claim 19 or Claim 21, wherein the lipophilic moiety is a palmitic acid derivative.
23. The compound represented by Formula I:

T-L-P,

or pharmaceutically acceptable salts thereof, wherein:

P is a peptide comprising SEQ ID Nos. 2-37, wherein

L is a linking moiety bonded to P at an N-terminal nitrogen of an N-terminal amino-acid residue selected from: C\*(O), C\*(S), S\*(O)<sub>2</sub>, N(R<sup>13</sup>)S\*(O), N(R<sup>13</sup>)S\*(O)<sub>2</sub>, N(R<sup>13</sup>)C\*(O), N(R<sup>13</sup>)C\*(S), OC\*(O), OC\*(S), SC\*(O), SC\*(S), C\*(=NH), and N(R<sup>13</sup>)C\*(=NH); wherein L is bonded to P at the atom marked with an asterisk (\*) and R<sup>13</sup> is selected from: H, D, (C<sub>1</sub>-C<sub>6</sub>)alkyl, (C<sub>2</sub>-C<sub>6</sub>)alkenyl, (C<sub>2</sub>-C<sub>6</sub>)alkynyl, (C<sub>1</sub>-C<sub>6</sub>)alkoxy, (C<sub>3</sub>-C<sub>9</sub>)cycloalkyl, 5-10 membered heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl; wherein said alkyl, alkenyl, alkynyl, alkoxy, cycloalkyl, heterocycloalkyl, aryl, aryloxy, heteroaryloxy, aralkyl, heteroaryl, and heteroaralkyl are optionally and independently substituted; and T is a lipophilic tether moiety bonded to L, wherein the C-terminal amino acid residue of P is functionalized by replacement of the acid moiety with C(O)NR<sub>3</sub><sup>1</sup>R<sub>4</sub><sup>1</sup>; R<sub>3</sub><sup>1</sup> is selected from hydrogen, C<sub>1</sub>-C<sub>10</sub> alkyl, (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>; R<sub>4</sub><sup>1</sup> is selected from (C<sub>1</sub>-C<sub>10</sub>)aralkyl, [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)OR<sub>2</sub> or [CH<sub>2</sub>CH<sub>2</sub>O]<sub>n</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)NR<sub>2</sub>;

n is 1-20

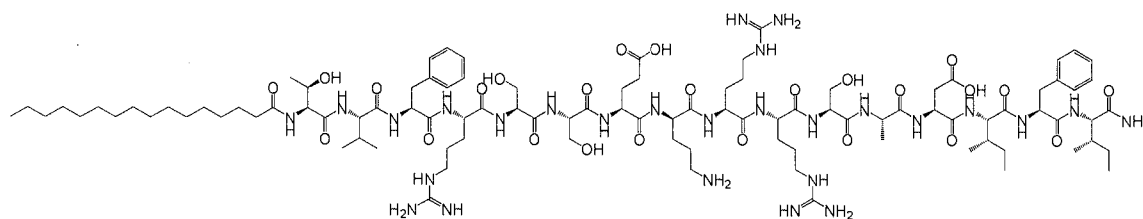
- 105 -

or  $-NR_3^1R_4^1$  is a non-aromatic nitrogen-containing heterocyclic group, wherein the non-aromatic group is optionally mono-or di-substituted at one or more substitutable carbon atoms with an  $R_5$ ;

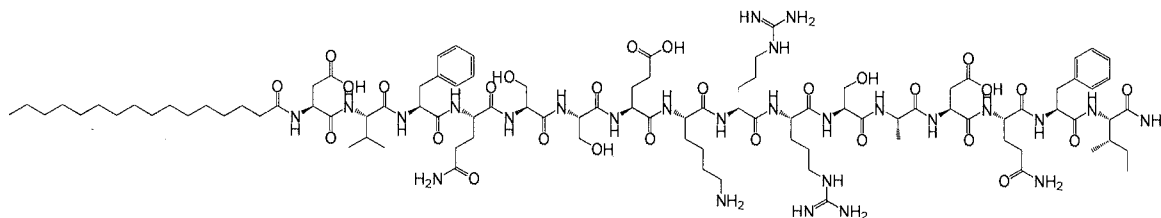
each  $R_5$  is independently halogen,  $-OH$ ,  $C_1-C_3$  alkyl,  $C_1-C_3$  haloalkyl,  $-NO_2$ ,  $-C_1-C_3$  alkoxy,  $-C_1-C_3$  haloalkoxy,  $-CN$ ,  $-NH_2$ ,  $-C_1-C_3$  alkylamino,  $-C_1-C_3$  dialkylamino,  $-C(O)NH_2$ ,  $-C(O)NH(C_1-C_3$  alkyl),  $-C(O)(C_1-C_3$  alkyl),  $-NHC(O)(C_1-C_3$  alkyl),  $-NHC(O)H$ ,  $-C(O)N(C_1-C_3$  alkyl)<sub>2</sub>,  $-NHC(O)O-(C_1-C_3$  alkyl),  $-C(O)OH$ ,  $-C(O)O-(C_1-C_3$  alkyl),  $-NHC(O)NH_2$ ,  $-NHC(O)NH(C_1-C_3$  alkyl),  $-NHC(O)N(C_1-C_3$  alkyl)<sub>2</sub>, or  $-SO_2NR_2$ .

24. A compound of Claim 23 selected from any one of Compound Nos 8, 12, 13, 15, 17, 30, and 36 or a pharmaceutically acceptable salt thereof.
25. A compound of Claim 23 selected from any one of Compound Nos 3, 14, 18, 21, 24, 25, 26, 27, 28, 29, and 32 or a pharmaceutically acceptable salt thereof.
26. A compound of Claim 23 selected from any one of Compound Nos 6, 7, 16, 19, 20, 33, 34 and 35 or a pharmaceutically acceptable salt thereof.
27. A compound of Claim 23 selected from:

Compound No. 8:

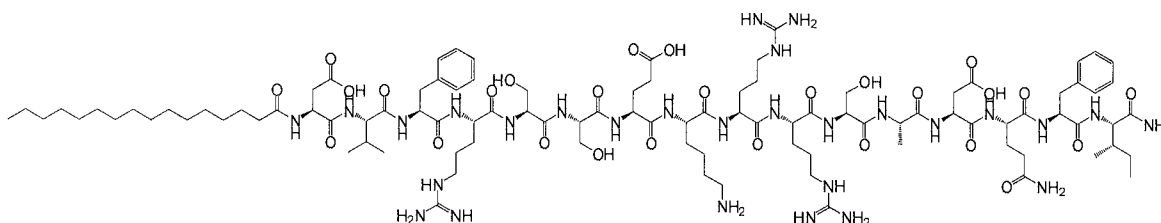


28. A compound of Claim 23 selected from:
- Compound No. 12:



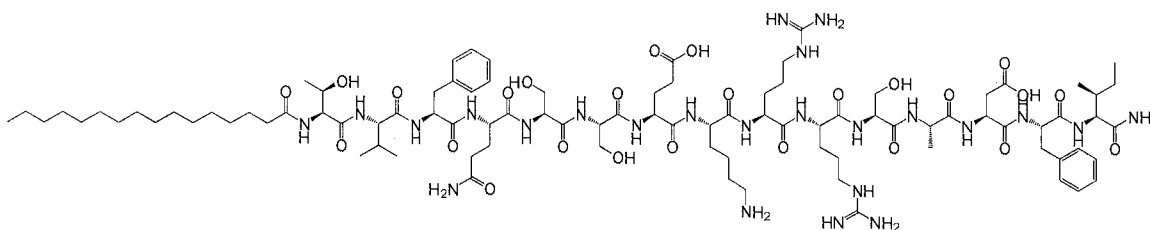
29. A compound of Claim 23 selected from:

Compound No. 13:



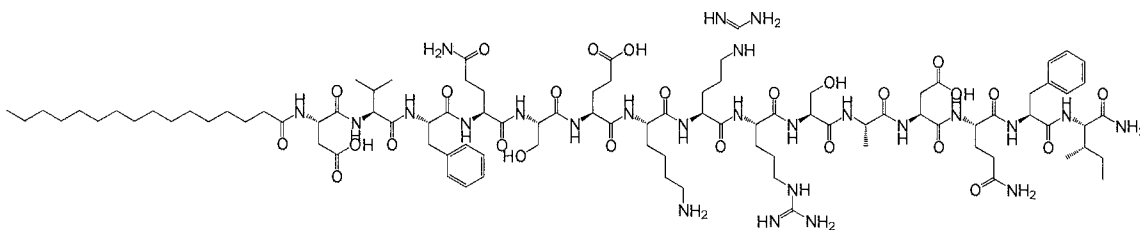
30. A compound of Claim 23 selected from:

Compound No. 15:



31. A compound of Claim 23 selected from:

Compound No. 17:

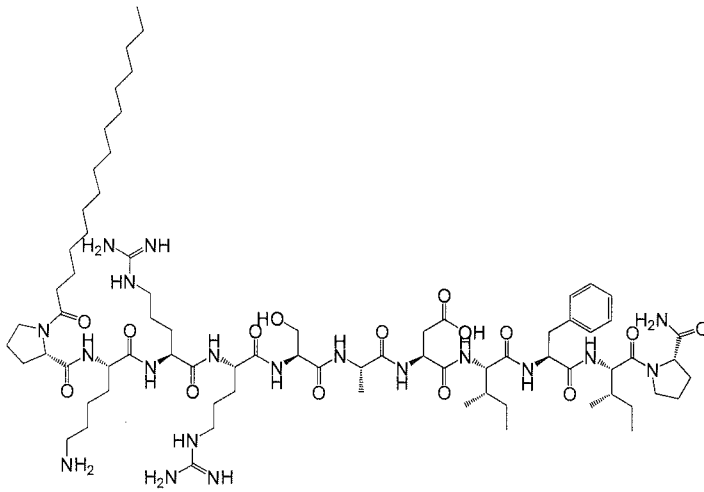


32. A compound of Claim 23 selected from:

Compound No. 30:

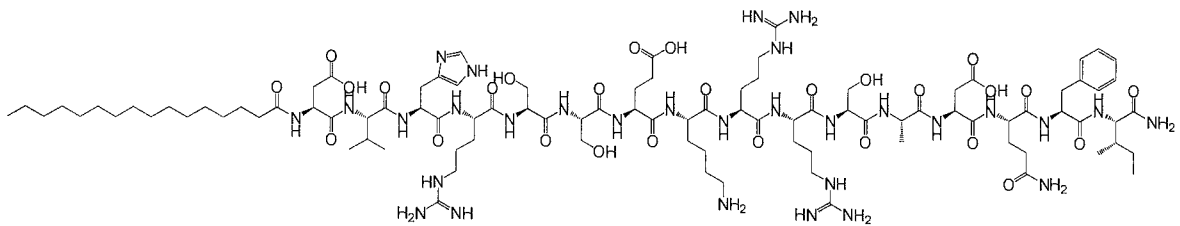
.....

- 107 -



33. A compound of Claim 23 selected from:

Compound No. 36:



34. The compound of Claim 23, wherein L is C\*(O).

35. The compound of Claim 23 or Claim 34, wherein the lipophilic moiety is a palmitic acid derivative.

36. A method of treating cardiovascular disease, cancer, diabetes, stem cell trafficking, fluid homeostasis, cell proliferation, immune function, obesity, metastatic disease, and HIV infection in a patient in need thereof comprising administering to said patient and effective amount of a compound of any one of Claims 1-35.

37. The method of Claim 36, wherein the cardiovascular disease, is selected from: hypertension and heart failure.

- 108 -

38. The method of Claim 37, wherein the heart failure is congestive heart failure or hypertrophic cardiomyopathy.
39. A pharmaceutical composition, comprising a compound of any one of Claims 1-35 and a pharmaceutically acceptable carrier.

Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hAPJ HEK cells)												
8	i1	9	TVFRSSE(d-orn)RRSADIFI	<p>Dose-response curve for compound 8. The y-axis represents cAMP concentration in nM, ranging from 0 to 40. The x-axis represents the logarithm of compound concentration in M, ranging from vehicle (veh) to -5. The data points show a sigmoidal decrease in cAMP levels as the concentration of compound 8 increases, reaching a plateau of approximately 8 nM at -6 M.</p> <table border="1"> <caption>Approximate data for Compound 8</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>20</td> </tr> <tr> <td>-9</td> <td>25</td> </tr> <tr> <td>-8</td> <td>14</td> </tr> <tr> <td>-7</td> <td>10</td> </tr> <tr> <td>-6</td> <td>8</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	20	-9	25	-8	14	-7	10	-6	8
Log10 [compound], M	cAMP (nM)															
veh	20															
-9	25															
-8	14															
-7	10															
-6	8															
12	i1	13	DVFQSSEkRRSADQFI	<p>Dose-response curve for compound 12. The y-axis represents cAMP concentration in nM, ranging from 0 to 30. The x-axis represents the logarithm of compound concentration in M, ranging from vehicle (veh) to -5. The data points show a sigmoidal decrease in cAMP levels as the concentration of compound 12 increases, reaching a plateau of approximately 2 nM at -6 M.</p> <table border="1"> <caption>Approximate data for Compound 12</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>20</td> </tr> <tr> <td>-9</td> <td>24</td> </tr> <tr> <td>-8</td> <td>12</td> </tr> <tr> <td>-7</td> <td>4</td> </tr> <tr> <td>-6</td> <td>2</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	20	-9	24	-8	12	-7	4	-6	2
Log10 [compound], M	cAMP (nM)															
veh	20															
-9	24															
-8	12															
-7	4															
-6	2															

FIG. 1A

Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hAPJ HEK cells)																
13	i1	14	DVFRSSEkRRSADQFI	<p>Dose-response curve for compound 13. The y-axis represents cAMP (nM) from 0 to 25. The x-axis represents Log10 [compound], M from veh to -5. The curve shows a sigmoidal decrease in cAMP levels as the concentration of compound 13 increases.</p> <table border="1"> <caption>Approximate data points for Compound 13</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>17</td> </tr> <tr> <td>-9</td> <td>20</td> </tr> <tr> <td>-8.5</td> <td>11</td> </tr> <tr> <td>-8</td> <td>5</td> </tr> <tr> <td>-7</td> <td>4</td> </tr> <tr> <td>-6.5</td> <td>2</td> </tr> <tr> <td>-6</td> <td>3</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	17	-9	20	-8.5	11	-8	5	-7	4	-6.5	2	-6	3
Log10 [compound], M	cAMP (nM)																			
veh	17																			
-9	20																			
-8.5	11																			
-8	5																			
-7	4																			
-6.5	2																			
-6	3																			
15	i1	16	TVFQSSEkRRSADFI	<p>Dose-response curve for compound 15. The y-axis represents cAMP (nM) from 0 to 40. The x-axis represents Log10 [compound], M from veh to -5. The curve shows a sigmoidal decrease in cAMP levels as the concentration of compound 15 increases.</p> <table border="1"> <caption>Approximate data points for Compound 15</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>21</td> </tr> <tr> <td>-9</td> <td>25</td> </tr> <tr> <td>-8.5</td> <td>20</td> </tr> <tr> <td>-8</td> <td>7</td> </tr> <tr> <td>-7</td> <td>8</td> </tr> <tr> <td>-6.5</td> <td>3</td> </tr> <tr> <td>-6</td> <td>3</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	21	-9	25	-8.5	20	-8	7	-7	8	-6.5	3	-6	3
Log10 [compound], M	cAMP (nM)																			
veh	21																			
-9	25																			
-8.5	20																			
-8	7																			
-7	8																			
-6.5	3																			
-6	3																			

FIG. 1B

Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hAPJ HEK cells)																		
17	ii	18	DVFQSEkRRSADQFI	<p>Dose-response curve for compound 17. The y-axis represents cAMP (nM) from 0 to 30. The x-axis represents Log10 [compound], M from veh to -5. The curve shows a decrease in cAMP from approximately 22 nM at veh to about 3 nM at 10<sup>-6</sup> M.</p> <table border="1"> <caption>Approximate data points for compound 17</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>22</td> </tr> <tr> <td>-9</td> <td>24</td> </tr> <tr> <td>-8.5</td> <td>24</td> </tr> <tr> <td>-8</td> <td>14</td> </tr> <tr> <td>-7.5</td> <td>6</td> </tr> <tr> <td>-7</td> <td>4</td> </tr> <tr> <td>-6.5</td> <td>3</td> </tr> <tr> <td>-6</td> <td>3</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	22	-9	24	-8.5	24	-8	14	-7.5	6	-7	4	-6.5	3	-6	3
Log10 [compound], M	cAMP (nM)																					
veh	22																					
-9	24																					
-8.5	24																					
-8	14																					
-7.5	6																					
-7	4																					
-6.5	3																					
-6	3																					
30	ii	31	PkRRSADIFIP	<p>Dose-response curve for compound 30. The y-axis represents cAMP (nM) from 0 to 25. The x-axis represents Log10 [compound], M from veh to -5. The curve shows a decrease in cAMP from approximately 17 nM at veh to about 6 nM at 10<sup>-6</sup> M.</p> <table border="1"> <caption>Approximate data points for compound 30</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>17</td> </tr> <tr> <td>-9</td> <td>18</td> </tr> <tr> <td>-8.5</td> <td>12</td> </tr> <tr> <td>-8</td> <td>9</td> </tr> <tr> <td>-7.5</td> <td>6</td> </tr> <tr> <td>-7</td> <td>6</td> </tr> <tr> <td>-6.5</td> <td>5</td> </tr> <tr> <td>-6</td> <td>6</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	17	-9	18	-8.5	12	-8	9	-7.5	6	-7	6	-6.5	5	-6	6
Log10 [compound], M	cAMP (nM)																					
veh	17																					
-9	18																					
-8.5	12																					
-8	9																					
-7.5	6																					
-7	6																					
-6.5	5																					
-6	6																					

FIG. 1C



Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hAPJ HEK cells)																
36	i1	37	DVHRSSEkRRSADQFI	<p>Detailed description of the graph: The graph plots cAMP concentration in nanomolar (nM) on the y-axis against the logarithm of compound concentration in molar (M) on the x-axis. The y-axis has major ticks at 0, 20, 40, 60, 80, and 100. The x-axis has labels for 'veh' (vehicle), -9, -8, -7, -6, and -5. Data points are shown as black circles with vertical error bars. A smooth sigmoidal curve is fitted to the data. The cAMP level starts at approximately 75 nM for the vehicle control and decreases to about 10 nM at a concentration of 10<sup>-6</sup> M.</p> <table border="1"> <caption>Estimated data points from the graph</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>~75</td> </tr> <tr> <td>-9</td> <td>~60</td> </tr> <tr> <td>-8.5</td> <td>~40</td> </tr> <tr> <td>-8</td> <td>~20</td> </tr> <tr> <td>-7.5</td> <td>~10</td> </tr> <tr> <td>-6.5</td> <td>~10</td> </tr> <tr> <td>-6</td> <td>~10</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	~75	-9	~60	-8.5	~40	-8	~20	-7.5	~10	-6.5	~10	-6	~10
Log10 [compound], M	cAMP (nM)																			
veh	~75																			
-9	~60																			
-8.5	~40																			
-8	~20																			
-7.5	~10																			
-6.5	~10																			
-6	~10																			

FIG. 1D

5/20

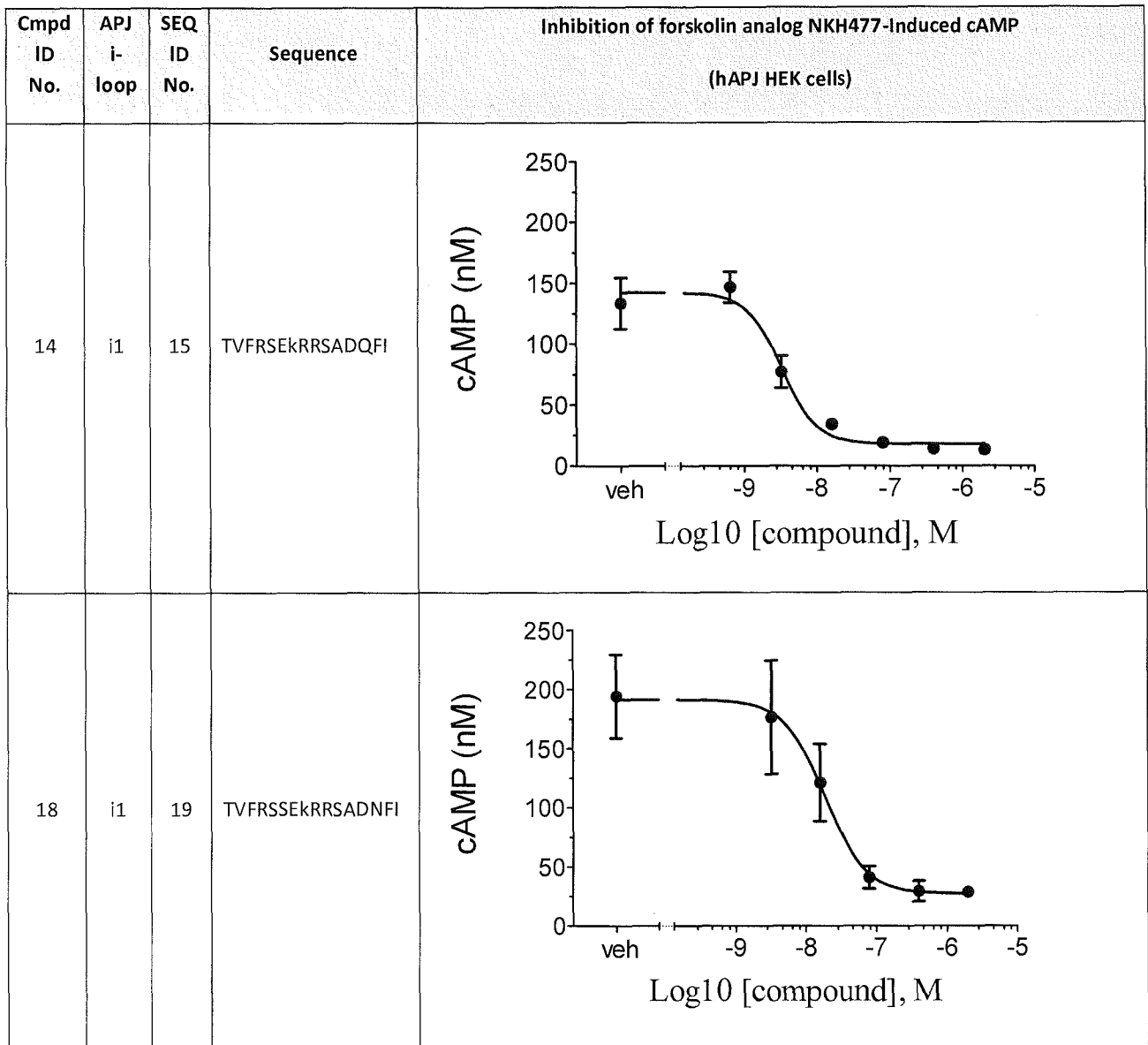


FIG. 2A

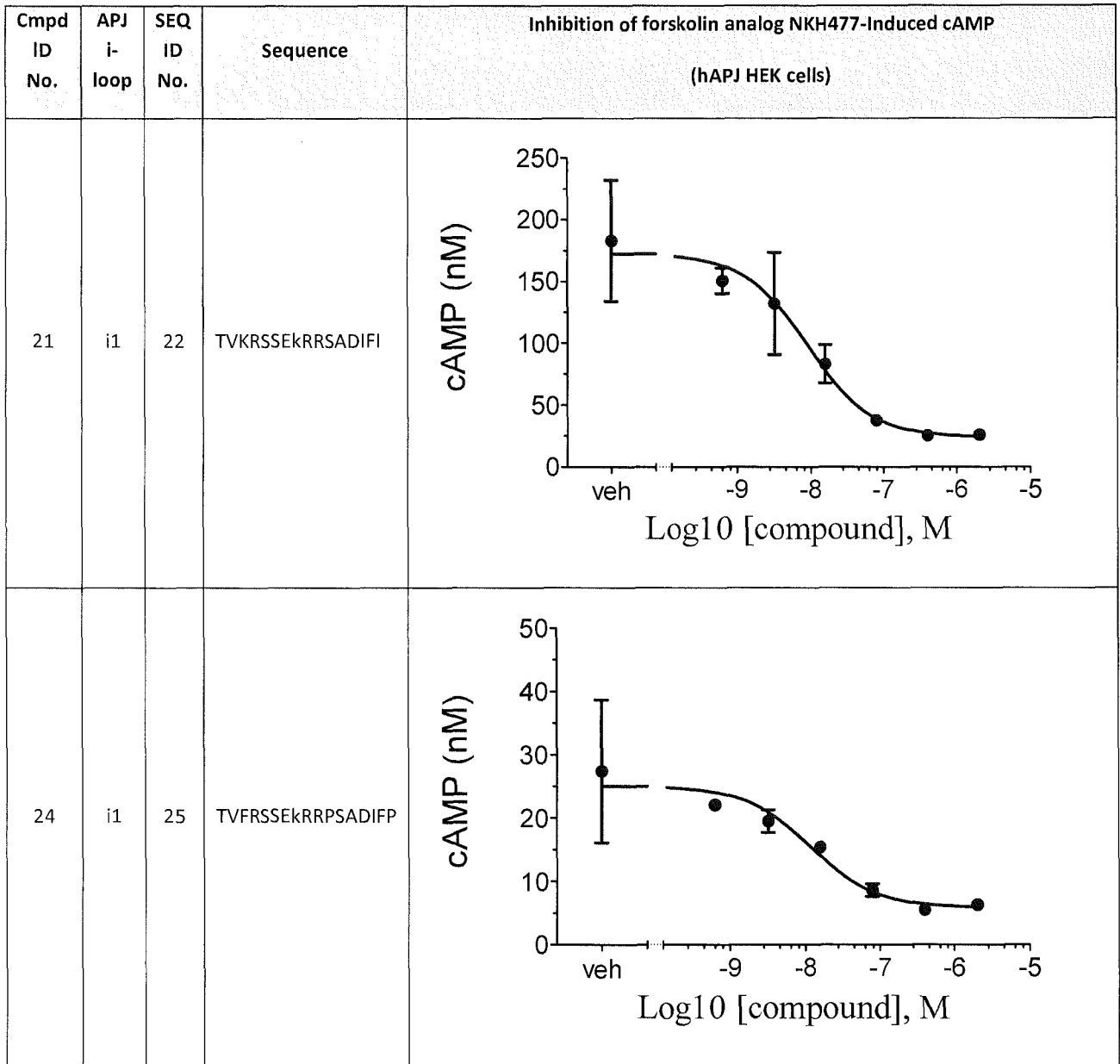


FIG. 2B

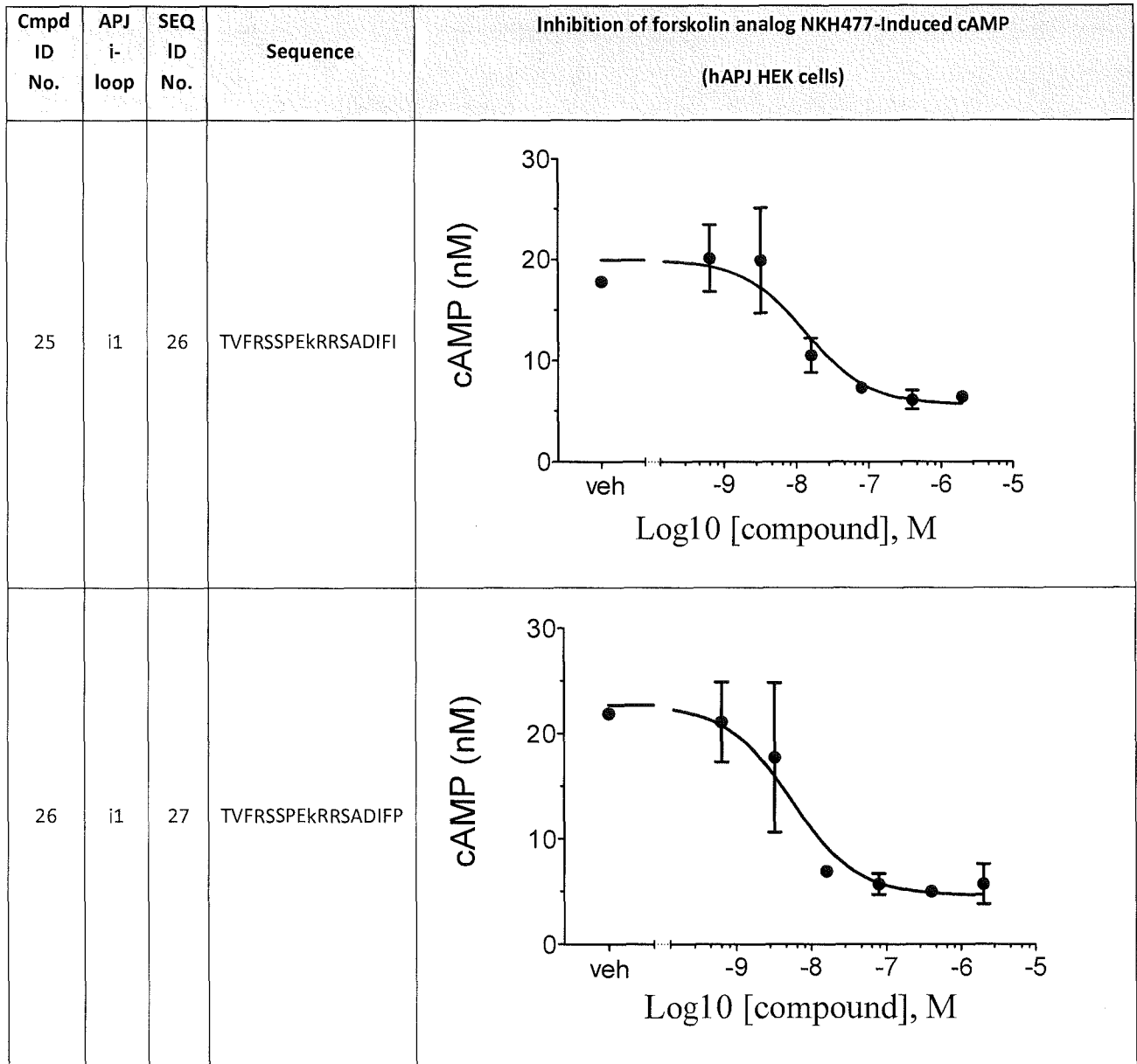


FIG. 2C

8/20

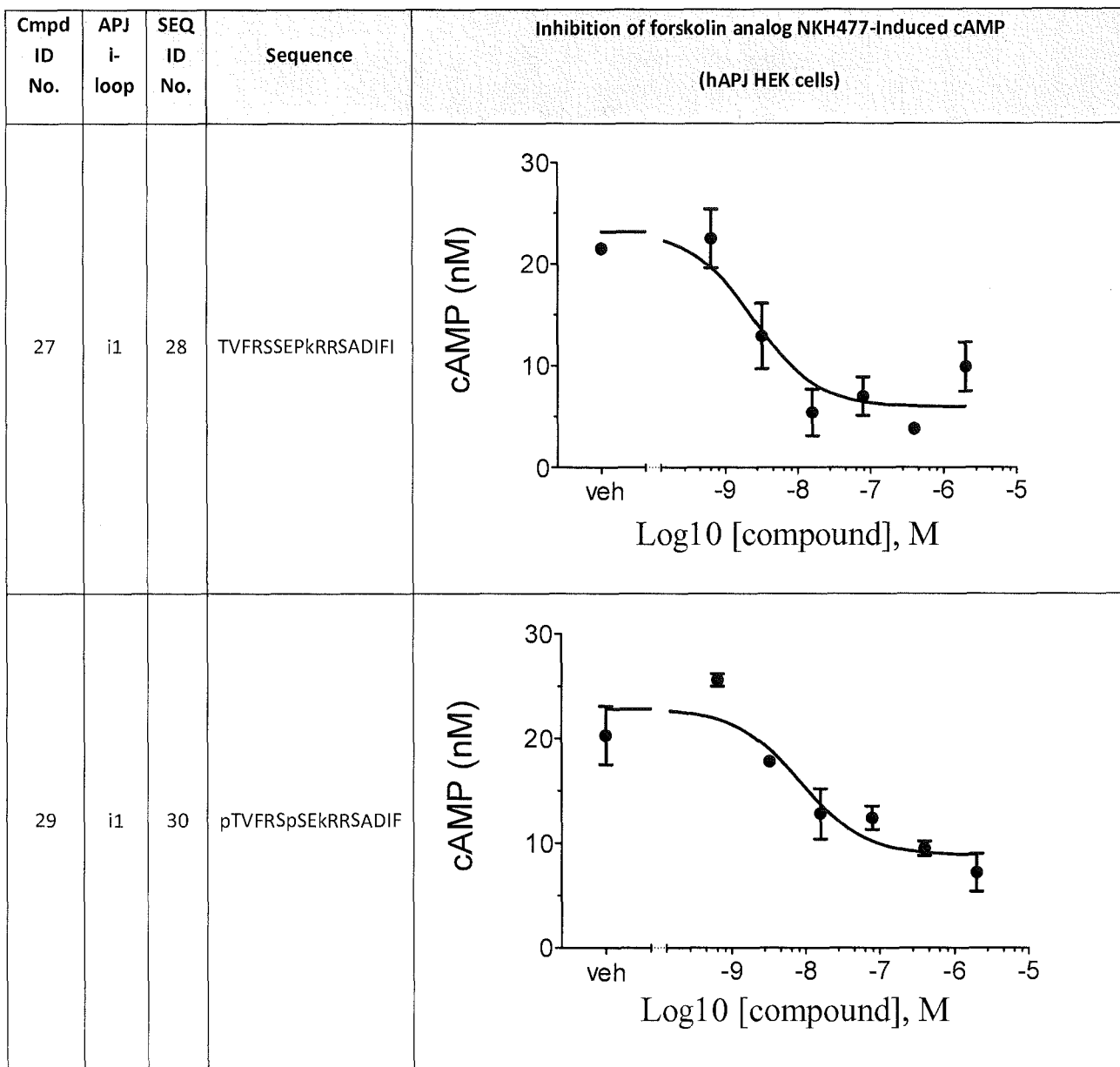


FIG. 2D

9/20

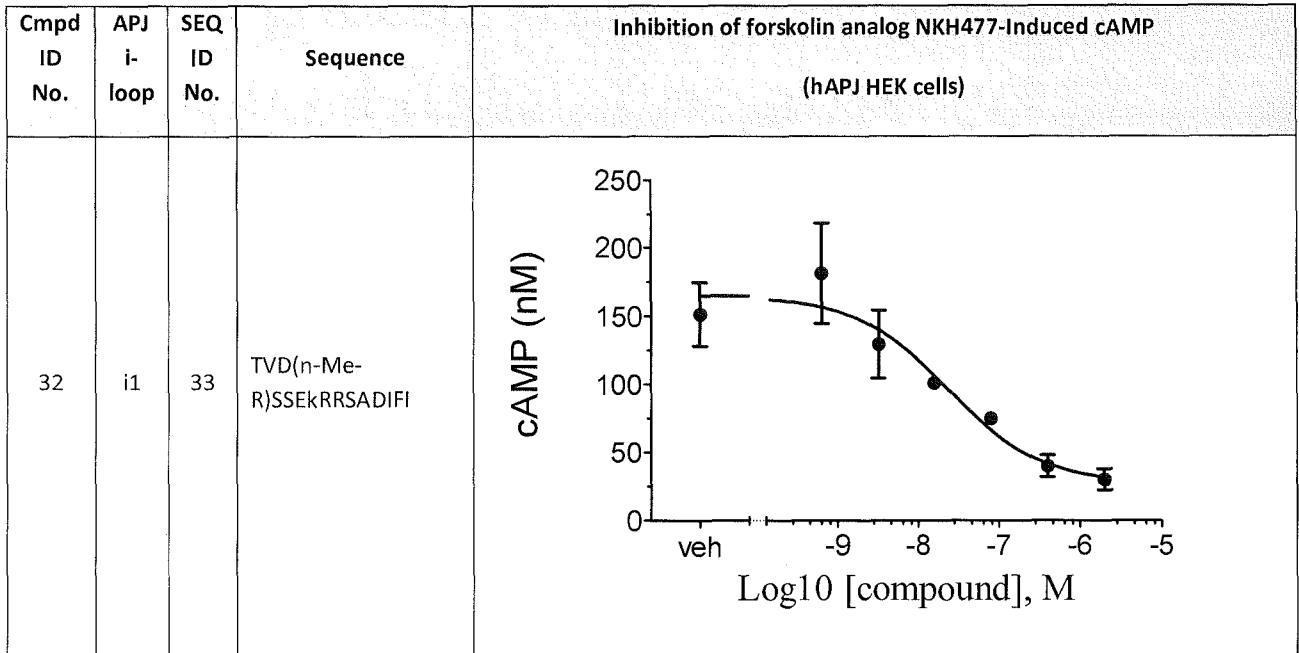


FIG. 2E

10/20

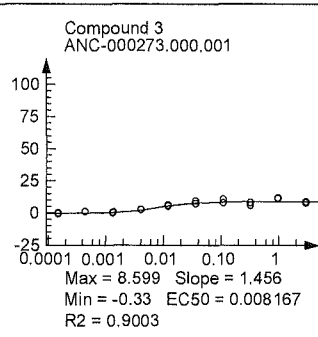
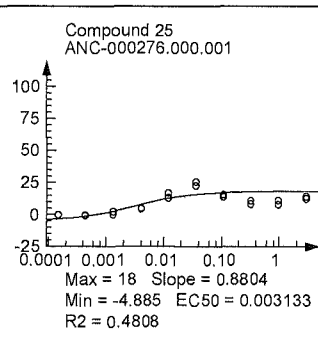
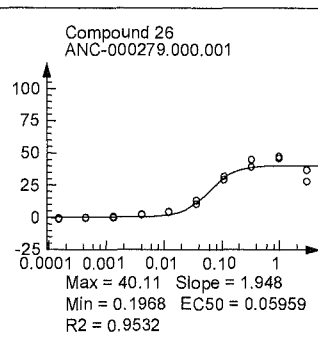
		β-arrestin Signaling		
Cmpd #	Sequence	EC <sub>50</sub> (uM)	Max Response <sup>1</sup>	Concentration Response Curve (CRC)
Apelin-13	N/A	0.0017	101.7	
3	PTVFRSPSEkRRSADIFI (SEQ ID NO: 4)	0.0082	11.0	<p>Compound 3 ANC-000273.000.001</p>  <p>Max = 8.599 Slope = 1.456 Min = -0.33 EC50 = 0.008167 R2 = 0.9003</p>
25	TVFRSSPEkRRSADIFI (SEQ ID NO: 26)	0.0031	12.6	<p>Compound 25 ANC-000276.000.001</p>  <p>Max = 18 Slope = 0.8804 Min = -4.885 EC50 = 0.003133 R2 = 0.4808</p>
26	TVFRSSPEkRRSADIFP (SEQ ID NO: 27)	0.0596	46.0	<p>Compound 26 ANC-000279.000.001</p>  <p>Max = 40.11 Slope = 1.948 Min = 0.1968 EC50 = 0.05959 R2 = 0.9532</p>

FIG. 3A

11/20

Cmpd #	Sequence	β-arrestin Signaling		
		EC <sub>50</sub> (uM)	Max Response <sup>1</sup>	Concentration Response Curve (CRC)
27	TVFRSSEPKRRSADIFI (SEQ ID NO: 28)	0.0081	23.7	<p>Compound 27 ANC-000278.000.001</p> <p>Max = 20 Slope = 2.714 Min = 0 EC50 = 0.00806 R2 = 0.7702</p>

<sup>1</sup>Relative to apelin-13

FIG. 3B



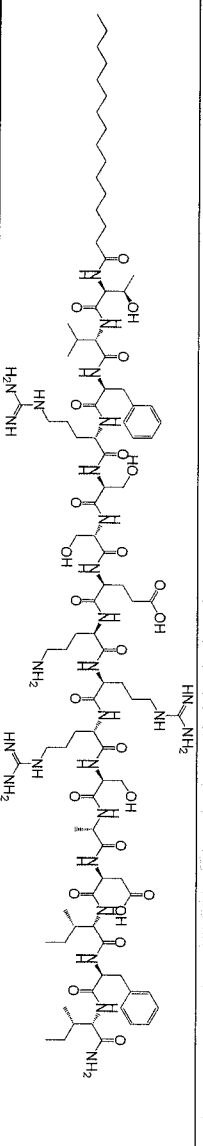
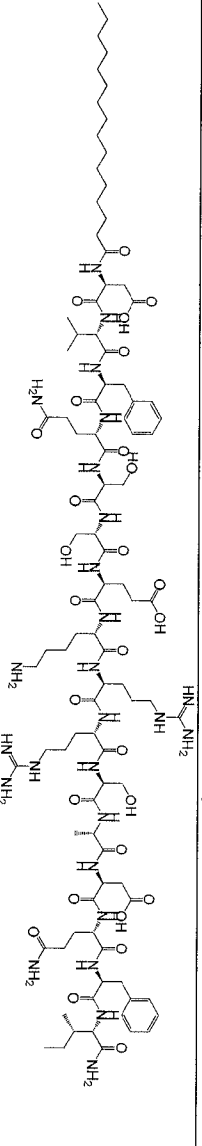
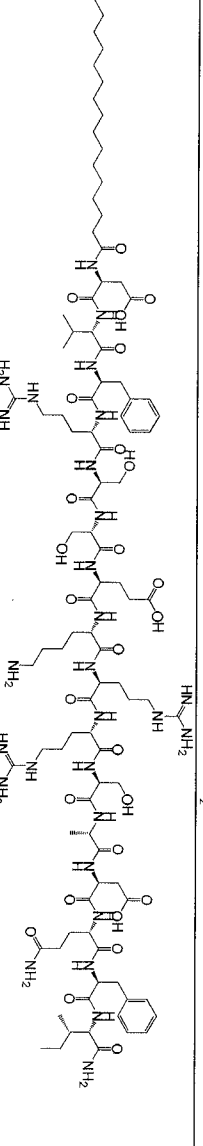
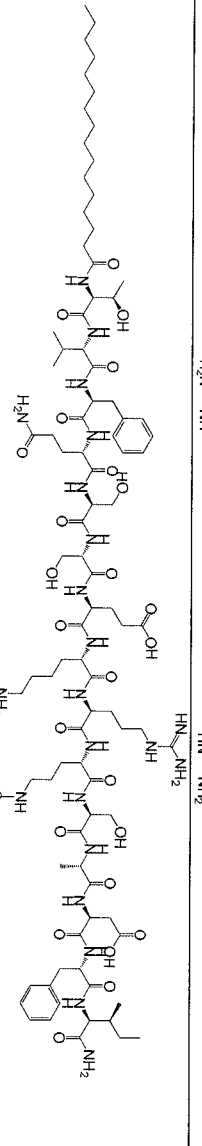
Cmpd ID No.	SEQ ID No.	Sequence	Structure
8	9	TVFRSSE(d-om)RRSADIFI	
12	13	DVFRSSEKR RSADQFI	
13	14	DVFRSSEKR RSADQFI	
15	16	TVFQOSEKR RSADFI	

FIG. 4A

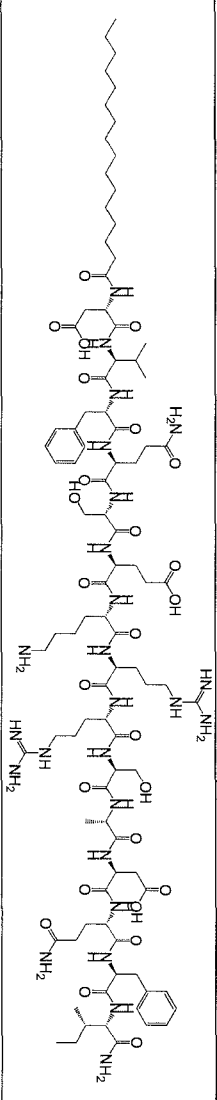
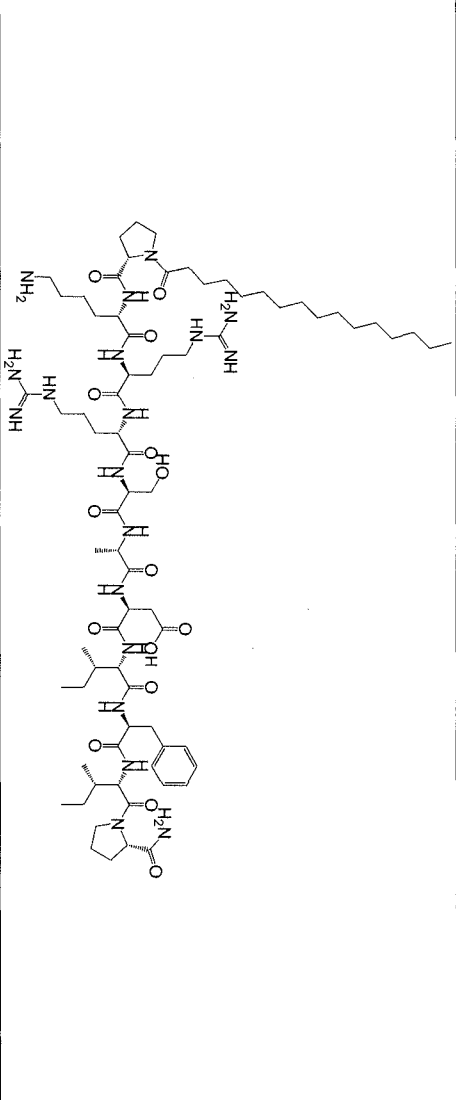
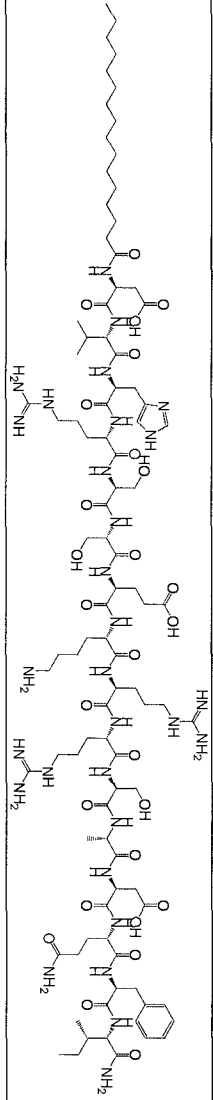
Cmpd ID No.	SEQ ID No.	Sequence	Structure
17	18	DYFQSEKRR SADQFI	
30	31	PKRRSADIFI P	
36	37	DYHRSSEKR RSADQFI	

FIG. 4B

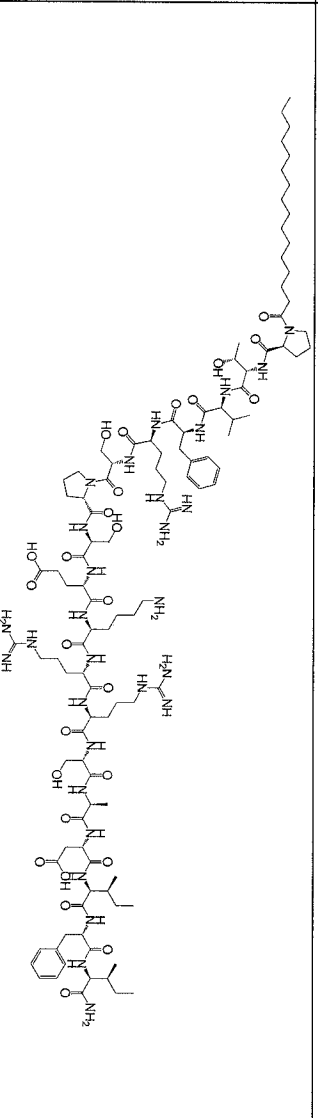
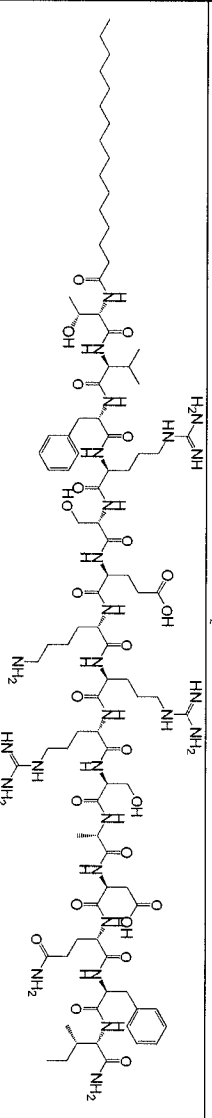
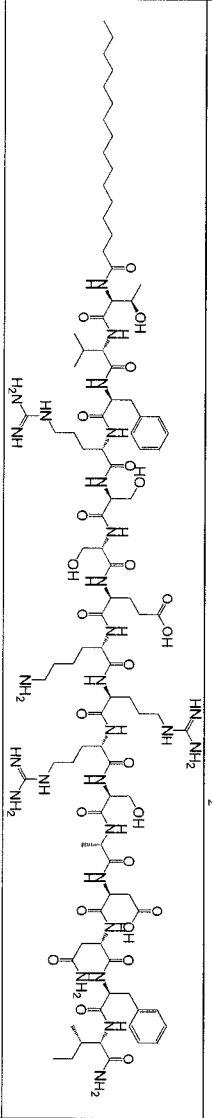
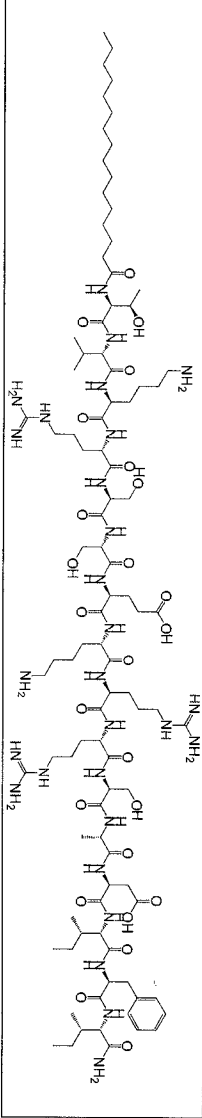
Cmpd ID No.	SEQ ID No.	Sequence	Structure
3	4	PTVFRSPSEKRRSADIFI	
14	15	TVFRSEKRRSADQFI	
18	19	TVFRSSEKRRSADNFI	
21	22	TVKRSSEKRRSADIFI	

FIG. 5A

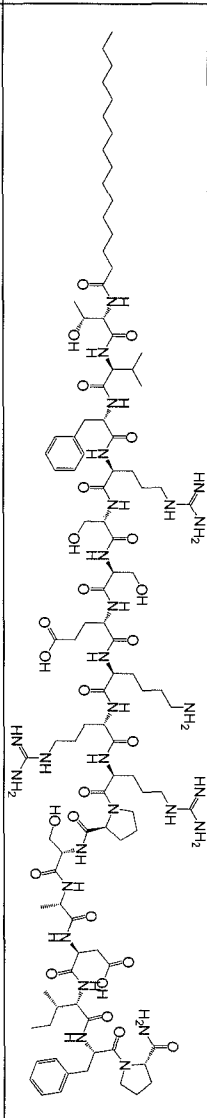
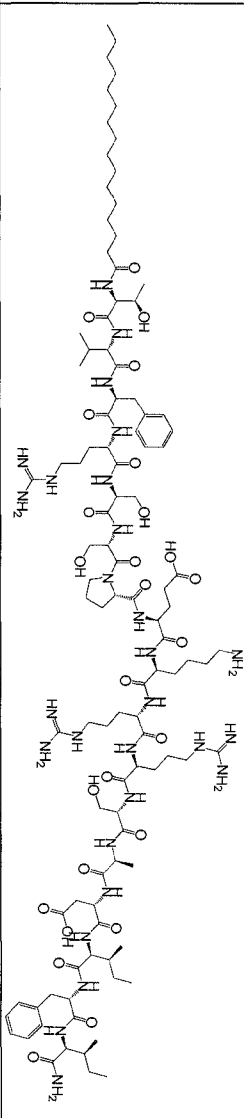
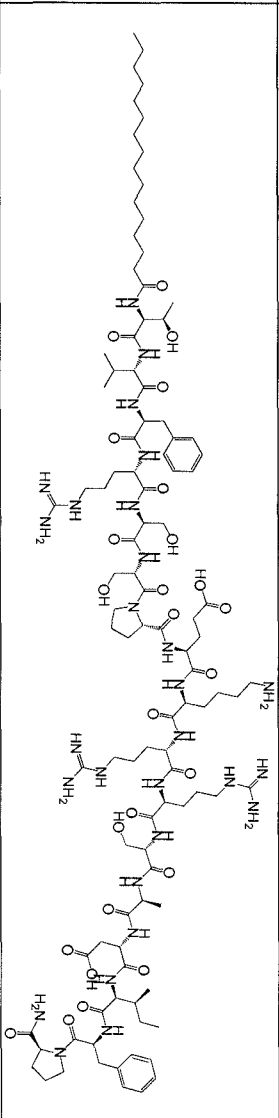
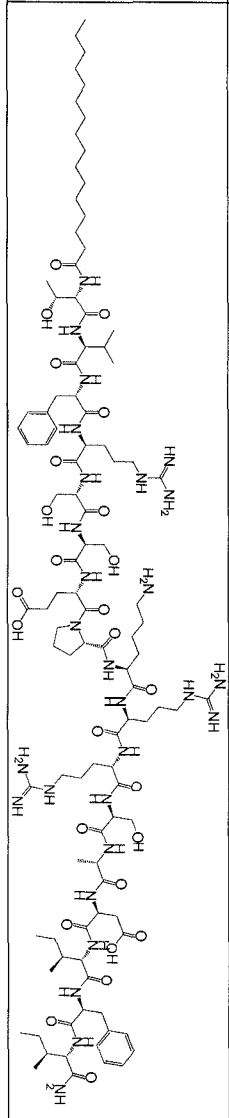
Cmpd ID No.	SEQ ID No.	Sequence	Structure
24	25	TVFRSSEKRRPPSADIFP	
25	26	TVFRSSPEKRRSADIFI	
26	27	TVFRSSPEKRRSADIFP	
27	28	TVFRSSEPKRRSADIFI	

FIG. 5B

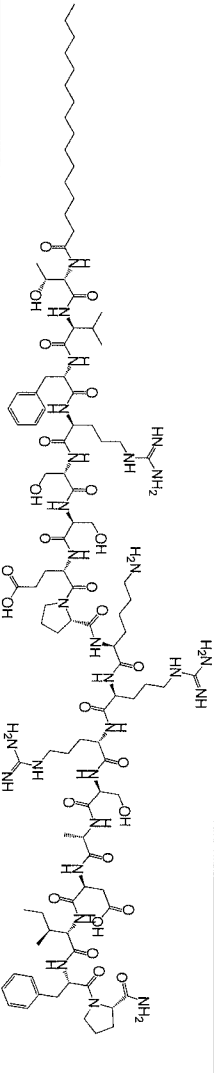
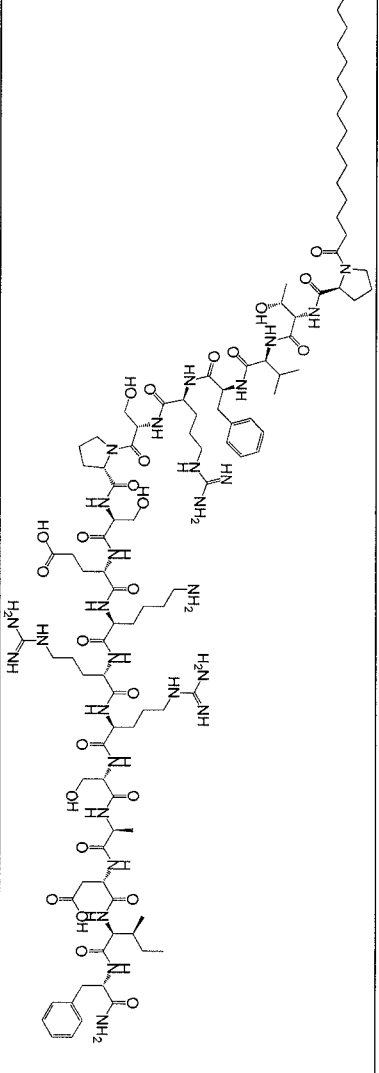
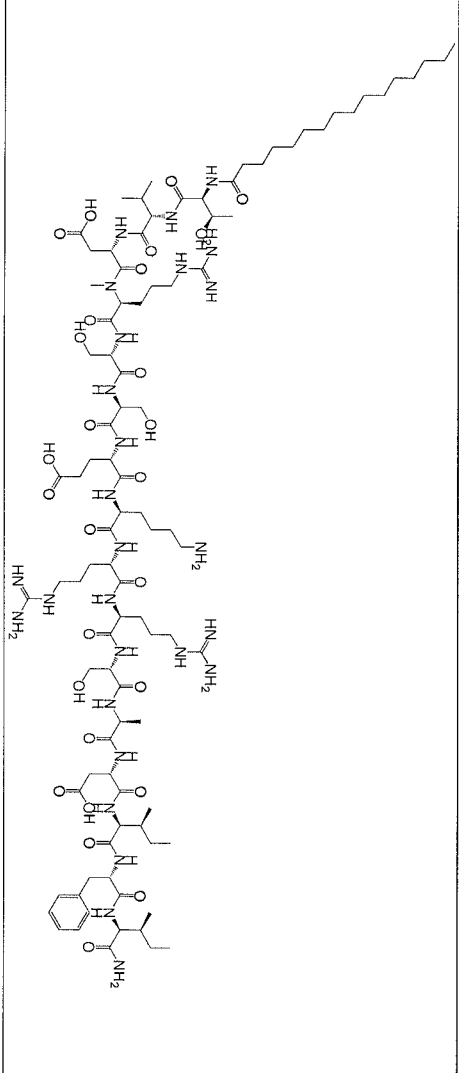
Cmpd ID No.	SEQ ID No.	Sequence	Structure
28	29	TVFRSSEPKRRSADIFP	 <p>The structure shows the peptide TVFRSSEPKRRSADIFP with a long C-terminal alkyl chain. The amino acid side chains are: Valine (V), Phenylalanine (F), Arginine (R), Serine (S), Serine (S), Glutamic acid (E), Lysine (K), Arginine (R), Arginine (R), Serine (S), Alanine (A), Isoleucine (I), and Phenylalanine (F). The N-terminus is a primary amine, and the C-terminus is a long alkyl chain.</p>
29	30	PTVFRSpSEKRRSADIF	 <p>The structure shows the peptide PTVFRSpSEKRRSADIF with a long C-terminal alkyl chain. The amino acid side chains are: Proline (P), Valine (V), Phenylalanine (F), Arginine (R), Serine (S), Serine (S), Glutamic acid (E), Lysine (K), Arginine (R), Arginine (R), Serine (S), Alanine (A), Isoleucine (I), and Phenylalanine (F). The N-terminus is a primary amine, and the C-terminus is a long alkyl chain.</p>
32	33	TVD(n-Me-R)SSEKRRSADIFI	 <p>The structure shows the peptide TVD(n-Me-R)SSEKRRSADIFI with a long C-terminal alkyl chain. The amino acid side chains are: Valine (V), Aspartic acid (D), Valine (V), Arginine (R), Serine (S), Serine (S), Glutamic acid (E), Lysine (K), Arginine (R), Arginine (R), Serine (S), Alanine (A), Isoleucine (I), and Phenylalanine (F). The N-terminus is a primary amine, and the C-terminus is a long alkyl chain.</p>

FIG. 5C

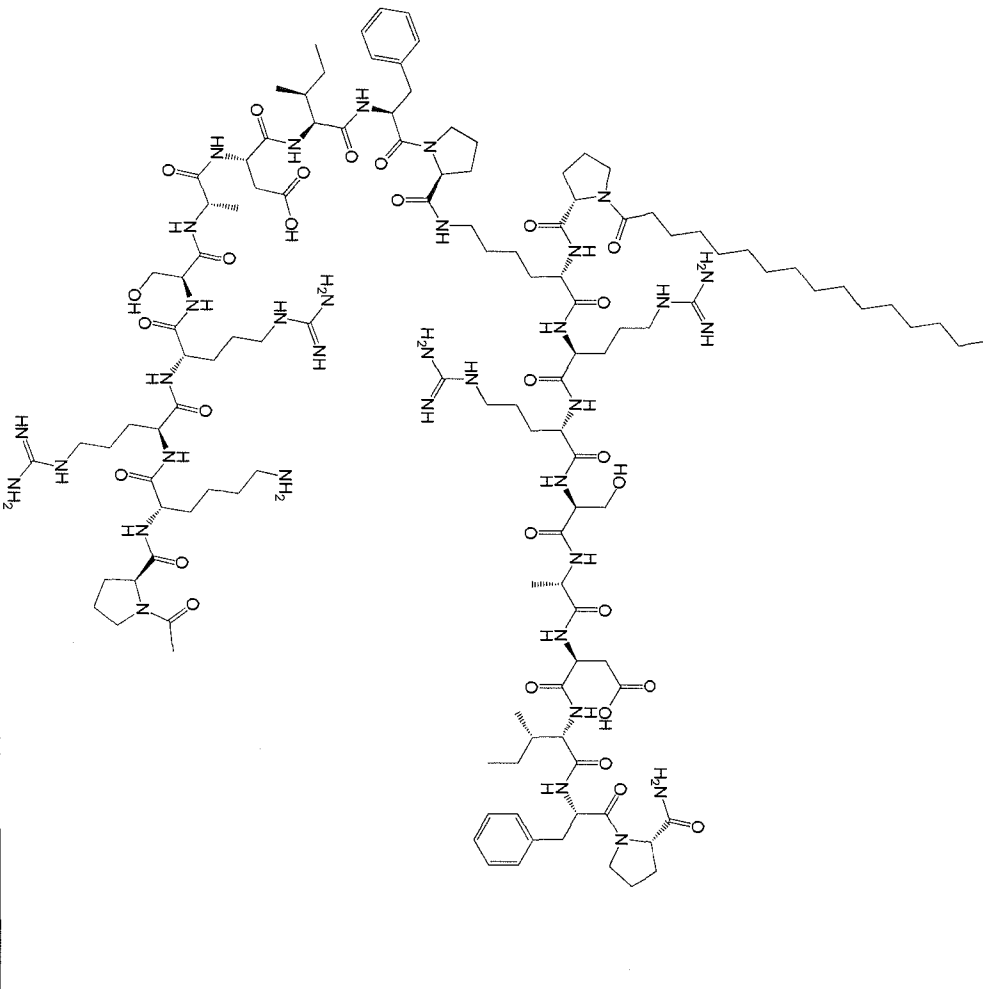
Cmpd ID No.	SEQ ID No.	Sequence	Structure
9	10	PK(PKRRSADIFP)RRSADIFP	

FIG. 5D

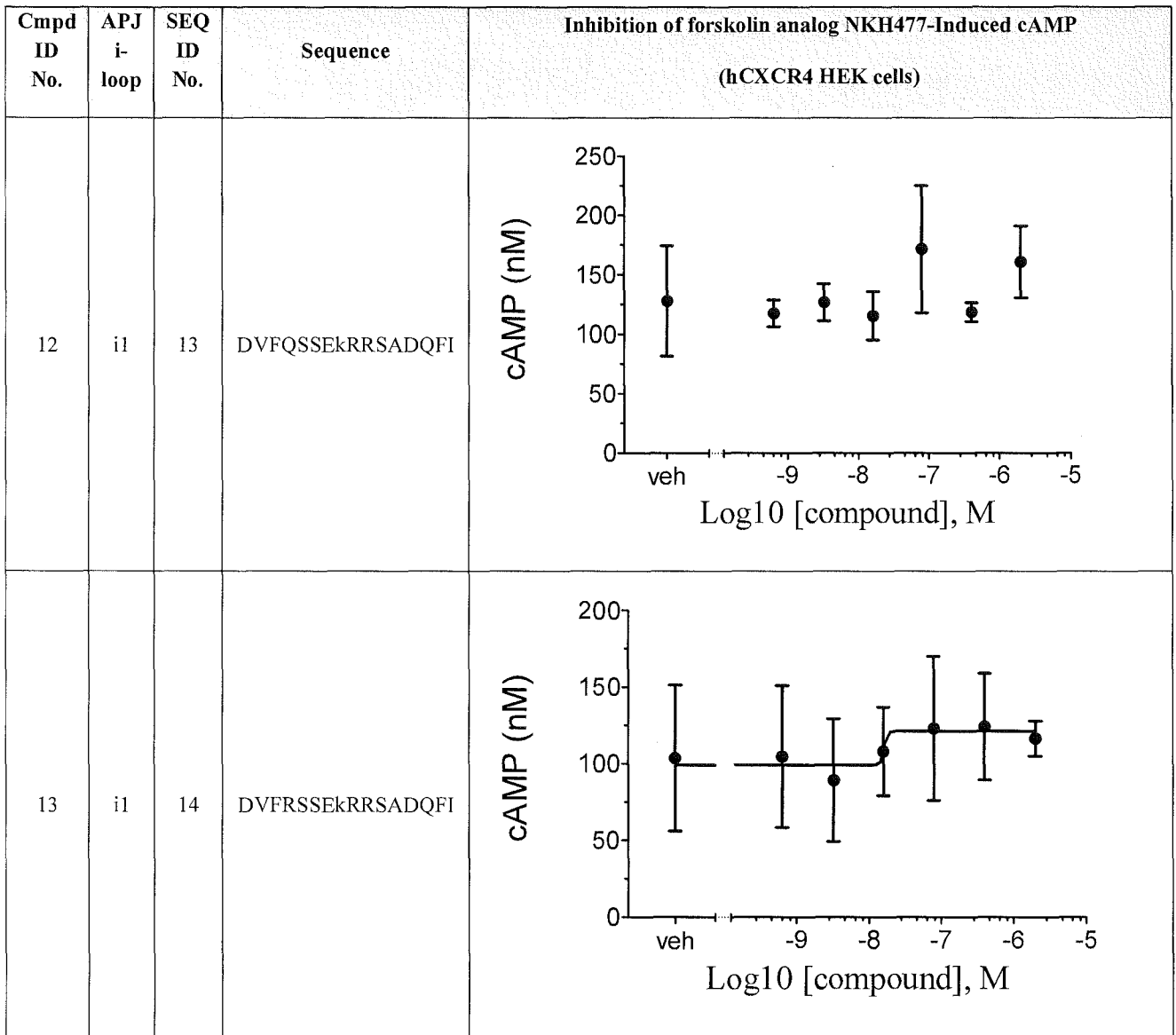


FIG. 6A

Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hCXCR4 HEK cells)														
14	i1	15	TVFRSEkRRSADQFI	<p>Detailed description: This graph shows the effect of compound 14 on forskolin-induced cAMP levels. The y-axis represents cAMP concentration in nM, ranging from 0 to 300. The x-axis represents the concentration of the compound in M on a log10 scale, with values from veh (vehicle) to -5. Data points are plotted with vertical error bars. A horizontal line is drawn across the graph at approximately 120 nM, indicating that the compound does not significantly inhibit the induced cAMP levels.</p> <table border="1"> <caption>Estimated data for Compound 14</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>150</td> </tr> <tr> <td>-9</td> <td>120</td> </tr> <tr> <td>-8</td> <td>80</td> </tr> <tr> <td>-7</td> <td>150</td> </tr> <tr> <td>-6</td> <td>140</td> </tr> <tr> <td>-5</td> <td>130</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	150	-9	120	-8	80	-7	150	-6	140	-5	130
Log10 [compound], M	cAMP (nM)																	
veh	150																	
-9	120																	
-8	80																	
-7	150																	
-6	140																	
-5	130																	
18	i1	19	TVFRSSEkRRSADNFI	<p>Detailed description: This graph shows the effect of compound 18 on forskolin-induced cAMP levels. The y-axis represents cAMP concentration in nM, ranging from 0 to 200. The x-axis represents the concentration of the compound in M on a log10 scale, with values from veh (vehicle) to -5. Data points are plotted with vertical error bars. A downward-sloping curve is fitted to the data, indicating that the compound inhibits the induced cAMP levels in a dose-dependent manner.</p> <table border="1"> <caption>Estimated data for Compound 18</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>120</td> </tr> <tr> <td>-9</td> <td>100</td> </tr> <tr> <td>-8</td> <td>80</td> </tr> <tr> <td>-7</td> <td>100</td> </tr> <tr> <td>-6</td> <td>100</td> </tr> <tr> <td>-5</td> <td>40</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	120	-9	100	-8	80	-7	100	-6	100	-5	40
Log10 [compound], M	cAMP (nM)																	
veh	120																	
-9	100																	
-8	80																	
-7	100																	
-6	100																	
-5	40																	

FIG. 6B



Cmpd ID No.	APJ i-loop	SEQ ID No.	Sequence	Inhibition of forskolin analog NKH477-Induced cAMP (hCXCR4 HEK cells)														
28	i1	29	TVFRSSEPKRRSADIFP	<p>The graph displays the effect of the compound on cAMP levels in hCXCR4 HEK cells. The y-axis represents cAMP concentration in nM, ranging from 0 to 250. The x-axis represents the logarithm of the compound concentration in M, ranging from vehicle (veh) to 10<sup>-5</sup> M. The data points show a dose-dependent increase in cAMP levels, with a fitted curve indicating a non-linear relationship. Error bars represent standard deviation.</p> <table border="1"> <caption>Estimated data from FIG. 6C</caption> <thead> <tr> <th>Log10 [compound], M</th> <th>cAMP (nM)</th> </tr> </thead> <tbody> <tr> <td>veh</td> <td>~75</td> </tr> <tr> <td>-9</td> <td>~95</td> </tr> <tr> <td>-8</td> <td>~90</td> </tr> <tr> <td>-7</td> <td>~105</td> </tr> <tr> <td>-6</td> <td>~125</td> </tr> <tr> <td>-5.5</td> <td>~130</td> </tr> </tbody> </table>	Log10 [compound], M	cAMP (nM)	veh	~75	-9	~95	-8	~90	-7	~105	-6	~125	-5.5	~130
Log10 [compound], M	cAMP (nM)																	
veh	~75																	
-9	~95																	
-8	~90																	
-7	~105																	
-6	~125																	
-5.5	~130																	

FIG. 6C

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/047232

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. (means)
    - on paper
    - in electronic form
  - b. (time)
    - in the international application as filed
    - together with the international application in electronic form
    - subsequently to this Authority for the purpose of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2014/047232

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K38/00 C07K7/08  
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
A61K C07K

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

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

EPO-Internal, WPI Data, BIOSIS, Sequence Search, EMBASE, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2013/106437 A1 (ANCHOR THERAPEUTICS INC [US]) 18 July 2013 (2013-07-18) the whole document	1-39
X	WO 2010/053545 A2 (ANCHOR THERAPEUTICS INC [US]; JANZ JAY [US]; KULIOPULOS ATHAN [US]; MC) 14 May 2010 (2010-05-14) Abstract, p. 27 "Compounds (T-L-P)"; compounds 1-108; p. 79 "Pharmaceutical compositions"; p. 86 "Methods of treatment"; claims	1-26, 34-39

Further documents are listed in the continuation of Box C.

See patent family annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search  18 November 2014	Date of mailing of the international search report  01/12/2014
---	--

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  López García, F
--	---

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/047232

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013106437 A1	18-07-2013	AU 2013208109 A1	14-08-2014
		CA 2860740 A1	18-07-2013
		EP 2802596 A1	19-11-2014
		WO 2013106437 A1	18-07-2013
-----			
WO 2010053545 A2	14-05-2010	AU 2009311640 A1	14-05-2010
		CA 2742528 A1	14-05-2010
		CN 102203117 A	28-09-2011
		CN 103396474 A	20-11-2013
		EP 2362878 A2	07-09-2011
		JP 2012507523 A	29-03-2012
		KR 20110091702 A	12-08-2011
		NZ 592738 A	25-01-2013
		RU 2011122482 A	20-12-2012
		US 2012028888 A1	02-02-2012
WO 2010053545 A2	14-05-2010		
-----			