PEPTIDOMIMETICS WITH GLUCAGON ANTAGONISTIC AND GLP-1 AGONISTIC ACTIVITIES

Field of Invention

The present invention relates to novel compounds of general formula (I), their tautomeric forms, their pharmaceutically acceptable salts and pharmaceutical compositions containing them.

$$A\text{-}Z_1\text{-}Z_2\text{-}Z_3\text{-}Z_4\text{-}Z_5\text{-}Z_6\text{-}Z_7\text{-}Z_8\text{-}Z_9\text{-}Z_{10}\text{-}Z_{11}\text{-}B \quad (I)$$

The present invention also relates to a process for preparing compounds of general formula (I), their tautomeric forms, their pharmaceutically acceptable salts and pharmaceutical compositions containing them.

Background to the invention

Diabetes is characterized by impaired insulin secretion from pancreatic β-cells, insulin resistance or both (Cavaghan, M.K., et al., J. Clin. Invest. 2000, 106, 329). Majority of type 2 diabetic patients can be treated with agents that reduces hepatic glucose production (glucagon antagonist), reduce glucose absorption form GIT, stimulate β-cell function (insulin secretagogues) or with agents that enhance the tissue sensitivity of the patients towards insulin (insulin sensitizes). The drugs presently used to treat type 2 diabetes include α-glucosidase inhibitors, insulin sensitizers, insulin secretagogues and K_{ATP} channel blocker (Chehade, J. M., et al., Drugs, 2000, 60, 95). However, almost one-half of type 2 diabetic subjects lose their response to these agents, over a period of time and thereby require insulin therapy. Insulin treatment has several drawbacks, it is injectable, produces hypoglycemia and causes weight gain (Burge, M.R., Diabetes Obes. Metab., 1999, 1, 199).

Problems with the current treatment necessitate new therapies to treat type 2 diabetes. In this regard, glucagon-like peptide-1 (GLP-1) agonist, which promote glucose-dependent insulin secretion in the pancreas and glucagon receptor antagonist, which inhibit hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis, were found to be therapeutically potential. Thus GLP-1 agonist and glucagon antagonist together were found to reduce the circulating glucose levels and represent useful therapeutic agents for the treatment and prevention of type 2 diabetes (Perry, T.A., et al., Trends Pharmacol. Sci., 2003, 24, 377).

Glucagon and GLP-1 are members of structurally related peptide hormone family (secretin family). Glucagon and GLP-1 constitute a highly homologous set of peptides because these two hormones originate from a common precursor, preproglucagon, which upon tissue-specific processing leads to production of GLP-1 predominantly in the intestine and glucagon in the pancreas (Jiang, G., et al., Am. J. Physiol. Endocrinol. Metab., 2003, 284, E671-678). The receptors for these two peptides are homologous (58 % identity) and belong to the class B family of G-protein coupled receptors (GPCRs). Class-B GPCRs is also called as the secretin receptor family, which consist of 15 peptide-binding receptors in humans. GPCR receptors comprise an extracellular N-terminal domain of 100-160 residues, connected to a juxtamembrane domain (J-domain) of seven membrane-spanning α-helices with intervening loops and a C-terminal tail (Brubaker, P. L., et al., Receptors Channels, 2002, 8, 179). Class B GPCRs are activated by endogenous peptide ligands of intermediate size, typically 30-40 amino acids (Hoare, S.R.J., Drug. Discovery Today, 2005, 10, 423; Gether, U., Endocrine Reviews, 2000, 21, 90).

Glucagon is a 29-amino acid peptide hormone processed from proglucagon in pancreatic α-cells by PC2. Glucagon acts via a seven transmembrane GPCRs, consisting of 485 amino acids. Glucagon is released into the bloodstream when circulating glucose is low. The main physiological role of glucagon is to stimulate hepatic glucose output, thereby leading to increase in glycemia (Tan, K., et al., Diabetologia, 1985, 28, 435). Glucagon provides the major counterregulatory mechanism for insulin in maintaining glucose homeostasis *in vivo*. Glucagon and its receptor represent potential targets for the treatment of diabetes. Antagonising glucagon action by blocking the action of the secreted glucagon at glucagon receptor (glucagon antagonist) or by inhibiting (suppressing) the glucagon production itself represents a new avenue for intervention of diabetes and metabolic disorders (Unson, C.G., et al., Peptides, 1989, 10, 1171; Parker, J. C., Diabetes, 2000, 49, 2079; Johnson, D. G., Science, 1982, 215, 1115; Ahn, J. M., JMC, 2001, 44(9), 1372-1379).

The GLP-1 (7-36) amide is a product of the preproglucagon gene, which is secreted from intestinal L-cells, in response to the ingestion of food. The physiological action of GLP-1 has gained considerable interest. GLP-1 exerts multiple action by stimulating insulin secretion from pancreatic β-cells, in a glucose dependent manner (insulinotropic action). GLP-1 lowers circulating plasma glucagon concentration, by

inhibiting its secretion (production) from α-cells (Drucker D. J., Endocrinology, 2001, 142, 521-527). GLP-1 also exhibits properties like stimulation of β-cell growth, appetite suppression, delayed gastric emptying and stimulation of insulin sensitivity (Nauck, M.A., Horm. Metab. Res., 2004, 36, 852). Currently, various analogs of GLP-1 and EX-4, such as Liraglutide / NN2211 (Novo Nordisk; Phase-III; WO 1998 008871), BIM 51077 (Ipsen; Phase-II; WO 2000 034331), CJC-1131 (ConjuChem; Phase-II; WO 2000 069911) and ZP-10 (Zealand and Aventis; Phase-II; WO 2001 004156) are in different stages of clinical development (Nauck M.A., Regulatory Peptides, 2004, 115, 13). Recently, BYETTA® (Exendin-4, AC 2933; US 5424286), has been launched in the US market (Amylin and Lilly). However, all the existing GLP-1 agonists are delivered by the parenteral route of administration, so the patient incompliance is major problem with the existing GLP-1 based therapy.

The effector system of glucagon and GLP-1 receptors is the Adenylyl Cyclase (AC) enzyme. Interaction of glucagon or GLP-1 agonist with glucagon or GLP-1 receptors (GLP-1 R) respectively causes activation of AC, which converts ATP to cAMP. Increase in the intracellular cAMP level raises the ratio of ADP/ATP, thereby initiating the cell depolarization (due to closure of K_{ATP} channel). Increase in the intracellular cAMP level also activates Protein Kinase (PK-A and PK-C), which raises the cystolic Ca^{2+} concentration, by opening of L-type of Ca^{2+} channel. An increase in the intracellular Ca^{2+} leads to exocytosis of insulin, in pancreatic β-cells and glucagon peptide in α-cells (Fehmann, H.C., Endocr. Rev., 1995, 16, 390).

GLP-1 and glucagon sequences alignment shown below represent the primary structural relationships:

Glucagon: NH_2-^{1}**HSQGTFTSD**^{9}YSKYLDSRRAQDFVQWLMNT^{29}-$CONH_2$ **(Seq. ID No: 1)**

GLP-1(7-36): NH_2-^{1}**HAEGTFTSD**^{9}VSSYLEGQAAKEFIAWLVKGR^{30}-$CONH_2$ **(Seq. ID No: 2)**

First N-terminal 1-9 residues of GLP-1 peptide, with C-terminal amide: $NH_3^{(+)}$-^{1}**HAE**$^{(-)}$**GTFTSD**$^{9(-)}$-$CONH_2$ **(Seq. ID No: 3)**: Net charge Negative

First N-terminal 1-9 residues of Glucagon peptide, with C-terminal amide: $NH_3^{(+)}$-^{1}**HSQGTFTSD**$^{9(-)}$-$CONH_2$ **(Seq. ID No: 4)**: Net charge Neutral

Single-letter abbreviations for amino acids can be found in Zubay, G., Biochemistry 2nd ed., 1988, MacMillan Publishing, New York, p. 33.

Native or synthetic GLP-1 peptides are rapidly metabolized by the proteolytic enzymes, such as dipeptidyl peptidase-IV (DPP-IV) into an inactive metabolite, thereby limiting the use of GLP-1 as a drug (Deacon, C. F., Regulatory Peptides, 2005, 128, 117). Similarly, several nonpeptidyl and peptidyl glucagon receptor antagonist of diverse structures have been reported over recent years, but none of them are in active development or under clinical trials (Kurukulasuriya, R., Expert Opinion Therapeutic Patents, 2005, 15, 1739; Lau, J., J. Med. Chem., 2007, 50, 113; Petersen, K. F. Diabetologia, 2001, 44, 2018; Cascieri, M. A., JBC, 1999, 274, 8694). It is believed that identifying nonpeptide ligands (especially agonist) for class B GPCRs is the principle bottleneck in drug discovery. HTS has apparently yielded few hits (US 2005/6927214; WO 2000/042026; US 2007/0043093), however, screening of those hits against corresponding receptors, especially under *in vivo* condition (animal models) prone to be false negatives (Murphy, K.G., PNAS, 2007, 104, 689).

Glucagon and GLP-1 both play major roles in overall glucose homeostasis (Drucker, D. J., J. Clin. Invest., 2007, 117, 24; Bollyky, J., J. Clin. Endocrinol. Metab., 2007, 92, 2879). Glucagon increases plasma glucose concentrations by stimulating gluconeogenesis and glycogenolysis in the liver while GLP-1 lowers plasma glucose concentrations mediated by glucose dependent insulin secretion (Mojsov, S., et al., JBC., 1990, 265, 8001). Knowing the importance of both glucagon peptide and GLP-1 in maintaining normal blood glucose concentrations, in the recent years, there has been considerable interest in identifying a single ligand, which act as glucagon receptor antagonists and GLP-1 receptor agonists (Claus, T. H., J. Endocrinology, 2007, 192, 371; Pan C.Q., JBC, 2006, 281, 12506).

Although identification of potent nonpeptide GLP-1 agonist may be difficult (Chen, D., PNAS, 2007, 104, 943; Knudsen, L. B., PNAS, 2007, 104, 937) but the design of a hybrid peptidomimetic acting as both glucagon antagonist and GLP-1 receptor agonist would likely to provide a novel approach for the treatment of type 2 diabetes (Claus, T.H., J. Endocrinology, 2007, 192, 371). Recently, series of chimeric peptides has been reported, which act as both GLP-1 receptor agonist and glucagon receptor antagonist, constructed mainly by combining the N-terminal residues of glucagon peptide (residues 1-26) with last C-terminal 4 residues of GLP-1 peptide (VKGR) (Pan C.Q., et al., US 6864069 B2; Pan C.Q., JBC, 2006, 281, 12506).

Structure-activity relationship (SAR) studies have been reported in the literature to determine the role of individual amino acids in both the glucagon and GLP-1

sequences (Runge, S., JBC, 2003, 278, 28005; Mann, R., Biochem. Soc. Trans., 2007, 35, 713). Glucagon and GLP-1 have no defined structure in aqueous solution, but in the presence of micelles or in the membrane mimetic environment, they adopt an alpha-helical structure in the mid-section, with flexible N- and C-terminal regions (Thornton, K., Biochemistry, 1994, 33, 3532; Neidigh, J. W., Biochemistry, 2001, 40, 13188). This suggests that the helical structure is required for binding of peptide ligands to their respective receptors. Mutations or deletion of amino acids in the N-terminal region of both the peptides results in receptor antagonists or inactive compounds, suggesting the importance of the N-terminus for receptor activation by both the glucagon and GLP-1 peptides (Hjorth, S.A., JBC., 1994, 269, 30121; Green, B. D., J. Mol. Endocrinology, 2003, 31, 529). *In vivo*, GLP-1 gets rapidly degraded by dipeptidyl-peptidase IV (DPP IV), a protease responsible for cleaving peptides containing proline or alanine residues in the penultimate N-terminal position, resulting in the inactive metabolites. Substitution of the DPP-IV susceptible sites, such as substitution of Ala at 2^{nd} position of GLP-1 peptide with D-Ala, Aib or Hfl (hexafluoroleucine) greatly improves plasma stability (Deacon, C. F., Diabetes, 1998, 47, 764; Meng, H., J. Med. Chem., 2008, 51, 7303-7307).

In the present investigation, we found that coupling of N-terminal sequence of glucagon peptide (first 1-9 residues, **Seq. ID. No. 4**) with a dipeptide of two unnatural amino acids resulted in the identification of novel class of peptidomimetics having both the glucagon antagonistic and GLP-1 agonistic activities, at varying degree of selectivity. To enhance the duration of action and stability against DPP-IV enzyme, we have site-specifically modified the hybrid peptidomimetics selectively at position Z_2 with unnatural amino acids such as D-Ala, Aib, α-methyl proline (α-Me-Pro), 1-amino-cyclopentanecarboxylic acid (APP) and 1-amino-cyclopropane carboxylic acid (ACP) and succeeded in identifying short peptidomimetics. Some of the peptidomimetics showed efficacy even by oral route of administration, while retaining both the glucagon antagonistic and GLP-1 agonistic activities.

Prior art and design strategy

A series of N-terminally modified GLP-1 modulators have been reported with general formula Xaa1-Xaa11, wherein Xaa1-Xaa9 represent the first 1-9 residues of GLP-1 peptide (HAEGTFTSD; **Seq. ID No. 3**), with some analogs wherein Xaa2 represents either Ala or are optionally replaced with Aib, Xaa3 represents amino acids with carboxylic acid side chain such as glutamic acid and aspartic acid, but not the Gln

(Q), which is conserved in N-terminal sequence of Glucagon peptide (HSQGTFTSD, **Seq. ID No. 4**). Xaa6 represents Phe or are optionally replaced with -α-Me-2F-Phe-, Xaa9 represent amino acids with carboxylic acid or amide side chains such as aspartic acid, glutamic acid, asparagine etc., Xaa10 represent substituted or unsubstituted biphenyl alanine (Bip) derivatives, and Xaa11 represent substituted or unsubstituted biphenyl alanine (Bip) derivatives or substituted or unsubstituted 2-amino-5-phenyl-pentanoic acid (APPA) derivatives (WO 2003/ 033671A2; US 2004/ 0127423 A1; WO 2004/ 094461 A2; US 2006 / 0004222 A1; WO 2006/ 014287 A1; WO 2006/127948 A2; WO 2007/082264 A2; US 2007/0021346 A1; US2007/0099835; US 2007/0238669A1; WO 2007/140284 A2; US 2006/7145040B2; US 2007/ 7238671 B2; US 2007/ 7238670 B2; US 2007/ 0287670 A1; US 2008/ 0045461 A1).

Earlier, a series of novel 11 amino acid peptidomimetics, mainly consist of N-terminal sequence of glucagon peptide (first 1-9 residues, **Seq. ID. No. 4**) as an activation component, covalently linked with a dipeptide of two unnatural amino acids (substituted or unsubstituted biphenyl alanine (Bip) derivatives), as binding component, which primarily showed glucose dependent insulin secretion has been reported. Furthermore, these peptidomimetics showed glucagon receptor antagonistic activity, along with the GLP-1 receptor agonistic activity (WO 2008/ 062457 A2).

In the present invention, novel peptidomimetics of formula (I) (hereinafter referred to as peptidomimetics) are reported. In this invention, instead of biphenyl alanine (Bip) based dipeptide binding component, we used substituted or unsubstituted Bip(OMe)-APPA based dipeptide derivatives as binding components. Surprisingly, instead of first 9 residues of N-terminal sequence of GLP-1 peptide (HAEGTFTSD; **Seq. ID No. 3**), when we attached this dipeptide to first 9 residues of N-terminal sequence of Glucagon peptide (^{1}HSQGTFTSD^{9}; **Seq. ID No: 4**), we found that this peptidomimetic (NH_3^+-HSQGTFTSD-Bip(OMe)-(APPA)-$CONH_2$; **Seq. ID No. 5**) primarily showed glucagon receptor antagonistic activity, along with the GLP-1 receptor agonistic activity, **Figure 1**.

Novel peptidomimetics of formula (I) primarily act as a glucagon receptor antagonist and also exhibit GLP-1R agonistic effects. Different peptidomimetics reported in this invention showed significant glucose dependent insulin secretion (*in vitro*) and reduce circulating glucose levels (*in vivo*), with different level of affinity/selectivity towards glucagon and GLP-1 receptors. Furthermore, in comparison with the previous peptidomemtics reported (for e.g. as in WO 2008/ 062457 A2), these

peptidomimetics, with substituted or unsubstituted Bip(OMe)-APPA based dipeptide derivatives as binding components showed increased stability to proteolytic cleavage, especially against DPP-IV, gastric and intestinal enzymes. Therefore, several of the peptidomimetics were found to be stable against GIT enzymes and acidic pH of stomach, with improved oral bioavailability, making them suitable candidate for the treatment / mitigation / prophylaxis of both type 1 and type 2 diabetes, metabolic disorders and related disorders.

Summary of the invention

The present invention describes a group of novel peptidomimetics that function both as an antagonist of the glucagon receptor and agonist of the GLP-1 receptor, having different degree of affinity/selectivity towards both the receptors and useful for reducing circulating glucose levels and for the treatment of diabetes. These peptidomimetics are defined by the general formula (I) as given below. The peptidomimetics of the present invention are useful in the treatment of the human or animal body, by regulation of insulin and glucagon action. Due to the incorporation of new and metabolically stable binding component, the peptidomimetics of present invention showed improved oral bioavailability and are therefore found to be suitable for the treatment/mitigation/regulation or prophylaxis of type 1 and type 2 diabetes and associated metabolic disorders.

Preferred embodiments

A preferred embodiment of the present invention is to provide novel peptidomimetics of general formula (I), their tautomeric forms, novel intermediates involved in their synthesis, their pharmaceutically acceptable salts, their pharmaceutically acceptable solvates and pharmaceutical compositions containing them or their mixtures, suitable for the treatment treatment/mitigation/regulation of diabetes.

In another preferred embodiment is provided a process for the preparation of novel peptidomimetics of general formula (I), their tautomeric forms, their pharmaceutically acceptable salts, pharmaceutically acceptable solvates and pharmaceutical compositions containing them.

In a further preferred embodiment, is provided pharmaceutical compositions containing peptidomimetics of general formula (I), their tautomeric forms, their pharmaceutically acceptable salts, solvates and their mixtures having pharmaceutically acceptable carriers, solvents, diluents, excipients and other media normally employed in their manufacture.

In a still further preferred embodiment is provided the use of the novel peptidomimetics of the present invention as antidiabetic agents, by administering a therapeutically effective and non-toxic amount of the peptidomimetics of formula (I), or their pharmaceutically acceptable compositions to the mammals those are in need of such treatment.

Abbreviations used

The following abbreviations are employed in the examples and elsewhere herein:

Aib = α-Aminoisobutyric acid,

ACN = Acetonitrile,

APPA = 2-Amino-5-phenylpentanoic acid,

ACPP= 2-Amino-5-(3-chlorophenyl)pentanoic acid,

ADMP= 2-Amino-5-(3,5-dimethylphenyl)pentanoic acid,

AMCB= 2-Amino-5-(4-chloro-2-methylphenyl)pentanoic acid,

2F-APPA= 2-Amino-5-(2-flurophenyl)pentanoic acid,

2,4-diF-APPA= 2-Amino-5-(2,4-diflurophenyl)pentanoic acid,

$2CF_3$-APPA= 2-Amino-5-(2-(trifluoromethyl)phenyl)pentanoic acid,

$2CF_3$,4F-APPA= 2-Amino-5-(4-fluro-2-(trifluoromethyl)phenyl)pentanoic acid,

2F, $4CF_3$-APPA= 2-Amino-5-(2-fluoro-4-(trifluoromethyl)phenyl)pentanoic acid,

2Cl-APPA= 2-Amino-5-(2-chlorophenyl)pentanoic acid,

2Cl, 4OMe-APPA= 2-Amino-5-(2-chloro-4-methoxyphenyl)pentanoic acid,

Bip = Biphenylalanine,

Bip(OMe)= 2'-ethyl-4'-methoxy-biphenylalanine,

α-Me-Bip(OMe)= α-methylated Bip(OMe),

N(Me)-Bip(OMe)= N-methylated Bip(OMe),

Bn = Benzyl,

Boc = tert-Butoxycarbonyl,

Bu^t= O-tert-butyl group,

cAMP= Adenosine 3',5'-cyclic monophosphate,

DCM = Dichloromethane,

DMF = N,N-Dimethylformamide,

DIPCDI= Di-isopropylcarbodiimide,

DIPEA= Diisopropylethylamine,

Et = Ethyl,

Et_2O = Diethyl ether,

Fmoc = Fluorenylmethoxycarbonyl,

g = Gram (s),

GLP-1R = Glucagon Like Peptide-1 Receptor,

Glucagon R= Glucagon receptor,

h = Hour (s),

Hfl = 5,5,5,5',5',5'-2S-hexafluoroleucine,

HOBt = Hydroxybenzotriazole,

HOAt= 7-Aza-hydroxybenzotriazole,

HBTU = 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl aminium hexafluorophosphate,

HPLC = High Performance Liquid Chromatography,

i.p.= intraperitonial,

L = Liter,

LC /MS = Liquid Chromatography / Mass Spectrometry,

Me = Methyl,

Min = minute (s),

mL = milliliter,

µl = microliter,

mg = milligram (s),

mmol = millimole (s),

MS= Mass Spectrometry,

PyBOP = Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate,

SPPS = Solid Phase Peptide Synthesis,

sc = subcutaneous,

TMS = Trimethylsilyl,

TIPS = Triisopropylsilane,

TFA = Trifluoroacetic acid,

TBTU= 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate,

Trt= Trityl group,

Brief description of the drawings

Figure 1 illustrates *in vitro* human Glucagon receptor antagonistic activity and GLP-1 receptor agonistic activity with **Seq. ID. 5**.

Figure 2 illustrates examples of orthogonally protected amino acids used in Fmoc based-solid phase peptide synthesis (SPPS) of peptidomimetics.

Figure 3 illustrates *in vitro* DRC and EC_{50} determination of Exendin (Figure A) and **Seq. ID No. 38** (Figure B), in H GLP-1 R assay.

Figure 4 illustrates *in vivo* glucose reduction in C57 mice, with **Seq. ID No. 38**, after intraperitonial (i.p) administration.

Figure 5 illustrates *in vivo* glucose reduction in C57 mice, with **Seq. ID No. 38**, after oral (p.o) administration.

Figure 6 illustrates *in vivo* glucose reduction in db/db mice, with **Seq. ID No. 38**, after oral (p.o) administration.

Figure 7 illustrates the serum insulin levels after single oral administration of vehicles / test peptidomimetics (**Seq. ID. No. 10, 20 and 25**), in C57BL/6J mice (*in vivo*).

Detailed description of the invention

In accordance with the present invention, synthetic peptidomimetics having the structural formula (I), which showed glucose dependent insulin secretion. Furthermore, it was found that these peptidomimetics showed glucagon receptor antagonistic activity, along with the GLP-1 receptor agonistic activity. These dual acting peptidomimetics exhibit increased stability to proteolytic cleavage, especially against DPP-IV (Dipeptidyl peptidase-IV) enzyme. Most of peptidomimetics were found to be stable in rat plasma upto 24 hours (*in vitro*), showed increased stability against GIT enzymes such as pepsin and acidic stomach pH and also against liver microsomes (*in vitro*). Due to increased metabolic stability, some of these peptidomimetics can also be delivered by oral routes of administration, for the treatment or prevention of diabetes and related metabolic disorders.

$$A\text{-}Z_1\text{-}Z_2\text{-}Z_3\text{-}Z_4\text{-}Z_5\text{-}Z_6\text{-}Z_7\text{-}Z_8\text{-}Z_9\text{-}Z_{10}\text{-}Z_{11}\text{-}B$$

(I)

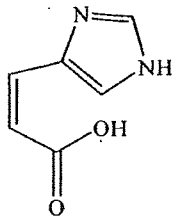
wherein, A represents the groups –NH-R_1, R_3-CO-, R_3-O-CO- or R_3-SO_2-, wherein R_1 represents hydrogen, or optionally substituted linear or branched (C_1-C_{10}) alkyl chain; R_3 is selected from linear or branched (C_1-C_{10}) alkyl, (C_3-C_6) cycloalkyl, aryl, heteroaryl or arylalkyl groups;

In a preferred embodiment, the aryl group is selected from phenyl, diphenyl, napthyl, indanyl, fluorenyl or biphenyl, groups; the heteroaryl group is selected from pyridyl, thienyl, furyl, imidazolyl, benzofuranyl groups;

B represents $-COOR_2$, $-CONHR_2$ or CH_2OR_2, wherein R_2 represents H, optionally substituted groups selected from linear or branched (C_1-C_{10}) alkyl group, aryl or aralkyl groups as defined earlier;

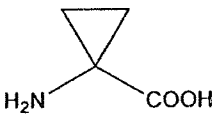
Z_1 to Z_{11} represent natural or unnatural amino acids, linked together by amide bond, unless and otherwise specified.

Z_1 represents L-Histidine (H), D-Histidine (*d*H) or Urocanic acid (UA);

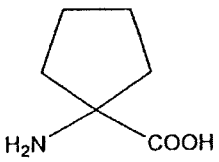


urocanic acid (UA)

Z_2 represents a naturally or unnaturally occurring amino acid selected from the group comprising of L-Serine (S), D-Serine (*d*S), L-Alanine (A), D-Alanine (*d*A), α-methyl proline (α-Me-Pro), α-amino-isobutyric acid (Aib), 1-amino cyclopropane carboxylic acid (ACP), 1-amino-cyclopentanecarboxylic acid (APP);

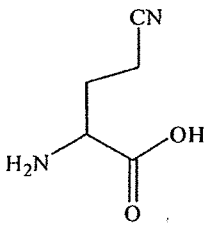


(ACP)

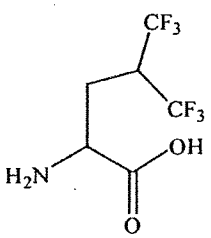


(APP)

Z_3 represents L-Glutamine (Gln; Q), D-Glutamine (*d*Q) or compounds of **formula II** (CNB or Hfl).



2-Amino-4-cyano-butyric acid (CNB)



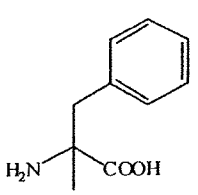
Hexafluoroleucine (Hfl)

Formula II

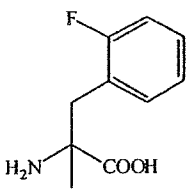
Z_4 represents Glycine (G) or the unnatural amino acids 1-amino cyclopropane carboxylic acid (ACP) or 1-amino-cyclopentanecarboxylic acid (APP);

Z_5 represents a naturally or nonnaturally occurring amino acid comprising a hydroxyl side chain; a preferred Z_5 is L-Threonine (T), D-Threonine (*d*T), L-Allo-threonine (allo-Thr; allo-T), D-Allo-threonine (*d*-allo-Thr; *d*-allo-T);

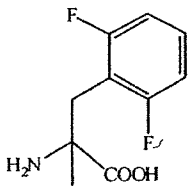
Z_6 represents a naturally or unnaturally occurring amino acid with a disubstituted alpha carbon having two side chains, wherein each of them may independently be an optionally substituted alkyl or aryl or an aralkyl group wherein the substituents may be selected from one or more alkyl groups or one or more halo groups. Preferred Z_6 represents phenylalanine (Phe; F), alpha-methyl-phenylalanine (-α-Me-Phe-), alpha-methyl-2-fluorophenylalanine (-α-Me-2F-Phe-) or alpha-methyl-2,6-diflurophenylalanine (-α-Me-2,6-F-Phe-) or 2-fluorophenylalanine (-2F-Phe-).



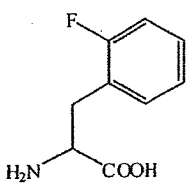
α-Me-Phe



α-Me-2F-Phe



α-Me-2,6-F-Phe

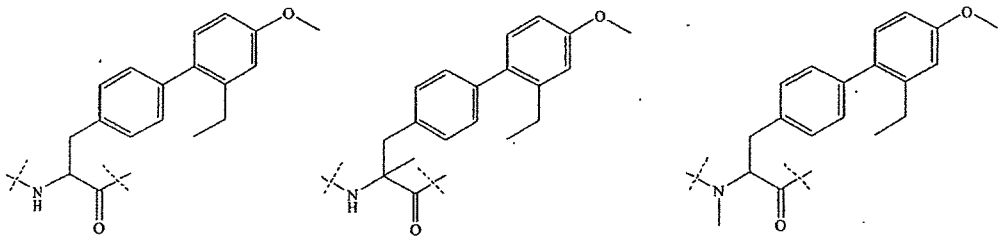


2F-Phe

Z_7 and Z_8 independently represents a naturally or non-naturally occurring amino acid comprising a hydroxyl side chain, preferred Z_7 and Z_8 are independently selected from Threonine, Serine, 1-amino cyclopropane carboxylic acid (ACP), as defined earlier;

Z_9 independently represent a naturally or nonnaturally occurring amino acid having an amino acid side chain comprising an acidic group. Preferred Z_9 is selected from L-Aspartic acid (D), D-Aspartic acid (*d*D) or compounds of **formula II**, as defined earlier.

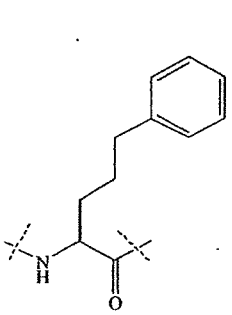
Z_{10} represents L or D unnatural amino acid of **formula III (a-c)**; selected from substituted or unsubstituted 2'-ethyl-4'-methoxy-biphenylalanine (Bip(OMe)) and its derivatives.



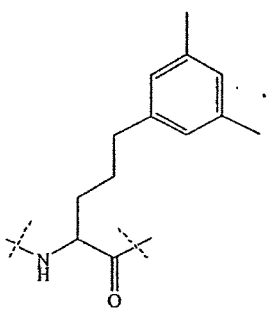
Bip(OMe) IIIa | α-Me-Bip(OMe) IIIb | (NMe)-Bip(OMe) IIIc

Z_{11} represents L or D unnatural amino acid of **formula IV (a-l)**; selected from substituted or unsubstituted 2-Amino-5-phenyl-pentanoic acid (APPA) and its derivatives.

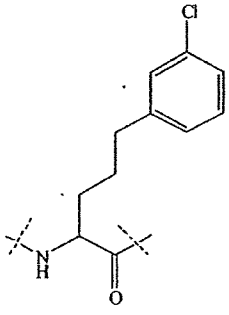
In a further embodiment the amide bond between Z_9 and Z_{10} or Z_{10} and Z_{11} or Z_9-Z_{11} may be N-methylated, represented by abbreviation '(NMe)', such as D-(NMe)-(Bip(OMe)); (Bip(OMe))-(NMe)-APPA; D-(NMe)-(Bip(OMe))-(NMe)-APPA; may be thioamide bond, represented by abbreviation 'C=S', such as D-(C=S)-(Bip(OMe)); Bip(OMe)-(C=S)-(APPA); D-(C=S)-(Bip(OMe))-(C=S)-(APPA) or the thioamide bond between Z_9 and Z_{10} or Z_{10} and Z_{11} or Z_9-Z_{11} may be reduced to '$-CH_2-$' group (desoxopeptide), such as D-(CH_2)-(Bip(OMe)); Bip(OMe)-(CH_2)-(APPA); D-(CH_2)-(Bip(OMe))-(CH_2)-(APPA).



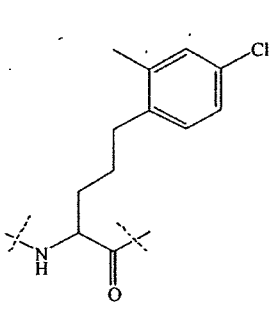
APPA
IVa



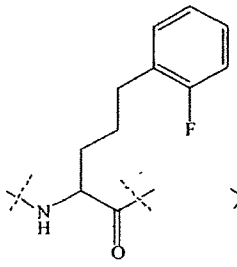
ADMP
IVb



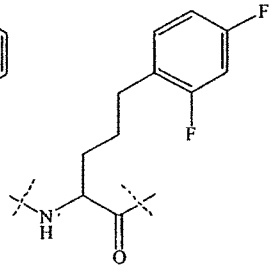
ACPP
IVc



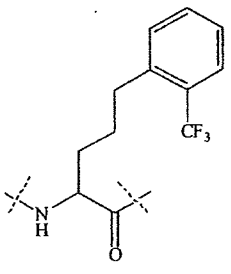
AMCB
IVd



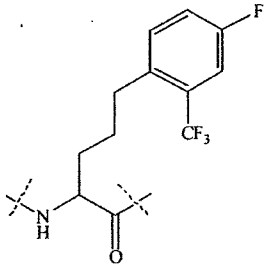
2F-APPA
IVe



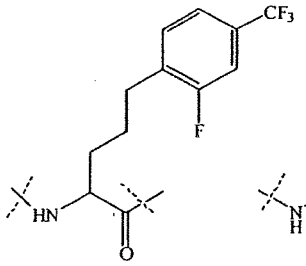
2,4-diF-APPA
IVf



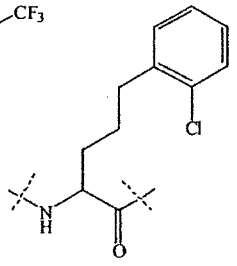
$2CF_3$-APPA
IVg



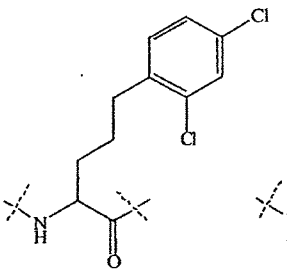
$2CF_3$, 4F-APPA
IVh



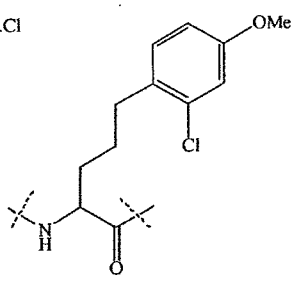
2F, $4CF_3$-APPA
IVi



2Cl-APPA
IVj



2,4-diCl-APPA
IVk



2Cl, 4OMe-APPA
IVl

Definitions:

The term 'natural amino acids' indicates all those twenty amino acids, which are present in nature.

The term 'unnatural amino acids' or 'non-natural amino acids' represents either replacement of L-amino acids with corresponding D-amino acids such as replacement of L-Ala with D-Ala and the like or suitable modifications of the L or D amino acids, amino alkyl acids, either by

- α-alkylation such as substitution of Ala with α-methyl Ala (Aib), replacement of Phe with alpha-methyl-phenylalanine (-α-Me-Phe-), alpha-methyl-2-fluorophenylalanine (-α-Me-2F-Phe-) or alpha-methyl-2,6-diflurophenylalanine (-α-Me-2,6-F-Phe-);
- substitution on the side chain of amino acid such as substitution of aromatic amino acid side chain with halogen, (C_1-C_3)alkyl, aryl groups, more specifically the replacement of Phe with alpha-methyl-2-fluorophenylalanine (-α-Me-2F-Phe-) or alpha-methyl-2,6-diflurophenylalanine (-α-Me-2,6-F-Phe-);
- N-methylation of amino acids, represented by abbreviation '(NMe)', such as (NMe)-Bip(OMe) or (NMe)-APPA;
- Replacement of amide bond with thioamide bond, represented by abbreviation 'C=S', chemically, amide to thioamide modification of dipeptide can be achieved by treatment of protected dipeptide, either in solution phase on solid support, using Lawssen's reagent. Furthermore, thioamide bond between dipeptide can be converted into '–CH_2-' group (desoxopeptide), using nickel boride reduction (Jr. Guziec, F.S., Tetrahedron Letters, 1990, 31(1), 23-26).

The various groups, radicals and substituents used anywhere in the specification are described in the following paragraphs.

The term "alkyl" used herein, either alone or in combination with other radicals, denotes a linear or branched radical containing one to ten carbons, such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *tert*-butyl, amyl, *t*-amyl, *n*-pentyl, *n*-hexyl, *iso*-hexyl, heptyl, octyl, decyl and the like.

The term "cycloalkyl" used herein, either alone or in combination with other radicals, denotes a radical containing three to seven carbons, such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like.

The term "aryl" or "aromatic" used herein, either alone or in combination with other radicals, denotes an aromatic system containing one, two or three rings wherein such rings may be attached together in a pendant manner or may be fused, such as phenyl, naphthyl, tetrahydronaphthyl, indane, biphenyl, and the like.

The term 'arylalkyl' denotes an alkyl group, as defined above, attached to an aryl, such as benzyl, phenylethyl, naphthylmethyl, and the like. The term "aryloxy" denotes an aryl radical, as defined above, attached to an alkoxy group, such as phenoxy, naphthyloxy and the like, which may be substituted.

The term "aralkoxy" denotes an arylalkyl moiety, as defined above, such as benzyloxy, phenethyloxy, naphthylmethyloxy, phenylpropyloxy, and the like, which may be substituted.

The term "heteroaryl" or "heteroaromatic" used herein, either alone or in combination with other radicals, denotes an aromatic system containing one, two or three rings wherein such rings may be attached together in a pendant manner or may be fused containing one or more hetero atoms selected from O, N or S.

The term "heteroaralkyl" used herein, either alone or in combination with other radicals, denotes a heteroaryl group, as defined above, attached to a straight or branched saturated carbon chain containing 1 to 6 carbons. The terms "heteroaryloxy", "heteroaralkoxy", "heterocycloxy denotes heteroaryl, heteroarylalkyl, groups respectively, as defined above, attached to an oxygen atom.

The term "acyl" used herein, either alone or in combination with other radicals, denotes a radical containing one to eight carbons such as formyl, acetyl, propanoyl, butanoyl, *iso*-butanoyl, pentanoyl, hexanoyl, heptanoyl, benzoyl and the like, which may be substituted.

The term "carboxylic acid" used herein, alone or in combination with other radicals, denotes a –COOH group, and includes derivatives of carboxylic acid such as esters and amides. The term "ester" used herein, alone or in combination with other radicals, denotes –COO- group, and includes carboxylic acid derivatives, where the ester moieties are alkoxycarbonyl, such as methoxycarbonyl, ethoxycarbonyl, and the like, which may be substituted.

Unless otherwise indicated, the term 'amino acid' as employed herein alone or as part of another group includes, without limitation, an amino group and a carboxyl group linked to the same carbon, referred to as 'α' carbon.

The absolute 'S' configuration at the 'α' carbon is commonly referred to as the 'L' or natural configuration. The 'R' configuration at the 'α' carbon is commonly referred to as the 'D' amino acid. In the case where both the 'α-substituents' is equal, such as hydrogen or methyl, the amino acids are Gly or Aib and are not chiral. The lower case '*d*' denotes the chirality of D-amino acids. In the specification, whenever any amino acid is described, it includes both the L & D forms, unless otherwise specified. Thus, **Table 2**, provides list of peptidomimetic prepared, in this invention, in which each of the amino acids could be with either 'L' or 'D'.

The term 'receptor antagonist' refers to compounds that inhibit the activation of receptor and generation of secondary messenger such as cyclic AMP either by competitive or non-competitive binding.

The term 'Glucagon receptor antagonist' refers to compounds that inhibit activation of glucagon receptor.

The term 'GLP-1 receptor modulator or agonist' refers to a compound that acts at the GLP-1 receptor to alter its ability to regulate downstream signaling events, such as cAMP production and insulin release. Example of receptor modulators includes agonist, partial agonist, inverse agonist and allosteric potentiators.

In accordance with the present invention, the synthetic isolated peptidomimetics described herein primarily act as a glucagon receptor antagonist. Furthermore, it was found that these peptidomimetics also act as GLP-1 receptor agonists. These synthetic peptidomimetics exhibit desirable *in vitro* glucagon receptor antagonist properties as well as GLP-1 receptor agonist activity in CHO cells transfected with human glucagon or GLP-1 receptor (H Glucagon R or HGLP-1R), in the range of 1- 100 nM concentration. H GLP-1 R agonistic activity is assessed by estimation of amount of cAMP released, while glucagon antagonistic activity was assessed by measuring the amount of cAMP production inhibited by the test peptidomimetics, in presence of glucagon peptide. Novel peptidomimetics exhibit desirable *in vitro* glucagon receptor antagonist activity in CHO cells transfected with human glucagon receptor, in the range of 1- 100 nM concentration. Some of the test peptidomimetics prepared showed glucose dependent insulin release and reduces fasting hyperglycemia, without causing hypoglycemia, when tested *in vivo*, in different diabetic animal models, such as hyperglycemic C57 mice, ob/ob and db/db mice, thus making them ideal therapeutic candidates for the treatment and prevention of type 2 diabetes. These new classes of peptidomimetics can be administered by oral or parenteral routes of administration.

The present invention provides peptidomimetics of formula (I) pharmaceutical compositions employing such peptidomimetics either alone or in combination and for methods of using such peptidomimetics. In particular, the present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a peptidomimetics of formula (I), alone or in combination(s), with a pharmaceutically acceptable carrier.

Further provided is a method for treating or delaying the progression or onset of diabetes, especially type 2 diabetes, including complications of diabetes, including

retinopathy, neuropathy, nephropathy and delayed wound healing and related diseases such as insulin resistance (impaired glucose homeostasis), hyperglycemia, hyperinsulinemia, elevated blood levels of fatty acids or glycerol, hyperlipidemia including hypertriglyceridemia, syndrome X, atherosclerosis and hypertension, wherein a therapeutically effective amount of a peptidomimetics of formula (I) or their combination(s) are administered to a mammal, example, human, a patient in need of treatment.

Preparation of the peptidomimetics:

Several synthetic routes can be employed to prepare the peptidomimetics of the present invention well known to one skilled in the art of peptide synthesis. The peptidomimetics of formula (I), where all symbols are as defined earlier can be synthesized using the methods described below, together with conventional techniques known to those skilled in the art of peptide synthesis, or variations thereon as appreciated by those skilled in the art. Referred methods include, but not limited to those described below.

The peptidomimetics thereof described herein may be produced by chemical synthesis using suitable variations of various solid-phase techniques generally known such as those described in G. Barany and R. B. Merrifield, "The peptides: Analysis, synthesis, Biology"; Volume 2- "Special methods in peptide synthesis, Part A", pp. 3-284, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980; and in J. M. Stewart and J. D. Young, "Solid-phase peptide synthesis" 2nd Ed., Pierce chemical Co., Rockford, Il, 1984.

The preferred strategy for preparing the peptidomimetics of this invention is based on the use of Fmoc-based SPPS approach, wherein Fmoc (9-Fluorenyl-methyl-methyloxycarbonyl) group is used for temporary protection of the α-amino group, in combination with the acid labile protecting groups, such as t-butyloxy carbonyl (Boc), tert-butyl (Bu^t), Trityl (Trt) groups (**Figure 2**), for temporary protection of the amino acid side chains (see for example E. Atherton and R.C. Sheppard, "The Fluorenylmethoxycarbonyl amino protecting group", in "The peptides: Analysis, synthesis, Biology"; Volume 9.- "Special methods in peptide synthesis, Part C", pp. 1-38, S. Undenfriend and J. Meienhofer, Eds., Academic Press, San Diego, 1987).

The peptidomimetics can be synthesized in a stepwise manner on an insoluble polymer support (resin), starting form the C-terminus of the peptide. In an embodiment, the synthesis is initiated by appending the C-terminal amino acid of the peptide to the

resin through formation of an amide, ester or ether linkage. This allows the eventual release of the resulting peptide as a C-terminal amide, carboxylic acid or alcohol, respectively.

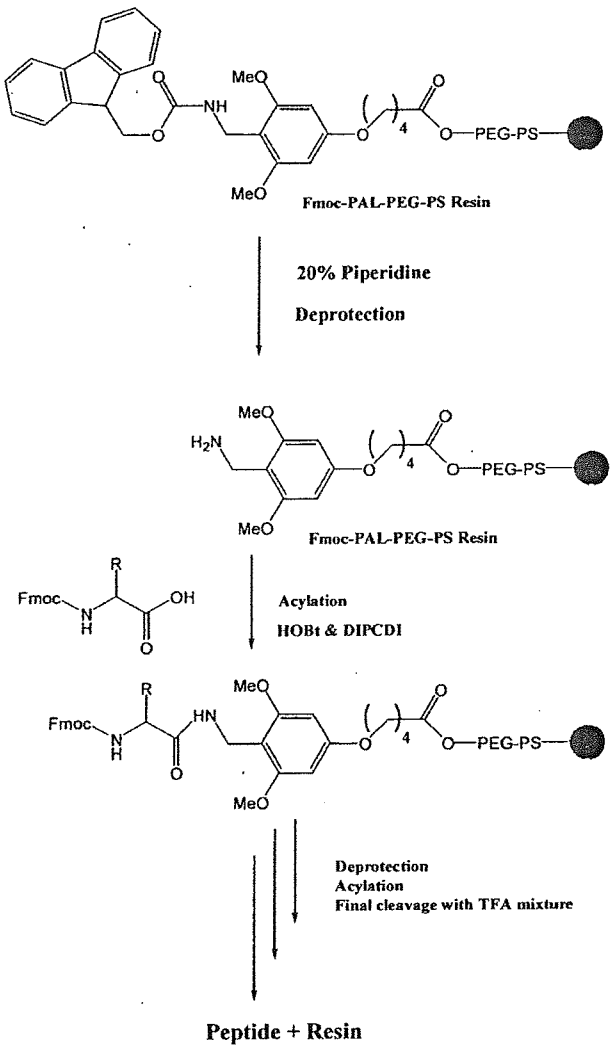
In the Fmoc-based SPPS, the C-terminal amino acid and all other amino acids used in the synthesis are required to have their α-amino groups and side chain functionalities (if present) differentially protected (orthogonal protection), such that the α-amino protecting group may be selectively removed during the synthesis, using suitable base such as 20% piperidine solution, without any premature cleavage of peptide from resin or deprotection of side chain protecting groups, usually protected with the acid labile protecting groups.

The coupling of an amino acid is performed by activation of its carboxyl group as an active ester and reaction thereof with unblocked α-amino group of the N-terminal amino acid appended to the resin. After every coupling and deprotection, peptidyl-resin was washed with the excess of solvents, such as DMF, DCM and diethyl ether. The sequence of α-amino group deprotection and coupling is repeated until the desired peptide sequence is assembled (**Scheme 1**). The peptide is then cleaved from the resin with concomitant deprotection of the side chain functionalities, using an appropriate cleavage mixture, usually in the presence of appropriate scavengers to limit side reactions. The resulting peptide is finally purified by reverse phase HPLC.

The synthesis of the peptidyl-resins required as precursors to the final peptides utilizes commercially available cross-linked polystyrene polymer resins (Novabiochem, San Diego, CA). Preferred for use in this invention are Fmoc-PAL-PEG-PS resin, 4-(2', 4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetyl-*p*-methyl benzhydrylamine resin (Fmoc-Rink amide MBHA resin), 2-chloro-Trityl-chloride resin or *p*-benzyloxybenzyl alcohol resin (HMP resin) to which the C-terminal amino acid may or may not be already attached. If the C-terminal amino acid is not attached, its attachment may be achieved by HOBt active ester of the Fmoc-protected amino acid formed by its reaction with DIPCDI. In case of 2-Chloro-trityl resin, coupling of first Fmoc-protected amino acid was achieved, using DIPEA. For the assembly of next amino acid, N-terminal protection of peptidyl resin was selectively deprotected using a solution of 10-20 % piperidine solution. After every coupling and deprotection, excess of amino acids and coupling reagents were removed by washing with DMF, DCM and ether. Coupling of the subsequent amino acids can be accomplished using HOBt or HOAT active esters

produced from DIPCDI/ HOBt or DIPCDI/HOAT, respectively. In case of some difficult coupling, especially coupling of those amino acids, which are hydrophobic or amino acids with bulky side chain protection, complete coupling can be achieved using a combination of highly efficient coupling agents such as HBTU, PyBOP or TBTU, with additives such as DIPEA.

The synthesis of the peptidomimetics described herein can be carried out by using batchwise or continuous flow peptide synthesis apparatus, such as CS-Bio or AAPPTEC peptide synthesizer, utilizing the Fmoc/t-butyl protection strategy. The non-natural non-commercial amino acids present at different position were incorporated into the peptide chain, using one or more methods known in the art. In one approach, a Fmoc-protected non-natural amino acid was prepared in solution, using appropriate literature procedures. For example, the Fmoc-protected α-methylated amino acids, such as Fmoc-Aib-OH, Fmoc-(α-Me-2F-Phe)-OH, Fmoc-(α-Me-2,6-F-Phe)-OH, were prepared using modified literature procedure (Boesten, W.H.J., et al., Org. Lett., 2001, 3(8), 1121; Kapadia, S. R., et al., JOC, 2001, 66, 1903-1905). Synthesis of N-Fmoc-2-amino-5-phenyl-pentanoic acid (Fmoc-APPA) and its derivatives, as listed in **Formula IV(a-l)**, were carried out using modified literature method (Betshrugge, J. V., Tetrahedron, 1998, 54, 1753-1762; WO 2003/087036). The Fmoc-Bip(OMe)-OH (2'-ethyl-4'-methoxy-biphenylalanine;2-amino-3-(2'-ethyl-4'-methoxy-biphenyl-4-yl)-propionic acid) were prepared by literature method (Kotha, S., et al., Tetrahedron 2002, 58, 9633; US 2006 / 0004222 A1) and the Fmoc-5,5,5,5',5',5'-2S-hexafluoroleucine (Fmoc-(Hfl)-OH) was prepared by reported procedure (Chiu, H. P., Cheng, R. P., Org. Lett., 2007, 9(26), 5517-5520). The resulting derivative was then used in the step-wise synthesis of the peptide. Alternatively, the required non-natural amino acid was built on the resin directly using synthetic organic chemistry procedures and a linear peptide chain were build.



Scheme 1: General Scheme for Fmoc-Based SPPS

The peptide-resin precursors for their respective peptidomimetics may be cleaved and deprotected using suitable variations of any of the standard cleavage procedures described in the literature (King, D. S., et al., Int. J. Peptide Protein Res., 1990, 36, 255). A preferred method for use in this invention is the use of TFA cleavage mixture, in the presence of water and TIPS as scavengers. Typically, the peptidyl-resin was incubated in TFA / Water /TIPS (94:3:3; V: V: V; 10 ml / 100 mg of peptidyl resin) for 1.5-2 hrs at room temperature. The cleaved resin is then filtered off, the TFA solution is concentrated or dried under reduced pressure. The resulting crude peptide is either precipitated or washed with Et_2O or is re-dissolved directly into DMF or 50 % aqueous acetic acid for purification by preparative HPLC.

Peptidomimetics with the desired purity can be obtained by purification using preparative HPLC. The solution of crude peptide is injected into a semi-Prep column

(Luna 10μ; C_{18}; 100 A°), dimension 250 X 50 mm and eluted with a linear gradient of ACN in water, both buffered with 0.1 % TFA, using a flow rate of 15 -50 ml /min with effluent monitoring by PDA detector at 220 nm. The structures of the purified peptidomimetics can be confirmed by Electrospray Mass Spectroscopy (ES-MS) analysis.

All the peptide prepared were isolated as trifluoro-acetate salt, with TFA as a counter ion, after the Prep-HPLC purification. However, some peptides were subjected for desalting, by passing through a suitable ion exchange resin bed, preferably through anion-exchange resin Dowex SBR P(Cl) or an equivalent basic anion-exchange resin. In some cases, TFA counter ions were replaced with acetate ions, by passing through suitable ion-exchange resin, eluted with dilute acetic acid solution. For the preparation of the hydrochloride salt of peptides, in the last stage of the manufacturing, selected peptides, with the acetate salt was treated with 4 M HCl. The resulting solution was filtered through a membrane filter (0.2 μm) and subsequently lyophilized to yield the white to off-white HCl salt. Following similar techniques and/or such suitable modifications, which are well within the scope of persons skilled in the art, other suitable pharmaceutically acceptable salts of the peptidomimetics of the present invention were prepared.

General method of preparation of peptidomimetics, using SPPS approach:

Assembly of peptidomimetics on resin:

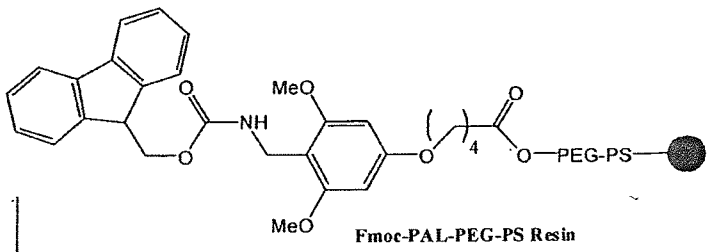
Sufficient quantity (50–100 mg) of Fmoc-PAL-PEG-PS resin or Fmoc-Rink amide MBHA resin, loading: 0.5-0.6 mmol / g was swelled in DMF (10-20 ml /100 mg of resin) for 2-10 minutes. The Fmoc-group on resin was then removed by incubation of resin with 10-20 % piperidine in DMF (10-30 ml / 100 mg of resin), for 10-30 minutes. Deprotected resin was filtered and washed excess of DMF, DCM and ether (50 ml X 4). Washed resin was incubated in freshly distilled DMF (1 ml / 100 mg of resin), under nitrogen atmosphere for 5 minutes. A 0.5 M solution of first Fmoc-protected amino acid (1-3 eq.), pre-activated with HOBt (1-3 eq.) and DIPCDI (1-2 eq.) in DMF was added to the resin and the resin was then shaken for 1-3 hrs, under nitrogen atmosphere. Coupling completion was monitored using a qualitative ninhydrin test. After the coupling of first amino acid, the resin was washed with DMF, DCM and Diethyl ether (50 ml X 4). For the coupling of next amino acid, firstly, the Fmoc-protection on first amino acid, coupled with resin was deprotected, using 20%

piperidine solution, followed by the coupling the Fmoc-protected second amino acid, using a suitable coupling agents, and as described above. The repeated cycles of deprotection, washing, coupling and washing were performed until the desired peptide chain was assembled on resin, as per general **Scheme 1** above.

Finally, the Fmoc-protected peptidyl-resin prepared above was deprotected by 20% piperidine treatment as described above and the peptidyl-resins were washed with DMF, DCM and Diethyl ether (50 ml X 4). Resin containing desired peptide was dried under nitrogen pressure for 10-15 minutes and subjected for cleavage/ deprotection.

Representative example of automated solid phase synthesis of peptide sequence ID. No. 37 (H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-$CONH_2$).

The linear peptide chain, H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-PAL-PEG-PS was assembled on an automated CS-Bio 536 PepSynthesiser™ using Fmoc solid phase peptide synthesis (SPPS) approach (**Scheme 2**). The Fmoc amino acids and the 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumtetrafluoroborate (TBTU) were packed together in vials and positioned in the amino acid module of the synthesizer. A stock solution of diisopropylethylamine (DIPEA; 0.9 M) and DMF were stored in reagent bottles, under dry nitrogen atmosphere. The resin, Fmoc-PAL-PEG-PS (0.38 mmol/g; 1g) was dried over P_2O_5, *in vacuo* (1 hr) and swollen in freshly distilled DMF (5 mL). The swollen resin was slurry packed into a glass column and positioned in the synthesizer. All the synthetic cycles were carried out at a flow rate of 5 mL min^{-1}, **Table 1**. The resin was washed with freshly distilled DMF for 10 minutes. Deprotection of Fmoc group was performed with 20% piperidine in DMF for 10 minutes and the deprotection was monitored by UV detection of the column effluent at 304 nm.



Fmoc-PAL-PEG-PS Resin

1) Piperidine (Fmoc deprotection)
2) Fmoc-APPA-OH (4 eq.); DMF; TBTU (3.9 eq.); DIPEA (8 eq); 2 h
3) Washing with DMF and DCM
4) Repeat step 1-3, with follwoing amino acids:
Fmoc-Bip(OMe)-OH
Fmoc-Asp(Bu^t)-OH
Fmoc-Ser(Bu^t)-OH
Fmoc-Thr(Bu^t)-OH
Fmoc-(α-Me-2F-Phe)-OH
Fmoc-Thr(Bu^t)-OH
Fmoc-Gly-OH
Fmoc-Gln(Trt)-OH
Fmoc-Aib-OH
Fmoc-His(Trt)-OH
5) Piperidine (Fmoc deprotection)
6) TFA cleavage
7) RP-HPLC purification

H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-$CONH_2$
(Seq. ID. No. 37)

Scheme 2: SPPS of Seq. ID. No. 37

Excess piperidine was removed by three auxiliary wash cycles and a distilled DMF wash cycle, with each cycle of 15 minutes. The amino group was treated with Fmoc-amino acid (4 equivalent), preactivated with TBTU (3.9 equivalent) in the presence of DIPEA (8 equivalent) and recycled for 120 minutes. The excess amino acid and soluble by-products were removed from column and loop by four auxiliary wash cycles and distilled DMF wash cycles, with each cycle of 10 minutes. Furthermore, synthetic cycles (deprotection, wash, acylation and wash) were repeated for complete assembly of linear peptide. Final deprotection cycle was performed with 20% piperidine in DMF for 15 minutes to remove the terminal Fmoc group, followed by wash cycle (10 X 4 minutes). Completed peptide-resin was filtered through sintered glass filter, washed three times successively with DMF, DCM, methanol, DMF and diethyl ether (100 mL each). Peptide-resin was dried *in vacuo* over P_2O_5 (2 hr) and stored at -20 ° C. Ninhydrin resin test was carried out to check the *N*-terminal free

amino group of resin bound peptide. Appearance of blue-purple colouration of the solution and the resin beads indicates the presence of free amino group on resin bound peptide and was considered to be a positive test.

Table 1. Automated cycles for solid phase peptide synthesis

Step	Function	Reagent/ Solvent	Number of cycles	Time (Minute)
1	Wash	Dimethylformamide (DMF)	1	10
2	Deprotection	20 % piperidine in DMF	2	15
3	Wash	DMF	3	15
4	Acylation	Amino acid; TBTU and diisopropylethylamine (in DMF)	Recycle	120
5	Wash	Dimethylformamide	4	10

Small-scale cleavage was carried out to assess the purity of resin bound peptide. The dried Peptide-resin (ca 10-mg) was treated with mixture (1 mL) of TFA, water, triisopropylsilane (95: 2.5: 2.5 v/v), for 90 minutes at room temperature with gentle occasional swirling. The resin was filtered, washed thoroughly with neat TFA (1 mL) and the entire filtrate was evaporated under reduced pressure. Residual TFA was azeotroped three times with diethyl ether (2 mL). Residue obtained was suspended in distilled water (2 mL) and the aqueous layer was extracted three times with diethyl ether (3 mL). The aqueous layer was separated and freeze-dried to yield the crude peptide H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-$CONH_2$. The lyophilised peptide H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-$CONH_2$ was dissolved in 0.1% aqueous TFA (ca 1mg /1 mL) and its purity was analyzed by analytical RP-HPLC and characterized by electrospray ionisation mass spectrometry (ESI-MS). Percent purity: 90 % (crude peptide). ESI-MS; Calcd. for H_2N-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)-$CONH_2$: 1465 (M^+), 1487 ($M+Na^+$) and 1503 ($M+K^+$); Found (m/z): 1465 (M^+), 1487 ($M+Na^+$) and 1503 ($M+K^+$).

Using above protocol and suitable variations thereof which are within the scope of a person skilled in the art, the peptidomimetics designed in the present invention were prepared, using Fmoc-SPPS approach. Furthermore, resin bound peptidomimetics were cleaved and deprotected, purified and characterized using following protocol.

Cleavage and deprotection:

The desired peptidomimetics were cleaved and deprotected from their respective peptidyl-resins by treatment with TFA cleavage mixture as follows. A solution of TFA / Water / Triisopropylsilane (95: 2.5: 2.5) (10 ml / 100 mg of peptidyl-resin) was added to peptidyl-resins and the mixture was kept at room temperature with occasional starring. The resin was filtered, washed with a cleavage mixture and the combined filtrate was evaporated to dryness. Residue obtained was dissolved in 10 ml of water and the aqueous layer was extracted 3 times with ether (20 ml each) and finally the aqueous layer was freeze-dried. Crude peptide obtained after freeze-drying was purified by preparative HPLC as follows:

Preparative HPLC purification of the crude peptidomimetics:

Preparative HPLC was carried out on a Shimadzu LC-8A liquid chromatograph. A solution of crude peptide dissolved in DMF or water was injected into a semi-Prep column (Luna 10μ; C_{18}; 100 A^0), dimension 250 X 50 mm and eluted with a linear gradient of ACN in water, both buffered with 0.1 % TFA, using a flow rate of 15 -50 ml / min, with effluent monitoring by PDA detector at 220 nm. A typical gradient of 20 % to 70 % of water-ACN mixture, buffered with 0.1 % TFA was used, over a period of 50 minutes, with 1% gradient change per minute. The desired product eluted were collected in a single 10-20 ml fraction and pure peptidomimetics were obtained as amorphous white powders by lyophilisation of respective HPLC fractions.

HPLC analysis of the purified peptidomimetics

After purification by preparative HPLC as described above, each peptide was analyzed by analytical RP-HPLC on a Shimadzu LC-10AD analytical HPLC system. For analytic HPLC analysis of peptidomimetics, Luna 5μ; C_{18}; 100 A°, dimension 250 X 4.6 mm column was used, with a linear gradient of 0.1% TFA and ACN buffer and the acquisition of chromatogram was carried out at 220 nm, using a PDA detector.

Characterization by Mass Spectrometry

Each peptide was characterized by electrospray ionisation mass spectrometry (ESI-MS), either in flow injection or LC/MS mode. Triple quadrupole mass

spectrometers (API-3000 (MDS-SCIES, Canada) was used in all analyses in positive and negative ion electrospray mode. Full scan data was acquired over the mass range of quadrupole, operated at unit resolution. In all cases, the experimentally measured molecular weight was within 0.5 Daltons of the calculated monoisotopic molecular weight. Quantification of the mass chromatogram was done using Analyst 1.4.1 software.

Utilizing the synthetic methods described herein along with other commonly known techniques and suitable variations thereof, the following novel peptidomimetics were prepared. This list is indicative of the various groups of peptidomimetics, which can be prepared according to the present invention, and are expected to at least include obvious variations of these peptidomimetics. However, such disclosure should not be construed as limiting the scope of the invention in any way. In **Table 2 (i-v)**, novel peptidomimetics of present invention are listed along with their corresponding Seq. ID. Numbers..

Table 2 (i): List of peptidomimetics prepared

Seq. ID. No.	Sequence of peptidomimetics
5	HSQGTFTSD-Bip(OMe)-(APPA)
6	HSQGTFTSD-Bip(OMe)-(ADMP)
7	HSQGTFTSD-Bip(OMe)-(ACPP)
8	HSQGTFTSD-Bip(OMe)-(AMCB)
9	H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(APPA)
10	H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(ADMP)
11	H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(ACPP)
12	H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(AMCB)
13	HAQGTFTSD-Bip(OMe)-(APPA)
14	HAQGTFTSD-Bip(OMe)-(ADMP)
15	HAQGTFTSD-Bip(OMe)-(ACPP)
16	HAQGTFTSD-Bip(OMe)-(AMCB)
17	H-Aib-QGTFTSD-Bip(OMe)-(APPA)
18	H-Aib-QGTFTSD-Bip(OMe)-(ADMP)
19	H-Aib-QGTFTSD-Bip(OMe)-(ACPP)
20	H-Aib-QGTFTSD-Bip(OMe)-(AMCB)
21	H-(ACP)-QGTFTSD-Bip(OMe)-(APPA)
22	H-(ACP)-QGTFTSD-Bip(OMe)-(ADMP)

23	H-(ACP)-QGTFTSD-Bip(OMe)-(ACPP)
24	H-(ACP)-QGTFTSD-Bip(OMe)-(AMCB)
25	H-(APP)-QGTFTSD-Bip(OMe)-(APPA)
26	H-(APP)-QGTFTSD-Bip(OMe)-(ADMP)
27	H-(APP)-QGTFTSD-Bip(OMe)-(ACPP)
28	H-(APP)-QGTFTSD-Bip(OMe)-(AMCB)
29	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(APPA)
30	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(ADMP)
31	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(ACPP)
32	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(AMCB)
33	H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(APPA)
34	H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(ADMP)
35	H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(ACPP)
36	H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(AMCB)
37	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
38	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ADMP)
39	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ACPP)
40	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(AMCB)
41	H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(APPA)
42	H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(ADMP)
43	H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(ACPP)
44	H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(AMCB)

Table 2 (ii): List of peptidomimetics prepared

Seq. ID. No.	Sequence of peptidomimetics
45	HSQGTFTSD-Bip(OMe)-(2F-APPA)
46	HSQGTFTSD-Bip(OMe)-(2,4-diF-APPA)
47	HSQGTFTSD-Bip(OMe)-(2CF_3-APPA)
48	HSQGTFTSD-Bip(OMe)-(2CF_3, 4F-APPA)
49	HSQGTFTSD-Bip(OMe)-(2F,4CF_3-APPA)
50	HSQGTFTSD-Bip(OMe)-(2Cl-APPA)
51	HSQGTFTSD-Bip(OMe)-(2,4-diCl-APPA)
52	HSQGTFTSD-Bip(OMe)-(2Cl, 4OMe-APPA)
53	HAQGTFTSD-Bip(OMe)-(2F-APPA)
54	HAQGTFTSD-Bip(OMe)-(2,4-diF-APPA)

55	HAQGTFTSD-Bip(OMe)-(2CF_3-APPA)
56	HAQGTFTSD-Bip(OMe)-(2CF_3,4F-APPA)
57	HAQGTFTSD-Bip(OMe)-(2F,4CF_3-APPA)
58	HAQGTFTSD-Bip(OMe)-(2Cl-APPA)
59	HAQGTFTSD-Bip(OMe)-(2,4-diCl-APPA)
60	HAQGTFTSD-Bip(OMe)-(2Cl,4OMe-APPA)
61	H-Aib-QGTFTSD-Bip(OMe)-(2F-APPA)
62	H-Aib-QGTFTSD-Bip(OMe)-(2,4-diF-APPA)
63	H-Aib-QGTFTSD-Bip(OMe)-(2CF_3-APPA)
64	H-Aib-QGTFTSD-Bip(OMe)-(2CF_3,4F-APPA)
65	H-Aib-QGTFTSD-Bip(OMe)-(2F,4CF_3-APPA)
66	H-Aib-QGTFTSD-Bip(OMe)-(2Cl-APPA)
67	H-Aib-QGTFTSD-Bip(OMe)-(2,4-diCl-APPA)
68	H-Aib-QGTFTSD-Bip(OMe)-(2Cl,4OMe-APPA)
69	H-(ACP)-QGTFTSD-Bip(OMe)-(2F-APPA)
70	H-(ACP)-QGTFTSD-Bip(OMe)-(2,4-diF-APPA)
71	H-(ACP)-QGTFTSD-Bip(OMe)-(2CF_3-APPA)
72	H-(ACP)-QGTFTSD-Bip(OMe)-(2CF_3,4F-APPA)
73	H-(ACP)-QGTFTSD-Bip(OMe)-(2F,4CF_3-APPA)
74	H-(ACP)-QGTFTSD-Bip(OMe)-(2Cl-APPA)
75	H-(ACP)-QGTFTSD-Bip(OMe)-(2,4-diCl-APPA)
76	H-(ACP)-QGTFTSD-Bip(OMe)-(2Cl, 4OMe-APPA)
77	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2F-APPA)
78	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2,4-diF-APPA)
79	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2CF_3-APPA)
80	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2CF_3,4F-APPA)
81	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2F,4CF_3-APPA)
82	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2Cl-APPA)
83	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2,4-diCl-APPA)
84	H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2Cl,4OMe-APPA)
85	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
86	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2,4-diF-APPA)
87	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2CF_3-APPA)
88	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2CF_3,4F-APPA)
89	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F,4CF_3-APPA)

90	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2Cl-APPA)
91	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2,4-diCl-APPA)
92	H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2Cl,4OMe-APPA)

Table 2 (iii): List of peptidomimetics prepared

Seq.ID. No.	Sequence of peptidomimetics
93	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
94	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
95	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
96	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
97	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
98	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
99	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
100	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
101	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
102	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
103	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
104	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
105	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
106	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
107	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
108	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
109	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
110	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
111	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
112	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
113	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
114	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
115	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
116	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
117	H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
118	H-Aib-(CNB)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
119	H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
120	H-Aib-(CNB)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
121	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(APPA))
122	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(APPA))
123	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(2F-APPA))
124	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(2F-APPA))
125	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA))
126	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA))
127	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA))
128	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA))
129	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA))
130	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA))

131	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA))
132	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA))
133	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(APPA))
134	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))- ((NMe)(APPA))
135	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(2F-APPA))
136	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(2F-APPA))
137	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA))
138	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA))
139	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(2F-APPA))
140	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(2F-APPA))
141	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA))
142	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA))
143	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))- ((NMe)(2F-APPA))
144	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(2F-APPA))
145	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
146	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
147	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
148	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
149	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
150	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))

Table 2 (iv): List of peptidomimetics prepared

Seq.ID. No.	Sequence of peptidomimetics
151	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
152	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
153	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
154	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
155	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
156	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-

	((NMe)(2F-APPA))
157	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(APPA)
158	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(APPA)
159	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(2F-APPA)
160	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(2F-APPA)
161	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA)
162	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA)
163	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA)
164	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA)
165	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA)
166	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA)
167	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA)
168	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA)
169	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(APPA)
170	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(APPA)
171	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
172	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
173	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA)
174	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA)
175	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
176	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
177	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA)
178	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA)
179	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
180	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
181	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(APPA)
182	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(APPA)
183	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(2F-APPA)
184	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(2F-APPA)
185	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA)
186	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA)
187	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA)
188	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA)
189	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA)
190	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA)
191	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA)
192	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA)
193	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe))-(CH_2)-(APPA)
194	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(APPA)
195	CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)

196	CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)
197	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)- (CH_2)-(APPA)
198	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)- (CH_2)-(APPA)
199	CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)
200	CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)
201	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA)
202	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA)
203	H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)
204	H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA)
205	CH_3CO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
206	CH_3OCO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
207	CH_3CO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
208	CH_3OCO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)

Table 2 (v): List of peptidomimetics prepared

Seq.ID. No.	Sequence of peptidomimetics
209	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA)
210	CH_3CO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
211	CH_3OCO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
212	CH_3CO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
213	CH_3OCO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
214	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA)
215	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA)
216	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA)
217	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA)
218	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA)
219	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA)
220	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA))
221	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA))
222	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA))
223	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA))
224	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
225	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
226	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
227	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA)
228	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA)
229	H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA)
230	H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(APPA)

231	H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(ADMP)
232	H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(ACPP)
233	H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(AMCB)
234	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA)
235	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ADMP)
236	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ACPP)
237	H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(AMCB)

***In vitro* and *in vivo* studies of novel peptidomimetics:**

The peptidomimetics prepared as described above were tested for

a) *In vitro* glucose-dependent insulin secretion (RIN5F cell assay screening protocol);
b) *In vitro* Human GLP-1 R agonist activity (Cyclic AMP determination);
c) *In vitro* human glucagon antagonist activity (Cyclic AMP determination);
d) Stability of peptidomimetics against DPP IV enzyme, human plasma, simulated gastric fluid, intestinal fluid and liver microsomes; and
e) Demonstration of *in vivo* efficacy of test compounds (peptidomimetics) in C57BL/6J mice (*in vivo*), using various *in vitro* and *in vivo* assays, as described below.

In vitro studies:

***In vitro* glucose-dependent insulin secretion (RIN5F cell assay screening protocol)**

RIN5F (Rat Insulinoma) cells were cultured in RPMI 1640 medium supplemented with sodium pyruvate (1 mM) HEPES and Glucose (4.5 g/L) in a humidified incubator (5 % CO_2), at 37 °C. After trypsinization, RIN5F cells were seeded at a concentration of 0.2 X 10^6 cells per well, in 12 well plates. The cells were grown overnight to 80 % confluence and insulin secretion experiments were performed as follows (Montrose-Rafizadeh C., et al., Mol. Cell. Endo. 1997, 130, 109.; Wang, X., et al., Endocrinology 2001, 5, 1820).

Cells were washed once with PBS solution followed by 40 min. incubation in fresh Krebs-Ringer Balanced Buffer containing NaCl (115 mmol/L), KCl (4.7 mmol/L), $CaCl_2$ (1.28 mmol/L), $MgSO_4.7H_2O$ (1.2 mmol/L), KH_2PO_4 (1.2 mmol/L), $NaHCO_3$ (10 mmol/ L) and HEPES (25 mmol/L), containing Glucose (1.1 mM) and B.S.A (0.5 %), pH 7.4. The buffer was replaced after 40 min. and the cells were incubated (37 °C) with the test peptidomimetics, at different concentration, for 30 min., both in the presence (16.7 mM) and absence (0 mM) of glucose load. The supernatant

was collected and the insulin amount was measured by ultra sensitive Rat insulin ELISA kit (Crystal Chem, IL). The protein was estimated in the supernatant using Bicinchoninic Acid kit, according to the manufacturer's protocol (Sigma Aldrich, MO). The total insulin content obtained in Pico-gram (pg) was divided with the total protein (μg) in order to normalize for differences in cell density between wells. *In vitro* glucose dependent insulin secretion activity of representative peptidomimetics are listed in **Table 3**.

Table 3. *In vitro* glucose dependent insulin secretion activity of representative peptidomimetics

Seq. ID. No.	**Conc. of test compd. (nM)**	**Insulin secretion (pg/μg/hr)***
Control 1 (0 mM glucose)		5.9±0.61
Control 2 (16.7 mM glucose)		10.2±0.51
Exendin-4	0.1 / 1 / 10	16.2±0.10 / 22.1±0.12 / 36.2±0.12
25	0.1 / 1 / 10	15.6±0.30 / 21.8±0.11 / 35.6±0.20
26	0.1 / 1 / 10	16.6±0.41 / 22.9±0.32 / 36.6±0.19
27	0.1 / 1 / 10	16.4±0.12 / 22.7±0.17 / 36.6±0.05
29	0.1 / 1 / 10	16.3±0.15 / 22.4±0.21 / 36.2±0.11
30	0.1 / 1 / 10	17.1±0.11 / 23.1±0.16 / 37.0±0.29
31	0.1 / 1 / 10	16.8±0.20 / 22.7±0.29 / 36.6±0.31
32	0.1 / 1 / 10	12.1±0.52 / 14.1±0.16 / 28.0±0.36
39	0.1 / 1 / 10	11.1±0.12 / 13.1±0.13 / 19.0±0.16
40	0.1 / 1 / 10	12.6±0.20 / 15.9±0.31 / 28.4±0.11
45	0.1 / 1 / 10	11.8±0.50 / 14.9±0.11 / 27.9±0.31
46	0.1 / 1 / 10	12.7±0.21 / 15.8±0.33 / 29.3±0.19
47	0.1 / 1 / 10	13.1±0.11 / 14.9±0.17 / 28.8±0.44
48	0.1 / 1 / 10	12.9±0.14 / 15.8±0.13 / 29.9±0.15
49	0.1 / 1 / 10	16.1±0.22 / 22.1±0.26 / 36.0±0.36
50	0.1 / 1 / 10	15.4±0.14 / 21.2±0.18 / 35.6±0.17
77	0.1 / 1 / 10	15.6±0.33 / 21.8±0.16 / 35.6±0.26
78	0.1 / 1 / 10	16.6±0.41 / 22.9±0.32 / 36.7±0.11
79	0.1 / 1 / 10	16.5±0.12 / 22.7±0.17 / 36.5±0.05
80	0.1 / 1 / 10	16.2±0.13 / 22.4±0.19 / 36.2±0.09

81	0.1 / 1 / 10	17.1±0.15 / 23.1±0.12 / 37.0±0.19
82	0.1 / 1 / 10	16.9±0.22 / 22.8±0.31 / 36.7±0.34
83	0.1 / 1 / 10	12.3±0.33 / 14.1±0.36 / 28.0±0.16
84	0.1 / 1 / 10	12.1±0.42 / 14.6±0.41 / 27.8±0.46
85	0.1 / 1 / 10	11.9±0.17 / 14.2±0.13 / 27.6±0.16
86	0.1 / 1 / 10	12.3±0.33 / 14.8±0.16 / 28.1±0.22
101	0.1 / 1 / 10	12.4±0.22 / 15.2±0.32 / 29.6±0.50
103	0.1 / 1 / 10	12.1±0.51 / 14.1±0.19 / 28.1±0.29
109	0.1 / 1 / 10	12.6±0.25 / 15.3±0.31 / 29.7±0.48
110	0.1 / 1 / 10	12.0±0.14 / 14.3±0.12 / 27.8±0.32
111	0.1 / 1 / 10	16.2±0.20 / 22.2±0.20 / 36.1±0.31
112	0.1 / 1 / 10	15.3±0.19 / 21.2±0.11 / 35.5±0.19
113	0.1 / 1 / 10	15.6±0.31 / 21.8±0.16 / 35.6±0.28
114	0.1 / 1 / 10	16.6±0.41 / 22.9±0.32 / 36.6±0.19
116	0.1 / 1 / 10	16.4±0.12 / 22.7±0.17 / 36.6±0.05
120	0.1 / 1 / 10	16.3±0.15 / 22.4±0.21 / 36.2±0.11
130	0.1 / 1 / 10	17.1±0.11 / 23.1±0.16 / 37.0±0.29
132	0.1 / 1 / 10	13.1±0.11 / 14.9±0.17 / 28.8±0.44
142	0.1 / 1 / 10	12.9±0.14 / 15.8±0.13 / 29.9±0.15
154	0.1 / 1 / 10	16.1±0.22 / 22.1±0.26 / 36.0±0.36
166	0.1 / 1 / 10	15.4±0.14 / 21.2±0.18 / 35.6±0.17
175	0.1 / 1 / 10	15.6±0.33 / 21.8±0.16 / 35.6±0.26
192	0.1 / 1 / 10	16.6±0.41 / 22.9±0.32 / 36.7±0.11
200	0.1 / 1 / 10	16.5±0.12 / 22.7±0.17 / 36.5±0.05
202	0.1 / 1 / 10	16.8±0.20 / 22.7±0.29 / 36.6±0.31
206	0.1 / 1 / 10	12.1±0.42 / 14.6±0.41 / 27.8±0.46
210	0.1 / 1 / 10	11.9±0.17 / 14.2±0.13 / 27.6±0.16
218	0.1 / 1 / 10	12.3±0.33 / 14.8±0.16 / 28.1±0.22
225	0.1 / 1 / 10	12.4±0.22 / 15.2±0.32 / 29.6±0.50
230	0.1 / 1 / 10	12.1±0.51 / 14.1±0.19 / 28.1±0.29
235	0.1 / 1 / 10	12.6±0.25 / 15.3±0.31 / 29.7±0.48
237	0.1 / 1 / 10	12.0±0.14 / 14.3±0.12 / 27.8±0.32

*In vitro glucose dependent (16.7-mM glucose load) insulin secretion with various concentrations of peptidomimetics were measured using Rat Insulinoma (RIN5F) cells. The total insulin content (pg) was divided with total protein (µg) to normalize difference in cell density between wells. n=3, values represent mean±.S.D. Basal insulin secretion was observed for all the test compounds at 0-mM glucose concentration.

***In vitro* Human GLP-1 R agonist activity (Cyclic AMP determination).**

The novel peptidomimetics were screened for Human GLP-1receptor (HGLP-1 R) agonist activity (*in vitro*), using the cAMP cell-based assay, in stably transfected CHO/ human GLP1R cells. The CHO-K1 cells (CRL 9618) were obtained from American Type Culture Collection (Rockville, MD). CHO cells were grown in Ham's F12 medium containing L-Glutamine (2mM), HEPES (25 mM), $NaHCO_3$ (1.1 g/L) and supplemented with NewBorn Calf Serum (NBCS; 10%), Penicillin (50 U /ml (v/v)) and Streptomycin (50 ug/ml (v/v)). Cells were split every 3 days 1:8.

Production of Stable CHO Cell Lines expressing the human GLP-1 Receptor.

The cDNA encoding the human GLP-1 receptor was isolated by RT- PCR according to standard protocol. The full-length cDNA was cloned in pcDNA3.1(+). For the production of CHO cell lines expressing the GLP-1 receptor, CHO cells were transfected with 10 µg of the expression plasmid pcDNA/hGLP-1R using $CaPO_4$ according to the standard protocol (Wheeler, M.B., et al., Endocrinology 1993, 133, 57.). Clones expressing the receptor were generated by G418 (800 µg/ml active, Sigma) selection. The stable clones were thereafter maintained at 500 ug/ml (G418). The selected clone was used between passages 9-25 for cAMP assays.

Determination of cAMP generation.

The CHO cells stably transfected with human GLP-1R were maintained in Ham's F12 + 10% NBCS + 500 ug/ml G418 upto a confluency of 70-75%. The cells were trypsinized using 2 ml of TPVG (0.25% trypsin, 0.53 mM EDTA, 1.38-mM glucose).

The trypsin was inactivated using Ham's F12 medium containing 10% NBCS and the cells were suspended in 2 ml of complete medium. 2 X 10^5 cells /well were then seeded in 12 well plate and the plates were incubated in humidified atmosphere at 37°C for 16 –18 h (Fehmann, H.C., et al., Peptides 1994, 15, 453). The next day the assay was proceeded, when the cells showed 90-95% confluency. The medium was aspirated off from the 12 well plate and the cells were washed once using Ham's F12 (plain). The cells were incubated at 37°C with 500 ul of Ham's F12 + 1% BSA+ 0.125 mM RO-20 for 30 min. After the incubation, the medium was aspirated off and fresh

medium (plain Ham's F12 + 1% BSA+ 0.25 mM RO-20) was added with 5ul of test compounds (peptidomimetics) that has been dissolved in water (MilliQ). The cells were incubated with the test compounds for 30 min in humidified atmosphere and 37°C. After the incubation, the medium was removed and cells were washed once with plain Ham's F12. Subsequently, the cells were lysed by adding 500 ul of ice cold 0.1 N HCl to each well and shaking for 30 minutes at 200 rpm. The cells were then scrapped, the lysate was collected in micro centrifuge tubes and centrifuged at 12000 rpm for 10 min to remove the debris. 300 ul of supernatant from each micro-centrifuge tube was then removed into a glass tube and dried under N_2 for 30 min, for cAMP estimation. The total cAMP was estimated from the sample according to the manufacturer's protocol using Cyclic AMP immunoassay kit (R&D systems, Minneapolis. MN). The remaining supernatant is used to determine the protein concentration using micro BCA (Sigma). Data is calculated as percent of control (Vehicle: water) and expressed as Mean ± SD. The *in-vitro* human GLP-1 receptor agonistic activities of representative peptidomimetics are listed in **Table 4.**

Table 4: *In vitro* Human GLP-1 R activity (cAMP release) of test compounds (peptidomimetics), shown as % activity with respect to control.

Seq. ID. No.	Concentration of test compounds				
	1nM	10 nM	100 nM	1 μM	10 μM
Exendin-4	88±0.11	95±0.10	99±0.04	99±0.08	99±0.07
11	99±0.03	99±0.01	99±0.06	99±0.09	99±0.11
16	99±0.11	99±0.13	99±0.16	99±0.06	99±0.10
19	99±0.12	99±0.08	99±0.11	99±0.12	99±0.16
22	99±0.02	99±0.26	99±0.31	99±0.60	99±0.08
29	96±0.09	99±0.07	99±0.04	99±0.01	99±0.08
30	38±0.12	78±0.15	86±0.18	95±0.03	98±0.09
32	39±0.11	80±0.09	88±0.06	96±0.14	99±0.19
39	40±0.09	81±0.07	87±0.04	95±0.01	98±0.08
40	38±0.11	77±0.16	85±0.11	94±0.08	97±0.05

42	39±0.10	81±0.08	89±0.09	96±0.11	99±0.16
45	51±0.03	86±0.40	91±0.21	99±0.32	99±0.21
47	55±0.16	89±0.05	93±0.09	99±0.02	99±0.04
50	60±0.12	92±0.15	98±0.18	99±0.03	99±0.09
62	38±0.12	78±0.15	86±0.18	95±0.03	98±0.09
68	39±0.11	80±0.09	88±0.06	96±0.14	99±0.19
72	45±0.022	84±0.46	90±0.41	99±0.66	99±0.03
77	40±0.09	81±0.07	87±0.04	95±0.01	98±0.08
79	62±0.11	94±0.09	99±0.06	99±0.14	99±0.19
80	66±0.022	97±0.46	99±0.41	99±0.66	99±0.03
81	69±0.09	98±0.07	99±0.04	99±0.01	99±0.08
82	78±0.12	99±0.15	99±0.18	99±0.03	99±0.09
84	38±0.11	77±0.16	85±0.11	94±0.08	97±0.05
101	41±0.09	82±0.07	87±0.04	95±0.01	98±0.08
110	38±0.11	77±0.16	85±0.11	94±0.08	97±0.05
112	39±0.10	81±0.08	89±0.09	96±0.11	99±0.16
114	82±0.22	87±0.12	92±0.14	99±0.22	99±0.26
116	55±0.16	88±0.13	92±0.11	99±0.07	99±0.09
120	99±0.12	99±0.15	99±0.18	99±0.03	99±0.09
130	39±0.10	81±0.08	89±0.09	96±0.11	99±0.16
140	51±0.03	86±0.40	91±0.21	99±0.32	99±0.21
144	55±0.16	89±0.05	93±0.09	99±0.02	99±0.04
154	60±0.12	92±0.15	98±0.18	99±0.03	99±0.09
156	86±0.11	99±0.09	99±0.06	99±0.14	99±0.19
166	96±0.02	99±0.46	99±0.41	99±0.66	99±0.03

168	96±0.09	99±0.07	99±0.04	99±0.01	99±0.08
178	39±0.12	80±0.15	86±0.18	95±0.03	98±0.09
180	40±0.11	81±0.09	89±0.06	96±0.14	99±0.19
185	46±0.022	85±0.46	90±0.41	99±0.66	99±0.03
190	41±0.09	82±0.07	87±0.04	95±0.01	98±0.08
194	38±0.11	77±0.16	85±0.11	94±0.08	97±0.05
196	39±0.10	81±0.08	89±0.09	96±0.11	99±0.16
198	82±0.22	87±0.12	92±0.14	99±0.22	99±0.26
200	55±0.16	88±0.13	92±0.11	99±0.07	99±0.09
202	99±0.12	99±0.15	99±0.18	99±0.03	99±0.09
203	86±0.11	99±0.09	99±0.06	99±0.14	99±0.19
204	96±0.02	99±0.46	99±0.41	99±0.66	99±0.03
205	96±0.09	99±0.07	99±0.04	99±0.01	99±0.08
206	39±0.12	80±0.15	86±0.18	95±0.03	98±0.09
207	40±0.11	81±0.09	89±0.06	96±0.14	99±0.19
210	46±0.022	85±0.46	90±0.41	99±0.66	99±0.03
216	93±0.11	99±0.09	99±0.06	99±0.14	99±0.19
220	93±0.10	99±0.46	99±0.41	99±0.66	99±0.03
224	99±0.06	99±0.06	99±0.08	99±0.10	99±0.12
228	99±0.11	99±0.13	99±0.16	99±0.06	99±0.10
230	99±0.12	99±0.08	99±0.11	99±0.12	99±0.16
235	99±0.02	99±0.26	99±0.31	99±0.60	99±0.08
237	96±0.09	99±0.07	99±0.04	99±0.01	99±0.08

Based upon, the *in-vitro* human GLP-1 receptor agonistic activity, EC_{50} values were determined for novel peptidomimetics and the comparative dose-response curves (DRC) for Exendin (EC_{50}= 0.56 nM) and **Seq. ID. No. 38**: H-Aib-QGT-(α-Me-2F-Phe)-

TSD-Bip(OMe)-(ADMP) (EC_{50}= 0.34 nM) are shown in **Figure 3** as representative example.

***In vitro* human glucagon antagonist activity (measurement of inhibition of amount of Cyclic AMP production, with test peptidomimetics).**

The novel peptidomimetics were screened for human glucagon receptor (H-glucagon-R) antagonistic activity (*in vitro*), using the cAMP cell-based assay, in stably transfected CHO/ human glucagon R cells. The CHO-K1 cells (CRL 9618) were obtained from American Type Culture Collection (Rockville, MD). CHO cells were grown in Ham's F12 medium containing L-Glutamine (2mM), HEPES (25 mM), $NaHCO_3$ (1.1 g/L) and supplemented with newborn Calf Serum (NBCS; 10%), Penicillin (50 U /ml (v/v)) and Streptomycin (50 ug/ml (v/v)). Cells were split every 3 days 1:8.

Production of Stable CHO Cell Lines expressing the human glucagon Receptor.

The cDNA encoding the human glucagon receptor was isolated by RT- PCR according to standard protocol. The full-length cDNA was cloned in pcDNA3.1(Invitrogen). For the production of CHO cell lines expressing the glucagon receptor, CHO cells were transfected with 10 μg of the expression plasmid pcDNA/ H-glucagon-R using $CaPO_4$ according to the standard protocol. Clones expressing the receptor were generated by G418 (800 μg/ml active, Sigma) selection. The stable clones were thereafter maintained at 500 ug/ml (G418). The selected clone was used between passages 9-25 for cAMP assays.

Determination of glucagon antagonistic activity by measuring amount of cAMP production inhibited after addition of test peptidomimetics along with glucagon peptide.

The CHO cells stably transfected with human glucagon R were maintained in Ham's F12 + 10% NBCS + 500 ug/ml G418 upto a confluency of 70-75%. The cells were trypsinized using 2 ml of TPVG (0.25% trypsin, 0.53 mM EDTA, 1.38-mM glucose). The trypsin was inactivated using Ham's F12 medium containing 10% NBCS and the cells were suspended in 2 ml of complete medium. 2 X 10^5 cells /well were then seeded in 12 well plate and the plates were incubated in humidified atmosphere at 37°C for 16 –18 h.The next day the assay was proceeded, when the cells showed 90-95% confluency. The medium was aspirated off from the 12 well plate and the cells were washed once using Ham's F12 (plain). The cells were incubated at 37°C with 500

ul of Ham's F12 + 1% BSA+ 0.125 mM RO-20 for 30 min. After the incubation, the medium was aspirated off and fresh medium (plain Ham's F12 + 1% BSA+ 0.25 mM RO-20) was added with 5ul of test compounds (peptidomimetics) that has been dissolved in water (MilliQ), followed by addition of glucagon peptide (as agonist). The cells were incubated with the peptidomimetics and glucagon peptide for 30 min in humidified atmosphere and 37 °C. After the incubation, the medium was removed and cells were washed once with plain Ham's F12. Subsequently, the cells were lysed by adding 500 ul of ice cold 0.1 N HCl to each well and shaking for 30 minutes at 200 rpm. The cells were then scrapped, the lysate was collected in micro centrifuge tubes and centrifuged at 12000 rpm for 10 min to remove the debris. 300 ul of supernatant from each micro-centrifuge tube was then removed into a glass tube and dried under N_2 for 30 min, for cAMP estimation. The total cAMP was estimated from the sample according to the manufacturer's protocol using Cyclic AMP immunoassay kit (R&D systems, Minneapolis. MN). The remaining supernatant is used to determine the protein concentration using micro BCA (Sigma). Data is calculated as percent of control (Vehicle: water) and expressed as Mean + SD. The *in-vitro* human glucagon receptor antagonistic activities of representative peptidomimetics are listed in **Table 5.**

Table 5: *In vitro* Human Glucagon receptor antagonistic activity of test compounds (peptidomimetics) shown as inhibition of cAMP production (pmol/ml/µg prt) of Glucagon peptide, by the test compounds, incubated at different concentration, along with saturated concentration of glucagon peptide.

Seq. ID. No.	Concentration of test compounds				
	1nM	10 nM	100 nM	1 µM	10 µM
Glucagon	23±0.01	36±0.09	36.1±0.08	37.2±0.11	36.9±0.02
5	6±0.02	5±0.04	3±0.14	0	0
9	5±0.02	3±0.15	0	0	0
11	3±0.04	0	0	0	0
19	0	0	0	0	0
22	17±0.12	15±0.13	12±0.13	8±0.16	6±0.12
26	15±0.11	12±0.13	8±0.09	4±0.01	2±0.08

28	14±0.02	11±0.03	7±0.22	3±0.12	0
29	20±0.06	18±0.09	16±0.01	14±0.03	12±0.01
30	22±0.03	21±0.05	20±0.07	18±0.04	18±0.03
31	6±0.02	5±0.03	2±0.61	0	0
40	18±0.07	16±0.01	12±0.03	8±0.02	6±0.08
45	15±0.11	12±0.13	8±0.09	4±0.01	2±0.08
49	10±0.03	8±0.03	6±0.22	2±0.13	0
51	5±0.09	3±0.11	0	0	0
65	10±0.12	8±0.02	6±0.04	4±0.05	1±0.02
72	9±0.11	6±0.12	5±0.14	3±0.22	0
77	20±0.06	18±0.09	16±0.01	14±0.03	12±0.01
78	22±0.03	21±0.05	20±0.07	18±0.04	18±0.03
79	6±0.02	5±0.03	2±0.61	0	0
84	4±0.01	2±0.17	0	0	0
110	18±0.07	16±0.01	12±0.03	8±0.02	6±0.08
112	6±0.01	5±0.02	2±0.13	0	0
114	4±0.02	2±0.15	0	0	0
116	2±0.03	0	0	0	0
120	0	0	0	0	0
130	5±0.04	3±0.02	1±0.11	0	0
132	15±0.11	12±0.13	8±0.09	4±0.01	2±0.08
142	10±0.03	8±0.03	6±0.22	2±0.13	0
144	5±0.09	3±0.11	0	0	0
150	10±0.12	8±0.02	6±0.04	4±0.05	1±0.02
154	9±0.11	6±0.12	5±0.14	3±0.22	0
156	5±0.01	2±0.17	0	0	0

166	19±0.03	17±0.08	15±0.01	14±0.02	12±0.11
168	21±0.11	19±0.02	18±0.07	18±0.03	16±0.02
170	6±0.05	5±0.04	2±0.16	0	0
172	4±0.16	2±0.11	0	0	0
177	0	0	0	0	0
178	0	0	0	0	0
188	5±0.04	3±0.15	1±0.15	0	0
189	3±0.12	1±0.12	0	0	0
191	4±0.05	2±0.13	2±0.12	0	0
192	4±0.09	2±0.12	0	0	0
194	18±0.12	16±0.11	12±0.13	8±0.12	6±0.18
195	15±0.11	12±0.13	8±0.09	4±0.01	2±0.08
197	14±0.02	11±0.03	7±0.22	3±0.12	0
199	4±0.06	3±0.11	0	0	0
200	10±0.16	8±0.12	6±0.14	4±0.15	1±0.12
202	9±0.14	6±0.14	5±0.13	3±0.24	0
204	6±0.05	5±0.04	2±0.16	0	0
205	4±0.16	2±0.11	0	0	0
206	0	0	0	0	0
207	0	0	0	0	0
208	5±0.04	3±0.15	1±0.15	0	0
212	10±0.12	8±0.02	6±0.04	4±0.05	1±0.02
218	9±0.11	6±0.12	5±0.14	3±0.22	0
225	5±0.01	2±0.17	0	0	0
228	19±0.03	17±0.08	15±0.01	14±0.02	12±0.11
237	21±0.11	19±0.02	18±0.07	18±0.03	16±0.02

Stability of peptidomimetics against DPP IV enzyme, human plasma, simulated gastric fluid, intestinal fluid and liver microsomes.

Different peptidomimetics (final concentration 2 μM) were incubated with either DPP IV (1: 25 mU) or pooled human plasma (7.5 μL) or simulated gastric fluid (pH 1.5; composition HCl, NaCl and Pepsin) or simulated intestinal fluid (pH 7.5) or human liver microsomes, for 0, 2, 4, 6, 12 and 24 h (37 °C; 50 mM triethanolamine-HCl buffer; pH 7.8). Concentrations of DPP IV enzyme/ human plasma/ simulated gastric fluid/ simulated intestinal fluid/ human liver microsomes were selected in preliminary experiments to provide degradation of approximately 50% of Exendin within 2-4 h, therefore allowing time-dependent degradation to be viewed over 24 h. Reactions were terminated by the addition of TFA/H_2O (15 mL, 10% (v/v)). The reaction products were then applied to a Vydac C_{18} analytical column (4.6 x 250-mm) and the major degradation fragment separated from intact peptidomimetic. The column was equilibrated with TFA/H_20, at a flow rate of 1 mL/min. Using 0.1% (v/v) TFA in 70% acetonitrile/H_2O, the concentration of acetonitrile in the eluting solvent was raised from 0% to 28% over 10 min and from 28% to 42% over 30 min. The absorbance was monitored at 206 nm using UV detector and peaks were collected manually prior to ESI-MS analysis. Area under the curve was measured for test peptidomimetics and their metabolites and percentage degradation were calculated at each time point over a period of 24 h. Stability study results of selected peptidomimetics, against DPP IV enzyme, human plasma, simulated gastric fluid, intestinal fluid and liver microsomes (*in vitro*) are listed in **Table 6**.

Table 6: Stability study results of selected peptidomimetics against DPP IV enzyme, human plasma, simulated gastric fluid, intestinal fluid and liver microsomes (*in vitro*)

Seq. ID. No.	DPP IV enzyme[a]	Human plasma[b]	Simulated gastric fluid[c]	Simulated intestinal fluid[d]	liver microsomes[e]
EX-4	88 (6.1)	87 (6.1)	100 (0.4)	100 (0.2)	100 (0.2)
7	71(8)	69(8)	11 (7)	43 (6)	77 (2)
8	00 (>24)	00 (>24)	00 (>24)	00 (>24)	35 (5)
12	76(9)	78(8)	14 (8)	43 (6)	80 (1)
14	74(9)	75(8)	12 (8)	46 (6)	83 (1)

16	70(9)	71(8)	14 (8)	40 (6)	78 (1)
19	86(9)	70(8)	15 (8)	41 (6)	77 (1)
27	00 (>24)	00 (>24)	00 (>24)	00 (>24)	31 (5)
29	00 (>24)	00 (>24)	00 (>24)	00 (>24)	32 (5)
30	00 (>24)	00 (>24)	00 (>24)	00 (>24)	33 (5)
31	76(10)	78(9)	100 (0.5)	100 (0.5)	100 (0.5)
32	75(10)	77(9)	100 (0.5)	100 (0.5)	100 (0.5)
45	77(10)	80(9)	100 (0.5)	100 (0.5)	100 (0.5)
47	76(9)	78(8)	12 (8)	55 (6)	79 (1)
49	75(9)	77(8)	14 (8)	45 (6)	81 (1)
50	77(9)	80(8)	13 (8)	50 (6)	82 (1)
65	76(9)	78(8)	14 (8)	43 (6)	80 (1)
68	74(9)	75(8)	12 (8)	46 (6)	83 (1)
70	70(9)	71(8)	14 (8)	40 (6)	78 (1)
72	86(9)	70(8)	15 (8)	41 (6)	77 (1)
74	76(10)	78(9)	100 (0.5)	100 (0.5)	100 (0.5)
77	74(10)	75(9)	100 (0.5)	100 (0.5)	100 (0.5)
78	70(10)	71(9)	100 (0.5)	100 (0.5)	100 (0.5)
79	86(10)	70(9)	100 (0.5)	100 (0.5)	100 (0.5)
80	72(10)	70(9)	100 (0.5)	100 (0.5)	100 (0.5)
81	00 (>24)	00 (>24)	50 (4)	00 (>24)	86 (2)
82	00 (>24)	00 (>24)	55 (4)	00 (>24)	84 (2)
84	00 (>24)	00 (>24)	45 (4)	00 (>24)	85 (2)
110	00 (>24)	00 (>24)	43 (4)	00 (>24)	84 (2)
112	00 (>24)	00 (>24)	49 (4)	00 (>24)	82 (2)
114	00 (>24)	00 (>24)	52 (4)	00 (>24)	81 (2)
116	00 (>24)	00 (>24)	43 (4)	00 (>24)	84 (2)
120	00 (>24)	00 (>24)	41 (4)	00 (>24)	80 (2)
130	76(9)	78(8)	12 (8)	55 (6)	79 (1)
132	75(9)	77(8)	14 (8)	45 (6)	81 (1)
142	77(9)	80(8)	13 (8)	50 (6)	82 (1)
144	76(9)	78(8)	14 (8)	43 (6)	80 (1)
154	74(9)	75(8)	12 (8)	46 (6)	83 (1)
156	70(9)	71(8)	14 (8)	40 (6)	78 (1)
166	86(9)	70(8)	15 (8)	41 (6)	77 (1)

168	72(9)	70(8)	12 (8)	42 (6)	78 (1)
178	00 (>24)	00 (>24)	00 (>24)	00 (>24)	35 (5)
180	00 (>24)	00 (>24)	50 (4)	00 (>24)	86 (2)
185	00 (>24)	00 (>24)	55 (4)	00 (>24)	84 (2)
190	00 (>24)	00 (>24)	45 (4)	00 (>24)	85 (2)
194	00 (>24)	00 (>24)	43 (4)	00 (>24)	84 (2)
198	00 (>24)	00 (>24)	49 (4)	00 (>24)	82 (2)
200	00 (>24)	00 (>24)	52 (4)	00 (>24)	81 (2)
201	00 (>24)	00 (>24)	43 (4)	00 (>24)	84 (2)
202	00 (>24)	00 (>24)	00 (>24)	00 (>24)	33 (5)
203	00 (>24)	00 (>24)	00 (>24)	00 (>24)	31 (5)
204	00 (>24)	00 (>24)	00 (>24)	00 (>24)	32 (5)
205	00 (>24)	00 (>24)	00 (>24)	00 (>24)	33 (5)
206	00 (>24)	00 (>24)	00 (>24)	00 (>24)	32 (5)
207	00 (>24)	00 (>24)	00 (>24)	00 (>24)	26 (5)
208	76(10)	78(9)	100 (0.5)	100 (0.5)	100 (0.5)
210	75(10)	77(9)	100 (0.5)	100 (0.5)	100 (0.5)
212	77(10)	80(9)	100 (0.5)	100 (0.5)	100 (0.5)
214	76(10)	78(9)	100 (0.5)	100 (0.5)	100 (0.5)
215	00 (>24)	00 (>24)	00 (>24)	00 (>24)	32 (5)
219	00 (>24)	00 (>24)	00 (>24)	00 (>24)	33 (5)
221	00 (>24)	00 (>24)	00 (>24)	00 (>24)	32 (5)
224	00 (>24)	00 (>24)	00 (>24)	00 (>24)	26 (5)
226	74(10)	75(9)	100 (0.5)	100 (0.5)	100 (0.5)
229	70(10)	71(9)	100 (0.5)	100 (0.5)	100 (0.5)
230	86(10)	70(9)	100 (0.5)	100 (0.5)	100 (0.5)
234	72(10)	70(9)	100 (0.5)	100 (0.5)	100 (0.5)
236	00 (>24)	00 (>24)	00 (>24)	00 (>24)	35 (5)

a: % degradation of peptidomimetics in 24h when incubated with DPP-IV enzyme and values in bracket represent half-life ($t_{1/2}$), in h;
b: % degradation of peptidomimetics in 24h when incubated with human plasma and values in bracket represent half-life ($t_{1/2}$), in h;
c: % degradation of peptidomimetics in 24h when incubated with simulated gastric fluid and values in bracket represent half-life ($t_{1/2}$), in h;
d: % degradation of peptidomimetics in 24h when incubated with simulated intestinal fluid and values in bracket represent half-life ($t_{1/2}$), in h;
e: % degradation of peptidomimetics in 24h when incubated with liver microsomes and values in bracket represent half-life ($t_{1/2}$), in h.

***In vivo* efficacy studies:**

Demonstration of in vivo efficacy (antihyperglycaemic/ antidiabetic activity) of test compounds (peptidomimetics) in C57BL/6J or db/db mice, both by parenteral (i.p) and oral routes of administration.

Animals

Acute single dose 120-min time-course experiments were carried out in male C57BL/6J or db/db mice, age 8-12 weeks, bred in-house. Animals were housed in groups of 6 animals per cage, for a week, in order to habituate them to vivarium conditions (25 ± 4 °C, 60-65 % relative humidity, 12: 12 h light: dark cycle, with lights on at 7.30 am). All the animal experiments were carried out according to the internationally valid guidelines following approval by the 'Zydus Research Center animal ethical committee'.

Procedure

The *in- vivo* glucose lowering properties of some of the test compounds (peptidomimetics) and Exendin-4 were evaluated in C57BL/6J (mild hyperglycemic) or db/db animal models as described below. Two days prior to the study, the animals were randomised and divided into 5 groups (n = 6), based upon their fed glucose levels. On the day of experiment, food was withdrawn from all the cages, water was given *ad-libitum* and were kept for overnight fasting. Vehicle (normal saline) / test / standard compounds were administered intraperitoneally (i.p.) or orally, on a body weight basis. Soon after the 0 min. blood collection from each animal, the subsequent blood collections were done at 30, 60 and 120 or upto 240 min., via retro-orbital route, under light ether anesthesia (Chen, D., et al., Diabetes Obesity Metabolism, 2005, *7*, 307. Kim, J. G. et al., Diabetes, 2003, *52*, 751).

Blood samples were centrifuged and the separated serum was immediately subjected for the glucose estimation. Serum for insulin estimation was stored at -70 °C until used for the insulin estimation. The glucose estimation was carried out with DPEC-GOD/POD method (Ranbaxy Fine Chemicals Limited, Diagnostic division, India), using Spectramax-190, in 96-microwell plate reader (Molecular devices Corporation, Sunnyvale, California). Mean values of duplicate samples were calculated using Microsoft excel and the Graph Pad Prism software (Ver 4.0) was used to plot a 0 min base line corrected line graph, area under the curve (0-120 min AUC) and base line corrected area under the curve (0 min BCAUC). The AUC and BCAUC obtained from

graphs were analyzed for one way ANOVA, followed by Dunnett's post test, using Graph Pad prism software. Furthermore, the insulin estimation was carried out using rat / mouse insulin ELISA kit (Linco research, Missouri USA). Changes in the blood glucose levels, at 0, 30, 60 and 120 min, with selected peptidomimetics are shown in **Table 7** (via ip route of administration) and **Table 8** (via oral route of administration) respectively.

Table 7: Acute single dose 120-min time-course experiments, in male C57BL/6J mice (*in vivo* glucose reduction); n=8, all values are Mean ± SEM, via intraperitonial (i.p.) route of administration.

Treatment group	0 min			30 min			60 min			120 min		
C57 control	183	±	1.2	185	±	2.1	186	±	3.1	181	±	1.1
Exendin (2 nM/kg, i.p)	182	±	1.3	111	±	2.2	128	±	3.2	145	±	6.1
Seq. ID. 6 (20 nM/kg, i.p)	182	±	2.1	121	±	2.9	130	±	3.1	138	±	2.4
Seq. ID. 12 (30 nM/kg, i.p)	181	±	4.1	117	±	3.8	117	±	3.0	117	±	1.6
Seq. ID. 15 (30 nM/kg, i.p)	180	±	3.2	119	±	3.2	117	±	3.1	118	±	1.8
Seq. ID. 18 (30 nM/kg, i.p)	183	±	3.6	118	±	3.3	116	±	3.2	117	±	1.9
Seq. ID. 23 (30 nM/kg, i.p)	182	±	4.8	118	±	2.8	116	±	2.6	118	±	1.2
Seq. ID. 29 (30 nM/kg, i.p)	180	±	1.2	116	±	3.2	116	±	3.1	118	±	1.8
Seq. ID. 30 (30 nM/kg, i.p)	183	±	3.6	117	±	3.3	116	±	3.2	117	±	1.4
Seq. ID. 32 (20 nM/kg, i.p)	182	±	3.3	120	±	3.6	127	±	3.4	145	±	2.1
Seq. ID. 40 (20 nM/kg, i.p)	180	±	3.1	122	±	3.9	129	±	2.2	140	±	2.9
Seq. ID. 45 (20 nM/kg, i.p)	179	±	2.9	121	±	3.0	129	±	3.8	142	±	1.6
Seq. ID. 48 (20 nM/kg, i.p)	182	±	3.3	120	±	3.6	127	±	3.4	145	±	2.1
Seq. ID. 50 (20 nM/kg, i.p)	181	±	3.0	121	±	3.4	128	±	2.8	141	±	2.3
Seq. ID. 55 (20 nM/kg, i.p)	180	±	3.1	122	±	3.9	129	±	2.2	140	±	2.9
Seq. ID. 60 (20 nM/kg, i.p)	179	±	2.9	121	±	3.0	129	±	3.8	142	±	1.6
Seq. ID. 70 (20 nM/kg, i.p)	182	±	2.8	122	±	3.2	128	±	3.4	141	±	1.7
Seq. ID. 75 (5 nM/kg, i.p)	181	±	3.0	123	±	3.8	128	±	3.2	146	±	1.8
Seq. ID. 77 (20 nM/kg, i.p)	183	±	4.9	120	±	2.8	129	±	3.0	139	±	2.0
Seq. ID. 78 (10 nM/kg, i.p)	182	±	2.1	111	±	2.1	112	±	2.8	113	±	2.2
Seq. ID. 80 (10 nM/kg, i.p)	183	±	2.2	113	±	2.8	113	±	2.9	114	±	2.0
Seq. ID. 84 (10 nM/kg, i.p)	182	±	2.3	110	±	2.3	112	±	3.0	112	±	1.9
Seq. ID. 90 (10 nM/kg, i.p)	181	±	2.5	111	±	3.4	113	±	3.1	110	±	1.8
Seq. ID. 92 (10 nM/kg, i.p)	180	±	4.2	113	±	3.2	112	±	2.4	110	±	1.9
Seq. ID. 94 (10 nM/kg, i.p)	182	±	4.1	112	±	3.1	113	±	2.6	111	±	1.8
Seq. ID. 96 (50 nM/kg, i.p)	181	±	5.1	120	±	3.8	128	±	2.2	149	±	1.6
Seq. ID. 100 (50 nM/kg, i.p)	180	±	5.2	119	±	3.2	129	±	2.6	148	±	1.8

Seq. ID. 103 (50 nM/kg, i.p)	182	±	5.3	121	±	3.3	127	±	2.8	149	±	2.0
Seq. ID. 108 (50 nM/kg, i.p)	183	±	5.1	122	±	3.1	130	±	2.3	148	±	2.2
Seq. ID.110 (5 nM/kg, i.p)	180	±	5.2	120	±	2.9	138	±	2.4	149	±	2.0
Seq. ID. 112 (50 nM/kg, i.p)	181	±	5.3	119	±	3.0	128	±	2.6	147	±	1.9
Seq. ID. 114 (5 nM/kg, i.p)	179	±	5.0	120	±	3.6	129	±	2.0	148	±	1.7
Seq. ID. 116 (50 nM/kg, i.p)	184	±	5.0	122	±	3.8	128	±	2.1	149	±	2.3
Seq. ID. 120 (30 nM/kg, i.p)	182	±	5.1	118	±	2.1	116	±	2.2	118	±	2.4
Seq. ID. 130 (30 nM/kg, i.p)	181	±	5.2	119	±	2.6	117	±	2.1	119	±	2.3
Seq. ID. 132 (30 nM/kg, i.p)	180	±	5.3	118	±	2.8	118	±	2.4	118	±	2.1
Seq. ID. 142 (30 nM/kg, i.p)	183	±	4.9	117	±	2.5	116	±	2.3	119	±	2.0
Seq. ID. 144 (30 nM/kg, i.p)	182	±	4.8	118	±	2.8	116	±	2.6	118	±	1.2
Seq. ID. 154 (30 nM/kg, i.p)	181	±	4.1	117	±	3.8	117	±	3.0	117	±	1.6
Seq. ID. 156 (30 nM/kg, i.p)	180	±	3.2	119	±	3.2	117	±	3.1	118	±	1.8
Seq. ID. 166 (30 nM/kg, i.p)	183	±	3.6	118	±	3.3	116	±	3.2	117	±	1.9
Seq. ID. 168 (20 nM/kg, i.p)	182	±	3.3	120	±	3.6	127	±	3.4	145	±	2.1
Seq. ID. 170 (20 nM/kg, i.p)	181	±	3.0	121	±	3.4	128	±	2.8	141	±	2.3
Seq. ID. 180 (20 nM/kg, i.p)	180	±	3.1	122	±	3.9	129	±	2.2	140	±	2.9
Seq. ID. 185 (20 nM/kg, i.p)	179	±	2.9	121	±	3.0	129	±	3.8	142	±	1.6
Seq. ID. 186 (20 nM/kg, i.p)	182	±	2.8	122	±	3.2	128	±	3.4	141	±	1.7
Seq. ID. 187 (10 nM/kg, i.p)	181	±	3.0	123	±	3.8	128	±	3.2	146	±	1.8
Seq. ID. 188 (10 nM/kg, i.p)	183	±	4.9	120	±	2.8	129	±	3.0	139	±	2.0
Seq. ID. 190 (10 nM/kg, i.p)	182	±	2.1	121	±	2.9	130	±	3.1	138	±	2.4
Seq. ID. 192 (10 nM/kg, i.p)	180	±	2.5	110	±	2.6	111	±	3.2	112	±	2.3
Seq. ID. 193 (10 nM/kg, i.p)	182	±	2.1	111	±	2.1	112	±	2.8	113	±	2.2
Seq. ID. 194 (10 nM/kg, i.p)	183	±	2.2	113	±	2.8	113	±	2.9	114	±	2.0
Seq. ID. 195 (10 nM/kg, i.p)	182	±	2.3	110	±	2.3	112	±	3.0	112	±	1.9
Seq. ID. 196 (50 nM/kg, i.p)	181	±	2.5	111	±	3.4	113	±	3.1	110	±	1.8
Seq. ID. 197 (50 nM/kg, i.p)	180	±	4.2	113	±	3.2	112	±	2.4	110	±	1.9
Seq. ID. 198 (50 nM/kg, i.p)	182	±	4.1	112	±	3.1	113	±	2.6	111	±	1.8
Seq. ID. 199 (50 nM/kg, i.p)	181	±	5.1	120	±	3.8	128	±	2.2	149	±	1.6
Seq. ID. 200 (50 nM/kg, i.p)	180	±	5.2	119	±	3.2	129	±	2.6	148	±	1.8
Seq. ID. 201 (5 nM/kg, i.p)	182	±	5.3	121	±	3.3	127	±	2.8	149	±	2.0
Seq. ID. 202 (50 nM/kg, i.p)	183	±	5.1	122	±	3.1	130	±	2.3	148	±	2.2
Seq. ID. 204 (10 nM/kg, i.p)	180	±	5.2	120	±	2.9	138	±	2.4	149	±	2.0
Seq. ID. 205 (50 nM/kg, i.p)	181	±	5.3	119	±	3.0	128	±	2.6	147	±	1.9
Seq. ID. 206 (50 nM/kg, i.p)	179	±	5.0	120	±	3.6	129	±	2.0	148	±	1.7
Seq. ID. 207 (50 nM/kg, i.p)	184	±	5.0	122	±	3.8	128	±	2.1	149	±	2.3
Seq. ID. 208 (30 nM/kg, i.p)	181	±	5.3	119	±	3.0	128	±	2.6	147	±	1.9
Seq. ID. 215 (10 nM/kg, i.p)	179	±	5.0	120	±	3.6	129	±	2.0	148	±	1.7

Seq. ID. 225 (10 nM/kg, i.p)	184	±	5.0	122	±	3.8	128	±	2.1	149	±	2.3
Seq. ID. 235 (10 nM/kg, i.p)	182	±	5.1	118	±	2.1	116	±	2.2	118	±	2.4

Table 8: Acute single dose 120-min time-course experiments, in male C57BL/6J mice (*in vivo* glucose reduction), with selected peptidomimetics; n=8, all values are Mean ± SEM, via oral route of administration

Treatment group	0 min			30 min			60 min			120 min		
C57 control	185	±	2.1	189	±	1.1	191	±	3.2	179	±	1.4
Seq. ID. 11 (2 µM/kg, oral)	184	±	2.2	118	±	3.3	112	±	4.4	110	±	2.9
Seq. ID. 15 (2 µM/kg, oral)	183	±	2.4	116	±	3.1	112	±	4.8	110	±	2.5
Seq. ID. 18 (2 µM/kg, oral)	185	±	2.3	115	±	3.8	113	±	4.9	109	±	2.2
Seq. ID. 24 (2 µM/kg, oral)	183	±	2.3	119	±	3.0	112	±	4.3	109	±	2.6
Seq. ID. 28 (2 µM/kg, oral)	183	±	2.1	118	±	3.4	113	±	4.6	111	±	2.8
Seq. ID. 29 (2 µM/kg, oral)	184	±	2.5	118	±	3.6	113	±	4.2	110	±	2.4
Seq. ID. 30 (2 µM/kg, oral)	183	±	2.4	119	±	3.1	112	±	4.8	110	±	2.5
Seq. ID. 31 (2 µM/kg, oral)	185	±	2.3	116	±	3.8	113	±	4.9	109	±	2.2
Seq. ID. 32 (2 µM/kg, oral)	181	±	2.5	117	±	3.3	112	±	4.0	108	±	2.9
Seq. ID. 44 (1 µM/kg, oral)	182	±	2.5	116	±	3.1	113	±	4.1	110	±	2.8
Seq. ID. 54 (2 µM/kg, oral)	184	±	2.2	118	±	3.3	112	±	4.4	110	±	2.9
Seq. ID. 77 (2 µM/kg, oral)	183	±	2.1	118	±	3.4	113	±	4.6	111	±	2.8
Seq. ID. 80 (0.5 µM/kg, oral)	184	±	2.5	115	±	3.6	113	±	4.2	110	±	2.4
Seq. ID. 84 (2 µM/kg, oral)	183	±	2.4	116	±	3.1	112	±	4.8	110	±	2.5
Seq. ID. 116 (2 µM/kg, oral)	185	±	2.1	115	±	2.8	112	±	4.9	111	±	2.2
Seq. ID. 120 (2 µM/kg, oral)	184	±	2.5	115	±	1.6	113	±	3.2	110	±	2.4
Seq. ID. 130 (2 µM/kg, oral)	183	±	2.4	116	±	3.1	112	±	4.8	110	±	2.5
Seq. ID. 142 (2 µM/kg, oral)	185	±	2.3	115	±	3.8	113	±	4.9	109	±	2.2
Seq. ID. 154 (2 µM/kg, oral)	181	±	2.5	117	±	3.3	112	±	4.0	108	±	2.9
Seq. ID. 168 (1.5 µM/kg, oral)	183	±	2.0	117	±	1.1	112	±	1.1	110	±	1.8
Seq. ID. 180 (2 µM/kg, oral)	184	±	2.2	118	±	3.3	112	±	4.4	110	±	2.9
Seq. ID. 190 (2 µM/kg, oral)	183	±	2.1	118	±	3.4	113	±	4.6	111	±	2.8
Seq. ID. 202 (2 µM/kg, oral)	184	±	2.5	115	±	3.6	113	±	4.2	110	±	2.4
Seq. ID. 207 (2 µM/kg, oral)	183	±	2.4	116	±	3.1	112	±	4.8	110	±	2.5
Seq. ID. 225 (2 µM/kg, oral)	183	±	2.3	119	±	3.0	112	±	4.3	109	±	2.6

Some of the baseline corrected serum glucose levels, as representative figures are shown, after acute treatment with **Seq. ID. No. 38** (H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ADMP)), at different doses (2 /5 /8 /10 /15 nM /Kg), in C57 mice, via ip

route of administration (**Figure 4**), via oral route of administration at 100 / 200 / 500 / 1000/ 2000 nM/ Kg dose (**Figure 5**) or in db/db via oral route of administration at 1000 and 2000 nM / kg dose (**Figure 6**). The **Figure 7** represents the change in serum insulin levels after acute oral administration of vehicles / test compounds (**Seq. ID. No. 10** (H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(ADMP)), **20** (H-Aib-QGTFTSD-Bip(OMe)-(AMCB)) and **25** (H-(APP)-QGTFTSD-Bip(OMe)-(APPA)), in C57BL/6J mice at 0.5 / 1 / 2 μM/ Kg dose (*in vivo*).

Overview on *in vitro* and *in vivo* results of peptidomimetics:

As described above, all the peptidomimetics prepared in the present invention were evaluated *in vitro* and *in vivo* and the data of selected peptidomimetics were presented in above section as examples of representative peptidomimetics. In RIN 5F (rat insulinoma) cell based assay, all the peptidomimetics showed only glucose-dependent insulin secretion, in the range of 1-10 nM concentration (**Table 3**), thereby these class of peptidomimetics are likely to be devoid of hyperglycemic episodes, which is commonly observed with other class of insulin secretagogues, such as sulfonylureas. In human glucagon receptor assay, *in vitro* antagonistic activity of peptidomemtics were estimated by measuring the inhibition of amount of cAMP production, with test peptidomemtics, when incubated along with the glucagon peptide. As shown in the **Table 5**, in general, all the peptidomimetics showed significant glucagon receptor antagonistic activity, in the range of 1nM to 1000 nM. In HGLP-1R assay, the novel peptidomimetics showed concentration dependent cAMP production (*in vitro* GLP-1 agonist activity), in the range of 1-100 nM concentration (**Table 4**). This dual nature of peptidomimetics (antagonist of the glucagon receptor and agonist of the GLP-1 receptor), make them ideal candidate for the safe and effective treatment of type 2 diabetes and associated metabolic disorders.

Stability study results of selected peptidomimetics against DPP-IV enzyme, human plasma, simulated gastric and intestinal fluid and liver microsomes, indicates that most of the peptidomimetics are stable against DPP-IV enzyme, when incubated upto 24 hrs. Similarly, in human plasma, simulated gastric and intestinal fluid, most of the peptidomimetics were found to be stable, when incubated upto 24 hrs. Incubation of peptidomimetics with liver microsomes showed significant stability and only 26-35% degradation were observed in 24 hrs, indicated that some of the peptidomimetics could be delivered by oral route of administration.

In vivo antihyperglycaemic / antidiabetic activity of peptidomimetics, both by parenteral and oral route of administration were determined in C57 or db/db mice,

using acute-single-dose 120/ 240-min time course experiment. As shown in **Table 7**, most of the peptidomimetics are active via i.p. route of administration, in the dose range of 5-50 nM, while orally, some of the selected peptidomimetics (**Table 8**) are active in the range of 0.5-2 μM/kg dose. Thus novel peptidomimetics exhibit glucagon antagonistic and GLP-1 agonistic activity and are orally bioavailable, which make them ideal candidate for the safe and effective treatment of type 2 diabetes and associated metabolic disorders.

Utilities:

In a preferred embodiment, the present invention provides a method of making a peptidomimetic, that function both as an antagonist of the glucagon receptor and agonist of the GLP-1 receptor having different degree of affinity/ selectivity towards both the receptors and useful for reducing circulating glucose levels and for the treatment of diabetes.

The synthetic peptidomimetics described in the present embodiment exhibit desirable *in vitro* glucagon antagonistic and GLP-1 agonist activity in CHO cells transfected with human glucagon or HGLP-1R, in nM concentration, and *in vivo*, some of the peptidomimetics showed glucose dependent insulin release and reduces fasting hyperglycemia, without causing hypoglycemia, when tested in different diabetic animal models, such as hyperglycemic C57 mice and db / db mice.

Novel peptidomemtics of present invention showed increased stability against various proteolytic enzymes and due to increased stability and short chain length, such peptidomimetics can also be delivered by oral route of administration, along with other invensive and non-invensive routes of administration.

The novel peptidomimetics of the present invention can be formulated into suitable pharmaceutically acceptable compositions by combining with suitable excipients as are well known.

The pharmaceutical composition is provided by employing conventional techniques. Preferably the composition is in unit dosage form containing an effective amount of the active component, that is, the peptidomimetics of formula (I) either alone or combination, according to this invention.

The quantity of active component, that is, the peptidomimetics of formula (I) according to this invention, in the pharmaceutical composition and unit dosage form thereof may be varied or adjusted widely depending upon the particular application

method, the potency of the particular peptidomimetics and the desired concentration. Generally, the quantity of active component will range between 0.5 % to 90 % by weight of the composition.

Accordingly, the peptidomimetics of the present invention can be administered to mammals, preferably humans, for the treatment of a variety of conditions and disorders, including, but not limited to, treating or delaying the progression or onset of diabetes (preferably type II, impaired glucose tolerance, insulin resistance and diabetic complications, such as nephropathy, retinopathy, neuropathy and cataracts), hyperglycemia, hyperinsulinemia, hypercholesterolemia, elevated blood levels of free fatty acids or glycerol, hyperlipidemia, hypertriglyceridemia, wound healing, tissue ischemia, atherosclerosis, hypertension, intestinal diseases (such as necrotizing enteritis, microvillus inclusion disease or celic disease). The peptidomimetics of the present invention may also be utilized to increase the blood levels of high-density lipoprotein (HDL).

In addition, the conditions, diseases collectively referenced to as 'Syndrome X' or metabolic syndrome as detailed in Johannsson G., J., Clin. Endocrinol. Metab., 1997, 82, 727, may be treated employing the peptidomimetics of the invention. The peptidomimetics of the present invention may optionally be used in combination with suitable DPP-IV inhibitors for the treatment of some of the above disease states either by administering the compounds sequentially or as a formulation containing the peptidomimetics of the present invention along with a suitable DPP-IV inhibitors.

No adverse effects were observed for any of the mentioned peptidomimetics of invention. The compounds of the present invention showed good glucose serum-lowering activity in the experimental animals used. These peptidomimetics are used for the testing/ prophylaxis of diseases caused by hyperinsulinemia, hyperglycemia such as NIDDM, metabolic disorders, since such diseases are inter-linked to each other.

We claim:

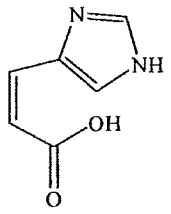
1. An isolated peptidomimetics having a sequence of Formula (I), including its tautomers, solvates

$$A\text{-}Z_1\text{-}Z_2\text{-}Z_3\text{-}Z_4\text{-}Z_5\text{-}Z_6\text{-}Z_7\text{-}Z_8\text{-}Z_9\text{-}Z_{10}\text{-}Z_{11}\text{-}B$$

(I)

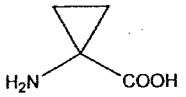
wherein,

A represents the groups –NH-R_1, R_3-CO-, R_3-O-CO- or R_3-SO_2-, wherein R_1 represents hydrogen, or optionally substituted linear or branched (C_1-C_{10}) alkyl chain; R_3 is selected from linear or branched (C_1-C_{10}) alkyl, (C_3-C_6) cycloalkyl, aryl, heteroaryl or arylalkyl groups; B represents –$COOR_2$, -$CONHR_2$ or CH_2OR_2, wherein R_2 represents H, optionally substituted groups selected from linear or branched (C_1-C_{10}) alkyl, aryl or aralkyl groups; Z_1 to Z_{11} represent natural or unnatural amino acids, linked together by amide bond, wherein Z_1 represents L-Histidine (H), D-Histidine (*d*H) or Urocanic acid (UA);

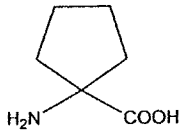


urocanic acid (UA)

Z_2 represents a naturally or unnaturally occurring amino acids selected from the group comprising of L-Serine (S), D-Serine (*d*S), L-Alanine (A), D-Alanine (*d*A), α-methyl proline (α-Me-Pro), α-amino-isobutyric acid (Aib), 1-amino cyclopropane carboxylic acid (ACP), 1-amino-cyclopentanecarboxylic acid (APP);

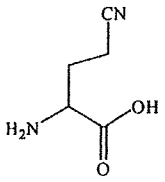


(ACP)

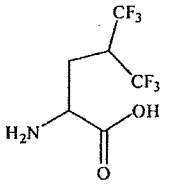


(APP)

Z_3 represents L-Glutamine (Gln; Q), D-Glutamine (*d*Q) or compounds of **formula II** (CNB or Hfl);



2-Amino-4-cyano-butyric acid (CNB)



Hexafluoroleucine (Hfl)

Formula II

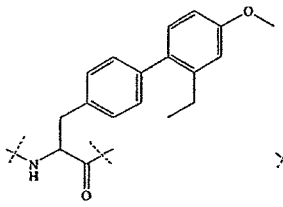
Z_4 represents Glycine (G) or the unnatural amino acids 1-amino cyclopropane carboxylic acid (ACP) or 1-amino-cyclopentanecarboxylic acid (APP);

Z_5 represents a naturally or nonnaturally occurring amino acid comprising a hydroxyl side chain; a preferred Z_5 is L-Threonine (T), D-Threonine (*d*T), L-Allo-threonine (allo-Thr; allo-T), D-Allo-threonine (*d*-allo-Thr; *d*-allo-T);

Z_6 represents a naturally or unnaturally occurring amino acid having a disubstituted alpha carbon having two side chains, wherein each of them may independently be an optionally substituted alkyl or aryl or an aralkyl group wherein the substituents may be selected from one or more alkyl groups or one or more halo groups;

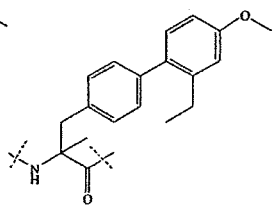
Z_7 and Z_8 independently represents a naturally or non-naturally occurring amino acid comprising a hydroxyl side chain;

Z_9 independently represent a naturally or nonnaturally occurring amino acid having an amino acid side chain comprising an acidic group; Z_{10} represents L or D unnatural amino acid of **formula III (a-c)**;



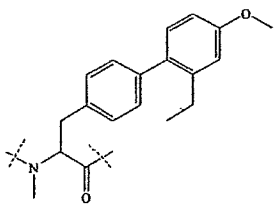
Bip(OMe)

IIIa



α-Me-Bip(OMe)

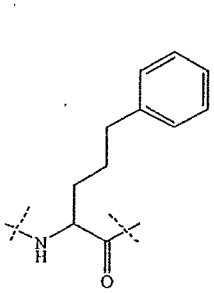
IIIb



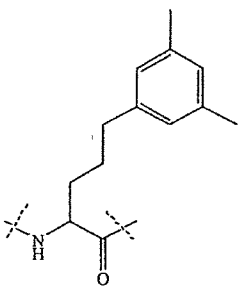
N(Me)-Bip(OMe)

IIIc

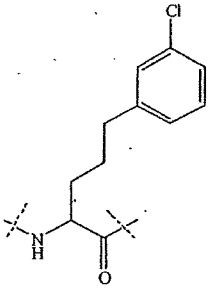
Z_{11} represents L or D unnatural amino acid of **formula IV (a-l);**



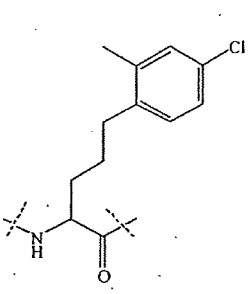
APPA
IVa



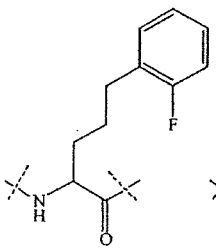
ADMP
IVb



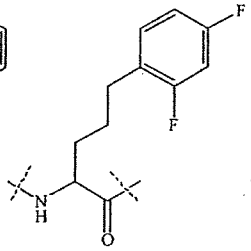
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IVc



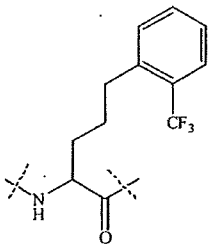
AMCB
IVd



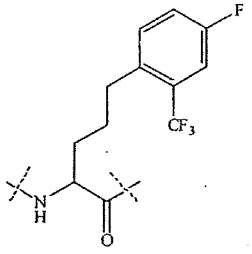
2F-APPA
IVe



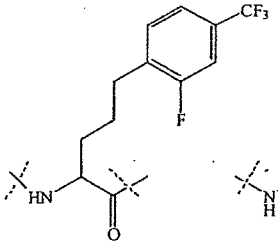
2,4-diF-APPA
IVf



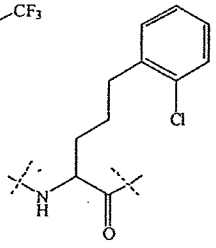
$2CF_3$-APPA
IVg



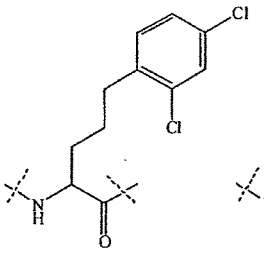
$2CF_3$, 4F-APPA
IVh



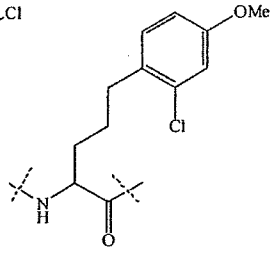
2F, $4CF_3$-APPA
IVi



2Cl-APPA
IVj



2,4-diCl-APPA
IVk



2Cl, 4OMe-APPA
IVl

2. A compound of formula 1 as claimed in claim 1, wherein Z_6 represents the groups Phe (F), alpha-methyl-phenylalanine (-α-Me-Phe-), alpha-methyl-2-fluorophenylalanine (-α-Me-2F-Phe-) or alpha-methyl-2,6-diflurophenylalanine (-α-Me-2,6-F-Phe-) or 2-fluorophenylalanine (-2F-Phe-).
3. A compound of formula 1 as claimed in claim 1 wherein each of Z_7 & Z_8 is selected from threonine, serine, 1-amino cyclopropane carboxylic acid.

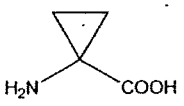
4. A compound of formula 1 as claimed in claim 1 wherein, Z_9 is selected from L-Aspartic acid (D), D-Aspartic acid (*d*D) or compounds of formula II.
5. A compound of formula 1 as claimed in claim 1 wherein the aryl group is selected from phenyl, napthyl, indanyl, fluorenyl or biphenyl, groups; the heteroaryl group is selected from pyridyl, thienyl, furyl, imidazolyl, benzofuranyl groups;
6. A compound of formula (I) of claim 1, wherein the amide bond between Z_9 and Z_{10} or Z_{10} and Z_{11} or Z_9-Z_{11} is further N-methylated, represented by '(NMe)'.
7. A compound of formula (I) of claim 1 wherein the amide bond between Z_9 and Z_{10} or Z_{10} and Z_{11} or Z_9-Z_{11} further is a thioamide bond.
8. A compound of formula (I) of claim 7 wherein further the thioamide bond between Z_9 and Z_{10} or Z_{10} and Z_{11} or Z_9-Z_{11} is reduced to a '$-CH_2-$' bond.
9. A compound of formula 1

$$A\text{-}Z_1\text{-}Z_2\text{-}Z_3\text{-}Z_4\text{-}Z_5\text{-}Z_6\text{-}Z_7\text{-}Z_8\text{-}Z_9\text{-}Z_{10}\text{-}Z_{11}\text{-}B$$

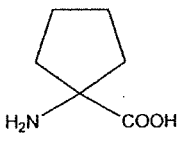
(I)

wherein,

'A' represents the groups $-NH\text{-}R_1$, $R_3\text{-}CO\text{-}$, $R_3\text{-}O\text{-}CO\text{-}$ or $R_3\text{-}SO_2\text{-}$, wherein R_1 represents hydrogen, or optionally substituted linear or branched (C_1-C_{10}) alkyl chain; R_3 is selected from linear or branched (C_1-C_{10}) alkyl, (C_3-C_6) cycloalkyl, aryl, heteroaryl or arylalkyl groups; B represents $-COOR_2$, $-CONHR_2$ or CH_2OR_2, wherein R_2 represents H, optionally substituted groups selected from linear or branched (C_1-C_{10}) alkyl group, aryl or aralkyl groups; Z_1 represents L-Histidine (H), D-Histidine (*d*H) or Urocanic acid (UA); Z_2 is selected from L-Serine, D-Serine, L-alanine, D-alanine, α-amino-isobutyric acid, 1-amino cyclopropane carboxylic acid;

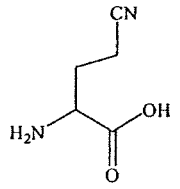


(ACP)

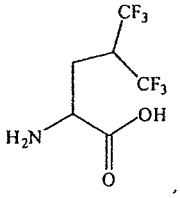


(APP)

Z_3 represents L-Glutamine (Gln; Q), D-Glutamine (*d*Q) or compounds of **formula II** (CNB or Hfl).



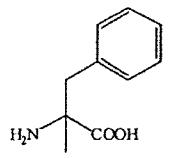
2-Amino-4-cyano-butyric acid (CNB)



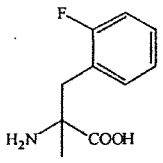
Hexafluoroleucine (Hfl)

Formula II

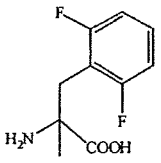
Z_4 represents Glycine (G) or the unnatural amino acids 1-amino cyclopropane carboxylic acid (ACP) or 1-amino-cyclopentanecarboxylic acid (APP); Z_5 represents L-Threonine (T), D-Threonine (*d*T), L-Allo-threonine (allo-Thr; allo-T), D-Allo-threonine (*d*-allo-Thr; *d*-allo-T); Z_6 represents phenylalanine (Phe; F), alpha-methyl-phenylalanine (-α-Me-Phe-), alpha-methyl-2-fluorophenylalanine (-α-Me-2F-Phe-), alpha-methyl-2,6-diflurophenylalanine (-α-Me-2,6-F-Phe-) or 2-fluorophenylalanine (-2F-Phe-);



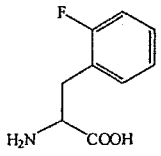
α-Me-Phe



α-Me-2F-Phe

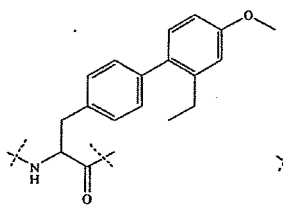


α-Me-2,6-F-Phe



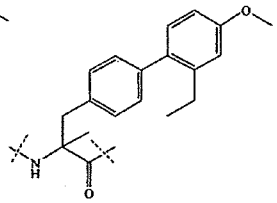
2F-Phe

Z_7 and Z_8 are independently selected from Threonine, Serine, 1-amino cyclopropane carboxylic acid (ACP); Z_9 is selected from L-Aspartic acid (D), D-Aspartic acid (*d*D) or compounds of **formula II**; Z_{10} represents L or D unnatural amino acid of **formula III (a-c)**



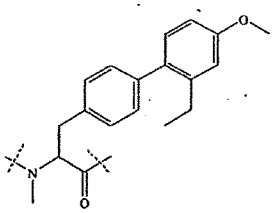
Bip(OMe)

IIIa



α-Me-Bip(OMe)

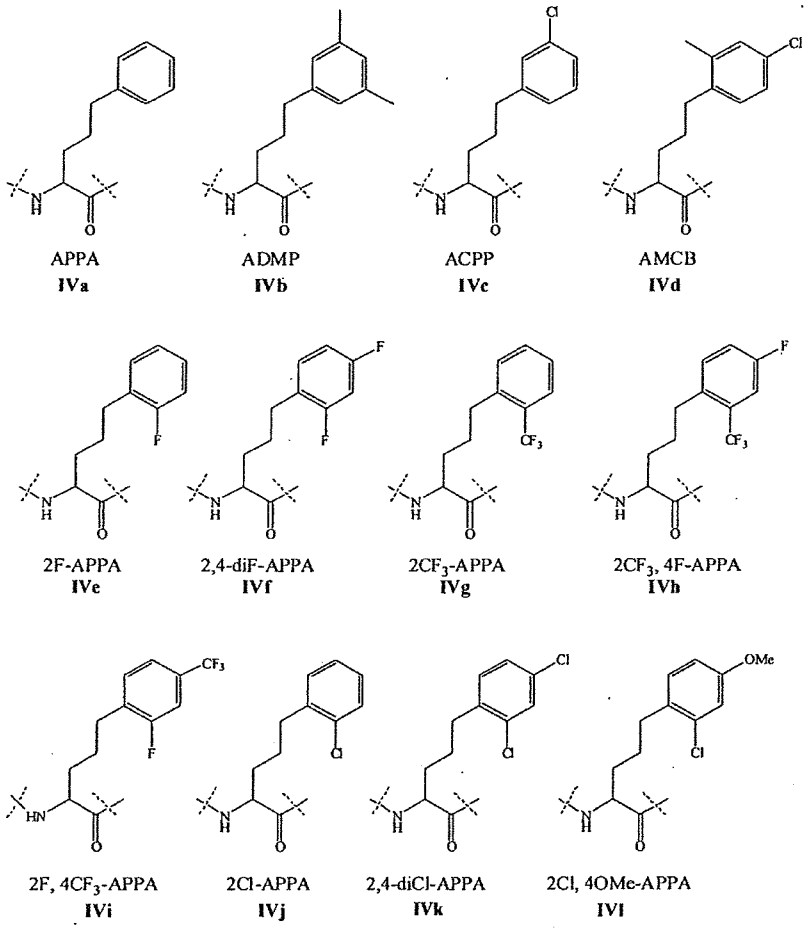
IIIb



(NMe)-Bip(OMe)

IIIc

Z_{11} represents L or D unnatural amino acid of **formula IV (a-l);**



10. A compound of formula 1 selected from

HSQGTFTSD-Bip(OMe)-(APPA);

HSQGTFTSD-Bip(OMe)-(ADMP);

HSQGTFTSD-Bip(OMe)-(ACPP);

HSQGTFTSD-Bip(OMe)-(AMCB);

H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(APPA);

H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(ADMP);

H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(ACPP);

H-(α-Me-Pro)-QGTFTSD-Bip(OMe)-(AMCB);

HAQGTFTSD-Bip(OMe)-(APPA);

HAQGTFTSD-Bip(OMe)-(ADMP);

HAQGTFTSD-Bip(OMe)-(ACPP);

HAQGTFTSD-Bip(OMe)-(AMCB);

H-Aib-QGTFTSD-Bip(OMe)-(APPA);

H-Aib-QGTFTSD-Bip(OMe)-(ADMP);
H-Aib-QGTFTSD-Bip(OMe)-(ACPP);
H-Aib-QGTFTSD-Bip(OMe)-(AMCB);
H-(ACP)-QGTFTSD-Bip(OMe)-(APPA);
H-(ACP)-QGTFTSD-Bip(OMe)-(ADMP);
H-(ACP)-QGTFTSD-Bip(OMe)-(ACPP);
H-(ACP)-QGTFTSD-Bip(OMe)-(AMCB);
H-(APP)-QGTFTSD-Bip(OMe)-(APPA);
H-(APP)-QGTFTSD-Bip(OMe)-(ADMP);
H-(APP)-QGTFTSD-Bip(OMe)-(ACPP);
H-(APP)-QGTFTSD-Bip(OMe)-(AMCB);
H-Aib-(CNB)-GTFTSD-Bip(OMe)-(APPA);
H-Aib-(CNB)-GTFTSD-Bip(OMe)-(ADMP);
H-Aib-(CNB)-GTFTSD-Bip(OMe)-(ACPP);
H-Aib-(CNB)-GTFTSD-Bip(OMe)-(AMCB);
H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(APPA);
H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(ADMP);
H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(ACPP);
H-Aib-QGT-(α-Me-Phe)-TSD-Bip(OMe)-(AMCB);
H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);
H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ADMP);
H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ACPP);
H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(AMCB);
H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(APPA);
H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(ADMP);
H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(ACPP);
H-Aib-QGT-(2F-Phe)-TSD-Bip(OMe)-(AMCB);
HSQGTFTSD-Bip(OMe)-(2F-APPA);
HSQGTFTSD-Bip(OMe)-(2,4-diF-APPA);
HSQGTFTSD-Bip(OMe)-(2CF_3-APPA);
HSQGTFTSD-Bip(OMe)-(2CF_3, 4F-APPA);
HSQGTFTSD-Bip(OMe)-(2F,4CF_3-APPA);
HSQGTFTSD-Bip(OMe)-(2Cl-APPA);
HSQGTFTSD-Bip(OMe)-(2,4-diCl-APPA);
HSQGTFTSD-Bip(OMe)-(2Cl, 4OMe-APPA);
HAQGTFTSD-Bip(OMe)-(2F-APPA);
HAQGTFTSD-Bip(OMe)-(2,4-diF-APPA);

HAQGTFTSD-Bip(OMe)-(2CF_3-APPA);

HAQGTFTSD-Bip(OMe)-(2CF_3,4F-APPA);

HAQGTFTSD-Bip(OMe)-(2F,4CF_3-APPA);

HAQGTFTSD-Bip(OMe)-(2Cl-APPA);

HAQGTFTSD-Bip(OMe)-(2,4-diCl-APPA);

HAQGTFTSD-Bip(OMe)-(2Cl,4OMe-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2F-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2,4-diF-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2CF_3-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2CF_3,4F-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2F,4CF_3-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2Cl-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2,4-diCl-APPA);

H-Aib-QGTFTSD-Bip(OMe)-(2Cl,4OMe-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2F-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2,4-diF-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2CF_3-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2CF_3,4F-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2F,4CF_3-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2Cl-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2,4-diCl-APPA);

H-(ACP)-QGTFTSD-Bip(OMe)-(2Cl, 4OMe-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2F-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2,4-diF-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2CF_3-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2CF_3,4F-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2F,4CF_3-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2Cl-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2,4-diCl-APPA);

H-Aib-(CNB)-GTFTSD-Bip(OMe)-(2Cl,4OMe-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2,4-diF-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2CF_3-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2CF_3,4F-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F,4CF_3-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2Cl-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2,4-diCl-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2Cl,4OMe-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-(CNB)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-QGT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-(CNB)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(APPA));

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(APPA));

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(2F-APPA));

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-((NMe)(2F-APPA));

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)- ((NMe)(APPA));

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)- ((NMe)(APPA));

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA));

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA));

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA));
H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA));
CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(APPA));
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CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA));
CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA));
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CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(2F-APPA));
H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))- ((NMe)(APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(APPA));
H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))- ((NMe)(2F-APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));
CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))- ((NMe)(APPA));
CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TS-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));
CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));
CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));
H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));
CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(C=S)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)- (CH_2)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)- (CH_2)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe))-(CH_2)-(APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(APPA);

CH_3CO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3OCO-H-Aib-QGT-(α-Me-2F-Phe)-TSD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)- (CH_2)-(APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)- (CH_2)-(APPA);

CH_3CO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3OCO-H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA);

H-Aib-QG-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

H-Aib-(CNB)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(2F-APPA);

CH_3CO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-(CNB)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(CH_2)-Bip(OMe)-(CH_2)-(APPA);

CH_3CO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3OCO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

CH_3CO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

CH_3OCO-H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(CH_2)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-(C=S)-Bip(OMe)-(C=S)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(C=S)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(APPA));

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-S-(NMe)D-((NMe)Bip(OMe))-((NMe)(2F-APPA));

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(APPA));

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-((NMe)(2F-APPA));

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-TSD-Bip(OMe)-(2F-APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(APPA);

H-Aib-(Hfl)-G-T(allo)-(α-Me-2F-Phe)-T(allo)-SD-Bip(OMe)-(2F-APPA);

H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(APPA);

H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(ACPP);

H-Aib-(Hfl)-GT-(α-Me-Phe)-TSD-Bip(OMe)-(AMCB);

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(APPA);

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ADMP);

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(ACPP);

H-Aib-(Hfl)-GT-(α-Me-2F-Phe)-TSD-Bip(OMe)-(AMCB).

11. A pharmaceutical composition comprising compounds of formula (I) as claimed in any preceding claims, and suitable pharmaceutically acceptable carrier(s).

12. The compounds of formula (I) or their pharmaceutical compositions as claimed in any preceding claims, which act as an antagonist of the glucagon receptor.

13. The compounds of formula (I) or their pharmaceutical compositions as claimed in any preceding claims which further acts as an agonist of GLP-I receptor.

14. The compounds or their pharmaceutical compositions as claimed in any preceding claims, useful for the treatment or prevention of diseases caused by hyperlipidaemia, hypercholesteremia, hyperglycemia, hyperinsulinemia, elevated blood levels of free fatty acids or glycerol, hypertriglyceridemia, delayed wound healing, impaired glucose tolerance, leptin resistance, insulin resistance or other diabetic complications.

15. Use of a compound of formula (I) as defined in any one of the preceding claims in the manufacture of a medicament for preventing or treating diseases caused by hypercholesteremia, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance, leptin resistance, insulin resistance or other diabetic complications.

16. Use of a compound of formula (I) as defined in any one of claims 1-14 in the manufacture of a medicament for preventing or treating type 2 diabetes, impaired glucose tolerance, dyslipidaemia, hypertension, atherosclerosis, hyperlipidaemia, coronary artery disease, cardiovascular disorders and other diseases wherein insulin resistance is the underlying pathophysiological mechanism.

17. Use of a compound of formula (I), as claimed in any one of the preceding claims and a pharmaceutically acceptable carrier, diluent, excipient or solvent in the manufacture of a medicine for treating or preventing diseases caused by hypercholesteremia, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance, leptin resistance, insulin resistance or other diabetic complications, or for treating or preventing type 2 diabetes, impaired glucose tolerance, dyslipidaemia, hypertension, atherosclerosis,

wherein insulin resistance is the underlying pathophysiological mechanism.

18. A compound of formula (I) as claimed in any one of claims 1-14 or a pharmaceutical composition or medicine containing said compound for use as a medicament for the treatment of disease wherein insulin resistance is the underlying pathophysiological mechanism.