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(71) Applicant *(for all designated States except US)*: **ZEALAND PHARMA A/S** [DK/DK]; Smedeland 36, DK-2600 Glostrup (DK).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **JUST, Rasmus** [DK/DK]; Egegade 8, 1, DK-2200 Copenhagen (DK). **LOECHEL, Steven, Charles** [DK/DK]; Duevej 58, 4.th., DK-2000 Frederiksberg (DK). **ØSTERLUND, Torben** [DK/SE]; Orkestervägen 28, S-224 72 Lund (SE). **RIBER, Ditte** [DK/DK]; Degnemose Allé 64, DK-2700 Brønshøj (DK). **FOSGERAU, Keld** [DK/DK]; Lyngholmvej 45, DK-2720 Vanløse (DK).

(74) Agents: **RUSKIN, Barbara, A.** et al.; Ropes & Gray LLP, 1211 Avenue of the Americas, New York, NY 10036 (US).

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(54) Title: GLU-GLP-1 DUAL AGONIST SIGNALING-SELECTIVE COMPOUNDS

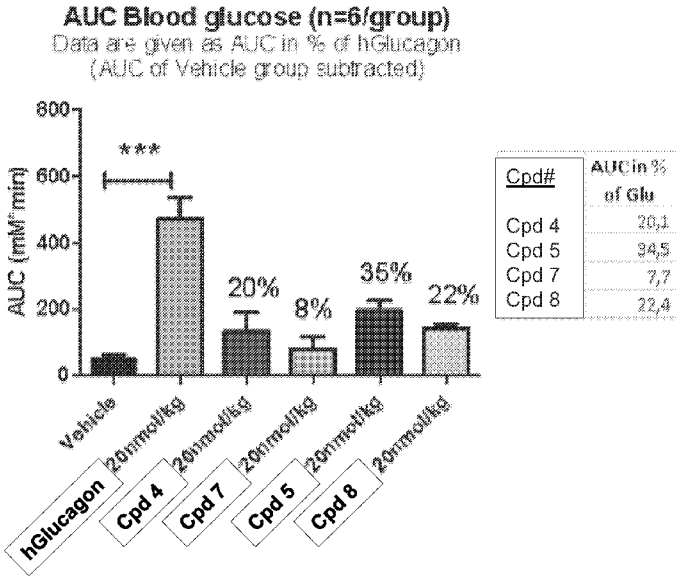

Figure 13

(57) Abstract: The present application relates to methods of screening Glu-GLP1 dual agonists (GGDAs) that activate alternative intracellular signalling pathways through the Glucagon receptor (Glu R). Compounds have been identified as partial agonists rather than full agonists, eliciting a submaximal response, presumably caused by generation of an alternative conformational state of the receptor induced by the ligand-receptor interaction. The compounds may be useful in the treatment of metabolic diseases, e.g., diabetes, obesity, and related glucose metabolism diseases, or other conditions characterized by excess body weight.

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GLU-GLP-1 DUAL AGONIST SIGNALING-SELECTIVE COMPOUNDS

Cross Reference to Other Applications

[0001] This application claims priority from U.S. Provisional Application No. 61/484,422, filed May 10, 2011, the disclosure of which is incorporated herein by reference in its entirety.

Background of the Invention

[0002] Obesity and diabetes are globally increasing health problems and are associated with various diseases, particularly cardiovascular disease (CVD), obstructive sleep apnea, stroke, peripheral artery disease, microvascular complications and osteoarthritis.

[0003] There are 246 million people worldwide with diabetes, and by 2025 it is estimated that 380 million will have diabetes. Many have additional cardiovascular risk factors including high/aberrant LDL and triglycerides and low HDL.

[0004] Cardiovascular disease accounts for about 50% of the mortality in people with diabetes, and the morbidity and mortality rates relating to obesity and diabetes underscore the medical need for efficacious treatment options. Pre-proglucagon is a 158 amino acid precursor polypeptide that is differentially processed in the tissues to form a number of structurally related proglucagon-derived peptides, including glucagon (Glu), glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), and oxyntomodulin (OXM). These molecules are involved in a wide variety of physiological functions, including glucose homeostasis, insulin secretion, gastric emptying and intestinal growth, as well as regulation of food intake.

[0005] Glucagon is a 29-amino acid peptide that corresponds to amino acids 53 to 81 of pre-proglucagon and has the native sequence His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr. Oxyntomodulin (OXM) is a 37 amino acid peptide which includes the complete 29 amino acid sequence of glucagon with an octapeptide

carboxyterminal extension (amino acids 82 to 89 of pre-proglucagon, having the sequence Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala and termed "intervening peptide 1" or IP-1; the full sequence of human oxyntomodulin is, thus, His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-Lys-Arg-Asn-Arg-Asn-Asn-Ile-Ala). The major biologically active fragment of GLP-1 is produced as a 30-amino acid, C-terminally amidated peptide that corresponds to amino acids 98 to 127 of pre-proglucagon.

[0006] Glucagon helps maintain the level of glucose in the blood by binding to glucagon receptors on hepatocytes, causing the liver to release glucose (stored in the form of glycogen) through glycogenolysis. As these stores become depleted, glucagon stimulates the liver to synthesize additional glucose by gluconeogenesis. This glucose is released into the bloodstream, preventing the development of hypoglycemia. Additionally, glucagon has been demonstrated to increase lipolysis and decrease body weight. By contrast, insulin decreases blood glucose by the opposite mechanism. In fact, the glucose balance is, to a very large degree, determined by the balance between glucagon and insulin. Insulin resistance during the development of metabolic syndrome and diabetes onset results in an imbalance between the two signals, resulting in impaired blood glucose regulation. The further development of diabetes results in impaired (or, in type-1 diabetes, a lack of) insulin secretion from the pancreas and further impaired glucose regulation. GLP-1 decreases elevated blood glucose levels by improving glucose-stimulated insulin secretion from pancreas and promotes weight loss chiefly through decreasing food intake.

[0007] Oxyntomodulin is released into the blood in response to food ingestion and in proportion to meal calorie content. The mechanism of action of oxyntomodulin is not well understood. In particular, it is not known whether the effects of the hormone are mediated exclusively through the glucagon receptor and the GLP-1 receptor, or through one or more as-yet unidentified receptors. Other peptides have been shown to bind and activate both the glucagon and the GLP-1 receptor (Hjort et al, *Journal of Biological Chemistry*, 269, 30121-30124,1994) and to suppress body weight gain and reduce food intake (WO 2006/134340; WO

2007/100535; WO 2008/101017, WO 2008/152403, WO 2009/155257 and WO 2009/155258).

[0008] Stabilization of peptides has been shown to provide a better pharmacokinetic profile for several drugs. In particular, addition of one or more polyethylene glycol (PEG) or acyl groups has been shown to prolong half-life of peptides such as GLP-1 and other peptides with short plasma stability.

[0009] WO 00/55184A1 and WO 00/55119 disclose methods for acylation of a range of peptides, in particular GLP-1. Madsen et al (*J. Med. Chem.* 2007, 50, 6126-6132) describe GLP-1 acylated at position 20 (Liraglutide) and provide data on its stability. Stabilization of OXM by PEGylation and C-terminal acylation has also been shown to improve the pharmacokinetic profile of selected analogs in WO2007/100535, WO2008/071972 and in Druce, M R et al. *Endocrinology* 2009, 150(4), 1712-1721. In addition, it has recently been shown that PEGylation of glucagon analogs has a significant effect on the pharmacokinetic profile of the tested compounds (WO2008/101017) but also interferes with the potency of these compounds. For further discussion of glucagon analogs, see WO2011/006497 and U.S. Provisional Application No. 61/358,623. A glucagon analog, as used herein, refers to a peptide sequence derived from any substitutions, truncations, deletions, additions and/or conjugations of the native glucagon sequence having one or more of the biological activities of a native glucagon. Glucagon analogs of the present invention include, but are not limited to, peptides having the specific sequence substitutions described herein.

[0010] In addition to stabilization of peptides, signaling selectivity may also confer beneficial properties to drugs. Until recently , pharmacological theory has held that "intrinsic efficacy," *i.e.*, the inherent ability of a ligand to impart (or reduce) stimulus to a cell once that ligand is bound to its receptor, is a system-independent parameter that is constant for each ligand at a given receptor, irrespective of where or in what context that receptor is expressed. It is now recognized that this classical notion of intrinsic efficacy as an invariant constant cannot be complete. The data that have driven this conceptual shift derive from studies on drugs that, despite acting at the same receptor and in the same cellular background, differentially activate certain subsets of intracellular signaling

pathways to the relative exclusion of others. Importantly, the actual pathways that are activated vary in a drug-specific manner, a phenomenon that has been dubbed "ligand-directed signaling bias," or simply "ligand bias" or "signaling selectivity". Signaling selectivity also may be extended to drug-specific modulation of receptor regulatory processes (e.g. desensitization, phosphorylation, internalization), thus opening new possibilities for achieving selectivity in "sculpting" cellular responses for therapeutic benefit.

Summary of the Invention

[0011] Provided herein are methods of screening Glu-GLP-1 dual agonist (GGDA) compounds for preferential pathway activation or inhibition, comprising screening for a full glucagon receptor agonist in a first glucagon receptor assay, identifying a full glucagon receptor agonist, screening for a partial glucagon receptor agonist in a second, different, glucagon receptor assay, identifying a partial glucagon receptor agonist, screening for a full GLP-1 receptor agonist in a first GLP-1 receptor assay, and identifying a full GLP-1 receptor agonist. In some embodiments, the first and second glucagon receptor assays and the GLP-1 receptor assay are selected from the group consisting of a cAMP assay, a pERK assay, and a Ca2+ assay.

[0012] Also provided herein are compounds which exhibit selective pathway activation or inhibition, *e.g.*, compounds identified by the methods described above, and methods of treating or preventing a metabolic disease or condition (*e.g.*, diseases or conditions characterized by excess body weight, such as obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, pre-diabetes, insulin resistance, glucose intolerance, type 2 diabetes, type I diabetes, hypertension, atherogenic dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease) comprising administering a treatment-effective amount of such a GGDA compound.

[0013] Select embodiments of the invention include:

1. A method of screening GGDA compounds for preferential pathway activation or inhibition, comprising:

screening for a full glucagon receptor agonist in a first glucagon receptor assay,

identifying a full glucagon receptor agonist of a first pathway,

screening for a partial glucagon receptor agonist in a second glucagon receptor assay,

identifying a partial glucagon receptor agonist of a second pathway,

screening for a full GLP-1 receptor agonist in a first GLP-1 receptor assay, and

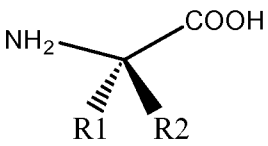
identifying a full GLP-1 receptor agonist of a GLP-1 receptor mediated pathway.

2. The method of embodiment 1, wherein the first and second glucagon receptor assays and the GLP-1 receptor assay are selected from the group consisting of: a cAMP assay, a pERK assay, and a Ca^{2+} assay.

3. The method of embodiments 1 or 2, wherein the GGDA is a partial glucagon receptor agonist as determined by a pERK assay or a Ca^{2+} assay.

4. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a compound of the formula His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr, wherein X2 is a linear or cyclic α,α-disubstituted amino acid, a lipophilic D-amino acid or polar D-amino acid.

5. The method of embodiment 4, wherein X2 is an α,α-disubstituted amino acid having the formula I:

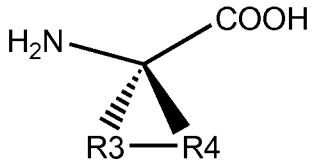


(formula I)

wherein R1 and R2, independently, are selected from the group consisting of $-CH_3$ (methyl), $-CH_2CH_3$ (ethyl), $-CH_2CH_2CH_3$ (1-propyl) and $-CH_2Ph$ (benzyl).

6. The method of embodiment 5, wherein X2 is Aib.

7. The method of embodiment 4, wherein X2 is a cyclic α,α-disubstituted amino acid having the formula II:

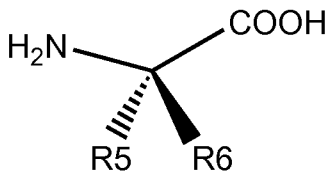


(formula II)

wherein R3 and R4, independently, are selected from the group consisting of -CH_2-, -CH_2CH_2-, -$CH_2CH_2CH_2$- and -$CH_2CH_2CH_2CH_2$-.

8. The method of embodiment 7, wherein X2 is selected from the group consisting of Ac3c, Ac4c, Ac5c, Ac6c, and Ac7c.

9. The method of embodiment 4, wherein X2 is a lipophilic D-amino acid having the formula III:

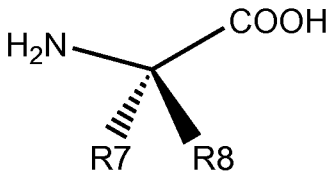


(formula III)

wherein R5 is hydrogen, and R6 is selected from the group consisting of -CH_3 (methyl), -$CH_2CH(CH_3)_2$ (2-methyl-1-propyl), -$CH(CH_3)(CH_2CH_3)$ (2-butyl), -$CH(CH_3)_2$ (2-propyl) and -CH_2Ph (benzyl).

10. The method of embodiment 9, wherein the lipophilic D-amino acid is selected from the group consisting of D-Ala, D-Leu, D-Ile, D-Val and D-Phe.

11. The method of embodiment 4, wherein X2 is a polar D-amino acid having the formula IV:



(formula IV)

wherein wherein R7 is hydrogen, and R8 is selected from the group consisting of -CH_2OH (hydroxymethyl), -$CH(CH_3)(OH)$ (1-hydroxyethyl) and -$CH_2CH_2SCH_3$ (2-methylthioethyl).

12. The method of embodiment 11, wherein X2 is not D-Ser.

13. The method of embodiment 11, wherein the polar D-amino acid is selected from the group consisting of D-Thr and D-Met

14. The method of embodiment 4, wherein X2 is glycine.

15. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a compound of the formula V:

R^1-X-Z-R^2 (formula V);

wherein

R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

X is a peptide which has the formula VI:

His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-X16-X17-Arg-Ala-X20-Asp-Phe-Ile-Glu-Trp-Leu-X27-X28-X29 (formula VI);

wherein

X2 is selected from Ser and Aib;

X16 is selected from Glu and Y;

X17 is selected from Arg and Y;

X20 is selected from Lys and Y;

X27 is selected from Leu and Y;

X28 is selected from Ser and Y or absent;

X29 is Ala or absent;

wherein at least one of X16, X17, X20, X27 and X28 is Y;

wherein each residue Y is independently selected from Lys, Cys and Orn;

wherein the side chain of at least one amino acid residue Y of X is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Y; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Y via Z^2;

and Z is absent or is a sequence of 1-20 amino acid units independently selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Cys, Glu, Lys, Arg, Dbu, Dpr and Orn;

or a pharmaceutically acceptable salt thereof.

16. The method of embodiment 15, wherein the GGDA compound comprises a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDERRAKDFIEWLKSA ;

HSQGTFTSDYSKYLDERRAKDFIEWLLSA;

HSQGTFTSDYSKYLDERRAKDFIEWLLKA;

HSQGTFTSDYSKYLDKRRAKDFIEWLLSA;

HSQGTFTSDYSKYLDEKRAKDFIEWLLSA; and

H-Aib-QGTFTSDYSKYLDEKRAKDFIEWLLSA.

17. The method of embodiment 15, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDERRAKDFIEWL-K(Hexadecanoyl-isoGlu)-SA-NH_2;

HSQGTFTSDYSKYLDERRA-K(Hexadecanoyl-isoGlu)-DFIEWLLSA-NH_2;

HSQGTFTSDYSKYLDERRAKDFIEWLL-K(Hexadecanoyl-isoGlu)-A-NH_2;

HSQGTFTSDYSKYLD-K(Hexadecanoyl-isoGlu)-RRAKDFIEWLLSA-NH_2;

HSQGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA-NH_2; and

H-Aib-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA-NH_2.

18. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a compound of the formula VII:

R^1-Z^3-R^2 (formula VII)

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula VIII:

His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-X12-Tyr-Leu-Asp-X16-X17-Ala-Ala-X20-X21-Phe-Val-X24-Trp-Leu-X27-X28-Ala-X30 (formula VIII);

wherein

X2 is selected from Aib and Ser;

X12 is selected from Lys, Arg or Leu;

X16 is selected from Arg and X;

X17 is selected from Arg and X;

X20 is selected from Arg, His and X;

X21 is selected from Asp and Glu;

X24 is selected from Ala and X;

X27 is selected from Leu and X;

X28 is selected from Arg and X;

X30 is X or is absent;

wherein at least one of X16, X17, X20, X24, X27, X28, and X30 is Xa;

and wherein each residue Xa is independently selected from the group consisting of Glu, Lys, Ser, Cys, Dbu, Dpr and Orn;

wherein the side chain of at least one residue Xa is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Xa; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Xa via Z^2;

with the proviso that Z^3 is not HSQGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA.

19. The method of embodiment 18, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDKKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAKDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLKRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLKA;

HSQGTFTSDYSRYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSLYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLRAK;

HSQGTFTSDYSKYLDSKAAHDFVEWLLSAK

HSQGTFTSDYSKYLDSKAAHDFVEWLKSA;

HSQGTFTSDYSKYLDSKAAHDFVKWLLRA;

HSQGTFTSDYSKYLDSCAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSCAAHDFVEWLLSA;

HSQGTFTSDYSKYLDSKAACDFVEWLLRA;

HSQGTFTSDYSKYLDKSAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSAK;

H-Aib-QGTFTSDYSKYLDSKAARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAKDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLKA

H-Aib-QGTFTSDYSKYLDSKAAKDFVAWLLSA

H-Aib-QGTFTSDYSKYLDSKAAHDFVAWLLKA;

H-Aib-QGTFTSDYSKYLDKKAAHDFVAWLLRA;

H-Aib-QGTFTSDYSRYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVKWLLSA;

H-Aib-QGTFTSDYSLYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSCAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAACDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDK()KAAE()DFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVE()WLLK()A

H-Aib-QGTFTSDYSKYLDSKAAK()DFVE()WLLRA;

H-Aib-QGTFTSDYSKYLDSK()AAHE()FVEWLLKA; and

H-Aib-QGTFTSDYSKYLDSK()AAKE()FVEWLLRA;

wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

20. The method of embodiment 18, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLD-K*-KAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAA-K*-DFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K*-RA;

HSQGTFTSDYSKYLDSKAAHDFVEWLL-K*-A;

HSQGTFTSDYSRYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSLYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLRA-K*;

HSQGTFTSDYSKYLDSKAAHDFVEWLLSA-K*;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K*-SA;

HSQGTFTSDYSKYLDSKAAHDFV-K*-WLLRA;

HSQGTFTSDYSKYLDS-C*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLDS-C*-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDSKAA-C*-DFVEWLLRA;

HSQGTFTSDYSKYLD-K*-SAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSA-K*;

H-Aib-QGTFTSDYSKYLDS-K*-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAA-K*-DFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLL-K*-A;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLKA;

H-Aib-QGTFTSDYSKYLDSKAA-K*-DFVAWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVAWLL-K*-A;

H-Aib-QGTFTSDYSKYLD-K*-KAAHDFVAWLLRA;

H-Aib-QGTFTSDYSRYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFV-K*-WLLSA;

H-Aib-QGTFTSDYSLYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-C*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAA-C*-DFVEWLLRA;

H-Aib-QGTFTSDYSKYLD-S*-KAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDK()K*AAE()DFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSK*AAHDFVE()WLLK()A

H-Aib-QGTFTSDYSKYLDSK*AAK()DFVE()WLLRA;

H-Aib-QGTFTSDYSKYLDSK()AAHE()FVEWLLK*A; and

H-Aib-QGTFTSDYSKYLDSK()AAK*E()FVEWLLRA;

wherein "*" indicates the position of a lipophilic substituent on the residue immediately to the left of the "*", and wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

21. The method of embodiment 18, wherein Z^3 has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLD-K(Hexadecanoyl-γ-Glu)-KAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K(Hexadecanoyl-γ-Glu)-RA;

HSQGTFTSDYSKYLDSKAA-K(Hexadecanoyl-γ-Glu)-DFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-γ-Glu)-A;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-γ-Glu)-A;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLKA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVE()WLLK()A;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAA-K(Hexadecanoyl-γ-Glu)-DFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Dodecanoyl-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[3-aminopropanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[8-aminooctanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-ε-Lys)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K([2-Butyloctanoyl]-γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-[4-Aminobutanoyl])-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-E)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K([2-Butyloctanoyl]-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[4-Aminobutanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA; and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-E)-AAHDFVEWLLSA;

wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

22. The method of embodiment 18, wherein Z^3 comprises a sequence selected from the group consisting of:

H-Aib-QGTFTSDYS-K(Hexadecanoyl-isoGlu)-YLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLD-K(Hexadecanoyl-isoGlu)-KAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAA-K(Hexadecanoyl-isoGlu)-DFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFV-K(Hexadecanoyl-isoGlu)-WLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoLys)-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAKDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHEFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAEDFVEWLLSA; and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLEA.

23. The method of any one of embodiments 19-21, wherein () represents a lactam ring between the side chains of the two residues.

24. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a compound of the formula IX:

R^1-Z^3-R^2 (formula IX);

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula X

His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-X17-Ala-Ala-His-Asp-Phe-Val-Glu-Trp-Leu-Leu-X28 (formula X);

wherein

X17 is Xb

X28 is Ser or absent;

wherein Xb is selected from the group consisting of Glu, Lys, and Cys;

and wherein the side chain of Xb is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Xb; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Xb via Z^2.

25. The method of embodiment 24, wherein Z^3 has a sequence selected from the group consisting of:

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLS and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLL.

26. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a compound of the formula XI:

R^1-Z^3-R^2 (formula XI);

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula XII:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-X17-Ala-Ala-His-Asp-Phe-Val-Glu-Trp-Leu-Leu-Ser-Ala (formula XII);

wherein

X17 is X;

wherein X is selected from the group consisting of Glu, Lys, and Cys;

and wherein the side chain of X is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of X; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of X via Z^2.

27. The method of embodiment 26, wherein Z^3 has a sequence:

H-Aib-EGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA.

28. The method of any one of embodiments 1-3, wherein the GGDA compound comprises a sequence selected from the group consisting of:
H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA;
HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA;

HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA; and H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA.

29. The method of embodiments 1 or 2, wherein the GGDA compound is a full glucagon receptor agonist as determined by a pERK assay or a Ca^{2+} assay.

30. A method of treating or preventing a metabolic disease or condition, e.g., a disease or condition caused or characterized by excess body weight, e.g., obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, pre-diabetes, insulin resistance, glucose intolerance, type 2 diabetes, type I diabetes, hypertension, atherogenic dyslipidaemia, atherosclerois, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease, comprising administering a treatment-effective amount of a GGDA compound identified by a method of any one of embodiments 1-6.

31. A GGDA compound having the formula:

H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA (Compound 17).

32. A GGDA compound having the formula:

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 18).

33. A GGDA compound having the formula:

HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 19).

34. A GGDA compound having the formula:

H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 20).

Brief Description of the Drawings

[0014] Figure 1 shows the sequences of certain Glu-GLP1 dual agonist (GGDA) compounds employed in the context of the present invention, and data for full (F) or partial (P) agonism of the Glucagon Receptor (Glu R) and GLP-1 Receptor (GLP-1 R) pCa^{2+} and pERK pathways. Aib, α-aminoisobutyric acid; an asterix (*) represents a site wherein the lysine is attached to hexadecanoyl-γ-Glu.

[0015] Figure 2 shows dosage response curves for the GLP-1 R pCa^{2+} assay.

[0016] Figure 3 shows dosage response curves for the GLP-1 R pERK pathway.

[0017] Figure 4 shows dosage response curves for the Glucagon R pCa^{2+} assay.

[0018] Figure 5 shows dosage response curves for the Glucagon R pERK pathway.

[0019] Figure 6 shows EC_{50} values for the Glucagon R and GLP-1 R cAMP pathway.

[0020] Figure 7 shows dosage response curves for the GLP-1 R true Ca^{2+} assay.

[0021] Figure 8 shows dosage response curves for the Glucagon R true Ca^{2+} assay.

[0022] Figure 9 shows a head to head comparison of Aib in position 2 of Compound 9 versus serine in position 2 (Compound 17) which demonstrates that a GGDA with serine has reduced Glucagon Receptor efficacy relative to endogenous human Glucagon. The Aib in position 2 further reduces the *in vitro* efficacy significantly and turns the compound into a partial agonist with reduced EC_{50} and reduced E_{max}. This observation is found in both HEK293 (Human embryonic kidney cells) (top figure 9) and CHO-K1 (Chinese Hamster Ovary cells) (bottom Figure 9) based cellular assay systems recombinantly expressing the human Glucagon Receptor, albeit being more pronounced in the CHO cellular background.

[0023] Figure 10 shows a head to head comparison of Aib in position 2 of Compound 7 versus serine in position 2 (Compound 4) which demonstrates that a GGDA with serine has reduced Glucagon Receptor efficacy relative to endogenous human Glucagon. The Aib in position 2 further reduces the *in vitro* efficacy significantly and turns the compound into a partial agonist with reduced EC_{50} and reduced E_{max}. This observation is found in both HEK293 and CHO based cellular

assay systems recombinantly expressing the human Glucagon Receptor, albeit being more pronounced in the CHO cellular background.

[0024] Figure 11 shows a head to head comparison of Aib in position 2 of Compound 8 versus serine in position 2 (Compound 5) which demonstrates that a GGDA with serine has reduced Glucagon Receptor efficacy relative to endogenous human Glucagon. The Aib in position 2 further reduces the *in vitro* efficacy significantly and turns the compound into a partial agonist with reduced EC_{50} and reduced E_{max}. This observation is found in both HEK293 and CHO based cellular assay systems recombinantly expressing the human Glucagon Receptor, albeit being more pronounced in the CHO cellular background.

[0025] Figure 12 shows GGDAs with signaling selective properties identified with the GluR calcium assay. The GGDA Cpd 17 has a serine in position 2 (Ser2) and has a slight reduced potency compared to human glucagon but still with full agonistic properties. By contrast, substituting the Ser2 for Aib (Cpd 9) or Glycine (Cpd 19) or D-ala (Cpd 20) further reduces the potency (EC_{50}) but also turns the compounds into partial agonists with significantly reduced E_{max}.

[0026] Figure 13 shows shows *in vivo* profiling of GGDAs with full (F) versus partial (P) agonism at the Glucagon Receptor when assayed for intracellular calcium release. The compounds were compared head to head with a natural serine in position 2 versus an Aib in position 2, i.e. Compound 4 versus Compound 7 and Compound 5 versus Compound 8.

[0027] Figure 14 shows the data relating to Figure 12. Compounds were profiled with cAMP, intracellular calcium release and phospho-ERK1/2 (pERK), respectively. All compounds were full and potent (EC_{50} under 1 nM) agonists for the cAMP pathway, whereas the compounds differentiate into full (F) and partial (P) agonists at both the Calcium release and pERK pathways activated via ligand binding to the Glucagon receptor.

[0028] Figure 15 shows the data relating to Figure 11. Compounds were profiled with cAMP, intracellular calcium release and phospho-ERK1/2 (pERK), respectively. All compounds were full and potent (EC_{50} under 1 nM) agonists for the cAMP pathway, whereas the compounds differentiate into full and partial

agonists at both the Calcium release and pERK pathways activated via ligand binding to the Glucagon receptor.

Detailed Description of the Invention

[0029] We have discovered unique properties associated with some Glu-GLP1 dual agonists (GGDAs) when assaying for their activation of alternative intracellular signalling pathways through the Glucagon receptor (Glu R). While most GGDAs vary just in their potency at the Glu R, four compounds have been identified as partial agonists rather than full agonists, eliciting a submaximal response, *i.e.*, the E_{max} of each compound is lower than E_{max} for the control compound of the receptor, presumably caused by generation of an alternative conformational state of the receptor induced by the ligand-receptor interaction. Of the 20 GGDAs profiled at multiple pathways so far, six (Compound 7, Compound 8, Compound 9, Compound 10, Compound 19 and Compound 20) can be functionally separated into a group of partial Glucagon agonists by measures of the alternative signaling pathways (e.g., MAPK activation and intracellular Ca^{2+} release), whereas they are full agonists by measures of the classical signaling pathway of cellular cAMP accumulation.

Agonism and antagonism

[0030] Full and partial agonism and antagonism associated with methods and compositions of the invention may be determined by any means known to one of skill in the art. In particular, the skilled worker may determine full and partial agonism and antagonism by calculating the E_{max} value of a test compound and comparing this value to a reference standard. In some embodiments, a test compound is a full agonist for one or more pathways being used in a test for agonism. The ratio of test:reference E_{max} values for a partial agonist or antagonist may be about 0.95 or less. In some embodiments, this ratio is about 0.95 or less, about 0.90 or less, about 0.85 or less, about 0.80 or less, about 0.75 or less, about 0.70 or less, about 0.65 or less, about 0.60 or less, about 0.55 or less, about 0.50 or less, about 0.45 or less, about 0.40 or less, about 0.35 or less, about 0.30 or less, about 0.25 or less, about 0.20 or less, about 0.15 or less, about 0.10 or less, about

0.05 or less, or about 0.001. In preferred embodiments, the ratio is about 0.50 or less, about 0.45 or less, about 0.40 or less, about 0.35 or less, about 0.30 or less, about 0.25 or less, about 0.20 or less, about 0.15 or less, about 0.10 or less, about 0.05 or less, or about 0.00. The ratio of test:reference E_{max} values for a full agonist or antagonist may be about 0.95 or greater. In some embodiments, this ratio is about 0.95 or greater or about 1.00.

Preferential pathway activation and inhibition

[0031] GGDA compounds employed in the context of the invention exhibit preferential pathway activation, preferential pathway inhibition, or can work in either direction depending on the particular pathway. Exemplary pathways include MAPK (pERK1/2), release of intracellular calcium, β-arrestin 1 and 2 recruitment and signaling, inositol phosphate turnover, CaMKII, and calcineurin. One of skill in the art will be able to select other pathways based on the desired properties of a GGDA compound. Preferential pathway activation, as used herein, refers to the activation of two or more pathways by a GGDA compound, wherein the GGDA compound is a full agonist for at least one of the two or more pathways and a partial agonist, partial antagonist, or full antagonist for, or has no effect on, at least one of the other pathways. Preferential pathway activation also may refer to the activation of two or more pathways by a GGDA compound, wherein the GGDA compound is a partial agonist for at least one of the two or more pathways and a full agonist, partial antagonist, or full antagonist for, or has no effect on, at least one of the other pathways. Preferential pathway inhibition, as used herein, refers to the inhibition of two or more pathways by a GGDA compound, wherein the GGDA compound is a full antagonist for at least one of the two or more pathways and a partial antagonist, partial agonist, or full agonist for, or has no effect on, at least one of the other pathways. Preferential pathway inhibition also may refer to the inhibition of two or more pathways by a GGDA compound, wherein the GGDA compound is a partial antagonist for at least one of the two or more pathways and a partial antagonist, partial agonist, or full agonist for, or has no effect on, at least one of the other pathways. In general, the GGDA concept holds the important feature of GLP1R and GluR opposing each others' actions in glucose

control. The GLP1R component of the GGDA prevents or opposes GluR mediated hyperglycemia and therefore mitigates the hyperglycemic risk associated with Glucagon receptor activation while maintaining the GluR mediated additional body weight reduction as compared to a GLP1R agonist alone. Further to this, a GGDA compound may confer beneficial physiological effects associated with or imparted by the GGDA's preferential pathway inhibition. For example, administering a partial GGDA Ca^{2+} agonist to an individual may maintain a high level of glucose control (reflected in improved HbA1c measurements) in the individual, while also inducing weight loss, albeit to a lesser extent than a full GGDA Ca^{2+} agonist, and in this manner result in an overall superior diabetes treatment. Also, the acute effects of partial GluR agonists on the liver differ from the acute effects of full GluR agonists. For example, less glucose is released in response to a GGDA being a partial GluR agonist as compare to a GGDA with full GluR agonist properties. The reduced acute lived glucose release by a GGDA with partial GluR properties can lead to less glucose fluctuations i.e. both in number and intensity and, thus, reduced HbA1c levels implying overall improved glucose control. This occurs while still maintaining a significant body weight loss by the remaining glucagon effects on the liver and in the adipose tissue. The full GluR agonists may produce a greater loss in body weight, but be inferior in terms of blood glucose control and, therefore likely not reduce HbA1c to the same extent as the GGDAs with partial GluR agonistic properties. A partial GGDA Ca2+ agonist, thus, may be preferred for the treatment of Diabetes e.g., for blood glucose control and weight loss, whereas a full GGDA Ca^{2+} agonist may be preferred for the treatment of obesity.

GGDA compounds

[0032] In some embodiments, the GGDA compounds employed in the context of the present invention are glucagon analogs that are Glucagon R-GLP-1 R dual agonists. Exemplary dual agonists are described in, e.g., WO2008/101017. The majority of those compounds are more similar in length to glucagon than to oxyntomodulin (OXM), being around 29 amino acids long, and so can be regarded as analogs of glucagon. Others, however are longer. Further examples of dual agonists are described in WO2009/155257 and WO2009/155258. Still further dual

agonists are described in WO2008/152403, WO2011/006497, PCT/GB2008/004132, PCT/GB2008/004121, PCT/GB2008/004157, PCT/GB2008/004130, U.S. Provisional Application No. 61/358,623, and European Patent Application No. 09251780.4. Any of the dual agonists described in the aforementioned documents that exhibit preferential pathway activation or inhibition may be suitable for use in the methods of the invention as described herein. Each of these patent publications is hereby incorporated by reference in its entirety.

[0033] In some embodiments, a GGDA compound employed in the context of the invention exhibits preferential receptor signaling pathway activation. In some embodiments, a GGDA compound employed in the context of the invention exhibits preferential receptor signaling pathway inhibition. In some embodiments, a GGDA compound employed in the context of the invention exhibits preferential receptor signaling pathway activation and/or inhibition along different pathways. In certain embodiments, a GGDA compound is a glucagon analog, and the compound has been affirmatively selected based on defined preferential pathway activation and/or inhibition. In certain of these embodiments, the defined preferential pathway activation or inhibition is determined by measuring maximal efficiency (E_{Max}) values of a test GGDA compound in comparison to a control compound. In certain of these embodiments, the E_{Max} values of a GGDA compound and a control compound are compared to provide a ratio. In general, E_{Max} values for GGDA compounds employed in the context of the invention will have statistically significant differences from E_{Max} values for a control compound at a particular receptor. A ratio between E_{Max} values for GGDA compounds employed in the context of the invention and E_{Max} values for a control compound can be in the range of: about 0.95 or less, about 0.90 or less, about 0.85 or less, about 0.80 or less, about 0.75 or less, about 0.70 or less, about 0.65 or less, about 0.60 or less, about 0.55 or less, about 0.50 or less, about 0.45 or less, about 0.40 or less, about 0.35 or less, about 0.30 or less, about 0.25 or less, about 0.20 or less, about 0.15 or less, about 0.10 or less, about 0.05 or less, and about 0.00. In some embodiments, a ratio between E_{Max} values for a GGDA compound employed in the context of the invention and E_{Max} values for a control compound for a first pathway

will have a statistically significant difference from a ratio between E_{Max} values for a GGDA compound employed in the context of the invention and E_{Max} values for a control compound for a second pathway. In some embodiments, a GGDA compound employed in the context of the invention is selected from the group consisting of: Compound 4, Compound 5, Compound 6, Compound 7, Compound 8, Compound 9, Compound 10, Compound 11, Compound 12, Compound 13, Compound 14, Compound 15, Compound 16, Compound 17, Compound 18, Compound 19, and Compound 20 (see table below for sequences). In certain of these embodiments, the GGDA compound is selected from the group consisting of: Compound 7, Compound 8, Compound 9, Compound 10, Compound 17, Compound 18, Compound 19, and Compound 20. The skilled worker will understand that a GGDA compound may be identified by a method of the invention and subsequently modified or optimized for one or more particular properties, including stability, solubility, half life, reduced immunogenicity, agonist or antagonist activity, reducing side effects upon administration, and pharmacokinetic or pharmacodynamic properties. Types of modifications include, for example, mutations (e.g., additional, deletion, or substitution), acylation, and PEGylation.

[0034] A GGDA compound also may be a prodrug. Prodrugs may have benefits such as improved absorption, distribution, metabolism, and excretion profiles. For example, one pharmacokinetic parameter that may be altered by generating a GGDA compound in prodrug format is increased half life. A GGDA prodrug may remain in prodrug form for at least about 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 24 hours, 36 hours, 48 hours, 60 hours, or 72 hours. In some embodiments the half life of a prodrug is about 100 hours or greater, for example, a half life of up to about 168, 336, 504, 672, or 720 hours. In some embodiments, a GGDA prodrug is converted to the active form at physiological conditions through a non-enzymatic reaction. In some embodiments the non-enzymatic activation $t_{1/2}$ of the prodrug is between 1 and 100 hrs, for example, between 12 and 72 hours between 12 and 48 hours, or between 48 and 72 hours. A $t_{1/2}$ may be measured by

incubating the prodrug in a phosphate buffer solution (e.g., PBS) at 37 °C and a pH of 7.2.

Compound	Sequence
hGlucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
1	HSQGTFTSDYSKYLDRARADDFVAWLKST
2	HSQGTFTSDYSKYLDSKAAHDFVEWLLRA
3	HSQGTFTSDYSKYLDERRAKDFIEWLLSA
4	HSQGTFTSDYSKYLDS-Lys(isoGlu(Palmitoyl))-AAHDFVEWLLRA
5	HSQGTFTSDYSKYLDE-K(isoGlu(Palmitoyl))-RAKDFIEWLLSA
6	HSQGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-isoGlu)-A
7	H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLRA
8	H-Aib-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA
9	H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA
10	H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-isoGlu)-A
11	HSQGTFTSDYSKYLDRARAEDFVAWLKST
12	HSQGTFTSDYSKYLDRARADDFVAWLERA
13	HSQGTFTSDYSKYLDSKAARDFVRWLKLA
14	HSQGTFTSDYSKYLDSRRAQDFVQWLMNTKRNRNNIA(oxyntomodulin)
15	H-DSer-QGTFTSDYSKYLDSKAAHDFVEWLLRA
16	H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLRA
17	H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA
18	HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA
19	HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA
20	H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA

[0035] In some embodiments, a GGDA compound of the invention does not comprise a D-amino acid, for example, D-Ser. In particular embodiments, a GGDA compound of the invention does not comprise D-Ser at an amino acid position corresponding to amino acid 2 of native glucagon. Without wishing to be bound by theory, substituting an L amino acid for a D amino acid at an amino acid

position corresponding to amino acid 2 of native glucagon may not confer preferential pathway activation or inhibition.

[0036] It should be noted that, as used herein in relation to compounds of the invention, –NH2 and $–NH_2$ are used interchangeably, and a terminal –NH2 or $–NH_2$ group represents an amino group. In some embodiments, a GGDA compound may comprise a C-terminal amino group. For example, a GGDA compound may have the sequence H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA-NH_2, HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA-NH_2, HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA-NH_2, or H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA-NH_2. In some embodiments, a GGDA compound may comprise an N-terminal hydrogen (H-).

Methods of screening

[0037] In some embodiments, the invention provides a method of screening GGDA compounds for preferential pathway activation or inhibition. In certain embodiments, the methods comprise screening for a full glucagon receptor agonist in a first glucagon receptor assay and identifying a full glucagon receptor agonist of a first pathway, screening for a partial glucagon receptor agonist in a second glucagon receptor assay and identifying a partial glucagon receptor agonist of a second pathway, and screening for a full GLP-1 receptor agonist in a first GLP-1 receptor assay and identifying a full GLP-1 receptor agonist of a GLP-1 receptor mediated pathway. In some embodiments, the GLP-1 receptor mediated pathway may or may not be the same pathway as the first pathway or the second pathway. In some embodiments, the first and second glucagon receptor assays and the GLP-1 receptor assay are selected from the group consisting of: a cAMP assay, a pERK assay, a Ca^{2+} assay, a β-arrestin 1 assay, a β-arrestin 2 assay, a receptor internalization assay, an inositol phosphate turnover assay, a CaMKII assay, and a calcineurin assay. In some embodiments, the GGDA is a partial glucagon receptor agonist as determined by a cAMP assay, a pERK assay, a Ca^{2+} assay, a β-arrestin 1

assay, a β-arrestin 2 assay, a receptor internalization assay, an inositol phosphate turnover assay, a CaMKII assay, and a calcineurin assay.

Lipophilic substituents

[0038] One or more of the amino acid side chains in a compound employed in the context of the invention may be conjugated to a lipophilic substituent Z^1. Without wishing to be bound by theory, it is thought that the lipophilic substituent binds albumin in the blood stream, thus shielding the compounds employed in the context of the invention from enzymatic degradation which can enhance the half-life of the compounds. The lipophilic substituent may also modulate the potency of the compound, *e.g.*, with respect to the glucagon receptor and/or the GLP-1 receptor.

[0039] In certain embodiments, only one amino acid side chain is conjugated to a lipophilic substituent. In other embodiments, two amino acid side chains are each conjugated to a lipophilic substituent. In yet further embodiments, three or even more amino acid side chains are each conjugated to a lipophilic substituent. When a compound contains two or more lipophilic substituents, they may be the same or different.

[0040] The lipophilic substituent Z^1 may be bonded covalently to an atom in the amino acid side chain, or alternatively may be conjugated to the amino acid side chain by a spacer Z^2.

[0041] The term "conjugated" is used herein to describe the covalent attachment of one identifiable chemical moiety to another, and the structural relationship between such moieties. It should not be taken to imply any particular method of synthesis. The spacer Z^2, when present, is used to provide a spacing between the compound and the lipophilic moiety.

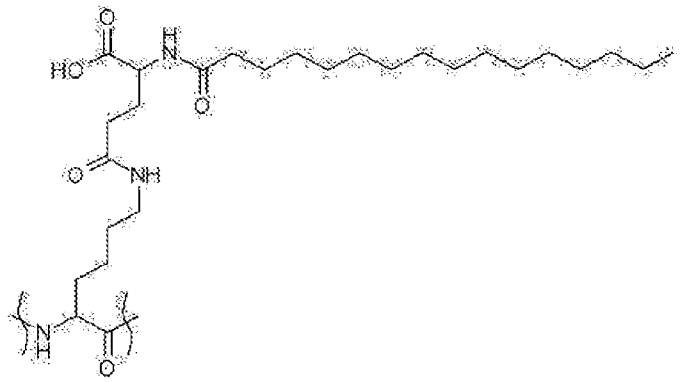
[0042] The lipophilic substituent may be attached to the amino acid side chain or to the spacer via an ester, a sulphonyl ester, a thioester, an amide or a sulphonamide. Accordingly it will be understood that preferably the lipophilic substituent includes an acyl group, a sulphonyl group, an N atom, an O atom or an S atom which forms part of the ester, sulphonyl ester, thioester, amide or sulphonamide. Preferably, an acyl group in the lipophilic substituent forms part of

an amide or ester with the amino acid side chain or the spacer. The lipophilic substituent may include a hydrocarbon chain having 10 to 24 carbon (C) atoms, e.g. 10 to 22 C atoms, e.g. 10 to 20 C atoms. Preferably it has at least 11 C atoms, and preferably it has 18 C atoms or fewer. For example, the hydrocarbon chain may contain 12, 13, 14, 15, 16, 17 or 18 carbon atoms. The hydrocarbon chain may be linear or branched and may be saturated or unsaturated. From the discussion above it will be understood that the hydrocarbon chain is preferably substituted with a moiety which forms part of the attachment to the amino acid side chain or the spacer, for example an acyl group, a sulphonyl group, an N atom, an O atom or an S atom. Most preferably the hydrocarbon chain is substituted with acyl, and accordingly the hydrocarbon chain may be part of an alkanoyl group, for example a dodecanoyl, 2-butyloctanoyl, tetradecanoyl, hexadecanoyl, heptadecanoyl, octadecanoyl or eicosanoyl group. As mentioned above, the lipophilic substituent Z^1 may be conjugated to the amino acid side chain by a spacer Z^2. When present, the spacer is attached to the lipophilic substituent and to the amino acid side chain. The spacer may be attached to the lipophilic substituent and to the amino acid side chain independently by an ester, a sulphonyl ester, a thioester, an amide or a sulphonamide. Accordingly, it may include two moieties independently selected from acyl, sulphonyl, an N atom, an O atom or an S atom. The spacer may consist of a linear C1-10 hydrocarbon chain or more preferably a linear C1-5 hydrocarbon chain. Furthermore, the spacer may be substituted with one or more substituents selected from C1-6 alkyl, C1-6 alkyl amine, C1-6 alkyl hydroxy and C1-6 alkyl carboxy.

[0043] The spacer may be, for example, a residue of any naturally occurring or unnatural amino acid. For example, the spacer may be a residue of Gly, Pro, Ala, Val, Leu, Ile, Met, Cys, Phe, Tyr, Trp, His, Lys, Arg, Gln, Asn, α-Glu, γ-Glu, ε-Lys, Asp, Ser, Thr, Gaba, Aib, β-Ala (i.e. 3-aminopropanoyl), 4-aminobutanoyl, 5-aminopentanoyl, 6-aminohexanoyl, 7-aminoheptanoyl, 8-aminooctanoyl, 9-aminononanoyl, 10-aminodecanoyl or 8-amino-3,6-dioxaoctanoyl. In certain embodiments, the spacer is a residue of Glu, γ-Glu, ε -Lys, β-Ala (i.e. 3-aminopropanoyl), 4-aminobutanoyl, 8- aminooctanoyl or 8-amino-3,6-dioxaoctanoyl. In the present invention, γ-Glu and isoGlu are used

interchangeably. The amino acid side chain to which the lipophilic substituent is conjugated is preferably a side chain of a Glu, Lys, Ser, Cys, Dbu, Dpr or Orn residue. For example, it may be a side chain of a Lys, Glu or Cys residue. Where two or more side chains carry a lipophilic substituent, they may be independently selected from these residues. Thus, the amino acid side chain includes a carboxy, hydroxyl, thiol, amide or amine group, for forming an ester, a sulphonyl ester, a thioester, an amide, or a sulphonamide with the spacer or lipophilic substituent.

[0044] An example of a lipophilic substituent comprising a lipophilic moiety Z^1 and spacer Z^2 is shown in the formula below:



[0045] Here, the side chain of a Lys residue is covalently attached to a γ-Glu spacer (Z^2) via an amide linkage. A hexadecanoyl group (Z^1) is covalently attached to the γ -Glu spacer via an amide linkage. This combination of lipophilic moiety and spacer, conjugated to a Lys residue, may be referred to by the short-hand notation K(Hexadecanoyl-γ-Glu), *e.g.*, when shown in formulae of specific compounds. γ-Glu may also be referred to as isoGlu, and a hexadecanoyl group as a palmitoyl group. Thus it will be apparent that the notation (Hexadecanoyl-γ-Glu) is equivalent to the notations (isoGlu(Palm)) or (isoGlu(Palmitoyl)) as used for example in PCT/GB2008/004121.

[0046] The skilled person will be well aware of suitable techniques for preparing the compounds employed in the context of the invention. For examples of suitable chemistry, see, e.g., WO98/08871, WO00/55184, WO00/55119, Madsen et al (*J. Med. Chem.* 2007, 50, 6126-32), and Knudsen et al. 2000 (*J. Med Chem.* 43, 1664-1669).

Non-proteinogenic amino acids

[0047] One or more of the amino acids of a compound of the invention may be a non-proteinogenic amino acid. Non-proteinogenic amino acids may include those amino acids not encompassed by the 20 "standard" amino acids used in protein synthesis, *e.g.*, alanine, arginine, aspartate, asparagine, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine. Examples of non-proteinogenic amino acids include, but are not limited to, para amino benzoic acid (PABA), 2-amino benzoic acid, anthranilic acid, p-hydroxybenzoic acid (PHBA), 3-amino benzoic acid, 4-aminomethyl benzoic acid, 4-amino salicylic acid (PAS), 4-amino cyclohexanoic acid 4-amino-phenyl acetic acid, 4-amino-hippuric acid, 4-amino-2-chlorobenzoic acid, 6-aminonicotinic acid, methyl-6-aminonicotinate, 4-amino methyl salicylate, 2-amino thiazole-4-acetic acid, 2-amino-4-(2-aminophenyl)-4-oxobutanoic acid (L-kynurenine), O-methyl serine, acetylamino alanine, β-alanine, β-(acetylamino)alanine, β-aminoalanine, β-chloroalanine, citrulline, homocitrulline, hydroxyproline, homoarginine, homoserine, homotyrosine, homoproline, ornithine, 4-amino-phenylalanine, sarcosine, biphenylalanine, homophenylalanine, 4-nitro-phenylalanine, 4-fluoro-phenylalanine, 2,3,4,5,6-pentafluoro-phenylalanine, norleucine, cyclohexylalanine, *N*-methyl-alanine, *N*-methyl-glycine, *N*-methyl-glutamic acid, tert-butylglycine, α-aminobutyric acid, α-aminoisobutyric acid (AIB), 2-aminoisobutyric acid, 2-aminoindane-2-carboxylic acid, selenomethionine, lanthionine, dehydroalanine, γ-aminobutyric acid, naphthylalanine, aminohexanoic acid, phenylglycine, pipecolic acid, 2,3-diaminoproprionic acid, tetrahydroisoquinoline-3-carboxylic acid, taurine, tert-leucine, tert-butylalanine, cyclohexylglycine, diethylglycine, and dipropylglycine.

C-terminal amidation

[0048] The major biologically active fragment of GLP-1 is produced as a 30-amino acid, peptide GLP-1-(7-36), which may be C-terminally amidated GLP-1-(7-36)NH_2. In some embodiments, a GGDA compound employed in the context of

the present invention may similarly comprise a C-terminal modification, *e.g.*, amidation.

Clinical utility

[0049] The compounds employed in the context of the invention may provide an attractive treatment option for metabolic diseases including obesity and diabetes mellitus (diabetes). Diabetes comprises a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Acute signs of diabetes include excessive urine production, resulting compensatory thirst and increased fluid intake, blurred vision, unexplained weight loss, lethargy, and changes in energy metabolism. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, notably the eyes, kidneys, nerves, heart and blood vessels. Diabetes is classified into type 1 diabetes, type 2 diabetes and gestational diabetes on the basis of pathogenetic characteristics. Type 1 diabetes accounts for 5-10% of all diabetes cases and is caused by auto-immune destruction of insulin-secreting pancreatic β-cells.

[0050] Type 2 diabetes accounts for 90-95% of diabetes cases and is a result of a complex set of metabolic disorders. Type 2 diabetes is the consequence of endogenous insulin production becoming insufficient to maintain plasma glucose levels below the diagnostic thresholds.

[0051] Gestational diabetes refers to any degree of glucose intolerance identified during pregnancy.

[0052] Pre-diabetes includes impaired fasting glucose and impaired glucose tolerance and refers to those states that occur when blood glucose levels are elevated but below the levels that are established for the clinical diagnosis for diabetes.

[0053] A large proportion of people with type 2 diabetes and pre-diabetes are at increased risk of morbidity and mortality due to the high prevalence of additional metabolic risk factors, including abdominal obesity (excessive fat tissue around the abdominal internal organs), atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and/or high LDL cholesterol,

which foster plaque buildup in artery walls), elevated blood pressure (hypertension) a prothrombotic state (e.g. high fibrinogen or plasminogen activator inhibitor-1 in the blood), and/or a proinflammatory state (e.g., elevated C-reactive protein in the blood).

[0054] Conversely, obesity confers an increased risk of developing pre-diabetes, type 2 diabetes as well as, e.g., certain types of cancer, obstructive sleep apnea and gall-bladder disease. Dyslipidemia is associated with increased risk of cardiovascular disease. High Density Lipoprotein (HDL) is of clinical importance since an inverse correlation exists between plasma HDL concentrations and risk of atherosclerotic disease. The majority of cholesterol stored in atherosclerotic plaques originates from LDL and hence elevated concentrations Low Density Lipoproteins (LDL) is closely associated with atherosclerosis. The HDL/LDL ratio is a clinical risk indictor for atherosclerosis and coronary atherosclerosis in particular.

[0055] Compounds employed in the context of the invention act as GluGLP-1 dual agonists (GGDAs) at the Glucagon and GLP-1 receptors. The dual agonist may combine the effect of glucagon *e.g.*, on fat metabolism with the effect of GLP-1 *e.g.*, on blood glucose levels and food intake. They may therefore act to accelerate elimination of excessive adipose tissue, induce sustainable weight loss, and improve glycemic control. Dual GluGLP-1 agonists may also act to reduce cardiovascular risk factors such as high cholesterol, and high LDL-cholesterol or abnormal HDL/LDL ratios.

[0056] The compounds of the present invention can therefore be used as pharmaceutical agents for preventing weight gain, promoting weight loss, reducing excess body weight or treating obesity (e.g. by control of appetite, feeding, food intake, calorie intake, and/or energy expenditure), including morbid obesity, as well as associated diseases and health conditions including but not limited to obesity linked inflammation, obesity linked gallbladder disease and obesity induced sleep apnea. The compounds employed in the context of the invention may also be used for treatment of insulin resistance, glucose intolerance, pre-diabetes, increased fasting glucose, type 2 diabetes, hypertension, dyslipidemia (or a combination of these metabolic risk factors), glucagonomas, congestive heart

failure, atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease and stroke. These are all conditions which can be associated with obesity. However, the effects of the compounds employed in the context of the invention on these conditions may be mediated in whole or in part via an effect on body weight, or may be independent thereof.

[0057] The compounds of the invention also can be used as pharmaceutical agents in a method of converting liver stem or progenitor cells into functional pancreatic cells, of preventing beta-cell deterioration and of stimulating beta-cell proliferation, of suppressing plasma blood levels of norepinepherine, of inducing an inotropic response and of increasing cardiac contractility, of improving nutrition via a non-alimentary route, (e.g., via intravenous, subcutaneous, intramuscular, peritoneal, or other injection or infusion rout), of pre-treating a subject to undergo an endoscopic procedure, and of modulating triglyceride levels, in a subject in need thereof.

Pharmaceutical compositions

[0058] The compounds of the present invention, or salts or solvates thereof, may be formulated as pharmaceutical compositions prepared for storage or administration, which typically comprise a therapeutically effective amount of a compound employed in the context of the invention, or a salt or solvate thereof, in a pharmaceutically acceptable carrier.

[0059] The therapeutically effective amount of a compound of the present invention will depend on the route of administration, the type of mammal being treated, and the physical characteristics of the specific mammal under consideration. These factors and their relationship to determining this amount are well known to skilled practitioners in the medical arts. This amount and the method of administration may be tailored to achieve optimal efficacy, and may depend on such factors as weight, diet, concurrent medication and other factors, well known to those skilled in the medical arts. The dosage sizes and dosing regimen most appropriate for human use may be guided by the results obtained by the present invention, and may be confirmed in properly designed clinical trials.

[0060] An effective dosage and treatment protocol may be determined by conventional means, starting with a low dose in laboratory animals and then increasing the dosage while monitoring the effects, and systematically varying the dosage regimen as well. Numerous factors may be taken into consideration by a clinician when determining an optimal dosage for a given subject. Such considerations are known to the skilled person. The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro ed. 1985). For example, sterile saline and phosphate- buffered saline at slightly acidic or physiological pH may be used. Suitable pH buffering agents may be phosphate, citrate, acetate, tris/hydroxymethyl)aminomethane (TRIS), *N*-Tris(hydroxymethyl)methyl-3-aminopropanesulphonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, which is a preferred buffer, arginine, lysine, or acetate or mixtures thereof. The term further encompasses any agents listed in the US Pharmacopeia for use in animals, including humans.

[0061] The term "pharmaceutically acceptable salt" refers to the salt of the compounds. Salts include pharmaceutically acceptable salts such as acid addition salts and basic salts. Examples of acid addition salts include hydrochloride salts, citrate salts and acetate salts. Examples of basic salts include salts where the cation is selected from alkali metals, such as sodium and potassium, alkaline earth metals such as calcium, and ammonium ions $^{+}N(R^3)_3(R^4)$, where R^3 and R^4 independently designate optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, optionally substituted aryl, or optionally substituted heteroaryl. Other examples of pharmaceutically acceptable salts are described in "Remington's Pharmaceutical Sciences" ,17th edition. Ed. Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, U.S.A., 1985 and more recent editions, and in the Encyclopaedia of Pharmaceutical Technology.

[0062] "Treatment" is an approach for obtaining beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of

disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures in certain embodiments. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. By treatment is meant inhibiting or reducing an increase in pathology or symptoms (e.g. weight gain, hyperglycemia) when compared to the absence of treatment, and is not necessarily meant to imply complete cessation of the relevant condition.

[0063] The pharmaceutical compositions may be in unit dosage form. In such form, the composition is divided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparations, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms. It may be provided in single dose injectable form, for example in the form of a pen. Compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Subcutaneous or transdermal modes of administration may be particularly suitable for the compounds described herein.

G-protein coupled receptors

[0064] In some embodiments, the methods of the invention may be used to screen for a compound with preferential receptor signaling pathway activation and/or inhibition along different pathways mediated by a G-protein coupled

receptor (GPCR). In some embodiments, the invention provides a compound with preferential receptor signaling pathway activation and/or inhibition along different GPCR-mediated pathways. In preferred embodiments, the GPCR shares structural similarities to the Glucagon Receptor. For example, a GPCR may have a Cα root-mean-square deviation (rmsd) of less than about 2.0 Å, about 1.5 Å, about 1.0 Å, about 0.9 Å, about 0.8 Å, about 0.7 Å, about 0.6 Å, about 0.5 Å, about 0.4 Å, about 0.3 Å, about 0.2 Å, or about 0.1 Å with respect to the Glucagon receptor. The skilled worker also will be able to determine structural similarity based on known techniques.

[0065] In certain embodiments, the GPCR is a Class B GPCR. Exemplary Class B GPCRs include, *e.g.*, Glucagon family receptors, Calcitonin receptors, Corticotropin-Releasing Factor receptors, Parathyroid Hormone receptors, VIP and PACAP receptors, and Class B orphan receptors. One of skill in the art would be able to substitute assays described herein with assays to measure signaling of one or more of these known Class B GPCRs using methods and reagents known in the art.

Combination therapy

[0066] The compound employed in the context of the invention may be administered as part of a combination therapy with an agent for treatment of diabetes, obesity, dyslipidemia, or hypertension.

[0067] In such cases, the two active agents may be given together or separately, and as part of the same pharmaceutical formulation or as separate formulations. Thus the compound employed in the context of the invention (or the salt thereof) may be used in combination with an antidiabetic agent including but not limited to metformin, a sulfonylurea, a glinide, a DPP-IV inhibitor, a glitazone, or insulin. In certain embodiments, the compound or salt or solvate thereof is used in combination with one or more of insulin, DPP-IV inhibitor, sulfonylurea or metformin, particularly sulfonylurea or metformin, for achieving adequate glycemic control. In certain preferred embodiments the compound (or salt or solvate thereof) is used in combination with insulin or an insulin analog for achieving adequate glycemic control. Examples of insulin analogs include but are

not limited to Lantus®, NovoRapid®, Humalog®, NovoMix®, Actraphane HM®, Levemir® and Apidra®.

[0068] The compound or salt thereof may further be used in combination with an anti-obesity agent including but not limited to a glucagon-like peptide receptor 1 agonist, peptide YY or analog thereof, cannabinoid receptor 1 antagonist, lipase inhibitor, melanocortin receptor 4 agonist, or melanin concentrating hormone receptor 1 antagonist.

[0069] The compound or salt thereof may be used in combination with an anti-hypertension agent including but not limited to an angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, angiotensin II receptor biased ligands, diuretics, beta-blocker, or calcium channel blocker.

[0070] The compound or salt thereof may be used in combination with an anti-dyslipidemia agent including but not limited to a statin, a fibrate, a niacin and/or a cholesterol absorbtion inhibitor.

Examples

[0071] The following examples demonstrate certain embodiments of the present invention. However, it is to be understood that these examples neither purport nor are they intended to be wholly definitive as to conditions and scope of this invention. The examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The following examples are presented for illustrative purpose, and should not be construed in any way as limiting the scope of this invention.

[0072] Disclosed are glucagon-GLP1 dual agonist (GGDA) compounds that exhibit signaling selectivity, and methods for screening these compounds. Signaling selectivity may be, for example, preferential pathway activation or preferential pathway inhibition, or both. The GGDA compounds may be useful for the treatment and/or prevention of diseases or conditions caused or characterized by excess body weight, including, but not limited to, obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, pre-diabetes, insulin resistance, glucose intolerance, type 2 diabetes, type I diabetes, hypertension, atherogenic

dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease, and stroke or microvascular disease.

[0073] While some embodiments of the invention have been described by way of illustration, it will be apparent that the invention can be put into practice with many modifications, variations and adaptations, and with the use of numerous equivalents or alternative solutions that are within the scope of persons skilled in the art, without departing from the spirit of the invention or exceeding the scope of the claims.

[0074] All publications, patents, and patent applications referred to herein are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

Example 1

[0075] The methods used in the instant invention are described below, except where expressly indicated otherwise.

General synthesis of acylated GGDA compounds

[0076] Solid phase peptide synthesis was performed on a CEM Liberty Peptide Synthesizer using standard Fmoc chemistry. TentaGel S Ram resin (1 g; 0.25 mmol/g) was swelled in NMP (10 ml) prior to use and transferred between tube and reaction vessel using DCM and NMP.

Coupling

[0077] An Fmoc-amino acid in NMP/DMF/DCM (1:1:1 ; 0.2 M; 5 ml) was added to the resin in a CEM Discover microwave unit together with HATU/NMP (0.5 M; 2 ml) and DIPEA/NMP (2.0 M; 1 ml). The coupling mixture was heated to 75°C for 5 min while nitrogen was bubbled through the mixture. The resin was then washed with NMP (4 x 10 ml).

Deprotection

[0078] Piperidine/NMP (20%; 10 ml) was added to the resin for initial deprotection and the mixture was heated by microwaves (30 sec; 40°C). The reaction vessel was drained and a second portion of piperidine/NMP (20%; 10 ml)

was added and heated (75°C; 3 min.) again. The resin was then washed with NMP (6 x 10 ml).

Side chain acylation

[0079] Fmoc-Lys(ivDde)-OH or alternatively another amino acid with an orthogonal side chain protective group was introduced at the position of the acylation. The N-terminal of the peptide backbone was then Boc-protected using Boc2O or alternatively by using a Boc-protected amino acid in the last coupling. While the peptide was still attached to the resin, the orthogonal side chain protective group was selectively cleaved using freshly prepared hydrazine hydrate (2-4%) in NMP for 2 x 15 min. The unprotected lysine side chain was first coupled with Fmoc-Glu-OtBu or another spacer amino acid, which was deprotected with piperidine and acylated with a lipophilic moiety using the peptide coupling methodology as described above. Abbreviations employed are as follows:

ivDde: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)3-methyl-butyl

Dde: 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl

DCM: dichloromethane

DMF: *N*,*N*-dimethylformamide

DIPEA: diisopropylethylamine

EtOH: ethanol

Et2O: diethyl ether

HATU: *N*-[(dimethylamino)-1H-1,2,3-triazol[4,5-b]pyridine-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide

MeCN: acetonitrile

NMP: *N*-methylpyrrolidone

TFA: trifluoroacetic acid

TIS: triisopropylsilane

Cleavage

[0080] The resin was washed with EtOH (3 x 10 ml) and Et2O (3 x 10 ml) and dried to constant weight at room temperature (r.t.). The crude peptide was cleaved from the resin by treatment with TFA/TIS/water (95/2.5/2.5; 40 ml, 2 h; r.t.). Most of the TFA was removed at reduced pressure and the crude peptide was

precipitated and washed three times with diethylether and dried to constant weight at room temperature.

HPLC purification of the crude peptide

[0081] The crude peptide was purified to greater than 90% by preparative reverse phase HPLC using a PerSeptive Biosystems VISION Workstation equipped with a C-18 column (5 cm; 10 μm) and a fraction collector and run at 35 ml/min with a gradient of buffer A (0.1% TFA, aq.) and buffer B (0.1% TFA, 90% MeCN, aq.). Fractions were analyzed by analytical HPLC and MS and relevant fractions were pooled and lyophilized. The final product was characterized by HPLC and MS.

Generation of cell lines expressing human glucagon- and GLP-1 receptors

[0082] The cDNAs encoding either the human glucagon receptor (GluR) (primary accession number P47871) or the human glucagon-like peptide 1 receptor (GLP-1 R) (primary accession number P43220) were cloned from the cDNA clones BC104854 (MGC:132514/IMAGE:8143857) or BC112126 (MGC:138331/IMAGE:8327594), respectively. The DNA encoding the Glu-R or the GLP-1 R was amplified by PCR using primers encoding terminal restriction sites for subcloning. The 5'-end primers additionally encoded a near Kozak consensus sequence to ensure efficient translation. The fidelity of the DNA encoding the Glucagon-R and the GLP-1 R was confirmed by DNA sequencing. The PCR products encoding the Glucagon-R or the GLP-1 R were subcloned into a mammalian expression vector containing a neomycin (G418) resistance marker.

[0083] The mammalian expression vectors encoding the Glu-R or the GLP-1 R were transfected into HEK293 or CHO-K1 cells by a standard calcium phosphate transfection method. Cells were seeded 48 hours after transfection for limited dilution cloning and selected with 1 mg/ml G418 in the culture medium. Three weeks later, surviving colonies of Glucagon-R and GLP-1 R expressing cells were picked, propagated, and tested in the Glucagon-R and GLP-1 R efficacy assays as described below. Clonal Glucagon-R expressing GLP-1 R expressing cell lines were validated for receptor expression and functionality and chosen for compound profiling.

Glucagon receptor and GLP-1 Receptor assays

cAMP assay

[0084] Glucagon receptor and GLP1 receptor expressing cell lines (HEK293 and CHO based) were seeded at 30,000 cells per well in 96-well microtiter plates coated with 0.01 % poly-L-lysine, and grown for 1 day in culture in 200 µl growth medium. On the day of analysis, growth medium was removed and the cells washed once with 200 µl Tyrode buffer containing 100 µM IBMX. Cells were incubated in 100 µl Tyrode buffer containing increasing concentrations of test peptides and 100 µM IBMX for 15 min at 37^0 C. The reaction was stopped by lysing the cells by addition of 80 µl Lysis/Detection Buffer (0.1 % w/v BSA, 5 mM HEPES, 0.3 % v/v Tween-20 in deionized water). The cAMP content was estimated using the AlphaScreen® cAMP Assay Kit from Perkin Elmer according to manufacturer instructions. Exendin-4 and human Glucagon is used as reference compound in the assay. Test compounds inducing an increase in the intracellular level of cAMP can be tested in this assay, and the response normalized relative to the reference control to calculate the EC_{50} and % Efficacy from the concentration response curve using the 4-parameter logistic (4PL) nonlinear model for curve fitting. Results are summarized in Figure 6.

Phospho-ERK1/2 assay (pERK)

In vitro effects of GGDA compounds employed in the context of the invention were estimated by measuring pERK (using the AlphaScreen™ SureFire pERK assay) in HEK293 or CHO cells stably expressing human Glucagon-R or human GLP-1 R. The assays (AlphaScreen™ SureFire p-ERK assay) were performed as follows:

Day 1: Seeding of cells

The human Glucagon-R or human GLP-1 R expressing cells in question were seeded at 30,000 cells/well in 100 µl growth medium [DMEM, 10% FCS, Penicillin (100 IU/ml), Streptomycin (100 µg/ml)] in a 96-well plate coated with poly-D-lysine. The cells were incubated in an incubator (37°C, 5% CO_2) for 24 hours.

Day 2: Change to serum-free medium

The growth medium was changed to 80 µl of serum-free medium [DMEM, Penicillin (100 IU/ml), Streptomycin (100 µg/ml)] per well, and incubation of the cells was continued for 19 hours in the incubator (37°C, 5% CO_2).

Day 3: Peptide conjugate stimulation and AlphaScreen™ SureFire p-ERK assay

1. 20 µl of serum-free medium containing different concentrations of GGDA compounds was added (performed in triplicate for each concentration), and the cells were incubated for 5 min at room temperature.
2. The stimulation medium was discarded by quickly turning the plate upside down, and 60 µl 1x lysis buffer (from the SureFire assay kit) was added per well.
3. The plate was shaken on a plate-shaker for 5 min and then placed on ice.
4. SureFire P-ERK assay: 4 µl of each supernatant was transferred to a 384 well proxiplate (Perkin-Elmer).
5. 4 µl of each of the two control lysates (unstimulated and stimulated) were added to the proxiplate in duplicate.
6. 60 parts reaction buffer, 10 parts activation buffer, 1 part acceptor beads and 1 part donor beads were mixed (reaction buffer + activation buffer + beads). 7 µl of the latter reaction buffer + activation buffer + beads per well were added in the proxiplate, the mix being resuspended carefully before addition to the wells.
7. The plate was incubated for 2 hours in a dark box in a 22°C incubator.
8. The plate was analyzed on an Envision™ light-emission plate reader (Perkin-Elmer) using the appropriate reading program.

Results are shown in Figures 3 and 5 and summarized in Figures 1, 14 and 15.

[0085] The GGDA compounds employed in the context of the invention were tested in the above-described assays. Human GLP-1(7-36) and Exendin-4(1-39) were used as positive controls in the human GLP-1 receptor (hGLP-1 R) activation efficacy assay, whereas human Glucagon (1-29) was used as the control compound for the human glucagon receptor

Example 2

[0070] Screening of peptides for promiscuous calcium (Ca^{2+}) signaling was performed at Millipore using their GPCRProfiler® Services. The respective

receptors of the G-protein coupled receptor (GPCR) family are expressed together with Gq-proteins in their proprietary ChemiScreen™ Ca^{2+} Optimized GPCR Cell Lines, that mediate cellular signaling to elevation of intracellular Ca^{2+}. The Ca^{2+} is measured by fluorescence using the FLIPR®Tetra platform from Molecular Devices (FLIPR assay). Since the receptors are "promiscuously" coupled to intracellular calcium signaling by the introduced Gq-proteins, these assays are referred to as "promiscuous calcium coupled assays" (pCa^{2+}). The response is dependent on concentration of peptide and is obtained at two different concentration of peptide or a series of concentrations. Each sample compound was diluted into assay buffer (1x Hank's Balanced Salt Solution (HBSS) with 20mM HEPES and 2.5mM Probenecid) at an appropriate concentration to obtain final concentrations. The former is to determine agonist and antagonist activities compared to control compounds, the latter to determine EC_{50} and E_{max} for the peptides.

[0086] A FLIPR assay (calcium activation) was conducted to analyze the dose response of the GGDA compounds for agonist activities on the human Glucagon receptor and GLP-1 receptor. Figures 2 and 4 illustrate the results of such analyses on the two receptors, respectively. A summary of the pCa^{2+} are shown in Figure 1 together with the pERK results. F indicates full agonism (>) 75% control) and P indicates partial agonism (< 75% of control).

[0087] Using our Glucagon- and GLP1-receptor HEK293 cell lines, the non-promiscuous calcium signaling also was assessed using the FDSS/μCELL calcium measurement instrument from Hamamatsu Photonics. These "true" Ca^{2+} (tCa^{2+}) assays were also performed to test the Ca^{2+} pathway in connection with the GLP-1 receptor and the Glucagon receptor in HEK293 cells (Figures 7 and 8, respectively). "True" Ca^{2+} assays differ from the promiscuous Ca^{2+} signaling assay described above. In the tCa^{2+} assays, the receptor is challenged in the context of native coupling in cells. The partial Glucagon receptor agonism of two compounds is shown in both pCa^{2+} and tCa^{2+}, and the other compounds are full agonists in both calcium assays (Figures 1 and 8).

[0088] HEK293 cells stably expressing the human Glucagon receptor or human GLP-1 receptor were seeded 48 hours in advance of experiment at a density of 15,000 cells/well in black view plates, coated with Poly-L-lysine. After 24 hours

the growth media was flicked off and replaced with 100 µl/well clear DMEM (No phenol red) reduced serum (2%) growth media. One hour prior to experimentation, 100 µl Calcium dye Fluo-4 direct (Invitrogen #F10471) was added on top of the GM for 1 hour incubation at 37°C with 5% CO_2 in the incubator. Compounds were prepared in duplicate masterplates and 20 µl transferred to the cell plate. Readout was started 10 seconds prior to compound transfer, and continued 300 seconds after compound addition with the following Settings; Excitation wave length: 470_495 Emission wave length: 515_575 Gain: 160 Exposure Time: 0.40 Excitation Intensity: 50.

[0089] The Fluo-4 direct kit (Invitrogen #F10471) general experimental protocol was performed as follows:

1. Store Fluo-4 Direct™ calcium assay buffer (Component C) at 5°C.

2. Prepare 250 mM stock solution of probenecid by adding 1 mL of Fluo-4 Direct™ calcium assay buffer to each 77 mg vial of water-soluble probenecid (Component B) Vortex until dissolved. Store any unused probenecid stock solution at ≤–20°C for up to six months.

3. Prepare the 2X Fluo-4 Direct™ calcium reagent loading solution with a final probenecid concentration of 5 mM for the kit that you are using, as follows: Add 10 mL Fluo-4 Direct™ calcium assay buffer and 200 µL 250 mM probenecid stock solution to one bottle of Fluo-4 Direct™ calcium reagent (Component A). This 2X Fluo-4 Direct™ calcium reagent loading solution is sufficient for two microplates. Note: Vortex and allow the solution to sit for five minutes to ensure that the reagent is completely dissolved and then vortex again. Ensure that reagent is completely dissolved before proceeding with loading cells.

4. Remove microplates containing cells from the incubator. Add an equal volume of 2X Fluo-4 Direct™ calcium reagent loading solution directly to wells containing cells in culture medium.

5. Incubate plates at 37°C for 60 minutes.

6. Measure fluorescence using instrument settings appropriate for excitation at 494 nm and emission at 516 nm.

Example 3

[0090] Compounds disclosed herein are useful in the treatment of a disease or condition caused or characterized by excess body weight, e.g., the treatment and/or prevention of obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, pre-diabetes, insulin resistance, glucose intolerance, type 2 diabetes, type I diabetes, hypertension, atherogenic dyslipidaemia, atherosclerosis, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease. In particular, compounds are useful in reducing body weight, decreasing food intake, reducing fat depots (*e.g.*, mesenteric adipose tissue depots, subcutaneous adipose tissue depots, epidymal adipose tissue depots, and retroperitoneal adipose tissue depots), reducing liver weight, reducing plasma cholesterol levels, reducing plasma LDL levels, increasing plasma HDL levels, reducing plasma triglyceride levels, reducing blood glucose levels, stabilizing blood glucose levels, and reducing plasma insulin levels.

[0091] One major benefit of compounds employed in the context of the invention is that adverse effects and undesirable pharmacokinetic parameters associated with other treatments are reduced or eliminated. Without wishing to be bound by theory, we hypothesize that adverse effects and undesirable pharmacokinetic parameters may be caused by activation or inhibition of pathways one or more pathways in response to the treatment. The compounds disclosed herein may be designed so as to preferentially activate, or preferentially inhibit, one or more pathways associated with an adverse effect or undesirable pharmacokinetic parameter, thereby reducing or eliminating the effect or parameter.

[0092] Methods useful in determining *in vivo* effects of the compounds employed in the context of the invention are as follows.

Pharmacokinetic parameters

[0093] Mice are given 100 nmol of a compound per kg as i.v. or s.c. bolus, and plasma samples collected up to 240 min post-dose. Samples are collected from three mice at each time point. The plasma samples are analyzed for the presence of the compound using LC/MS/MS (10-1000 nM).

[0094] Alternatively, mice are given a single subcutaneous dose of 100 nmol/kg of a compound. Blood samples are taken after 5 and 30 min and after 1, 2, 4, 6, 16 and 24 hours. At each time point, samples from two mice are taken. Plasma samples are analyzed for the compound after solid phase extraction (SPE) by liquid chromatography mass spectrometry (LC-MS/MS).

Measurement of insulin

[0095] The db/db mouse model has previously been used to assess the β-cell preserving effects of potential therapeutic candidates (Rolin, B. et al., *Am. J. Physiol. Endocrinol. Metab.* 283: E745-E752 (2002)). Several studies have demonstrated a correlation between pancreatic Insulin content and β-cell mass (Rolin, B. et al. (loc.cit.); Suarez-Pinzon, W.L. et al., *Diabetes* 54: 2596-2601 (2005); Suarez-Pinzon W.L. et al., *Diabetes* 57: 3281-3288 (2008)).

[0096] Six week old db/db (BKS.Cg-m +/+ Leprdb/J) female mice (Taconic Europe A/S, Lille Skensved, Denmark) are acclimatized to their new environment and given *ad libitum* access to normal chow and water. Mice are housed in pairs in a light-, temperature- and humidity-controlled room. The progression of diabetes is followed for 2 weeks by monitoring blood glucose levels, and then before treatment the diabetic mice are randomized according to their blood glucose levels into treatment groups (n = 10/group). Animals are then mock-injected subcutaneously (sc) with 100 µl vehicle (once daily) for a period of three days to acclimatize the animals to handling and injections. Following randomization and mock injection, animals are treated (sc, twice daily) for 16 days with control or test compounds, or with vehicle (PBS buffer; injection volume 5 ml/kg). Daily injections take place between 8:00 and 9:00 hours, and between 15:00 and 16:00 hours, with fresh solutions prepared immediately before dosing.

[0097] Blood samples (200 µl) are obtained from the orbital plexus and placed in EDTA coated tubes before dosing (day 1), and at day 8 and day 16 of the treatment. Each blood sample is centrifuged, and plasma (100 µl) is stored at -80°C for later analysis. Blood samples for blood glucose determinations are taken from the tail vein. Following the last day of dosing, all animals are sacrificed (day 16) by CO2 anesthesia, followed by cervical dislocation. The pancreas from each

animal is immediately isolated, weighed, and stored for later analysis of Insulin content.

[0098] Whole blood glucose concentration (mM) is determined by the immobilized glucose oxidase method (Elite Autoanalyser, Bayer, Denmark). Plasma C-peptide is determined using a rat C-peptide radioimmunoassay kit (Linco/Millipore, kit RCP-21K). Pancreatic Insulin content is determined using a rat Insulin radioimmunoassay kit (Linco/Millipore, kit R1-13).

Measurement of insulin (6 week study)

[0099] 125 db/db (BKS.Cg-m +/+ Leprdb/J) female mice (6 weeks at arrival) are obtained from Taconic Eu-rope A/S. At day 4, blood is collected from semi-fasted animals for determination of baseline plasma C-peptide, plasma insulin, blood glucose, and HbA1c levels. Animals are then stratified into 5 treatment groups of n = 20 based on baseline plasma C-peptide and HbA1c levels. Animals are injected s.c. with 100 µl of vehicle twice daily for at least 3 days to acclimatize the animals to handling and experimental procedures.

[0100] Animals are then injected s.c. twice daily with peptides or vehicle for a total of 42 days. The daily injections take place between 08:00-09:00 h and 15:00-16:00 h with freshly prepared solutions. The last day of dosing is day 42 in the morning.

[0101] The study is terminated on day 42. Animals are semi-fasted, and they receive the final dose in the morning. Blood is sampled for determination of plasma C-peptide, plasma insulin, blood glucose, and HbA1c. After the blood sampling, animals are euthanized using CO2 followed by cervical dislocation. The pancreas is isolated, weighed, divided into 3 pieces, and transferred to tubes containing 2 ml of cold acidic alcohol and analyzed for insulin content.

Measurement of blood glucose levels

[0102] 150 male db/db mice are obtained at an age of 5-6 weeks. The animals are housed (5 mice/cage) under controlled conditions (20-22°C, 55-85% humidity) following a 12:12-hrs light/dark cycle with a light on at 05:00 AM. The animals are fed ad libitum with standard Altromin No. 1324 diet and have free access to

acidified tap water. At the time of study start, the animals are 8-9 weeks old. All animals are acclimatized and handled daily for a minimum one week prior to the experiment.

Blood samples

[0103] Before treatment start, and on day 93 (before termination) in fasted mice (17 hrs) a blood sample (150 µl) is obtained from orbital plexus with an EDTA coated micro-pipette. Blood samples are taken into EDTA coated tubes and kept on ice. The blood sample is centrifuged and the resulting plasma (at least 50 µl) is stored (at -80°C) for later analysis of C-peptide and insulin level. Also, on day -10/12 (before treatment start), and day 93 (before termination) a blood sample (50 µl) obtained from orbital plexus is analyzed for BG (sticks) and HbA1c.

Stratification

[0104] On days -6 to -4 before the first drug dose, fasted animals (17 hrs) are subjected to an oral glucose tolerance test (OGTT, see below). The area under the blood glucose concentration curve obtained over a 240-minute period (AUC0-240; unit: mM*min) is used to stratify animals into 5 groups (A-E) of 26 animals each in order to obtain similar glucose tolerances in both groups. After the first 50 days of dosing (period 1) a second OGTT is performed. On the basis of this second OGTT test, each group of mice is stratified by AUC into two sub-groups displaying similar glucose tolerances.

Dosing

[0105] The animals are given one daily (QD) subcutaneous (SC) dose of vehicle (2 * n=26), control compound (n=26) or test compound (n=26) and dosed according for a period of 50 days as follows:

Substance Period 1	Substance Period 2	Route	Dose (nmol/kg/day)
Vehicle	Vehicle	SC once daily	0+0
Vehicle	Exendin-4		0+100
Vehicle	Test compound		0+100
Vehicle	Liraglutide		0+100
Control compound	Control compound		100+100

Substance Period 1	Substance Period 2	Route	Dose (nmol/kg/day)
Control compound	Vehicle		100+0
Liraglutide	Liraglutide		100+100
Liraglutide	Vehicle		100+0
Test compound	Test compound		100+100
Test compound	Vehicle		100+0

Dosing is performed between 02:00 and 03:00 PM every day, with an injection volume of 5 ml/kg. After 50 days of dosing the animals are stratified into 7 groups. This dosing regimen is continued for 40 days until animals are sacrificed on day 93.

Oral Glucose Tolerance Test (OGTT)

[0106] OGTT is performed on days -6/4, 50, 65, 78 and 91 of the treatment period on animals fasted overnight (17 hours) after the last injection of vehicle or compound. Blood samples are taken from the tip of the tail and blood glucose is measured. To prevent confounding food intake, the animals are kept fasted during all OGTTs. Immediately after the initial blood sample (t = 0, fasting blood glucose level) an oral dose (1 g/kg) of glucose (Glucose-monohydrat, SAD 500 g/l), dissolved MQ water is given (5 ml/kg, 0.2 g/ml), and the animals are returned to their home cages (t = 0). Then, BG levels are measured at t = 15, 30, 60, 120, and 240 minutes.

Fasting blood glucose

[0107] To further monitor the diabetic status of the animals, fasting blood glucose levels are measured after 8 hours of fasting on day 0, 37, 44, 58, 72, and 85. To minimize stress, animals are fasted during the day (from 06:00 AM when habitual consumption of food was low), and fasting blood glucose is determined at 02:00 PM.

HbA1c determination

[0108] It is possible to assess the long term effect of a compound on a subject's glucose level by determining the level of haemoglobin A1C (HbA1c). HbA1c is a glycated form of haemoglobin whose level in a cell reflects the average level of

glucose to which the cell has been exposed during its lifetime. In mice, HbA1c is a relevant biomarker for the average blood glucose level during the preceding 4 weeks, because conversion to HbA1c is limited by the erythrocyte's life span of approximately 47 days (Abbrecht & Littell, 1972; *J. Appl. Physiol.* 32, 443-445).

[0109] The HbA1c determination is based on Turbidimetric INhibition ImmunoAssay (TINIA) in which HbA1c in the sample reacts with anti-HbA1c to form soluble antigen-antibody complexes. Additions of polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex, which can be measured turbidimetrically. Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum, which is measured bichromatically during the preincubation phases. The final result is expressed as percent HbA1c of total hemoglobin (Cobas® A1C-2).

Cholesterol level determination

[0110] The assay is an enzymatic colorimetric method. In the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDLA and chylomicrons, which are resistant to PEG-modified enzymes. The HDL cholesterol is determined enzymatically by cholesterol esterase and cholesterol oxidase coupled with PEG to the amino groups. Cholesterol esters are broken down quantitatively to free cholesterol and fatty acids. HDL cholesterol is enzymatically oxidized to choles-4-en-3-one and H_2O_2, and the formed H_2O_2 is measured colorimetrically (Cobas®; HDLC3).

[0111] The direct determination of LDL takes advantage of the selective micellary solubilization of LDL by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). The combination of a sugar compound with detergent enables the selective determination of LDL in plasma. The test principle is the same as that of cholesterol and HDL, but due to the sugar and detergent only LDL-cholesterol esters are broken down to free cholesterol and fatty acids. Free cholesterol is then oxidized and the formed H2O2 is measured colorimetrically (LDL_C, Cobas®).

[0112] Additional methods are referred to in International Application Publication No. WO2011/006497 and U.S. Provisional Application No. 61/358,623, which are incorporated herein by reference.

Example 4

[0113] Functionally selective signaling for the GLP-1 receptor in a cardiac setting has not yet been demonstrated. A G protein coupled receptor can activate the ERK pathway by G protein-dependent or β-arrestin-dependent mechanisms. The classical G protein-dependent path relies on interactions between the G protein coupled receptor (GPCR) and the G protein, which is itself comprised of α, β, and γ subunits. As described above, GLP-1 R is a GPCR that can activate ERK, and therefore represents an attractive target for cardiovascular diseases. GGDA compounds disclosed herein which exhibit signal selectivity, thus, are expected to be useful in the treatment of cardiovascular diseases (e.g., by inducing cardioprotective effects).

Example 5

[0114] A head to head comparison of Aib in position 2 of a GGDA (Figures 9-12 and 14-15) versus the normal serine in position 2 demonstrates that a GGDA with serine has reduced Glucagon Receptor efficacy relative to endogenous human Glucagon. The Aib in position 2 reduces the in vitro efficacy significantly and turns the compound into a partial agonist with E_{max} of approximately 50%. For example, Figure 12 shows GGDAs with signaling selective properties identified with the GluR calcium assay. The GGDA Cpd 17 has a serine in position 2 (Ser2) and has a slight reduced potency compared to human glucagon but still with full agonistic properties. By contrast, substituting the Ser2 for Aib (Cpd 9) or Glycine (Cpd 19) or D-ala (Cpd 20) further reduces the potency (EC_{50}) but also turns the compounds into partial agonists with significantly reduced E_{max}. Importantly, each of these compounds is a full and potent (EC_{50}< 1 nM) agonist when assayed in an cAMP assay (see Figure 14). Therefore, the calcium assay can be used to discriminate compounds based on the signalling selective properties not visible when assaying for the G-protein mediated increase in cellular cAMP.

Example 6

[0115] 8 week old male Sprague-Dawley rats were maintained on a normal chow diet (Altromin) and domestic quality tap water with added citric acid to a pH of about 3.6. They were housed in groups of n = 4 in a light-, temperature-, and humidity-controlled room (12-hour light:12-hour dark cycle, lights on/off at 0600/1800 hours; 20-22 °C; 40-60% relative humidity). Animals were semi-fasted for 4 hours prior to the experiment (fasting at 6:00 AM, study at 10:00 AM). Bodyweight and blood glucose (BG) were then determined and the animals were anesthetized using a standard mixture of hyporm/dormicum given subcutaneously at 0.2 ml/100g body weight. Following 60 minutes of anesthesia, a baseline blood sample was taken, and BG was determined (Biosen procedure using 5µl capillary tubes). After, the test substance was injected by subcutaneous injection (SC) in a dose of 20 nmol/kg of bodyweight. Dosing volume was adjusted to approximately 5 ml/kg. Blood glucose was measured every 10 minutes for 2 hours at t = 0 (immediately before dosing of the test compound), 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minutes after SC injection of the test compound. The blood glucose levels were monitored for hGlucagon or head to head comparators of GGDAs with full (F) vs partial (P) agonism at the Glucagon Receptor as assayed by intracellular calcium release (Compound 4 (F) vs Compound 7 (P) and Compound 5 (F) vs Compound 8 (P)). The GGDAs all have reduced glucose release from the liver after administration which likely can be ascribed to the opposing actions of the GLP1 component of the compounds. A trend towards a reduction of blood glucose levels for the signaling selective partial GluR agonists can be seen (Cpd 4 (20%) to Cpd 7 (8%) and Cpd 5 (35%) to Cpd 8 (22%) (Figure 13). Data is given as area under the curve (AUC) for the blood glucose profiles (groups are mean of n=6 rats). *** represents $p<0.001$ ANOVA with Dunnets MC test.

What is Claimed is:

1. A method of screening GGDA compounds for preferential pathway activation or inhibition, comprising:

screening for a full glucagon receptor agonist in a first glucagon receptor assay,

identifying a full glucagon receptor agonist of a first pathway,

screening for a partial glucagon receptor agonist in a second glucagon receptor assay,

identifying a partial glucagon receptor agonist of a second pathway,

screening for a full GLP-1 receptor agonist in a first GLP-1 receptor assay, and

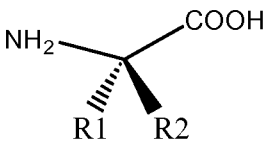
identifying a full GLP-1 receptor agonist of a GLP-1 receptor mediated pathway.

2. The method of claim 1, wherein the first and second glucagon receptor assays and the GLP-1 receptor assay are selected from the group consisting of: a cAMP assay, a pERK assay, and a Ca^{2+} assay.

3. The method of claims 1 or 2, wherein the GGDA is a partial glucagon receptor agonist as determined by a pERK assay or a Ca^{2+} assay.

4. The method of any one of claims 1-3, wherein the GGDA compound comprises a compound of the formula His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr, wherein X2 is a linear or cyclic α,α-disubstituted amino acid, a lipophilic D-amino acid or polar D-amino acid.

5. The method of claim 4, wherein X2 is an α,α-disubstituted amino acid having the formula I:

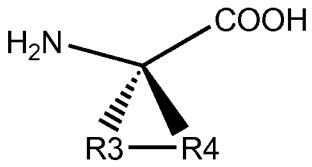


(formula I)

wherein R1 and R2, independently, are selected from the group consisting of -CH_3 (methyl), -CH_2CH_3 (ethyl), -$CH_2CH_2CH_3$ (1-propyl) and -CH_2Ph (benzyl).

6. The method of claim 5, wherein X2 is Aib.

7. The method of claim 4, wherein X2 is a cyclic α,α-disubstituted amino acid having the formula II:

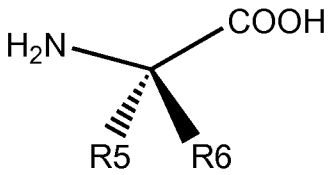


(formula II)

wherein R3 and R4, independently, are selected from the group consisting of -CH_2-, -CH_2CH_2-, -$CH_2CH_2CH_2$- and -$CH_2CH_2CH_2CH_2$-.

8. The method of claim 7, wherein X2 is selected from the group consisting of Ac3c, Ac4c, Ac5c, Ac6c, and Ac7c.

9. The method of claim 4, wherein X2 is a lipophilic D-amino acid having the formula III:

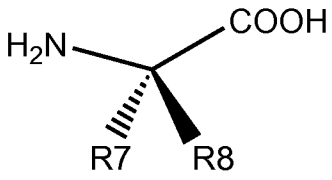


(formula III)

wherein R5 is hydrogen, and R6 is selected from the group consisting of -CH_3 (methyl), -$CH_2CH(CH_3)_2$ (2-methyl-1-propyl), -$CH(CH_3)(CH_2CH_3)$ (2-butyl), -$CH(CH_3)_2$ (2-propyl) and -CH_2Ph (benzyl).

10. The method of claim 9, wherein the lipophilic D-amino acid is selected from the group consisting of D-Ala, D-Leu, D-Ile, D-Val and D-Phe.

11. The method of claim 4, wherein X2 is a polar D-amino acid having the formula IV:



(formula IV)

wherein wherein R7 is hydrogen, and R8 is selected from the group consisting of -CH_2OH (hydroxymethyl), -$CH(CH_3)(OH)$ (1-hydroxyethyl) and -$CH_2CH_2SCH_3$ (2-methylthioethyl).

12. The method of claim 11, wherein X2 is not D-Ser.

13. The method of claim 11, wherein the polar D-amino acid is selected from the group consisting of D-Thr and D-Met

14. The method of claim 4, wherein X2 is glycine.

15. The method of any one of claims 1-3, wherein the GGDA compound comprises a compound of the formula V:

R^1-X-Z-R^2 (formula V);

wherein

R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

X is a peptide which has the formula VI:

His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-X16-X17-Arg-Ala-X20-Asp-Phe-Ile-Glu-Trp-Leu-X27-X28-X29 (formula VI);

wherein

X2 is selected from Ser and Aib;

X16 is selected from Glu and Y;

X17 is selected from Arg and Y;

X20 is selected from Lys and Y;

X27 is selected from Leu and Y;

X28 is selected from Ser and Y or absent;

X29 is Ala or absent;

wherein at least one of X16, X17, X20, X27 and X28 is Y;

wherein each residue Y is independently selected from Lys, Cys and Orn;

wherein the side chain of at least one amino acid residue Y of X is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Y; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Y via Z^2;

and Z is absent or is a sequence of 1-20 amino acid units independently selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Cys, Glu, Lys, Arg, Dbu, Dpr and Orn;

or a pharmaceutically acceptable salt thereof.

16. The method of claim 15, wherein the GGDA compound comprises a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDERRAKDFIEWLKSA ;

HSQGTFTSDYSKYLDERRAKDFIEWLLSA;

HSQGTFTSDYSKYLDERRAKDFIEWLLKA;

HSQGTFTSDYSKYLDKRRAKDFIEWLLSA;

HSQGTFTSDYSKYLDEKRAKDFIEWLLSA; and

H-Aib-QGTFTSDYSKYLDEKRAKDFIEWLLSA.

17. The method of claim 15, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDERRAKDFIEWL-K(Hexadecanoyl-isoGlu)-SA-NH_2;

HSQGTFTSDYSKYLDERRA-K(Hexadecanoyl-isoGlu)-DFIEWLLSA-NH_2;

HSQGTFTSDYSKYLDERRAKDFIEWLL-K(Hexadecanoyl-isoGlu)-A-NH_2;

HSQGTFTSDYSKYLD-K(Hexadecanoyl-isoGlu)-RRAKDFIEWLLSA-NH_2;

HSQGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA-NH_2; and

H-Aib-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA-NH_2.

18. The method of any one of claims 1-3, wherein the GGDA compound comprises a compound of the formula VII:

R^1-Z^3-R^2 (formula VII)

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula VIII:

His-X2-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-X12-Tyr-Leu-Asp-X16-X17-Ala-Ala-X20-X21-Phe-Val-X24-Trp-Leu-X27-X28-Ala-X30 (formula VIII);

wherein

X2 is selected from Aib and Ser;

X12 is selected from Lys, Arg or Leu;

X16 is selected from Arg and X;

X17 is selected from Arg and X;

X20 is selected from Arg, His and X;

X21 is selected from Asp and Glu;

X24 is selected from Ala and X;

X27 is selected from Leu and X;

X28 is selected from Arg and X;

X30 is X or is absent;

wherein at least one of X16, X17, X20, X24, X27, X28, and X30 is Xa;

and wherein each residue Xa is independently selected from the group consisting of Glu, Lys, Ser, Cys, Dbu, Dpr and Orn;

wherein the side chain of at least one residue Xa is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Xa; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Xa via Z^2;

with the proviso that Z^3 is not HSQGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA.

19. The method of claim 18, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDKKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAKDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLKRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLKA;

HSQGTFTSDYSRYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSLYLDSKAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLRAK;

HSQGTFTSDYSKYLDSKAAHDFVEWLLSAK

HSQGTFTSDYSKYLDSKAAHDFVEWLKSA;

HSQGTFTSDYSKYLDSKAAHDFVKWLLRA;

HSQGTFTSDYSKYLDSCAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSCAAHDFVEWLLSA;

HSQGTFTSDYSKYLDSKAACDFVEWLLRA;

HSQGTFTSDYSKYLDKSAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSAK;

H-Aib-QGTFTSDYSKYLDSKAARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAKDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLKA

H-Aib-QGTFTSDYSKYLDSKAAKDFVAWLLSA

H-Aib-QGTFTSDYSKYLDSKAAHDFVAWLLKA;

H-Aib-QGTFTSDYSKYLDKKAAHDFVAWLLRA;

H-Aib-QGTFTSDYSRYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVKWLLSA;

H-Aib-QGTFTSDYSLYLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSCAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAACDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDK()KAAE()DFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVE()WLLK()A

H-Aib-QGTFTSDYSKYLDSKAAK()DFVE()WLLRA;

H-Aib-QGTFTSDYSKYLDSK()AAHE()FVEWLLKA; and

H-Aib-QGTFTSDYSKYLDSK()AAKE()FVEWLLRA;

wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

20. The method of claim 18, wherein the GGDA compound has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLD-K*-KAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAA-K*-DFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K*-RA;

HSQGTFTSDYSKYLDSKAAHDFVEWLL-K*-A;

HSQGTFTSDYSRYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSLYLDS-K*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLLRA-K*;

HSQGTFTSDYSKYLDSKAAHDFVEWLLSA-K*;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K*-SA;

HSQGTFTSDYSKYLDSKAAHDFV-K*-WLLRA;

HSQGTFTSDYSKYLDS-C*-AAHDFVEWLLRA;

HSQGTFTSDYSKYLDS-C*-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDSKAA-C*-DFVEWLLRA;

HSQGTFTSDYSKYLD-K*-SAAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLLSA-K*;

H-Aib-QGTFTSDYSKYLDS-K*-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAA-K*-DFVAWLLRA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLL-K*-A;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K*-AAHDFVEWLLKA;

H-Aib-QGTFTSDYSKYLDSKAA-K*-DFVAWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVAWLL-K*-A;

H-Aib-QGTFTSDYSKYLD-K*-KAAHDFVAWLLRA;

H-Aib-QGTFTSDYSRYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFV-K*-WLLSA;

H-Aib-QGTFTSDYSLYLDS-K*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-C*-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAA-C*-DFVEWLLRA;

H-Aib-QGTFTSDYSKYLD-S*-KAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDK()K*AAE()DFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSK*AAHDFVE()WLLK()A

H-Aib-QGTFTSDYSKYLDSK*AAK()DFVE()WLLRA;

H-Aib-QGTFTSDYSKYLDSK()AAHE()FVEWLLK*A; and

H-Aib-QGTFTSDYSKYLDSK()AAK*E()FVEWLLRA;

wherein "*" indicates the position of a lipophilic substituent on the residue immediately to the left of the "*", and wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

21. The method of claim 18, wherein Z^3 has a sequence selected from the group consisting of:

HSQGTFTSDYSKYLD-K(Hexadecanoyl-γ-Glu)-KAAHDFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWL-K(Hexadecanoyl-γ-Glu)-RA;

HSQGTFTSDYSKYLDSKAA-K(Hexadecanoyl-γ-Glu)-DFVEWLLRA;

HSQGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-γ-Glu)-A;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFVEWLL-K(Hexadecanoyl-γ-Glu)-A;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLKA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVE()WLLK()A;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-γ-Glu)-AAHDFVEWLLRA;

H-Aib-QGTFTSDYSKYLDSKAA-K(Hexadecanoyl-γ-Glu)-DFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Dodecanoyl-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[3-aminopropanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[8-aminooctanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-ε-Lys)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K([2-Butyloctanoyl]-γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-[4-Aminobutanoyl])-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-E)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K([2-Butyloctanoyl]-γ-Glu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-[4-Aminobutanoyl])-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Octadecanoyl- γ-Glu)-AAHDFVEWLLSA; and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-E)-AAHDFVEWLLSA;

wherein "()" represents an intramolecular bond between the side chains of the two amino acid residues immediately preceding the "()".

22. The method of claim 18, wherein Z^3 comprises a sequence selected from the group consisting of:

H-Aib-QGTFTSDYS-K(Hexadecanoyl-isoGlu)-YLDSKAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLD-K(Hexadecanoyl-isoGlu)-KAAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAA-K(Hexadecanoyl-isoGlu)-DFVEWLLSA;

H-Aib-QGTFTSDYSKYLDSKAAHDFV-K(Hexadecanoyl-isoGlu)-WLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoLys)-AARDFVAWLLRA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAKDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHEFVEWLLSA;

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAEDFVEWLLSA; and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLEA.

23. The method of any one of claims 19-21, wherein () represents a lactam ring between the side chains of the two residues.

24. The method of any one of claims 1-3, wherein the GGDA compound comprises a compound of the formula IX:

R^1-Z^3-R^2 (formula IX);

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula X

His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-X17-Ala-Ala-His-Asp-Phe-Val-Glu-Trp-Leu-Leu-X28 (formula X);

wherein

X17 is Xb

X28 is Ser or absent;

wherein Xb is selected from the group consisting of Glu, Lys, and Cys;

and wherein the side chain of Xb is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of Xb; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of Xb via Z^2.

25. The method of claim 24, wherein Z^3 has a sequence selected from the group consisting of:

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLS and

H-Aib-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLL.

26. The method of any one claims 1-3, wherein the GGDA compound comprises a compound of the formula XI:

R^1-Z^3-R^2 (formula XI);

wherein R^1 is H, C_{1-4} alkyl, acetyl, formyl, benzoyl or trifluoroacetyl;

R^2 is OH or NH_2;

and Z^3 is a peptide having the formula XII:

His-Aib-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-X17-Ala-Ala-His-Asp-Phe-Val-Glu-Trp-Leu-Leu-Ser-Ala (formula XII);

wherein

X17 is X ;

wherein X is selected from the group consisting of Glu, Lys, and Cys;

and wherein the side chain of X is conjugated to a lipophilic substituent having the formula:

(i) Z^1, wherein Z^1 is a lipophilic moiety conjugated directly to the side chain of X; or

(ii) Z^1Z^2, wherein Z^1 is a lipophilic moiety, Z^2 is a spacer, and Z^1 is conjugated to the side chain of X via Z^2.

27. The method of claim 26, wherein Z^3 has a sequence:

H-Aib-EGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA.

28. The method of any one of claims 1-3, wherein the GGDA compound comprises a sequence selected from the group consisting of:
H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA;
HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA;

HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA; and H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA.

29. The method of claims 1 or 2, wherein the GGDA compound is a full glucagon receptor agonist as determined by a pERK assay or a Ca^{2+} assay.

30. A method of treating or preventing a metabolic disease or condition, e.g., a disease or condition caused or characterized by excess body weight, e.g., obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea, metabolic syndrome, pre-diabetes, insulin resistance, glucose intolerance, type 2 diabetes, type I diabetes, hypertension, atherogenic dyslipidaemia, atherosclerois, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or microvascular disease, comprising administering a treatment-effective amount of a GGDA compound identified by a method of any one of claims 1-6.

31. A GGDA compound having the formula:

H-DSer-QGTFTSDYSKYLDE-K(Hexadecanoyl-isoGlu)-RAKDFIEWLLSA (Compound 17).

32. A GGDA compound having the formula:

HSQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 18).

33. A GGDA compound having the formula:

HGQGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 19).

34. A GGDA compound having the formula:

H-DAla-QGTFTSDYSKYLDS-K(Hexadecanoyl-isoGlu)-AAHDFVEWLLSA (Compound 20).

	Peptide structure	Glucagon R		GLP-1 R	
		pCa^{2+}	pERK	pCa^{2+}	pERK
hGLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH2	No effect	-	F	-
hGlucagon	HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH	F	F	F (tCa^{++})	-
Compound 1	HSQGTFTSDYSKYLDRARADDFVAWLKST-NH2	F	-	F	-
Compound 2	HSQGTFTSDYSKYLDSKAAHDFVEWLLRA-NH2	F	F	F	F
Compound 3	HSQGTFTSDYSKYLDERRAKDFIEWLLSA-NH2	F	F	F	F
Compound 4	HSQGTFTSDYSKYLDS**K*** AAHDFVEWLLRA-NH2	F	F	P	F
Compound 5	HSQGTFTSDYSKYLDE**K*** RAKDFIEWLLSA-NH2	F	F	P	F
Compound 6	HSQGTFTSDYSKYLDSKAAHDFVEWLL**K*** A-NH2	F	F	P	F
Compound 7	H-**Aib**-QGTFTSDYSKYLDS**K*** AAHDFVEWLLRA-NH2	P	P	P	F
Compound 8	H-**Aib**-QGTFTSDYSKYLDE**K*** RAKDFIEWLLSA-NH2	P	P	P	F
Compound 9	H-**Aib**-QGTFTSDYSKYLDS**K*** AAHDFVEWLLSA-NH2	P	P	P	F
Compound 10	H-**Aib**-QGTFTSDYSKYLDSKAAHDFVEWLL**K*** A-NH2	P	P	P	F
Liraglutide	HAEGTFTSDVSSYLEGQAA**K*** EFIAWLVRGRG-OH	No effect	No effect	F (tCa^{++})	F

Figure 1

Compound	EC_{50} Potency Value (nM)
GLP-1 (7-36) amide	8.5
hGLP-1	0.29
Exendin-4	0.11
hGlucagon	820
Compound 2	2.1

Compound	EC_{50} Potency Value (nM)
GLP-1 (7-36) amide	7.6
Compound 1	350
Compound 11	120
Compound 12	2300
Compound 3	2.4

Compound	EC_{50} Potency Value (nM)
GLP-1 (7-36) amide	8.8
Compound 4	3.5
Compound 8	5.9
Compound 5	2.3
Compound 9	2.1

Compound	EC_{50} Potency Value (nM)
GLP-1 (7-36) amide	7.6
Compound 13	0.4
Compound 7	3.4
Compound 10	15
Compound 6	13

Figure 2

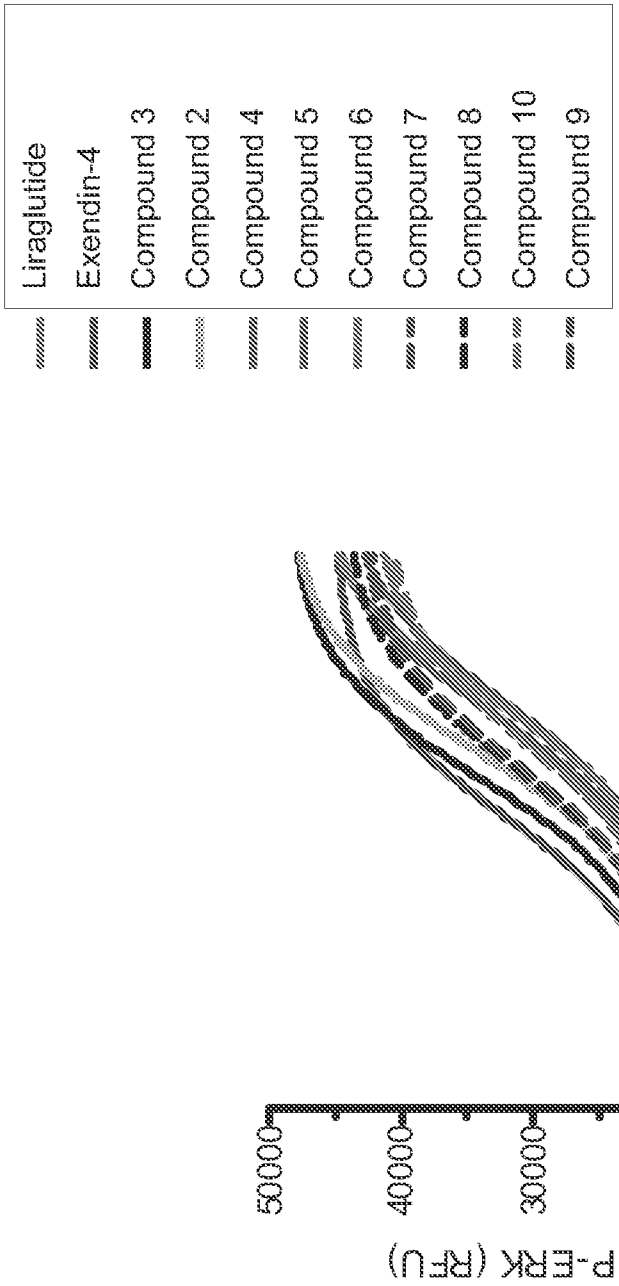

Liraglutide
Exendin-4
Compound 3
Compound 2
Compound 4
Compound 5
Compound 6
Compound 7
Compound 8
Compound 10
Compound 9
P-ERK (RFU)
50000
40000
30000
20000
0
-9
-8
-7
-6
-5
Log [M] peptide

Figure 3

Compound	EC_{50} Potency Value (nM)
Glucagon	8.8
hGLP-1	-
Exendin-4	-
hGlucagon	4.3
Compound 2	15

Compound	EC_{50} Potency Value (nM)
Glucagon	9.1
Compound 1	91
Compound 11	89
Compound 12	31
Compound 3	2.8

Compound	EC_{50} Potency Value (nM)
Glucagon	12
Compound 4	19
Compound 8	120
Compound 5	15
Compound 9	99

Compound	EC_{50} Potency Value (nM)
Glucagon	7.3
Compound 13	43
Compound 7	78
Compound 10	47
Compound 6	12

Figure 4

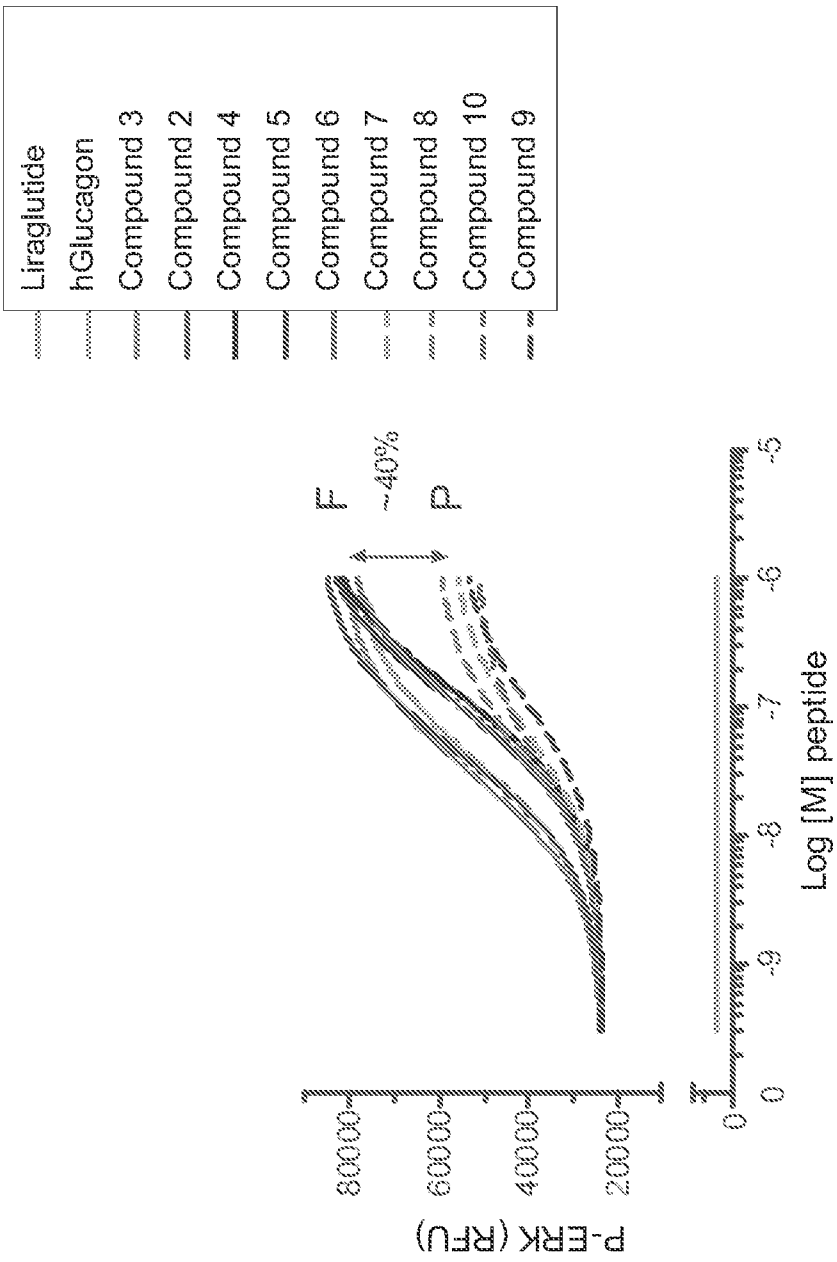
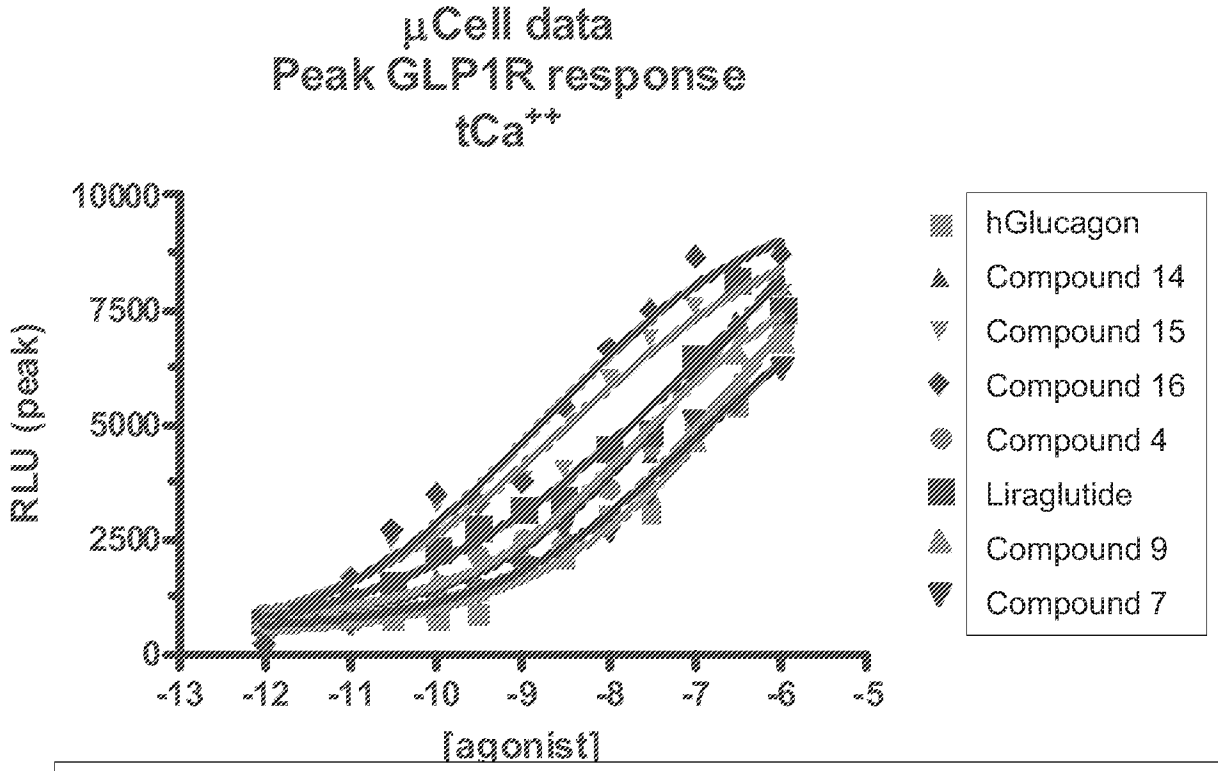

Liraglutide
hGlucagon
Compound 3
Compound 2
Compound 4
Compound 5
Compound 6
Compound 7
Compound 8
Compound 10
Compound 9
F
~40%
P
P-ERK (RFU)
80000
60000
40000
20000
0
-9
-8
-7
-6
-5
Log [M] peptide

Figure 5

cAMP data

Compound #	Glucagon-R EC_{50}	GLP1-R EC_{50}
Glucagon	0.027	2.271
Compound 1	0.794	0.407
Compound 2	0.051	0.052
Compound 3	0.064	0.063
Compound 4	0.082	0.103
Compound 5	0.183	0.133
Compound 6	0.333	0.396
Compound 7	0.162	0.089
Compound 8	0.156	0.126
Compound 9	0.215	0.207
Compound 10	0.159	0.113
Liraglutide	21.388	0.184

Figure 6



	hGlucagon	Compound 14	Compound 15	Compound 16	Compound 4	Liraglutide	Compound 9	Compound 7
Sigmoidal dose-response (variable slope)				Hit constraint				
Best-fit values								
Bottom	323.9	656.2	234.2	~ 1.699e-016	752.2	123.2	548.3	532.7
Top	15987	11314	9755	10079	8813	12819	11610	8444
LogEC50	-5.609	-7.018	-8.466	-8.773	-7.602	-6.908	-6.446	-7.124
HillSlope	0.3088	0.3727	0.3117	0.3307	0.4131	0.2476	0.3447	0.3789
EC50	2.461e-006	9.589e-008	3.418e-009	1.687e-009	2.503e-008	1.235e-007	3.584e-007	7.516e-008

Figure 7

	hGlucagon	Compound 14	Compound 15	Compound 16	Compound 4	Liraglutide	Compound 9	Compound 7
Sigmoidal dose-response (variable slope)						Not converged		
Best-fit values								
Bottom	534.0	633.6	625.7	577.0	590.7		631.4	622.3
Top	7761	7861	7636	7743	9964		6245	3782
LogEC50	-8.438	-7.605	-7.921	-7.410	-6.725		-6.131	-6.766
HillSlope	0.8500	0.8267	0.8341	0.6467	0.8439		0.9722	1.256
EC50	3.263e-009	2.464e-008	1.198e-008	3.886e-008	1.865e-007		7.399e-007	1.712e-007

Figure 8

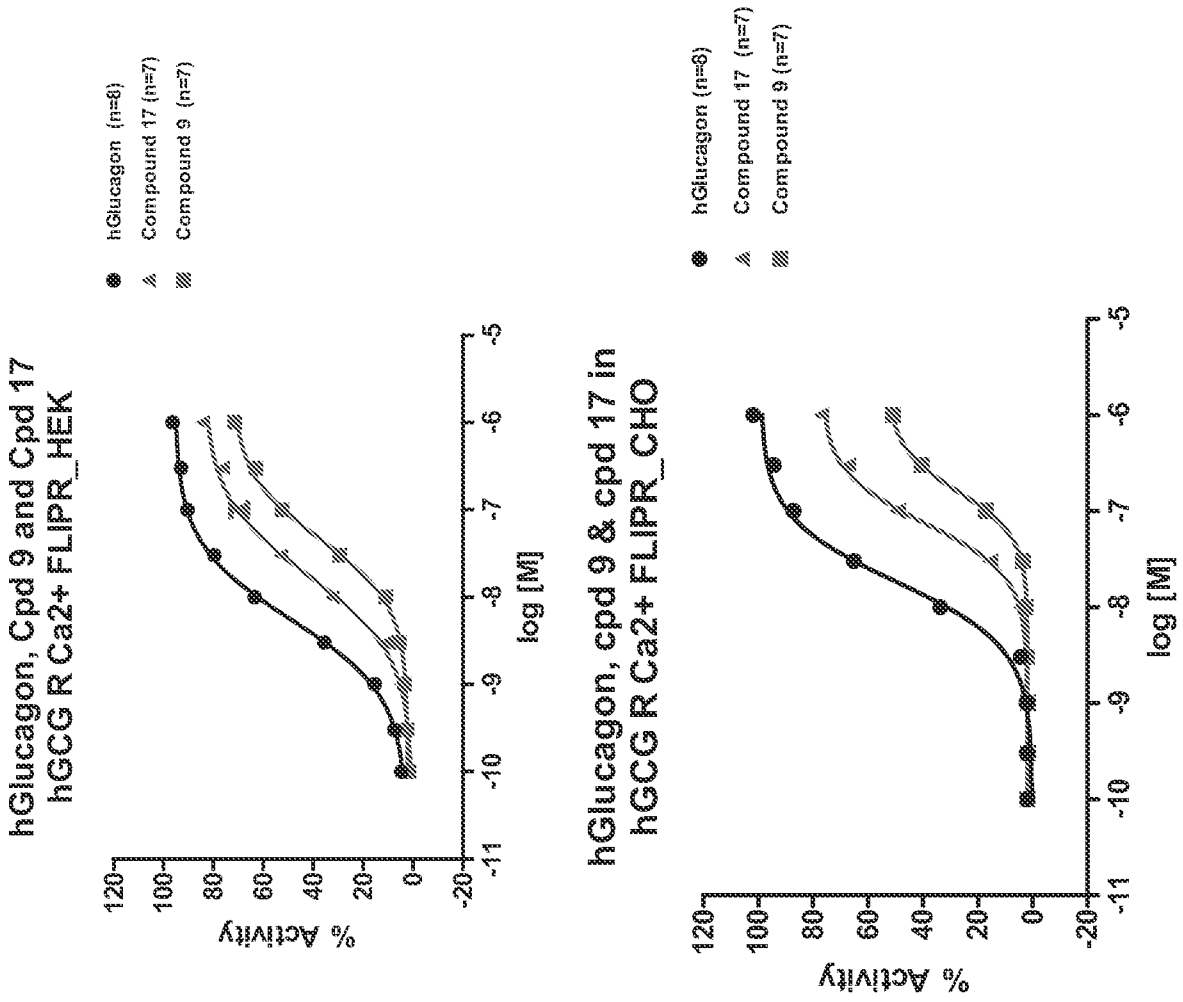

hGlucagon, Cpd 9 and Cpd 17
hGCG R Ca2+ FLIPR_HEK
hGlucagon (n=8)
Compound 17 (n=7)
Compound 9 (n=7)
% Activity
log [M]
hGlucagon, cpd 9 & cpd 17 in
hGCG R Ca2+ FLIPR_CHO
hGlucagon (n=8)
Compound 17 (n=7)
Compound 9 (n=7)
% Activity
log [M]

Figure 9

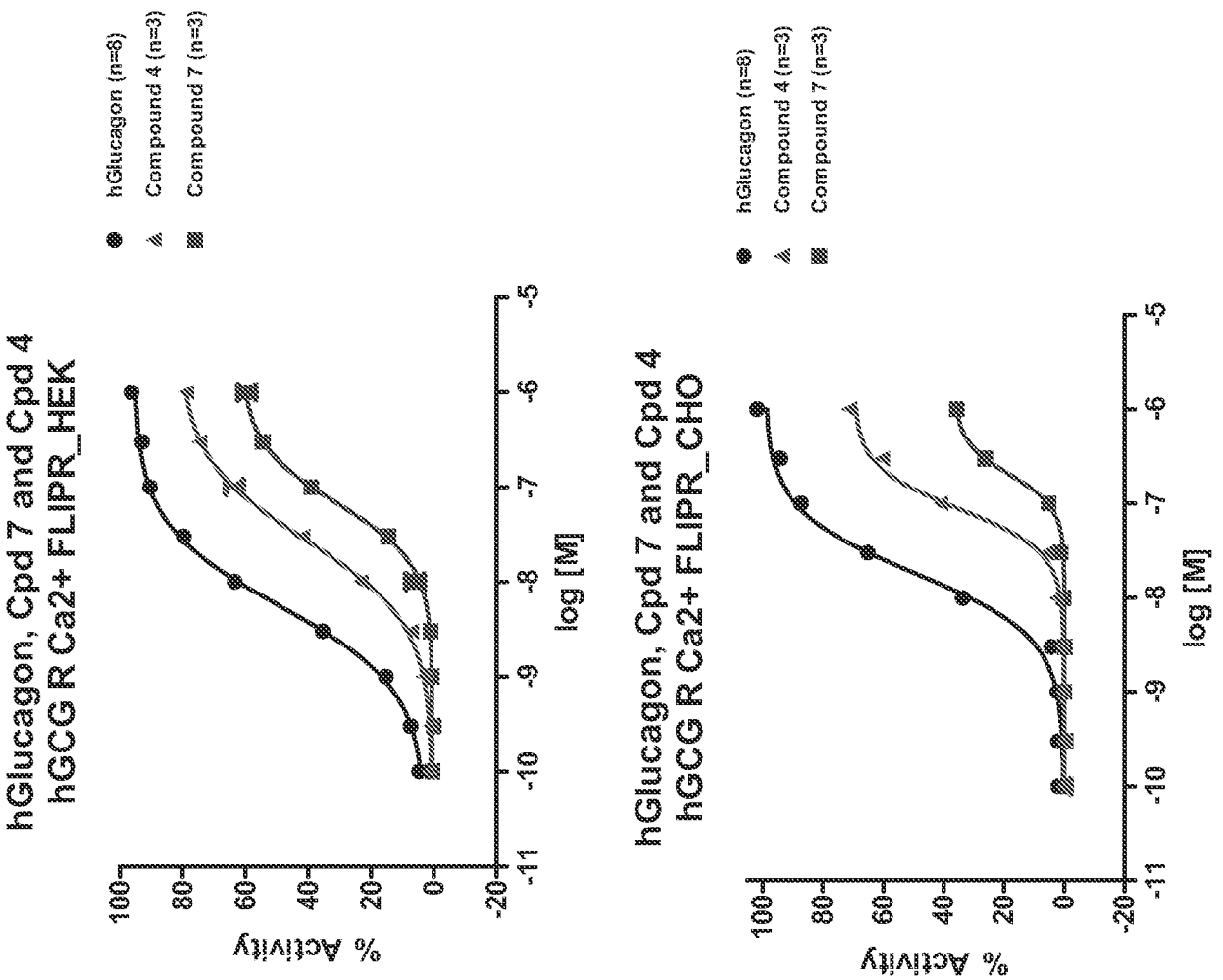

hGlucagon, Cpd 7 and Cpd 4
hGCG R Ca2+ FLIPR_HEK
hGlucagon (n=8)
Compound 4 (n=3)
Compound 7 (n=3)
% Activity
100
80
60
40
20
0
-20
-11
-10
-9
-8
-7
-6
-5
log [M]
hGlucagon, Cpd 7 and Cpd 4
hGCG R Ca2+ FLIPR_CHO
hGlucagon (n=8)
Compound 4 (n=3)
Compound 7 (n=3)
% Activity
100
80
60
40
20
0
-20
-11
-10
-9
-8
-7
-6
-5
log [M]

Figure 10

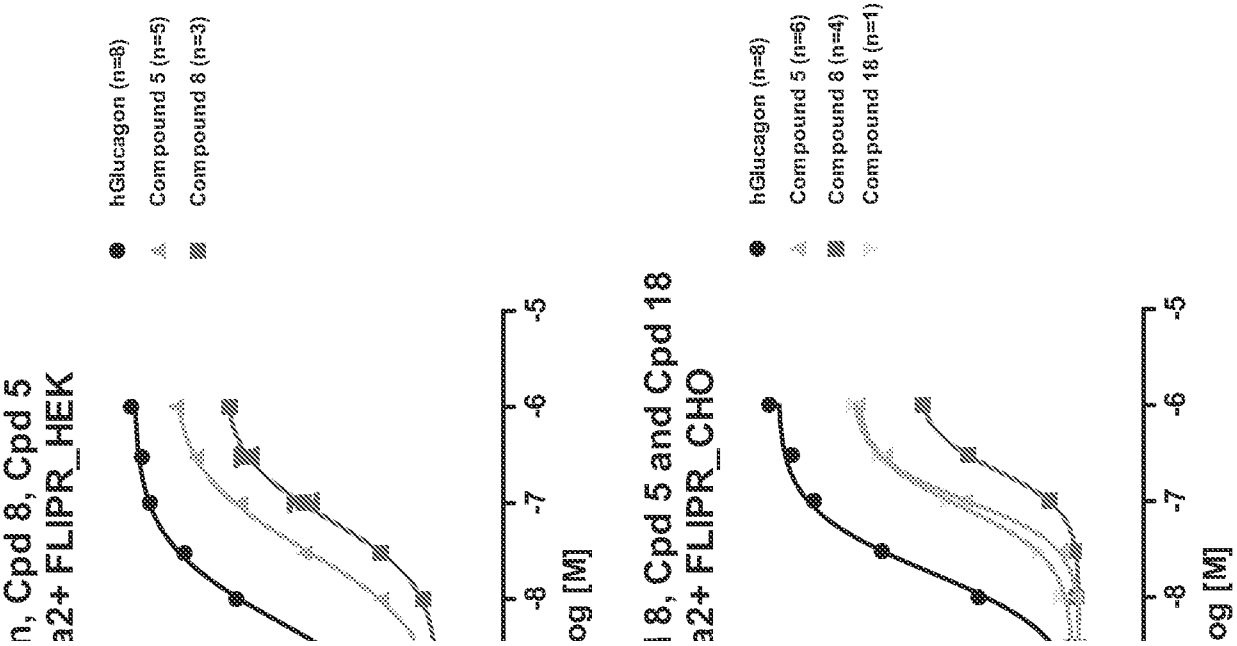

n, Cpd 8, Cpd 5
a2+ FLIPR_HEK
hGlucagon (n=8)
Compound 5 (n=5)
Compound 8 (n=3)
-8
-7
-6
-5
og [M]
l 8, Cpd 5 and Cpd 18
a2+ FLIPR_CHO
hGlucagon (n=8)
Compound 5 (n=6)
Compound 8 (n=4)
Compound 18 (n=1)
-8
-7
-6
-5
og [M]

Figure 11

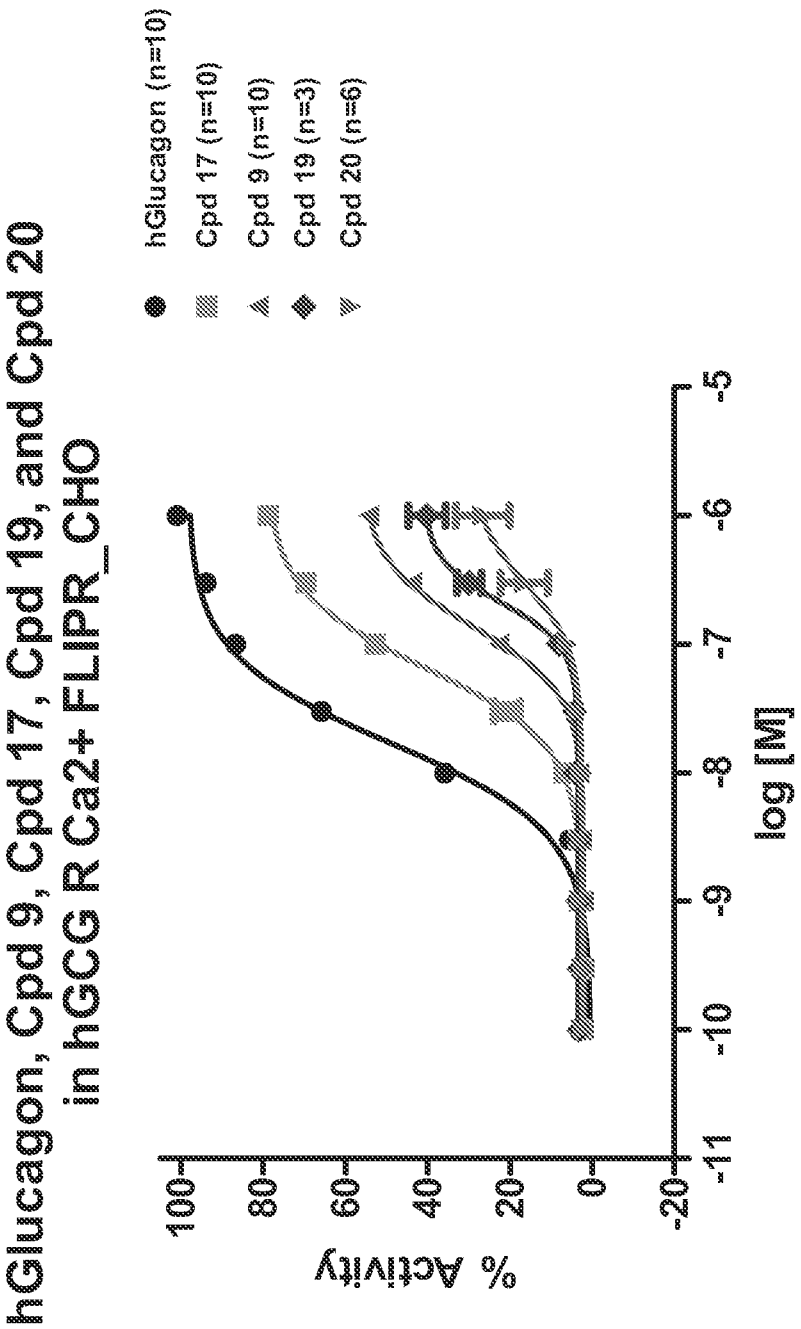

hGlucagon, Cpd 9, Cpd 17, Cpd 19, and Cpd 20
in hGCG R Ca2+ FLIPR_CHO
hGlucagon (n=10)
Cpd 17 (n=10)
Cpd 9 (n=10)
Cpd 19 (n=3)
Cpd 20 (n=6)
% Activity
100
80
60
40
20
0
-20
-11
-10
-9
-8
-7
-6
-5
log [M]

Figure 12

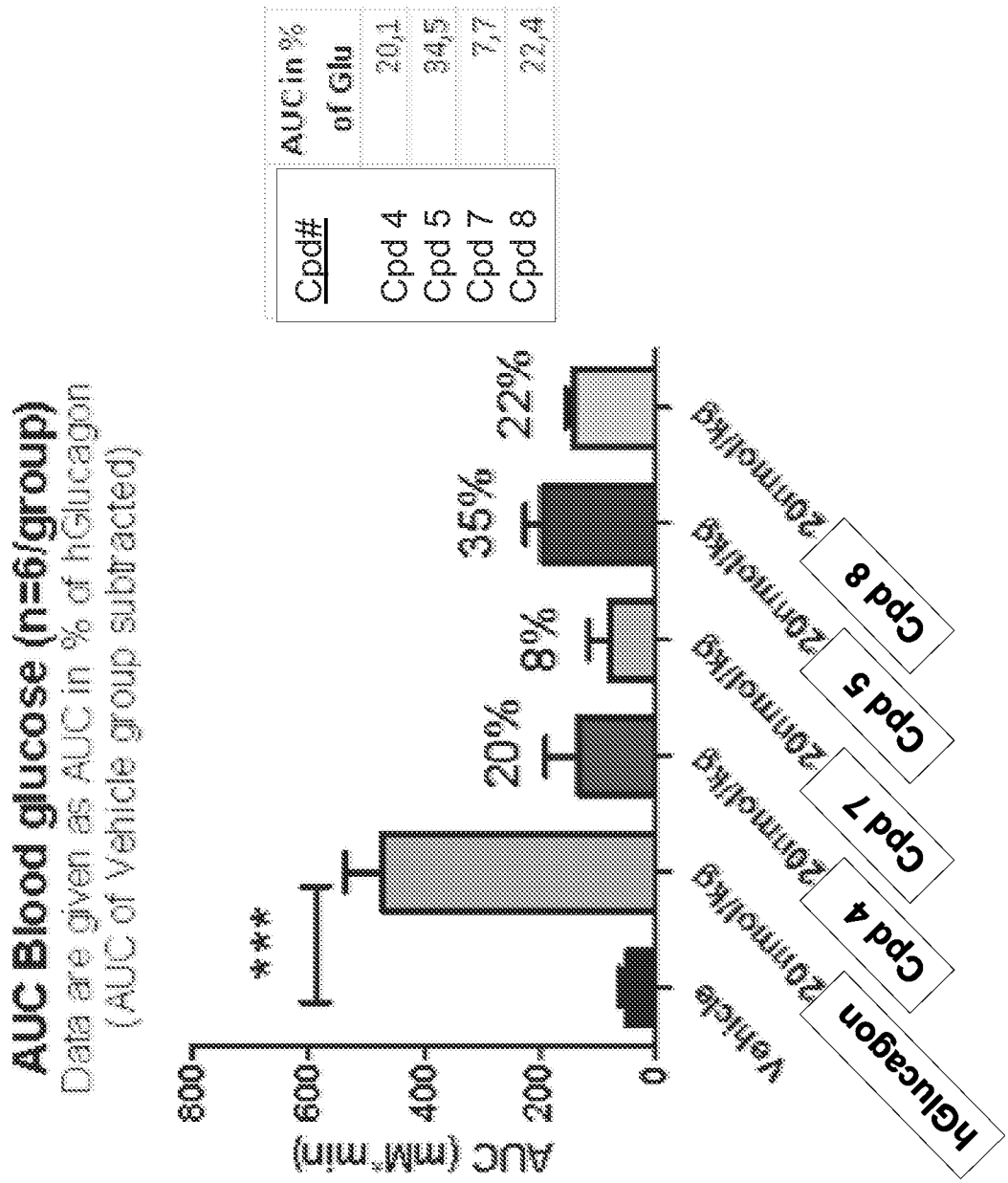

AUC Blood glucose (n=6/group)
Data are given as AUC in % of hGlucagon
(AUC of Vehicle group subtracted)
AUC (mM*min)
800
600
400
200
0
***
20%
8%
35%
22%
Vehicle
20nmol/kg
20nmol/kg
20nmol/kg
20nmol/kg
20nmol/kg
hGlucagon
Cpd 4
Cpd 7
Cpd 5
Cpd 8
Cpd#
AUC in % of Glu
Cpd 4 20,1
Cpd 5 34,5
Cpd 7 7,7
Cpd 8 22,4

Figure 13

compound	hGCG R cAMP_CHO				hGCG R Ca2+ FLIPR_CHO				hGCG R pERK_CHO			
	EC50_A vs (nM)	EC50_S pD (nM)	%Eff_S pD	EC50_n	EC50_A vs (nM)	EC50_S pD (nM)	%Eff_S pD	EC50_n	EC50_A vs (nM)	EC50_S pD (nM)	%Eff_S pD	EC50_n
hGlucagon	0.024	0.011	**106 (F)**	3.4	58	16	4	100 (F)	5	18	22	3	109 (F)	27	3
Compound 9	0.056	0.018	**107 (F)**	1.9	5	123	41	55 (P)	9	7	35	1	26 (P)	32	2
Compound 19	0.215	0.035	**105 (F)**	1.0	3	40% at 1000 nM (P)			3						
Compound 17	0.029	0.002	**103 (F)**	1.2	3	66	16	80 (F)	6	11	58	0	78 (F)	1	2
Compound 20	0.160	0.046	**105 (F)**	1.0	3	27% at 1000 nM (P)			6	6	129	17	51 (P)	8	2

Figure 14

Compound	hGCG R cAMP_CHO				hGCG R Ca2+ FLIPR_CHO				hGCG R mERK_CHO			
	EC50_Avg (nM)	EC50_SD (nM)	%Eff	%Eff_SD	EC50_Avg (nM)	EC50_SD (nM)	%Eff	%Eff_SD	EC50_Avg (nM)	EC50_SD (nM)	%Eff	%Eff_SD
H Glucagon	0.024	0.011	106	3.4	16	4	100	5	22	3	109	27
Cpd. 5	0.028	0.004	105	0.6	97	14	73	4	63		89	
Cpd. 8	0.041	0.006	104	2.3	113	21	60	5	53	14	57	8
Cpd 18	0.034	0.011	105	2.6	90	15	77	6				

	hGCG R cAMP_CHO				hGCG R Ca2+ FLIPR_CHO				hGCG R mERK_CHO			
	EC50_n				EC50_n				EC50_n			
H Glucagon	3				3				3			
Cpd. 5	1				1				1			
Cpd. 8	2				2				2			
Cpd 18	3				3							

Figure 15