

In one aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of R₁₀-DVX₁₁X₁₂YLX₁₅X₁₆QAX₁₉X₂₀EFX₂₃X₂₄WLVRRGPSSGAPPPSX₄₀-R₂₀ (SEQ ID NO: 97), wherein

- 5 R₁₀ is NH₂ or an N-terminal extension of 1 or 2 amino acids, wherein one of the amino acids of the N-terminal extension is acylated with a C14-C20 fatty acid or diacid,
 X₁₁ is Trp, dTrp or Ser;
 X₁₂ is Arg, Ser or acylated Lys;
 X₁₅ is Glu or dGlu;
10 X₁₆ is Glu, or dGlu;
 X₁₉ is Val;
 X₂₀ is Arg;
 X₂₃ is Ile, or dIle;
 X₂₄ is Glu, or Ala; and
15 X₄₀ is a bond or an acylated Lys; and
 R₂₀ is COOH or CONH₂, with the proviso that R₁₀ and X₁₂ cannot both comprise an acylated amino acid.

In another aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

- 20 DX₁₀X₁₁X₁₂YLX₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGPSSGAPPPS (SEQ ID NO: 98);
 wherein

- X₁₀ is Trp, dTrp or Val;
 X₁₁ is Trp, dTrp or Ser;
 X₁₂ is Arg, Lys or Ser;
25 X₁₅ is Glu or dGlu;
 X₁₆ is dGlu or Glu;
 X₂₃ is Ile or dIle;
 X₂₄ is Ala or Glu;

- said GLP-1 receptor antagonist being acylated with a C16-C18 fatty acid or C16-C18
30 fatty diacid.

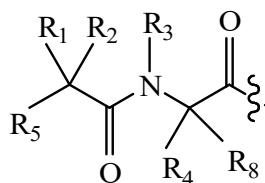
In another aspect, the present disclosure refers to a GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁RYLX₁₅X₁₆QAVREFX₂₃EWLVRRGGPSSGAPPSX₄₀R₂₀ (SEQ ID NO: 5),
wherein

- 5 X₁₀ is Trp, dTrp or Val;
X₁₁ is Trp, dTrp or Ser;
X₁₅ is Glu or dGlu
X₁₆ is dGlu or Glu;
X₂₃ is Ile or dIle;
10 X₄₀ is an acylated amino acid; and

R₂₀ is COOH or CONH₂.

In another aspect, the present disclosure refers to a derivative of the GLP-1 receptor antagonist as described above further comprising a dipeptide A-B:



- 15 linked to said GLP-1 antagonist through an amide bond wherein

R₁, R₂, R₄ and R₈ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄ alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W1)C₁-C₁₂ alkyl, wherein W1 is a heteroatom selected from the group consisting of N, S and O;

R₃ is selected from the group consisting of C₁-C₁₈ alkyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)NH₂, (C₁-C₁₈ alkyl)SH, (C₀-C₄ alkyl)(C₃-C₆)cycloalkyl, (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, and (C₁-C₄ alkyl)(C₃-C₉ heteroaryl) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine ring;

- 25 R₅ is NHR₆ or OH;
R₆ is H, C₁-C₈ alkyl; and
R₇ is selected from the group consisting of H and OH
wherein the chemical cleavage half-life (t_{1/2}) of A-B from said GLP-1 antagonist is at
30 least 1 hour to 1 week in PBS under physiological conditions.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising a GLP-1 receptor antagonist as disclosed herein, or a derivative as disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient.

- 35 In another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a patient suffering from hyperinsulinemic-induced hypoglycemia.

In accordance with one example a GLP-1 receptor antagonist peptide is provided comprising the amino acid sequence

Claims:

1. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of R₁₀-DVX₁₁X₁₂YLX₁₅X₁₆QAX₁₉X₂₀EFX₂₃X₂₄WLVRRGGPSSGAPPPSX₄₀-R₂₀ (SEQ ID NO: 97), wherein

R₁₀ is NH₂ or an N-terminal extension of 1 or 2 amino acids, wherein one of the amino acids of the N-terminal extension is acylated with a C14-C20 fatty acid or diacid,

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Ser or an acylated Lys;

X₁₅ is Glu or dGlu;

X₁₆ is Glu, or dGlu;

X₁₉ is Val;

X₂₀ is Arg;

X₂₃ is Ile, or dIle;

X₂₄ is Glu, or Ala; and

X₄₀ is a bond or an acylated Lys; and

R₂₀ is COOH or CONH₂, with the proviso that R₁₀ and X₁₂ cannot both comprise an acylated amino acid.

2. The GLP-1 receptor antagonist of claim 1 wherein said antagonist comprises the amino acid sequence of

R₁₀-DVX₁₁X₁₂YLX₁₅X₁₆QAX₁₉X₂₀EFX₂₃EWLVRRGGPSSGAPPPSX₄₀-R₂₀ SEQ ID NO: 23), wherein

R₁₀ is NH₂ or an N-terminal extension of 2 amino acids having the structure X₇X₈, wherein X₇ is an amino acid acylated with a C14-C20 fatty acid or diacid, and X₈ is Gly or a C₁-C₄ N-alkylated Gly;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Ser or an acylated Lys;

X₁₅ is Glu or dGlu;

X₁₆ is Glu, or dGlu;

X₁₉ is Val;

X₂₀ is Arg;

X₂₃ is Ile, or dIle; and

X₄₀ is an acylated Lys; and
R₂₀ is COOH or CONH₂.

3. The GLP-1 receptor antagonist of any one of claims 1-2 wherein R₁₀ is an acylated Lys and X₁₂ is Arg.

4. The GLP-1 receptor antagonist of any one of claims 1-2 wherein R₁₀ is NH₂ and X₁₂ is an acylated Lys.

5. The GLP-1 receptor antagonist of any one of claims 1-2 wherein R₁₀ is NH₂ and X₁₂ is Arg.

6. The GLP-1 receptor antagonist of claim 1 wherein the antagonist comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39.

7. The GLP-1 receptor antagonist of any one of claims 1-6 wherein the acylated amino acids of said GLP-1 antagonist are independently a Lys residue acylated with a C16-C18 fatty acid or C16-C18 fatty diacid directly linked to the Lys side chain or via a spacer comprising a

- i) gamma glutamic acid,
 - ii) minipege polymer: -[COCH₂(OCH₂CH₂)_kNH]-, wherein k is 2, 4, 6 or 8,
- or
- iii) any multiplicity or combination of i) or ii).

8. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁X₁₂YLX₁₅X₁₆QAVREFX₂₃X₂₄WLVRRGGPSSGAPPPS (SEQ ID NO: 98);

wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₂ is Arg, Lys or Ser;

X₁₅ is Glu or dGlu;

X₁₆ is dGlu or Glu;

X₂₃ is Ile or dIle;

X₂₄ is Ala or Glu;

said GLP-1 receptor antagonist being acylated with a C16-C18 fatty acid or C16-C18 fatty diacid.

9. A GLP-1 receptor antagonist, said antagonist comprising the amino acid sequence of

DX₁₀X₁₁RYLX₁₅X₁₆QAVREFX₂₃EWLVRGGPSSGAPPPSX₄₀R₂₀ (SEQ ID NO: 5), wherein

X₁₀ is Trp, dTrp or Val;

X₁₁ is Trp, dTrp or Ser;

X₁₅ is Glu or dGlu

X₁₆ is dGlu or Glu;

X₂₃ is Ile or dIle;

X₄₀ is an acylated amino acid; and

R₂₀ is COOH or CONH₂.

10. The GLP-1 receptor antagonist of claim 9 wherein X₁₁ is Trp or dTrp.

11. The GLP-1 receptor antagonist of claim 9 or 10 wherein the amino acid at position 12 is substituted with an acylated Lys.

12. The GLP-1 receptor antagonist of any one of claims 8-11 wherein

X₁₅ is dGlu;

X₁₆ is Glu; and

X₂₃ is Ile.

13. The GLP-1 receptor antagonist of any one of claims 8-11 wherein

X₁₅ is Glu;

X₁₆ is Glu; and

X₂₃ is Ile.

14 The GLP-1 receptor antagonist of any one of claims 8-13 wherein the N-terminal alpha amine of the GLP-1 receptor antagonist is covalently linked to said C16-C18 fatty acid or C16-C18 fatty diacid.

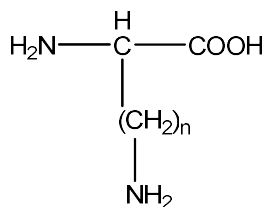
15 The GLP-1 receptor antagonist of claim 8 wherein said antagonist comprising the amino acid sequence of

DVSSYLEEQAVREFIEWLVRGGPSSGAPPPSK[C16]-NH₂ (SEQ ID NO: 70), wherein

K[C16] is a lysine residue acylated at its side chain with a C16 fatty acid or a C16 fatty diacid.

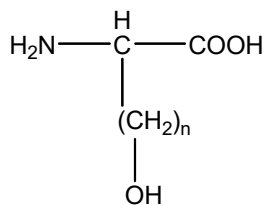
16. The GLP-1 receptor antagonist of any one of claims 10-15 wherein X₄₀ is an amino acid having an acyl group linked to the side chain of the amino acid.

17. The GLP-1 receptor antagonist of claim 16 wherein X₄₀ is an amino acid comprising a structure of Formula I, Formula II, or Formula III, wherein each of Formulae I, II, and III, is:



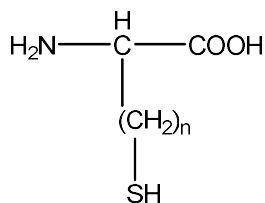
wherein n = 1 to 4

[Formula I];



wherein n = 1 to 4

[Formula II]; and



wherein n = 1 to 4

[Formula III].

18. The GLP-1 receptor antagonist of claim 15 or 16 wherein X₄₀ is an acylated lysine.

19. The GLP-1 receptor antagonist of any one of claims 10-18 wherein the acyl group of the acylated amino acid is selected from (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀CH₃, (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀CH₃, (C₁-C₄ alkyl)NH-CO(CH₂)₁₄₋₂₀COOH or (C₁-C₄ alkyl)NH-[spacer]-CO(CH₂)₁₄₋₂₀COOH.

20. The GLP-1 receptor antagonist of any one of claims 10-19 wherein the acyl group of the acylated amino acid is covalently linked to the amino acid side chain of the acylated amino acid via a spacer.

21. The GLP-1 receptor antagonist of any one of claims 16-20 wherein the spacer is an amino acid or dipeptide.

22. The GLP-1 receptor antagonist of any one of claims 16-20, wherein the spacer comprises a

- i) gamma glutamic acid,
- ii) minipeeg polymer: -[COCH₂(OCH₂CH₂)_kNH]-, wherein k is 2, 4, 6 or 8,
- iii) or any multiplicity or combination of i) and/or ii).

23. The GLP-1 receptor antagonist of any one of claims 10-22, wherein the acylated amino acid is linked to a C16 to C18 fatty acid or C16 to C18 fatty diacid, via a spacer comprising the structure:

-(gamma glutamic acid)_p-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_n-

or

-[COCH₂(OCH₂CH₂)_kNH]_q-(gamma glutamic acid)_p-[COCH₂(OCH₂CH₂)_kNH]_m-;

wherein k is an integer selected from 2, 4 or 8, m and n are independently an integer selected from 0, 1 or 2, and p and q are independently an integer selected from 1, 2, 4 or 8.

24. The GLP-1 receptor antagonist of 23, wherein the acylated amino acid is Lys having a C16 to C18 fatty acid linked to the lysine side chain via a spacer comprising the structure:



wherein k is 2, and p and q are independently an integer selected from 1 or 2.

25. The GLP-1 receptor antagonist of claim 10 wherein

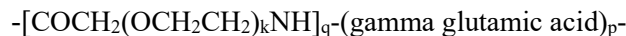
X₁₁ is Trp or dTrp;

X₁₅ is Glu or dGlu;

X₁₆ is Glu or dGlu;

X₂₃ is Ile or dIle; and

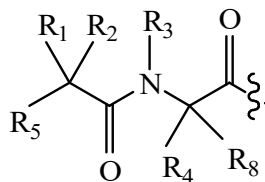
X₄₀ is Lys acylated with a C16 or C18 diacid via a spacer comprising the structure:



wherein k is 2, and p and q are independently an integer selected from 1 or 2.

26. The GLP-1 receptor antagonist of any one of claims 10-20 wherein R₂₀ is CONH₂.

27. A derivative of the GLP-1 receptor antagonist of any one of the claims 9-26 further comprising a dipeptide A-B:



linked to said GLP-1 antagonist through an amide bond wherein

R₁, R₂, R₄ and R₈ are independently selected from the group consisting of H, C₁-C₁₈ alkyl, C₂-C₁₈ alkenyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, (C₁-C₄

alkyl)NHC(NH₂⁺)NH₂, (C₀-C₄ alkyl)(C₃-C₆ cycloalkyl), (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, (C₁-C₄ alkyl)(C₃-C₉ heteroaryl), and C₁-C₁₂ alkyl(W₁)C₁-C₁₂ alkyl, wherein W₁ is a heteroatom selected from the group consisting of N, S and O;

R₃ is selected from the group consisting of C₁-C₁₈ alkyl, (C₁-C₁₈ alkyl)OH, (C₁-C₁₈ alkyl)NH₂, (C₁-C₁₈ alkyl)SH, (C₀-C₄ alkyl)(C₃-C₆)cycloalkyl, (C₀-C₄ alkyl)(C₂-C₅ heterocyclic), (C₀-C₄ alkyl)(C₆-C₁₀ aryl)R₇, and (C₁-C₄ alkyl)(C₃-C₉ heteroaryl) or R₄ and R₃ together with the atoms to which they are attached form a pyrrolidine ring;

R₅ is NHR₆ or OH;

R₆ is H, C₁-C₈ alkyl; and

R₇ is selected from the group consisting of H and OH

wherein the chemical cleavage half-life (t_{1/2}) of A-B from said GLP-1 antagonist is at least 1 hour to 1 week in PBS under physiological conditions.

28. The derivative of claim 27 wherein the dipeptide A-B is covalently linked to the N-terminal alpha amine of the GLP-1 antagonist amino acid sequence.

29. The derivative of claim 27 or 28 wherein

R₁ and R₈ are independently H or C₁-C₈ alkyl;

R₂ and R₄ are independently selected from the group consisting of H, C₁-C₈ alkyl, (C₁-C₄ alkyl)OH, (C₁-C₄ alkyl)SH, (C₂-C₃ alkyl)SCH₃, (C₁-C₄ alkyl)CONH₂, (C₁-C₄ alkyl)COOH, (C₁-C₄ alkyl)NH₂, and (C₁-C₄ alkyl)(C₆ aryl)R₇;

R₃ is C₁-C₆ alkyl;

R₅ is NH₂; and

R₇ is selected from the group consisting of hydrogen, and OH.

30. The derivative of claim 27 or 28, wherein

R₁, comprises a side chain selected from the group consisting of C₁-C₈ alkyl, (C₁-C₄ alkyl)OH, (C₁-C₄ alkyl)SH, (C₁-C₄ alkyl)COOH, and (C₁-C₄ alkyl)NH₂, wherein a C₁₆-C₃₀ fatty acid or C₁₆-C₃₀ diacid is covalently linked to said side chain, either directly or via a spacer selected from the group consisting of a gamma glutamic acid, a gamma glutamic acid-gamma glutamic acid dipeptide, and a gamma glutamic acid-[COCH₂(OCH₂CH₂)_kNH]_q-gamma glutamic acid, wherein

k is an integer selected from the range of 1-8; and

q is an integer selected from the range of 1-8;

R₂, R₄ and R₈ are independently H, or C₁-C₄ alkyl;

R₃ is C₁-C₆ alkyl; and

R₅ is NH₂.

31. The derivative of claim 27 or 28, wherein

R₁ is H, C₁-C₄ alkyl, (C₁-C₄ alkyl)OH or (C₁-C₄ alkyl)NH₂;

R₂ is H,

R₃ is C₁-C₄ alkyl;

R₄ is H, or C₁-C₄ alkyl;

R₅ is NH₂; and

R₈ is hydrogen.

32. The derivative of claim 27 or 28, wherein said dipeptide A-B

comprises an acylated Lys residue and an N-alkylated Gly residue, wherein said Lys and N-alkylated Gly residues are linked via a peptide bond.

33. The derivative of claim 27 or 28, wherein

R₁ is (C₄ alkyl)NH₂ or (C₄ alkyl)NH(mPeg-γE-diacid)-C18;

R₂, R₄ and R₈ are each H;

R₃ is C₁-C₄ alkyl; and

R₅ is NH₂;

R₅ is an amine.

34. A pharmaceutical composition comprising a GLP-1 receptor antagonist

of any one of claims 1-26, or a derivative of any one of claims 27-33 and a pharmaceutically acceptable carrier, diluent, or excipient.

35. Use of the pharmaceutical composition of claim 34 in the manufacture

of a medicament for treating a patient suffering from hyperinsulinemic-induced hypoglycemia.