

GLP-1/GIP DUAL AGONIST, PREPARATION METHOD AND USE THEREOF

TECHNICAL FIELD

The present invention relates to the field of medicine, and in particular relates to a GLP-1/GIP dual agonist and its use in the manufacture of a medicament for treating diabetes mellitus.

BACKGROUND ART

Type II diabetes mellitus is a kind of chronic metabolic disease clinically characterized by hyperglycemia caused by insulin resistance. Elevated blood glucose can increase the incidence and mortality of other diseases. All the methods commonly used to treat the disease at present, such as doing more exercise, controlling the diet, orally administering a hypoglycaemic drug and injecting a therapeutic drug can only temporarily maintain the blood glucose balance, and cannot fundamentally treat diabetes mellitus and various complex complications caused by hyperglycaemia. Therefore, it is of great clinical importance to extensively explore and develop a therapeutic drug for treating diabetes mellitus involving a novel hypoglycaemic mechanisms with high safety.

Glucagon-like peptide-1 (GLP-1) is a kind of hormone mainly produced by intestinal L cells and belongs to incretin. GLP-1 is an important incretin hormone secreted by intestinal L cells, and its main physiological functions in the body include stimulating the secretion and release of insulin, inhibiting the secretion of glucagon, promoting the proliferation of pancreatic β cells and inhibiting its apoptosis, inhibiting gastric emptying, promoting the generation of satiety and the like. The function of glucose-dependent insulinotropic polypeptide (GIP) is regulating blood glucose by promoting insulin and glucagon, which has also been widely confirmed and applied.

Compounds with GLP-1/GIP dual agonist activity have been disclosed in WO2013164483A1, WO2014192284A1 and WO2016111971A1.

Modifying a polypeptide with a fatty acid, through which the fatty acid chain is physically bond to an albumin subdomain/motif, can, for example, increase the half-life, thereby improving the pharmacokinetics of the peptide. Although the half-life of a peptide may be improved by use of a fatty acid, the specific prolonging effect and some side effects are still technical problems that need to be solved.

A series of adverse effects are produced by GLP-1. For example, there are concerns about the effects of GLP-1 receptor agonists on pancreatic and thyroid tissues, since animal studies and drug database analysis have shown that these drugs are associated with pancreatitis, pancreatic cancer and thyroid cancer. According to other cases reported, the use of GLP-1 (mainly Exenatide) is associated with the occurrence of acute kidney injury, mainly due to

haemodynamic disturbances caused by nausea, vomiting and diarrhoea. The most common symptom associated with the use of GLP-1 receptor agonists is gastrointestinal symptoms, mainly including nausea. Other common adverse reactions include injection site reactions, headache and nasopharyngitis and the like. (Filippatos TD, Panagiotopoulou TV, Elisaf MS. Adverse Effects of GLP-1 Receptor Agonists. *Rev Diabet Stud.* 2014 Fall-Winter;11(3-4):202-30. doi: 10.1900/RDS.2014.11.202. Epub 2015 Feb 10. PMID: 26177483; PMCID: PMC5397288.)

About the side effects of Tirzepatide (Mathiesen DS, Bagger JI, Bergmann NC, Lund A, Christensen MB, Vilsbøll T, Knop FK. The Effects of Dual GLP-1/GIP Receptor Agonism on Glucagon Secretion-A Review. *Int J Mol Sci.* 2019 Aug 22;20(17): 4092. doi: 10.3390/ijms20174092. PMID: 31443356; PMCID: PMC6747202.): at the highest dose, the number of adverse events caused by Tirzepatide exceeds that caused by Dulaglutide, especially gastrointestinal tract adverse events (66.0% for the subjects taken 15 mg Tirzepatide *versus* 42.6% for the subjects taken 1.5 mg Dulaglutide), and the total number of hypoglycemic episodes caused by Tirzepatide also exceeds that caused by Dulaglutide (7.5% for the subjects taken 15 mg Tirzepatide *versus* 3.7% for the subjects taken Dulaglutide)(Frias J.P., Nauck M.A., Van J., Kutner M.E., Cui X., Benson C., Urva S., Gimeno R.E., Milicevic Z., Robins D., et al. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in subjects with type 2 diabetes mellitus: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *Lancet.* 2018;392:2180–2193. doi: 10.1016/S0140-6736(18)32260-8). In order to avoid this problem, a titration strategy to reduce adverse events of Tirzepatide has been reported (Frias J.P., Nauck M.A., Van J., Benson C., Bray R., Milicevic Z., Haupt A., Robins D.A. 993-P: A 12-week, randomized, placebo-controlled study assessing the efficacy and safety of three dose-escalation algorithms of Tirzepatide, a novel dual GIP and GLP-1 receptor agonist, in subjects with type 2 diabetes mellitus. *Diabetes mellitus.* 2019;68:993. doi: 10.2337/db19-993-P). Moreover, as compared with the previously reported studies, the three-step dose escalation regimen for delivering 15 mg Tirzepatide seems ineffective in reducing the overall incidence of gastrointestinal side effects (Frias J.P., Nauck M.A., Van J., Kutner M.E., Cui X., Benson C., Urva S., Gimeno R.E., Milicevic Z., Robins D., et al. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in subjects with type 2 diabetes mellitus: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. *Lancet.* 2018;392:2180–2193. doi: 10.1016/S0140-6736(18)32260-8). In the subjects being administered 15 mg Tirzepatide, the incidence of hypoglycemic episodes also increased to about 16%. Although the treatment with Tirzepatide was still associated with desired weight loss (about 5.6 kg after 12 weeks), the high incidence of gastrointestinal side effects did raise an uncertainty about the clinical potential of Tirzepatide. In addition, the titration strategy given in the commercial product instruction for Tirzepatide lasts for a long time. The initial dosage is 2.5 mg, and the dosage is increased to 5 mg after 4 weeks. If the dosage needs to be increased

to control blood glucose, the current dosage should be maintained for 4 weeks before increasing a further 2.5 mg, which is quite inconvenient.

At present, there is still a need in medicine to provide new compounds, which are dual agonists of GIP and GLP-1 receptors, and have the possibility of administrating once a week, which offers a safer, more effective and more convenient option.

Summary of Invention

An object of the present invention is to provide some long-acting GLP-1/GIP dual agonist compounds. Compared with GIP/GLP-1 dual agonist compounds in the art, the compounds according to the present invention have a long half-life and/or a longer duration of action. The compounds according to the present invention have a stronger hypoglycemic effect. The compounds according to the present invention have less side effects, including but not limited to gastrointestinal reactions and heart safety risks and the like. The compounds according to the present invention could adopt shorter titration strategies. The compounds according to the present invention have a wider safety window. In addition, the compounds according to the present invention have a protective effect on the liver.

As a part of the present invention, the applicant has found that the selections in terms of the length, composition and bonding position of the fatty acid chains, and the linker between the peptide and the fatty acid chain, have an unexpected effect on prolonging the half-life of the polypeptide.

An embodiment of the present invention provides a compound of Formula (AI) and a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-A11-A12-A13-L-D-K-A17-A-Q-A20-E-F-V-K-W-L-L-K-A29-G-P-S-S-G-A-P-P-P-S-K Formula (AI);

wherein X₁ is Aib; X₂ is α MePhe;

A11 is Aib or Ala;

A12 is Ala、Ile、Lys、Phe or Pya(4);

A13 is Aib、Cha、Leu、 α MePhe or α MeTyr;

A17 is Gln or Ile;

A20 is Ala or Ser;

A29 is Gln or Gly;

1, 2, 3 or 4 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-

Z, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 24, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates.

In a further embodiment, the present invention provides a compound of Formula (AI) or a pharmaceutically acceptable salt thereof, wherein,

X₁ is Aib; X₂ is αMePhe;

A11 is Aib or Ala;

A12 is Ala, Ile, Lys, Phe or Pya(4);

A13 is Aib, Cha, Leu, αMePhe or αMeTyr;

A17 is Gln or Ile;

A20 is Ala or Ser;

A29 is Gln or Gly,

1, 2, 3 or 4 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates; in addition, the present invention further provides compounds wherein c is independently selected from 14, 16, 18 or 20.

The peptide of the above Formula (AI) or a pharmaceutically acceptable salt thereof, wherein A11 is Aib; and/or

the peptide of the above Formula (AI) or a pharmaceutically acceptable salt thereof, wherein A12 is Ile; and/or

the peptide of the above Formula (AI) or a pharmaceutically acceptable salt thereof, wherein A13 is Aib; and/or

the peptide of the above Formula (AI) or a pharmaceutically acceptable salt thereof, wherein A17 is Gln; and/or

the peptide of the above Formula (AI) or a pharmaceutically acceptable salt thereof, wherein A20 is Ala; and/or

the peptide of the above Formula (AI), or a pharmaceutically acceptable salt thereof, wherein A29 is Gly.

An embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; 1, 2, 3 or 4 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 24, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In some embodiments, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein 1 K selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 40 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z.

In some embodiments, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein 2 Ks selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16 and K at position 24 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 24 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-

ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 24 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 28 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z.

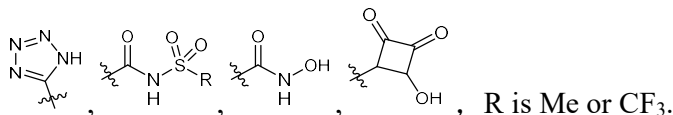
In some embodiments, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein 3 Ks selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified with three modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16, K at position 24 and K at position 28 are chemically modified with three modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16, K at position 24 and K at position 40 are chemically modified with three modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16, K at position 28 and K at position 40 are chemically modified with three modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 24, K at position 28 and K at position 40 are chemically modified with three modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z.

In some embodiments, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein 4 Ks selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified with four modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z. For example, K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified with four modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z.

In some embodiments, the present invention provides a compound of Formula (I), or a pharmaceutically acceptable salt thereof, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates, for example, but not limited to the instances where said Z may comprise a carboxylic acid (-

CO₂H) or carboxylic acid bioisosteres (for example , a phosphonic acid (-P(O)(OH)₂) or a sulfonic acid (-SO₂OH) group, preferably -CO₂H.

As for carboxylic acid bioisosteres, suitable carboxylic acid bioisosteres are known in the art. Preferably, the bioisosteres have protons with a pK_a similar to that of the corresponding carboxylic acids. Examples of suitable bioisosteres may include, but are not limited to, tetrazoles, acylsulfonamides, acylhydroxylamines and squaric acid derivatives as shown below:



In some embodiments, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, preferably 1, 2 or 3, b is independently selected from an integer of 1, 2, 3, 4 or 5, preferably 1, 2 or 3, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

An embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; 1 K or 2 Ks selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-Z, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates, preferably -CO₂H, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 24, and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; 1 K or 2 Ks selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40 are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-Z, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates, preferably -CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

An embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-

acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 16 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 40 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 40 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 and K at position 28 are chemically modified with two modifying chains through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 24 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 24 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 16 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

A further embodiment of the present invention provides a compound of Formula (I) and a pharmaceutically acceptable salt thereof,

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

In a further embodiment, the present invention provides a compound of Formula (I) or a pharmaceutically acceptable salt thereof, wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 and K at position 40 are chemically modified with two modifying chains through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20.

In all the above-mentioned embodiments, each a is independently an integer of 1 to 3, each b is independently an integer of 1 to 3, and each c is independently an integer of 12 to 22. In addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20. Among all the embodiments described above, preferred are the embodiments in which K at position 24 or K at position 28 is modified.

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified

through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:7).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₁₈-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:13).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:14).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:15).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-

acetyl)₃-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:16).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:8).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:17).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:18).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ -Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:19).

In an embodiment, the present invention provides a compound of the following Formula or a pharmaceutically acceptable salt thereof;

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:20).

The present invention further provides a method for increasing the action duration of the GLP-1/GIP dual agonist in a subject, characterized by using the acylated GLP-1/GIP dual agonist provided by the present invention as described above.

In an embodiment, the present invention provides a composition comprising the compound according to the present invention and a pharmaceutically acceptable carrier, diluent or excipient.

An embodiment provides a use of the compound according to any one of the above-mentioned embodiments in the manufacture of a medicament for treating or preventing a disease selected from hyperglycemia, impaired glucose tolerance, Type I diabetes mellitus, Type II diabetes mellitus, obesity, hypertension, Syndrome X, dyslipidemia, cognitive impairment, atherosclerosis, myocardial infarction, coronary heart disease and other cardiovascular diseases, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

An embodiment provides a use of the compound according to any one of the above-mentioned embodiments in the manufacture of a medicament for delaying or preventing the disease development of Type II diabetes mellitus.

An embodiment provides a use of the compound according to any one of the above-mentioned embodiments in the manufacture of a medicament for reducing food intake, reducing β -cell apoptosis, increasing β -cell function and β -cell volume, and/or restoring glucose sensitivity of β -cell.

In an embodiment, the present invention provides a method for treating Type II diabetes mellitus, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention.

In an embodiment, the present invention provides a method of improving blood glucose control in a subject suffering from Type II diabetes mellitus, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention as an adjunct to diet and exercise.

In an embodiment, the present invention provides a method for chronic weight management in a subject with an initial body mass index ≥ 27 and Type 2 diabetes, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention as an adjunct to a reduced-calorie diet and increased physical activity.

In an embodiment, the present invention provides a method for treating metabolic syndrome, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention. In a further embodiment, the present invention provides a method for treating dyslipidaemia, obesity and/or hepatic steatosis associated with insulin resistance and diabetes mellitus, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention. Additionally, the present invention further provides a method for treating frailty or increasing bone strength, comprising administering to a subject in need thereof, an effective amount of the compound according to the present invention.

In an embodiment, the present invention provides a compound of the present invention as an adjunct to diet and exercise for blood glucose control in a Type II diabetic subject. In an embodiment, the present invention provides a compound of the present invention for chronic weight management as an adjunct to a reduced-calorie diet and increased physical activity in a subject with an initial body mass index ≥ 27 and Type II diabetes.

Due to the above-mentioned activities of the compounds according to the present invention towards GLP-1 receptor and GIP receptor, the compound according to the present invention can be used as a medicament for treating or preventing various diseases, such as obesity. The compound according to the present invention can be used as a medicament for preventing or treating a disease, such as, symptomatic obesity, obesity based on simple obesity, obesity-related conditions or diseases, eating disorders, diabetes mellitus (for example, Type I diabetes mellitus, Type II diabetes mellitus, gestational diabetes mellitus, obesity diabetes mellitus), hyperlipidemia (e.g., hypertriglyceridemia, hypercholesterolemia, hyper-LDL-cholesterolemia, low HDL-cholesterolemia, postprandial hyperlipidemia), hypertension, heart failure, complications of diabetes mellitus [for example, neuropathy, nephropathy, retinopathy, diabetic cardiomyopathy, cataract, macroangiopathy, osteopenia, hyperosmolar diabetic coma], infectious disease (for example, respiratory tract infection, urinary tract infection, gastrointestinal infection, superficial soft tissue infection, lower extremity infection), diabetic gangrene, xerostomia, hypacusia, cerebrovascular disorder, peripheral blood circulation disorder, metabolic syndrome (with three or more conditions selected from the group consisting of hypertriglyceridemia (TG), low HDL-cholesterolemia (HDL-C), hypertension, abdominal obesity, and impaired glucose tolerance), sarcopenia, and the like.

Examples of symptomatic obesity include: endocrine obesity (e.g., Cushing's syndrome, hypothyroidism, insulinoma, obese Type II diabetes mellitus, pseudohypoparathyroidism, hypogonadism), central obesity (e.g. hypothalamic obesity, frontal lobe syndrome, Klein-Lewis

syndrome), genetic obesity (e.g., Prader-Willi syndrome, Laurence-Moon-Biedl's syndrome), drug-induced obesity (e.g., steroids, phenothiazines, insulin, sulfonylurea (SU) drugs, β -blocker induced obesity), and the like.

Examples of conditions or diseases associated with obesity include: glucose tolerance disorder, diabetes mellitus (especially Type II diabetes mellitus, obesity diabetes mellitus), abnormal lipid metabolism (synonymous with hyperlipidemia above), hypertension, heart failure, hyperuricemia, fatty liver (including non-alcoholic hepatitis), coronary heart disease (myocardial infarction, angina pectoris), cerebral infarction (cerebral thrombosis, transient ischemic attack), bone/joint disease (knee osteoarthritis, hip osteoarthritis, deformed spondylitis, low back pain), sleep apnea syndrome/Pickwick syndrome, menstrual disorders (abnormal menstrual cycle, abnormal menstruation and cycle, amenorrhea, abnormal menstrual symptoms), metabolic syndrome, and the like.

The compounds provided by the present invention have GLP-1/GIP dual agonist activity, a long half-life, and show a longer duration of drug effect.

The compounds according to the present invention can react with any of a variety of inorganic and organic acids to form pharmaceutically acceptable acid addition salts. The pharmaceutically acceptable salts and the methods commonly used for their preparation are well-known in the art.

The amino acid sequences of the present invention contain the standard single letter or three letter codes for twenty natural amino acids. In addition, "Aib" is α -aminoisobutyric acid, " α MePhe" is α -methylphenylalanine, "Pya(4)" is 4-pyridylalanine; "Cha" is cyclohexylalanine, " α MeTyr" is α -methyltyrosine.

"AEEA" as disclosed in the present invention is an abbreviation for 2-(2-amino-ethoxy)-ethoxy]-acetyl. "Ste" as disclosed in the present invention represents octadecanedioyl -C(O)-C₁₆H₃₂-C(O)- derived from octadecanedioic acid.

"Effective amount" or "therapeutically effective amount" as disclosed in the present invention refers to the amount or dose of the compound or a pharmaceutically acceptable salt thereof according to the present invention which, upon single or multiple dose administration to the subject, provides the desired effect in the subject under diagnosis or treatment.

"Minimum effective dose" as disclosed in the present invention refers to the minimum administration dose at which the drug effect is produced. Specifically, as the drug effect experiment of multiple administrations on db/db mice in the present invention is concerned, it refers to the minimum dose at which the blood glucose AUC of the group taken the test compound is significantly different ($p < 0.05$) from that of the Vehicle group.

"Efficacy dose" as disclosed in the present invention refers to the administration dose at which the drug effect is produced. Specifically, as the drug effect experiment of multiple

administrations on db/db mice in the present invention is concerned, it refers to the dose at which the blood glucose AUC of the group taken the test compound is significantly different ($p < 0.05$) from that of the Vehicle group.

"Vehicle group" as disclosed in the present invention refers to the vehicle control group.

The "AUC" as disclosed in the present invention refers to the area under the concentration-time curve, that is, the area surrounded by the drug concentration-time curve and the time axis. This parameter is an important indicator to evaluate the degree of drug absorption, which reflects the exposure characteristics of a drug in the body.

The "MRT" as disclosed in the present invention refers to the mean residence time, and is the average value that a drug molecule resides in the body and means the time required for 63.2% of the drug to be eliminated from the body.

The "MTD" as disclosed in the present invention refers to the maximum tolerated dose, which is the highest dose that does not cause the death of the test animals.

The "internal standard" as disclosed in the present invention refers to a specified amount of a certain pure compound added to the sample for correcting the errors due to signal fluctuations of instruments, personnel operations and the like.

The "inhibition of blood glucose AUC" as disclosed in the present invention is the reduction percentage of blood glucose AUC in the group taken the test compound as compared with the group taken the Vehicle. Inhibition of blood glucose AUC = $(\text{blood glucose AUC}_{\text{Vehicle}} - \text{blood glucose AUC}_{\text{test compound}}) / \text{blood glucose AUC}_{\text{Vehicle}}$.

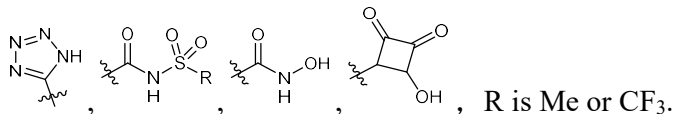
The "titration strategy" as disclosed in the present invention is a dose escalation method, that is, after the initial dose, the frequency for dose escalation and the dosage are adjusted to obtain the optimal administration dosage.

The "subject" as disclosed in the present invention includes humans or animals, preferably humans.

The "modifying chain" as disclosed in the present invention is $[\text{2-(2-amino-ethoxy)-ethoxy}]\text{-acetyl}_a\text{-(}\gamma\text{-Glu)}_b\text{-CO-(CH}_2\text{)}_c\text{-Z}$, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 22; preferably, wherein a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; in addition, the present invention further provides compounds wherein c is 14, 16, 18 or 20, wherein Z is independently selected from the group consisting of -CH_3 , carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates, for example, but not limited to the cases where said Z may comprise a carboxy acid ($\text{-CO}_2\text{H}$) or carboxylic acid

bioisosteres (for example , a phosphonic acid (-P(O)(OH)₂) or a sulfonic acid (-SO₂OH) group, preferably -CO₂H.

As for carboxylic acid bioisosteres, suitable carboxylic acid bioisosteres are well-known in the art. Preferably, the bioisosteres have protons with a pK_a similar to that of the corresponding carboxylic acid. Examples of suitable bioisosteres may include, but are not limited to, tetrazoles, acylsulfonamides, acylhydroxylamines and squaric acid derivatives, as shown below:



As for the peptides mentioned herein, according to conventional peptide designation, the left end is the N-terminus (amino-terminus) and the right end is the C-terminus (carboxyl-terminus). The C-terminus of the peptide can be any one of amide (-CONH₂), carboxyl (-COOH), carboxylate (-COO⁻), alkylamide (-CONHR') and ester (-COOR'), and R' is C₁₋₈ alkyl. In particular, amides (-CONH₂) are preferred.

The "coupling" or "incorporating" in the preparation process of the present invention refers to the process of adding a new amino acid to the combined amino acids or peptide.

Description of Drawings

Figures 1A-1B show blood glucose changes in db/db mice after administration. Figure 1A shows the blood glucose changes in db/db mice after a single subcutaneous administration of P007, P008, P014, P019, Tirzepatide and P001 at 10 nmol/kg; and Figure 1B shows the blood glucose changes in db/db mice after a single subcutaneous administration of P013, P015, P016, P017, P018, P020 and Tirzepatide at 10 nmol/kg.

Figures 2A-2C show blood glucose AUC in db/db mice after administration. Figure 2A shows the AUC from 0 to 48 hours after a single subcutaneous administration of P007, P008, P014, P019, Tirzepatide and P001 at 10 nmol/kg; Figure 2B shows the AUC from 0 to 72 hours after a single subcutaneous administration of P007, P008, P014, P019 and Tirzepatide at 10 nmol/kg; and Figure 2C shows the AUC from 0 to 56 hours after a single subcutaneous administration of P013, P015, P016, P017, P018, P020 and Tirzepatide at 10 nmol/kg. In Figure 2A, T-Test, *p<0.05, **p<0.01, ***p<0.001, vs. vehicle; §p<0.05, §§p<0.01, §§§p<0.001, vs. P001. In Figure 2B, T-Test, *p<0.05, **p<0.01, ***p<0.001, vs. vehicle; #p<0.05, ##p<0.01, vs. Tirzepatide. In Figure 2C, T-Test, *p<0.05, **p<0.01, ***p<0.001 vs. Vehicle; #p<0.05, vs. Tirzepatide.

Figures 3A-3D show changes in blood glucose in db/db mice after multiple administrations. Figure 3A compares the changes of blood glucose in db/db mice after multiple subcutaneous administrations of P014 and Tirzepatide; Figure 3B compares the changes of blood glucose in

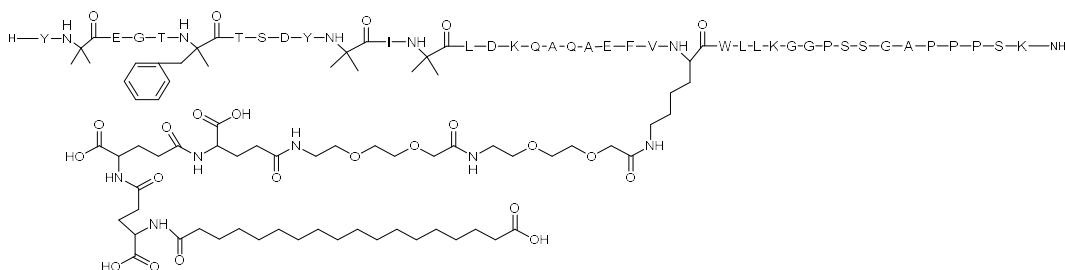
db/db mice after multiple subcutaneous administrations of P016 and Tirzepatide; Figure 3C compares changes in blood glucose in db/db mice after multiple subcutaneous administrations of P017 and Tirzepatide; and Figure 3D compares changes in blood glucose in db/db mice after multiple subcutaneous administrations of P020 and Tirzepatide.

The present invention also includes novel intermediates and methods useful in the synthesis of compounds or pharmaceutically acceptable salts thereof according to the present invention. The intermediates and compounds according to the present invention can be prepared by various methods known in the art. In particular, methods using chemical synthesis are exemplified in the Examples below. The specific synthetic steps of each pathway as described can be combined in various ways to prepare compounds or salts thereof according to the present invention. Reagents and starting materials are readily available to those skilled in the art. It should be understood that these examples are not intended to limit the scope of the present invention in any way. The mass spectrometer is performed on an Agilent 1260/6110 liquid chromatography mass spectrometer, and the scanning range is between 100 and 1500.

Example 1:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:15).



The above structure contains the standard single letter amino acid code with exception of residues Aib2, αMePhe6, Aib11, Aib13 and K24 where the structures of amino acid residues Aib2, αMePhe6, Aib11, Aib13 and K24 have been expanded.

(1) Synthesis of Peptide-resin Intermediate 1

Fmoc-Rink Linker-Nle-MBHA resin (S=0.49 mmol/g) is charged, swelled with an appropriate amount of DCM, washed with DCM for 2 to 3 times, de-protected with 20% PIP/DMF solution for 30 minutes, filtered and washed to obtain a NH₂-Rink linker-Nle-MBHA resin with Fmoc removed, which is suctioned to remove the solvent for further use.

4 equivalents of Fmoc-Lys(Boc)-OH and HOBt are charged respectively, and dissolved in an appropriate amount of DMF/DCM. Separately, 4 equivalents of DIC is charged and double diluted with DCM. The resultant diluent is slowly added to the DMF/DCM solution, and reacted under stirring at -5 to 0°C for no less than 60 minutes so as to be activated for further use.

The activated Fmoc-Lys(Boc)-OH solution is added to the NH₂-Rink linker-Nle-MBHA resin. The reaction temperature is controlled at 10 to 30°C. The coupling reaction is performed for 240 to 480 minutes. After filtration and washing, a Fmoc-Lys(Boc)-Rink linker-Nle-MBHA resin is obtained. The resin is de-protected with 20% PIP/DMF solution for 30 minutes. After filtration and washing, a Lys(Boc)-Rink linker-Nle-MBHA resin with Fmoc removed is obtained.

According to the reaction conditions as stated above, each amino acid is successively coupled starting from the second amino acid at the C-terminus to the N-terminus. If the coupling is incomplete (a color development reaction occurs), a second condensation is carried out using HBTU/DIEA to ensure that each amino acid is completely condensed. The couplings are successively performed in the following order: Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Pro-OH, Fmoc-Ala-OH.H₂O, Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Pro-OH, Fmoc-Gly-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Leu-OH, Fmoc-Trp(Boc)-OH, Fmoc-Lys(Mtt)-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Glu(OtBu)-OH.H₂O, Fmoc-Ala-OH.H₂O, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH.H₂O, Fmoc-Gln(Trt)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Aib-OH, Fmoc-Ile-OH, Fmoc-Aib-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc- α -Me-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Glu(OtBu)-Gly-OH, Fmoc-Aib-OH and Boc-Tyr(tBu)-OH.

The following resin is obtained: Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(Mtt)-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink Linker-Nle-MBHA resin.

When the above condensation is complete, the Mtt protecting groups are removed with 50% HFIP/DCM solution for 30 minutes. After washing and filtration, the following peptide-resin intermediate 1 is obtained:

Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink Linker-Nle-MBHA Resin.

(2) Modification Steps with Modifying chain

4 equivalents of Fmoc-AEEA-OH and HOBt are charged, and dissolved in an appropriate

amount of DMF/DCM. Separately, 4 equivalents of DIC is charged and double diluted with DCM. The diluent is slowly added to the DMF/DCM solution, and reacted under stirring at -5 to 0°C for no less than 60 minutes so as to be activated for further use.

The activated Fmoc-AEEA-OH solution is added to the previously swollen and washed peptide-resin intermediate 1. The reaction temperature is controlled at 10 to 30°C. The coupling reaction is performed for 240 to 480 minutes. The resin is filtrated and washed. The Fmoc protecting groups are removed with 20% PIP/DMF solution for 30 minutes. The resin is filtrated and washed. According to the reaction conditions as stated above, the activated Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH, Fmoc-Glu(α -OtBu)-OH, Fmoc-Glu(α -OtBu)-OH and mono-tert-butyl octadecanedioate are successively coupled on the resin, Fmoc deprotected, washed with DCM and dried to obtain the following P015 peptide-resin: Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(tBuO-Ste- γ -Glu(α -OtBu)- γ -Glu(α -OtBu)- γ -Glu(α -OtBu)-AEEA-AEEA)-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink-Linker-Nle-MBHA Resin.

The P015 peptide-resin is charged. 12-15 mL/g cleavage reagent for the peptide-resin (TFA:EDT:TIS:H₂O, 94:2:2:2 v/v) is added and reacted with stirring at 25±5°C for 4 hours. The reaction mixture is filtered with a sand core funnel. The filtrate is collected. The resin is washed with a small amount of TFA for three times, and the filtrates are combined and concentrated under reduced pressure. Methyl tert-butyl ether (MBTE) is added, and the resulting precipitate is washed with MBTE for 3 to 4 times, and dried by evaporating MBTE. The crude product is dried under reduced pressure at room temperature to constant weight to obtain the crude P015 product, which is subsequently purified and dried to obtain the P015 sample (the molecular weight determined by MS is 5255.2).

Example 2:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ -Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:19).

Fmoc-Aib-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Thr(tBu)-OH, Fmoc- α -Me-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Glu(OtBu)-Gly-OH, Fmoc-Aib-OH and Boc-Tyr(tBu)-OH.

The following resin is obtained:

Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(Boc)-Trp(Boc)-Leu-Leu-Lys(Mtt)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink Linker-Nle-MBHA Resin.

When the above condensation is complete, the Mtt protecting groups are removed with 50% HFIP/DCM solution for 30 minutes. After washing and filtration, the following peptide-resin intermediate 2 is obtained: Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(Boc)-Trp(Boc)-Leu-Leu-Lys-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink Linker-Nle-MBHA Resin.

(2) Modification Steps with Modifying chain

4 equivalents of Fmoc-AEEA-OH and HOBt are charged, and dissolve in an appropriate amount of DMF/DCM. Separately, 4 equivalents of DIC is charged and double diluted with DCM. The diluent is slowly added to the DMF/DCM solution, and reacted under stirring at -5 to 0°C for no less than 60 minutes so as to be activated for further use.

The activated Fmoc-AEEA-OH solution is added to the previously swollen and washed peptide-resin intermediate 2. The reaction temperature is controlled at 10 to 30°C. The coupling reaction is performed for 240 to 480 minutes. The resin is filtrated and washed. The Fmoc protecting groups are removed with 20% PIP/DMF solution for 30 minutes. The resin is filtrated and washed. According to the reaction conditions as stated above, the activated Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH and mono-tert-butyl octadecanedioate are successively coupled on the resin, Fmoc deprotected, washed with DCM and dried to obtain the following P019 peptide-resin: Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(tBuO-St ϵ - γ -Glu(α -OtBu)-AEEA-AEEA-AEEA)-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink-Linker-Nle-MBHA Resin.

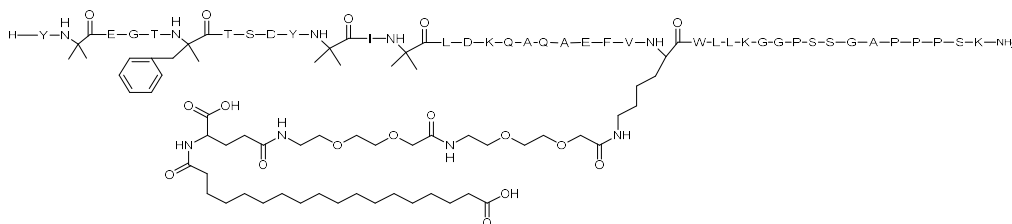
The P019 peptide-resin is charged. 12-15 mL/g cleavage reagent for the peptide-resin (TFA:EDT:TIS:H₂O, 94:2:2:2 v/v) is added and reacted with stirring at 25 $\pm$ 5°C for 4 hours. The reaction mixture is filtered with a sand core funnel. The filtrate is collected. The resin is washed with a small amount of TFA three times, and the filtrates are combined and concentrated under reduced pressure. Methyl tert-butyl ether (MBTE) is added, and the resulting precipitate

is washed with MBTE for 3 to 4 times, and dried by evaporating MBTE. The crude product is dried under reduced pressure at room temperature to constant weight to obtain the crude P019 product, which is subsequently purified and dried to obtain the P019 sample (the molecular weight determined by MS is 5142.0).

Example 3

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:7).



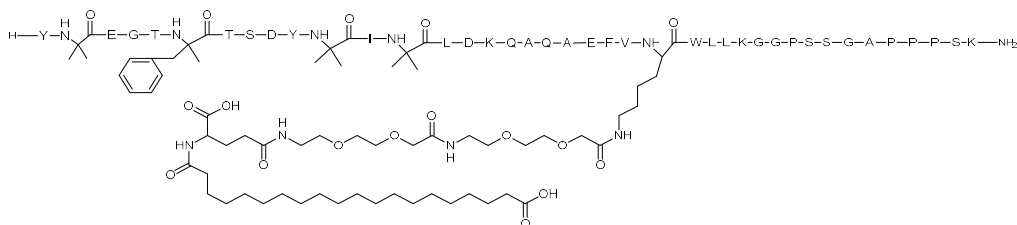
The above structure contains the standard single letter amino acid code with exception of residues Aib2, αMePhe6, Aib11, Aib13 and K24 where the structures of amino acid residues Aib2, αMePhe6, Aib11, Aib13 and K24 have been expanded.

Similar to the method described in Example 1 above, the peptide-resin intermediate 1 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 7 of the present invention (the molecular weight determined by MS is 4997.2). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α-OtBu)-OH and mono-tert-butyl octadecanedioate on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P007 peptide-resin. Other steps are similar.

Example 4

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₈-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:13).



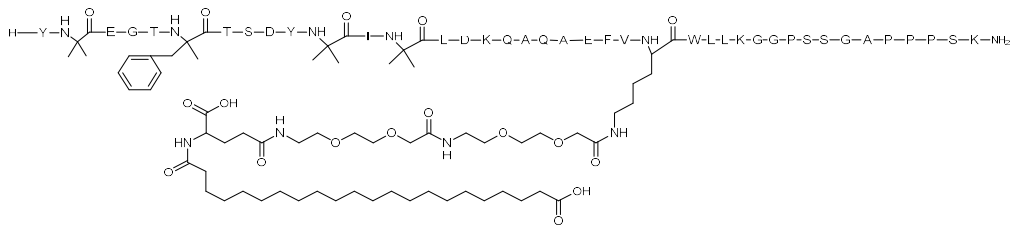
The above structure contains the standard single letter amino acid code with exception of residues Aib2, α MePhe6, Aib11, Aib13 and K24 where the structures of amino acid residues Aib2, α MePhe6, Aib11, Aib13 and K24 have been expanded.

Similar to the method described in Example 1 above, the peptide-resin intermediate 1 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 13 of the present invention (the molecular weight determined by MS is 5025.2). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH and eicosanedioic acid mono-tert-butyl ester on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P013 peptide-resin. Other steps are similar.

Example 5:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:14).



The above structure contains the standard single letter amino acid code with exception of residues Aib2, α MePhe6, Aib11, Aib13 and K24 where the structures of amino acid residues Aib2, α MePhe6, Aib11, Aib13 and K24 have been expanded.

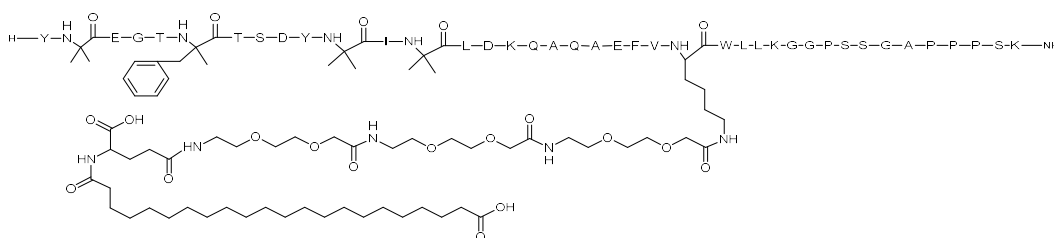
Similar to the method described in Example 1 above, the peptide-resin intermediate 1 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 14 of the present invention (the molecular weight determined by MS is 5052.4). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH and docosanedioic acid mono-tert-butyl ester on the

resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P014 peptide-resin. Other steps are similar.

Example 6:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:16).



The above structure contains the standard single letter amino acid code with exception of residues Aib2, αMePhe6, Aib11, Aib13 and K24 where the structures of amino acid residues Aib2, αMePhe6, Aib11, Aib13 and K24 have been expanded.

Similar to the method described in Example 1 above, the peptide-resin intermediate 1 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 16 of the present invention (the molecular weight determined by MS is 5198.0). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α-OtBu)-OH and docosanedioic acid mono-tert-butyl ester on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P016 peptide-resin. Other steps are similar.

Example 7:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

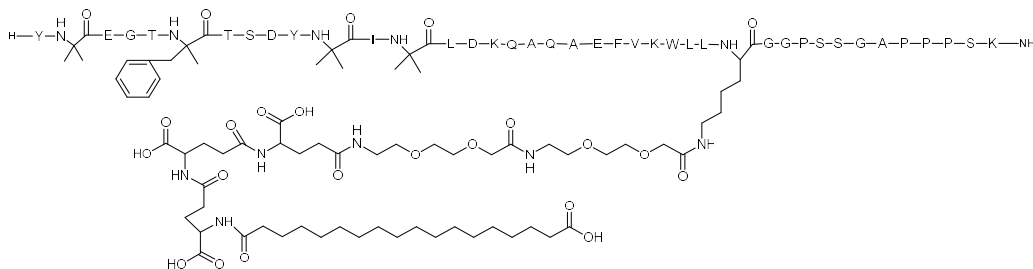
wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 are chemically modified through conjugation to the ε-amino of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:8).

Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH and docosanedioic acid mono-tert-butyl ester on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P017 peptide-resin. Other steps are similar.

Example 9:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ -Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:18).



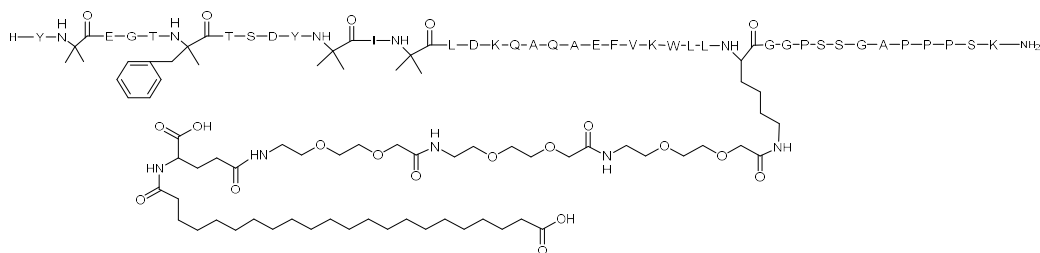
The above structure contains the standard single letter amino acid code with exception of residues Aib2, α MePhe6, Aib11, Aib13 and K28 where the structures of amino acid residues Aib2, α MePhe6, Aib11, Aib13 and K28 have been expanded.

Similar to the method described in Example 2 above, the peptide-resin intermediate 2 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 18 of the present invention (the molecular weight determined by MS is 5255.2). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH, Fmoc-Glu(α -OtBu)-OH, Fmoc-Glu(α -OtBu)-OH and mono-tert-butyl octadecanedioate on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P018 peptide-resin. Other steps are similar.

Example 10:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ -Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:20).



The above structure contains the standard single letter amino acid code with exception of residues Aib2, α MePhe6, Aib11, Aib13 and K28 where the structures of amino acid residues Aib2, α MePhe6, Aib11, Aib13 and K28 have been expanded.

Similar to the method described in Example 2 above, the peptide-resin intermediate 2 is used to carry out the preparation steps to synthesize the peptide of SEQ ID NO: 20 of the present invention (the molecular weight determined by MS is 5198.4). The difference is that the modification process with the modifying chain is sequentially coupling Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-AEEA-OH, Fmoc-Glu(α -OtBu)-OH and docosanedioic acid ester mono-tert-butyl on the resin. The resultant product is de-protected from Fmoc group, and washed with DCM to obtain P020 peptide-resin. Other steps are similar.

In some embodiments of the present invention, as for the preparation of the peptide-resin intermediate 3 (a peptide-resin intermediate wherein the modifying chain is bonded to the Lys at position 16), that is Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(Boc)-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys(Boc)-Rink Linker-Nle-MBHA Resin, the preparation method for peptide-resin intermediate 1 in Example 1 is referred to.

As for the preparation of the peptide-resin intermediate 4 (a peptide-resin intermediate wherein the modifying chain is bonded to the Lys at position 40), that is Boc-Tyr(tBu)-Aib-Glu(OtBu)-Gly-Thr(tBu)- α MePhe-Thr(tBu)-Ser(tBu)-Asp(OtBu)-Tyr(tBu)-Aib-Ile-Aib-Leu-Asp(OtBu)-Lys(Boc)-Gln(Trt)-Ala-Gln(Trt)-Ala-Glu(OtBu)-Phe-Val-Lys(Boc)-Trp(Boc)-Leu-Leu-Lys(Boc)-Gly-Gly-Pro-Ser(tBu)-Ser(tBu)-Gly-Ala-Pro-Pro-Pro-Ser(tBu)-Lys-Rink Linker-Nle-MBHA Resin, the preparation method for peptide-resin intermediate 1 in Example 1 is referred to.

Other dual agonist compounds in the present invention can be prepared according to the methods described above.

Related Assays

Provided below are the conditions and data in several assays for Examples.

I. IN VITRO FUNCTION

(I) In Vitro Binding Activity on Human GLP-1 and GIP Receptors

The in vitro binding potency of compounds according to the present invention to human GIP and GLP-1 receptors is evaluated by measuring the binding affinities K_i , using crude cellular membranes obtained from clonal cell lines over-expressing either the human GLP1R cDNA or human GIP-R cDNA.

1) In Vitro Binding Activity on Human GLP-1 Receptor

hGLP-1 and the compound of the present invention are dissolved in DMSO and stored at -80°C . 89 μL of membrane dissolved in binding buffer (50 mM Hepes, pH 7.4, 5 mM MgCl_2 , 5 mM EDTA, 0.005% TWEEN, 0.005% HSA) is transferred to a 96-well test plate (5 $\mu\text{g}/\text{well}$). The compound is serially diluted in DMSO, and then, 1 μL of the diluted compound or 100% DMSO is added to the test plate containing the membrane solution. 10 μL of [^{125}I] GLP-1 is further added (the final concentration in the reaction is 0.15 nM). The test plate is left at room temperature for 90 minutes. The membrane complexes are harvested into a GF/B plate pre-coated with 0.5% PEI by using a Cell Harvester, and washed for 3 times with 500 μL of pre-cooled elution buffer (50 mM Hepes, pH 7.4, 500 mM NaCl) at 4°C . After drying at 37°C for 2 hours, 50 μL of scintillation fluid is added to each well. The plate is read with a MicroBeta2 scintillation counter after sealing and settling for at least 1 hour so as to determine the membrane bound radioligand level.

The absolute IC_{50} concentration of the compound is derived by non-linear regression of the binding percent of [^{125}I]GLP-1 *versus* the concentration of the compound added. The IC_{50} concentration is converted to K_i (K_i is the inhibition constant) using the Cheng-Prusoff equation.

2) In Vitro Binding Activity on Human GIP Receptor

hGIP and the compound of the present invention are dissolved in DMSO and stored at -80°C . 98 μL of membrane dissolved in binding buffer (50 mM HEPES pH 7.4, 5 mM MgCl_2 , 1 mM CaCl_2 , 0.1% BSA, 0.005% Tween-20) is transferred to a 96-well test plate (15 $\mu\text{g}/\text{well}$). The compound is serially diluted in DMSO, and then, 2 μL of the diluted compounds or 100% DMSO is added to the test plate containing the membrane solution. 100 μL of [^{125}I]GIP is further added (the final concentration in the reaction is 0.0315 nM). The test plate is left at room temperature for 90 minutes. The membrane complexes are harvested into a GF/B plate pre-coated with 0.5% PEI by using a Cell Harvester, and washed for 3 times with 500 μL of pre-cooled elution buffer (50 mM Tris-HCl pH 7.4, 125 mM NaCl) at 4°C . After drying at 37°C for 2 hours, 50 μL of scintillation fluid is added to each well. The plate is read with a MicroBeta2 scintillation counter after sealing and settling for at least 1 hour so as to determine the membrane bound radioligand level.

The absolute IC_{50} concentration of the compound is derived by non-linear regression of the binding percent of [^{125}I]GIP *versus* the concentration of the compound added. The IC_{50} concentration is converted to K_i (K_i is the inhibition constant) using the Cheng-Prusoff equation.

Table 1. Receptor Binding Affinity, Ki Ratio

Compound	GLP-1R	GIPR
	Ki ratio	Ki ratio
GLP-1	1.00	NA
GIP	NA	1.00
P001	19.52	8.82
Tirzepatide	0.28	0.06
P007	0.47	NA
P008	1.09	0.16
P013	0.28	0.14
P014	0.37	0.08
P015	0.17	NA
P016	0.63	0.11
P017	0.56	0.04
P018	1.45	0.05
P019	0.93	0.09
P020	1.36	0.05

NA: not detected

Ki ratio: the ratio of the Ki value of the endogenous ligand to the Ki value of the test compound

The binding affinities of all compounds to the receptors are expressed by Ki ratios, and a larger Ki ratio of the compound indicates a stronger binding affinity. As compared with P001, the binding affinities of the compounds according to the present invention to both human GLP-1R and GIPR are decreased to a certain extent (see Table 1).

As compared with Tirzepatide, the binding affinities of the other 9 compounds according to the present invention, except for P015, to human GLP-1R are slightly stronger than that of Tirzepatide. In addition, the binding affinities of the five compounds, namely, P008, P013, P014, P016 and P019, to GIPR are also slightly stronger than that of Tirzepatide (see Table 1).

To sum up, the binding affinities to human GLP-1R and GIPR of the compounds with different modifying chains according to the present invention are lower than that of P001 in some degree, but are slightly stronger than or equivalent to that of Tirzepatide.

(II) Agonistic Activity on hGLP-1R and hGIPR

For compounds according to the present invention, the in vitro functional activities against human GIP and GLP-1 receptors are determined in HEK-293 clonal cell lines expressing these receptors.

1) Agonistic Activity on Human GLP-1 Receptor (cAMP Reporter Gene Assay)

The agonistic activities of the compounds according to the present invention on human GLP-1R are determined by cAMP reporter gene assay, and P001, Tirzepatide and endogenous ligand GLP-1 are used as references.

HEK293/CRE/GLP-1R cells are seeded in a 96-well plate at 50,000 cells/well (80 μ L/well), and incubated overnight in a 37°C, 5% CO₂ incubator. 20 μ L/well of the test medium (DMEM containing 0.1% casein) containing a compound (the compound according to the present invention, P001, Tirzepatide or GLP-1) is added to the 96-well plate, and continued to incubate in a 37°C, 5% CO₂ incubator for 6 hours and equilibrated to room temperature. The supernatant is removed. 50 μ L/well Bright-Glo reagent is added, shaken and lysed at room temperature for 10 minutes. The luminescence is read using an Envision microplate reader, and the luciferase activity is measured.

The response value to 100 nM GLP-1 is set as 100% response value, and GraphPad is used to perform nonlinear regression according to the response percentage and the concentration of the compound added to obtain the EC₅₀ value for each compound.

2) Agonistic Activity against Human GIP Receptor (LANCER Ultra cAMP Assay)

The agonistic activities of the compounds according to the present invention against human GIPR are determined by a LANCER Ultra cAMP Kit, and P001, Tirzepatide and endogenous ligand GIP are used as references.

The in vitro agonistic activities of the compounds according to the present invention against GIPR is determined in HEK293 cells stably expressing human GIPR (HEK293/GIPR cells). HEK293/GIPR cells are formulated in HBSS buffer (0.1% Casein, 500 μ M IBMX, 5 mM HEPES), and seeded in a 384-well cell culture plate at 1000 cells/well (5 μ L/well). 5 μ L of HBSS buffer containing 2 $\times$ compounds is added to the above-mentioned 384-well cell culture plate. After been sealed, the plate is placed into a 37°C, 5% CO₂ incubator for about 30 minutes. After the incubation is completed, 5 μ L of cAMP-Eu working solution and 5 μ L of cAMP-Ulight working solution are added sequentially, and the plate is shaken for uniform mixing. The incubation is performed for 1 hour at 25°C. The signal values at 665 nm and 615 nm are read on an Envision microplate reader. The ratio of the signal value at 665 nm to the signal value at 615 is calculated, and converted into a cAMP concentration by using the cAMP standard curve. The response value to 1 μ M GIP is set as 100% response value, and GraphPad is used to perform nonlinear regression according to the response percentage and the concentration of the compound added to obtain the EC₅₀ value for each compound.

Table 2. Agonist Activity on Human GLP-1 and GIP Receptors, EC₅₀ Values

Compound	GLP-1R	GIPR
	EC ₅₀ value	EC ₅₀ value
GLP-1	1.00	NA
GIP	NA	1.00
P001	52.09	7.13
Tirzepatide	0.74	0.51
P007	5.84	1.15
P008	4.79	1.67
P013	2.41	0.86
P014	1.73	0.25
P015	2.44	0.17
P016	1.56	0.56
P017	1.35	0.16
P018	3.63	2.55
P019	4.95	1.68
P020	1.41	0.22

NA: not detected

EC₅₀ value: the ratio of the EC₅₀ value of the endogenous ligand to the EC₅₀ value of the test compound

The agonistic activity of all compounds against human GLP-1 and GIP receptors is expressed by EC₅₀ value, and the larger the EC₅₀ value, the stronger the activity of the compound. As compared with P001, the compound according to the present invention shows a certain degree of decrease in the agonistic activity on human GLP-1R and GIPR (see Table 2).

As compared with Tirzepatide, all compounds according to the present invention have stronger agonistic activity on human GLP-1R. In addition, the agonistic activity of the six compounds, namely, P007, P008, P013, P016, P018 and P019, on human GIPR is also slightly stronger than that of Tirzepatide (see Table 2).

To sum up, the agonistic activity on human GLP-1R and GIPR of the compounds with different modifying chains according to the present invention is lower than that of P001 in some degree, but 6 compounds according to the present invention are stronger than Tirzepatide in terms of agonistic activity against human GLP-1R and GIPR.

As shown by in vitro function assay, since the 10 compounds according to the present invention do not differ significantly in activity, the compounds are further screened using a single administration hypoglycaemic assay in db/db mice.

II. Pharmacokinetics

(I) Pharmacokinetics Following Single Administration in SD Rat

After subcutaneous or intravenous administration to a SD rat, plasma is collected and the concentration of the drug in plasma is detected to demonstrate the in vivo pharmacokinetic properties of the compound according to the present invention. The compound is dissolved in a phosphate buffer, and filtered through a membrane filter (PTFE, 0.45 μm) to obtain a 25 nmol/mL solution of the compound. The compound is administered to male SD rats (230-268 g, $n=3$) at a dose of 50.0 nmol/kg (s.c.) or 25.0 nmol/kg (i.v.). About 150 μL of whole blood is collected from the internal jugular vein into an EDTA- K_2 anticoagulant tube at predose, 0.0833 (only for i.v.), 0.25, 0.5, 1, 2, 4, 6, 8, 24, 48, 72, 96 and 120 hours respectively. The blood samples are centrifuged at 1500 g for 10 minutes to obtain a plasma, which is stored at -90°C to -60°C for further analysis and assay.

Sample Treatment

(1) Method for Treating the Samples for Tirzepatide, P007, P008, P013 and P014

30.0 μL of thawed plasma sample is charged. 150 μL of acetonitrile solution (containing 5 $\text{ng}\cdot\text{mL}^{-1}$ Verapamil, 50 $\text{ng}\cdot\text{mL}^{-1}$ Glibenclamide, 200 $\text{ng}\cdot\text{mL}^{-1}$ Tolbutamide and 200 $\text{ng}\cdot\text{mL}^{-1}$ Diclofenac) are added to precipitate protein. The mixture is vortexed for 5 minutes, and centrifuged at 3700 rpm for 8 minutes. 70.0 μL of the supernatant is charged, into which 70.0 μL of 0.5% aqueous formic acid solution is added. The mixture is vortexed for 5 minutes. 15 μL of the mixture solution is sampled and injected into the LC-MS/MS to determine the drug concentration in plasma.

(2) Method for Treating the Samples for P015, P016, P017 and P018

20.0 μL of thawed plasma sample is taken. 60 μL of acetonitrile solution (containing 5 $\text{ng}\cdot\text{mL}^{-1}$ Verapamil, 50 $\text{ng}\cdot\text{mL}^{-1}$ Glibenclamide, 200 $\text{ng}\cdot\text{mL}^{-1}$ Tolbutamide and 200 $\text{ng}\cdot\text{mL}^{-1}$ Diclofenac) are added to precipitate protein. The mixture is vortexed for 1 minute, and centrifuged at 13000 rpm for 8 minutes. 60.0 μL of the supernatant is taken, into which 60.0 μL of 0.5% aqueous formic acid solution is added. The mixture is vortexed for 10 minutes. 10.0 μL of the mixture solution is sampled and injected into the LC-MS/MS to determine the drug concentration in plasma.

(3) Method for Treating the Samples for P019 and P020

30.0 μL of thawed plasma sample is taken. 150 μL of acetonitrile solution (containing 5 $\text{ng}\cdot\text{mL}^{-1}$ Verapamil, 50 $\text{ng}\cdot\text{mL}^{-1}$ Glibenclamide, 200 $\text{ng}\cdot\text{mL}^{-1}$ Tolbutamide and 200 $\text{ng}\cdot\text{mL}^{-1}$ Diclofenac)

are added to precipitate protein. The mixture is vortexed for 5 minutes, and centrifuged at 3700 rpm for 8 minutes. 70.0 μ L of the supernatant is taken, into which 70.0 μ L of 0.5% aqueous formic acid solution is added. The mixture is vortexed for 5 minutes. 10.0 μ L of the mixture solution is sampled and injected into the LC-MS/MS to determine the drug concentration in plasma.

Based on the drug concentration in plasmas of the compounds in SD rats, the pharmacokinetic parameters are calculated by applying the software Winnolin 8.2 in a non-compartmental model, and the results are shown in Table 3 and Table 4 below.

Table 3 Pharmacokinetic Parameters after Subcutaneous Injection of Different Polypeptide Compounds to Rats

PK parameter	Unit	Tirzepatide	P007	P008	P013	P014
Dosage	nmol/kg	50	50	50	50	50
T _{1/2}	h	11.4 $\pm$ 1.79	15.0 $\pm$ 2.89	18.7 $\pm$ 4.82	15.0 $\pm$ 0.737	14.8 $\pm$ 0.503
T _{max}	h	7.33 $\pm$ 1.15	7.33 $\pm$ 1.15	13.3 $\pm$ 9.24	24.0 $\pm$ 0	24.0 $\pm$ 0
C _{max}	nM	128 $\pm$ 42.8	169 $\pm$ 14.7	149 $\pm$ 20.5	142 $\pm$ 28.2	170 $\pm$ 16.4
AUC _{0-t}	nM·h	3610 $\pm$ 1200	6150 $\pm$ 776	5400 $\pm$ 1030	5740 $\pm$ 1070	6230 $\pm$ 651
AUC _{0-inf}	nM·h	3670 $\pm$ 1190	6630 $\pm$ 439	6680	6080 $\pm$ 1180	6620 $\pm$ 712
MRT _{0-t}	h	19.0 $\pm$ 0.265	21.4 $\pm$ 1.87	21.0 $\pm$ 1.85	26.2 $\pm$ 0.700	26.2 $\pm$ 0.781
MRT _{0-inf}	h	20.3 $\pm$ 0.755	25.7 $\pm$ 1.69	25.4	29.9 $\pm$ 1.33	30.0 $\pm$ 1.01

Table 4 Pharmacokinetic Parameters after Subcutaneous Injection of Different Polypeptide Compounds to Rats

PK parameter	Unit	P015	P016	P017	P018	P019	P020
Dosage	nmol/kg	50	50	50	50	50	50
T _{1/2}	h	16.1 $\pm$ 1.21	14.1 $\pm$ 0.153	18.7 $\pm$ 2.69	18.3 $\pm$ 6.77	10.9 $\pm$ 1.92	17.9 $\pm$ 0.57
T _{max}	h	24.0 $\pm$ 0	24.0 $\pm$ 0	24.0 $\pm$ 0	24.0 $\pm$ 0	13.3 $\pm$ 9.24	24.0 $\pm$ 0
C _{max}	nM	213 $\pm$ 21.3	119 $\pm$ 7.00	112 $\pm$ 15.5	175 $\pm$ 45.1	302 $\pm$ 89.9	139 $\pm$ 28.1
AUC _{0-t}	nM·h	8830 $\pm$ 862	4840 $\pm$ 270	4190 $\pm$ 242	7490 $\pm$ 1360	7910 $\pm$ 545	5620 $\pm$ 1690
AUC _{0-inf}	nM·h	9490 $\pm$ 1090	5070 $\pm$ 262	4530 $\pm$ 28.28	7940 $\pm$ 1230	8630 $\pm$ 601	7070 $\pm$ 28.3
MRT _{0-t}	h	26.8 $\pm$ 0.557	28.1 $\pm$ 1.12	27.9 $\pm$ 2.99	28.5 $\pm$ 4.45	17.1 $\pm$ 2.55	29.5 $\pm$ 4.68
MRT _{0-inf}	h	31.4 $\pm$ 1.35	31.1 $\pm$ 1.00	36.9 $\pm$ 3.46	33.4 $\pm$ 7.00	18.3 $\pm$ 2.76	37.1 $\pm$ 0.636

The pharmacokinetics study on rats by subcutaneously injecting the compounds according to

the present invention shows that: the half-lives of P007, P008, P013, P014, P015, P016, P017, P018 and P020 are greater than the half-lives of Tirzepatide (increased by about 23.7% to 64.0%). As compared with Tirzepatide, a commercially available dual-target product, the compounds according to the present invention modified by the modifying chain can provide a longer half-life in plasma. In addition, as compared with P001, the compound according to the present invention has a substantially longer half-life. The half-life is prolonged by more than 15 times, more than 20 times, or even more than 30 times.

(II) Pharmacokinetics in C57 mice

After subcutaneous or intravenous administration to C57 mice, plasma is collected and the drug concentration in plasma is determined to illustrate the in vivo pharmacokinetic properties of the compound according to the present invention. The compound is dissolved in a phosphate buffer containing 0.1% Tween 20, and filtered through a filter membrane (PTFE, 0.45 μm) to obtain a 15 nmol/mL compound solution. C57 mice (18-22 g) were given 30 nmol/kg subcutaneously (s.c.) or 15.0 nmol/kg intravenously (i.v.). About 100 μL of whole blood is collected from the orbit vein into an EDTA- K_2 anticoagulant tube at predose, 0.5, 1, 2, 4, 8, 24, 48 and 72 hours respectively. The blood samples are centrifuged at 1500 g for 10 minutes to obtain plasma, which is stored at -90°C to -60°C for further analysis and assay.

Sample Treatment

(1) Method for Treating the Samples for Tirzepatide, P014, P016 and P020

20.0 μL of thawed plasma sample is taken. 20 μL of 50% aqueous methanol solution (containing 100 ng mL^{-1} internal standard) and 60 μL of acetonitrile are added sequentially to precipitate protein. The mixture is vortexed for 5 minutes, and centrifuged at 3700 rpm for 8 minutes. 50.0 μL of the supernatant is taken, into which 50.0 μL of 0.5% aqueous formic acid solution is added. The mixture is vortexed for 5 minutes. 50 μL of the mixture solution is sampled and injected into the LC-MS/MS to determine the drug concentration in plasma.

Based on the drug concentration in plasmas of the compounds in C57 mice, the pharmacokinetic parameters are calculated by applying the software Winnolin 8.2 in a non-compartmental model, and the results are shown in Table 5 and Table 6 below.

Table 5 Average Pharmacokinetic Parameters after Single Subcutaneous Administration to Mice

PK Parameter	Unit	Tirzepatide	P014	P016	P020
C _{max}	ng/mL	871	851.84	433.67	835.67
T _{max}	hr	6	6	8	4.25
t _{1/2}	hr	9.21	20.33	23.05	19.89

AUC _(0-t)	ng·hr/mL	19413.9	27316.4	15914.8	18950.56
AUC _(0-∞)	ng·hr/mL	19704.7	30150.5	18160.6	21093.26
MRT _(0-t)	hr	14.48	22.94	25.98	21.84
MRT _(0-∞)	hr	15.29	30.28	35.91	29.36

Table 6 Average Pharmacokinetic Parameters after Single Intravenous administration to Mice

PK Parameter	Unit	Tirzepatide	P014	P016	P020
C ₀	ng/mL	1901.94	1943.93	1147.89	1819.74
t _{1/2}	hr	10.47	17.20	20.15	16.66
AUC _(0-t)	ng·hr/mL	12723.1	17458.9	11082.1	16281.7
AUC _(0-∞)	ng·hr/mL	12822.1	18245.6	12083.3	17369.9
MRT _(0-t)	hr	11.39	15.72	19.92	16.80
MRT _(0-∞)	hr	12.00	19.215	26.64	21.77

The pharmacokinetics test on mice by subcutaneously injecting the compounds according to the present invention shows that: the half-lives of P014, P016 and P020 are greater than the half-life of Tirzepatide (increased by 116.0% to 150.3%). That means that the half-lives of these compounds are significantly greater than that of Tirzepatide. As compared with Tirzepatide, a commercially available dual-target product, the compounds according to the present invention modified by the modifying chain can provide a longer half-life in plasma.

(III) Pharmacokinetics in Cynomolgus Monkeys

After subcutaneous or intravenous administration to cynomolgus monkeys, plasma is collected and the drug concentration in plasma is determined to illustrate the in vivo pharmacokinetic properties of the compound according to the present invention. The compound is dissolved in 1.4%PG in 8 mM Na₂HPO₄ Buffer (pH 7.39), and filtered through a filter membrane (PTFE, 0.45 μm) to obtain 25 and 12.5 nmol/mL solution of the compound respectively. About 500 μL of whole blood is collected from the cephalic vein or other appropriate veins into an EDTA-K₂ anticoagulant tube at predose, 2 hours, 12 hours, 24 hours, 32 hours, 48 hours, 3 days(72 hours), 4 days(96 hours), 6 days(144 hours), 9 days(216 hours), 12 days(288 hours), 15days(360 hours) and 18 days(432 hours) respectively. The blood samples are centrifuged at 2200 g for 10 minutes to obtain a plasma, which is stored at -90°C to -60°C for further analysis and assay.

Sample Treatment

(1) Method for Treating the Samples for Tirzepatide, P014, P016, P017 and P020

70.0 μ L of thawed plasma sample is taken. 70 μ L of acetonitrile (containing 1 ng/ml of the internal standard) is added to precipitate protein. The mixture is vortexed for 1 minute, and centrifuged at 14000 rpm for 10 minutes. 60.0 μ L of the supernatant is taken, into which 60.0 μ L of 0.5% aqueous formic acid solution is added. The mixture is vortexed for 5 minutes. 10 μ L of the mixture solution is sampled and injected into the LC-MS/MS to determine the drug concentration in plasma.

Based on the drug concentration in plasmas of the compounds in the cynomolgus monkeys, the pharmacokinetic parameters are calculated by applying the software Winnolin 8.2 in a non-compartmental model, and the results are shown in Table 7 and Table 8 below.

Table 7 Average Pharmacokinetic Parameters after Single Subcutaneous Administration to Cynomolgus Monkeys

PK Parameter	Unit	Tirzepatide	P014	P016	P017	P020
T _{max}	hr	22.67±10.10	16.00±6.93	40.00±13.90	48.00±24.00	22.70±10.10
C _{max}	ng/mL	1297.45± 300.00	951.00±283	2997.00± 481.00	1655.00± 295.00	1349.00±77.0 0
T _{1/2}	hr	62.54±6.07	52.70±6.45	60.40±12.80	44.30±4.80	67.40±9.47
AUC _(0-t)	hr*ng/ mL	148063.24± 38207.00	87690.00± 24556.00	319272.00± 41475.00	153476.00± 28919.00	161575.00± 10637.00
AUC _(0-∞)	hr*ng/ mL	150081.26± 39104.00	88432.00± 24213.00	322800.00± 44567.00	154121.00± 28747.00	163878.00± 12089.00
MRT _(0-t)	hr	99.62±6.26	74.20±13.83	96.50±15.90	76.40±12.70	97.50±9.67
MRT _(0-∞)	hr	104.89±7.58	77.40±13.46	101.00±19.2	77.80±12.20	103.00±13.10

Table 8 Average Pharmacokinetic Parameters after Single Intravenous Administration to Cynomolgus Monkeys

PK Parameter	Unit	Tirzepatide	P016
C ₀	ng/mL	1167.70±159.90	1699.50±334.94
T _{1/2}	hr	59.53±5.22	59.81±8.95
AUC _(0-t)	hr*ng/mL	57554.64±11825.48	78169.25±20321.82
AUC _(0-∞)	hr*ng/mL	58529.59±11927.99	80032.34±21063.24

MRT _(0-t)	hr	68.34±8.61	65.29±8.85
MRT _(0-∞)	hr	74.27±8.76	72.94±10.91

The pharmacokinetics study on cynomolgus monkeys by subcutaneously injecting the compounds according to the present invention shows that: the half-lives of P014, P016 and P020 are equivalent to that of Tirzepatide, and the T_{max}s of P016 and P017 are delayed as compared with that of Tirzepatide. As compared with Tirzepatide, a commercially available dual-target product, the compounds according to the present invention modified by the modifying chain can provide a comparable half-life in plasma, and the T_{max}s of P016 and P017 are 2 times that of Tirzepatide. The time to peak drug concentration is significantly delayed as compared to Tirzepatide, which may result in less/few gastrointestinal response or shorter titration time.

III. Pharmacodynamics Effects

(I) Pharmacodynamics Effects in db/db Mice is intended to studying the effects of the compounds according to the present invention on blood glucose in diabetic model mice (db/db mice).

In this study, db/db mice are subcutaneously single administered, and the changes in the blood glucose, the food intake, and the body weight of the mice are measured to illustrate the hypoglycemic effects of the compounds according to the present invention as well as the duration of the drug effect, and compared them with those of the positive controls of Tirzepatide and P001. In this study, 8-9 week old male db/db mice are used. The db/db mice are placed in an individual ventilated cage (IVC) facility with a controlled temperature (20-26 °C) and humidity (40-70%) and a controlled 12h:12 h light-dark cycle. The mice received food and water ad libitum. Blood is sampled at the tail tip, and the fasting blood glucose is measured by a Roche blood glucose meter. The mice are randomly divided into groups according to the initial blood glucose and initial body weight (n=6/group), and each group had similar body weight and blood glucose.

The compound according to the present invention (10 nmol/kg), or the positive controls of Tirzepatide (10 nmol/kg) and P001 (10 nmol/kg), are dissolved in a vehicle (PBS containing 0.1% Tween 20, pH 7.2-7.4). After a single subcutaneous administration, the random blood glucoses are recorded at the set time point (0 to 120 hours, the recording of the random blood glucose is stopped until there is no difference between the random blood glucose of the compound and that of the vehicle group), and the daily body weight and food intake are also recorded. The data results are statistically analyzed using GraphPad Prism8, and the statistical differences among the groups are analyzed following a T-Test. Significant differences are identified at p<0.05.

Hypoglycemic Duration: the longest time during which the random blood glucose of the

compound has a significant difference ($P<0.05$) vs. the vehicle group.

Table 9. Hypoglycemic Duration of the Compounds

Group	Compound	Hypoglycemic Duration(h)
Group 1	P001	32
	Tirzepatide	56
	P007	56
	P008	72
	P014	104
	P019	56
Group 2	Tirzepatide	48
	P013	72
	P015	32
	P016	104
	P017	72
	P018	48
	P020	104

As compared with P001, the hypoglycemic duration of the compounds according to the present invention is more than 24 hours (P007, P008, P014, P019), even more than 40 hours (P008, P014), and more than 72 hours (P014) (see Table 9, Figure 1A, and Figure 1B) longer than that of Tirzepatide.

As compared with Tirzepatide, the hypoglycemic duration of the compounds according to the present invention is equivalent or longer (P007, P008, P013, P014, P016, P017, P018, P019, and P020), even more than 16 hours (P008, P013, P014, P016, P017, P020), more than 24 hours (P013, P014, P016, P017, P020), more than 48 hours (P014, P016, P020), more than 56 hours (P016, P020) longer than that of P001 (see Table 9).

As compared with P001, based on the experiments according to the method described above, during the test period, the hypoglycemic effects of P008 ($p<0.01$), P014 ($p<0.001$) and P019 ($p<0.05$) are significantly better than that of P001 (blood glucose AUC, $P<0.05$, as shown in Figure 2A). The inhibition of the blood glucose AUC is 2.3, 2.6 and 2.2 times of that of P001 respectively; the duration of hypoglycemia effect is 40 hours, 72 hours and 24 hours longer than that of P001 respectively (Table 9). The hypoglycemic effect of P007 is comparable to that of P001, but the hypoglycemic duration is 24 hours longer than that of P001 (as shown in Figure 2A, and Table 9).

As compared with Tirzepatide: the hypoglycemic effects of P014 ($p < 0.05$, Figure 2B), P016 ($p < 0.05$, Figure 2C) and P020 ($p < 0.05$, Figure 2C) are stronger than that of Tirzepatide (blood glucose AUC, $p < 0.05$). The inhibitions of the blood glucose AUC are 1.7, 2.2 and 2.0 times that of Tirzepatide respectively. Moreover, they also show significantly longer duration of drug effects (48 hours, 56 hours and 56 hours longer than that of Tirzepatide respectively, Table 9). The hypoglycemic effects of P013 and P017 are comparable to that of Tirzepatide (Figure 2C), but the duration of drug effects is 24 hours longer than that of Tirzepatide (Table 9).

Surprisingly, as compared with P001, the acylated GLP-1/GIP agonists of the present invention significantly improve the hypoglycemic effect while maintaining the long-acting effect. The compounds according to the present invention with modifying chains in different forms can realize stronger and longer-lasting hypoglycemic effects than P001. Moreover, some compounds according to the present invention exhibit stronger and longer-lasting hypoglycemic effects than Tirzepatide.

Furthermore, in conjunction with performance of the binding and agonistic activity of the compounds according to the present invention at the targeted human GLP-1 and GIP receptors, it is surprisingly found that, although the compounds according to the present invention show a significantly reduced binding and agonistic activity at both the targeted human GLP-1 receptor and the targeted human GIP receptor as compared with P001, the hypoglycemic effects of the compounds according to the present invention are significantly better than that of P001.

(II) Multiple Administration Experiment on db/db Mice

In this study, db/db mice are subcutaneously administrated multiple times, and the changes in the blood glucose in the mice are determined to further illustrate the hypoglycemic effects of the compounds according to the present invention, and compared them with that of the positive control Tirzepatide. In this study, 7-8 week old male db/db mice are used. The db/db mice are placed in an individual ventilated cage (IVC) facility with a controlled temperature (20-26°C) and humidity (40-70%) and a controlled 12:12 h light-dark cycle. The mice received food and water ad libitum. Blood are sampled at the tail tip, and the fasting blood glucose is measured by a Roche blood glucose meter. The mice are randomly divided into groups according to the initial blood glucose and initial body weight ($n=6/\text{group}$), and each group had similar body weight and blood glucose.

The compounds according to the present invention (3, 10, 30 nmol/kg) or the positive control Tirzepatide (3, 10, 30 nmol/kg) is dissolved in a vehicle (PBS containing 0.1% Tween 20, pH 7.2-7.4). The mice are subcutaneously administrated every 3 days for 4 weeks. Subcutaneous administration is conducted on days 1, 4, 7, 10, 13, 16, 19, 22 and 25. Random blood glucose is recorded every 3 days throughout the study. On day 28, the fasting blood glucose is measured. Then the blood is collected by orbital blood sampling. After the orbital blood sampling, the animals are sacrificed, and the liver is taken and weighed. After 1 to 2 hours, the collected blood

is centrifuged at 3000 rpm for 10 min to separate the serum. Biochemical indicators such as triglyceride (TG), alanine aminotransferase (ALT), and total bilirubin (TBIL) are determined with a biochemical analyzer. The data results are statistically analyzed using GraphPad Prism8, and the statistical differences among the groups are analyzed following T-Test. Significant differences are identified at $p < 0.05$.

In the experiment performed as described in the above method, the compounds according to the present invention, such as P014, P016, P017 and P020, all show better blood glucose inhibition as compared with Tirzepatide at a dose of 3 nmol/kg. At a dose of 30 nmol/kg, the drug efficacy of P016 is superior to that of Tirzepatide (see Figures 3A-3D, and Table 10). P016, P017 and P020 significantly reduced liver weight in db/db mice at several doses, while Tirzepatide did not reduce liver weight at the three tested doses. The compounds according to the present invention, such as P014, P016, P017, P020 and Tirzepatide, can effectively reduce TG in the serum of the db/db mice (except for P017 and Tirzepatide groups at 3 nmol/kg), and the P016 and P020 groups at some doses show better drug efficacy than Tirzepatide (see Table 11). In addition, P014 and P016 can also reduce TBIL and/or ALT in serum.

Table 10. Fasting Blood Glucose and Blood Glucose AUC of db/db Mice

Group	D28 Fasting Blood Glucose (mmol/L)	AUC 4-25 day Glu (mmol/L·day)
Vehicle	18.34±3.45	493.71±40.3
P014 3 nmol/kg	10.22±1.58 ^{***}	294±40.43 ^{***}
P014 10 nmol/kg	10.72±1.75	214.4±33.73 ^{***}
P014 30 nmol/kg	13.38±3.38	190.62±26.47 ^{***}
P016 3 nmol/kg	10.42±1.46 ^{***}	256.38±22 ^{***###}
P016 10 nmol/kg	12.5±2.19	249.25±41.42 ^{**}
P016 30 nmol/kg	6.05±0.41 ^{***}	163.98±11.57 ^{***#}
P017 3 nmol/kg	11.68±1.7 [#]	303.78±33.24 ^{**#}
P017 10 nmol/kg	11.95±2.51	249.65±47.65 ^{**}
P017 30 nmol/kg	10.08±0.79 [*]	294.96±11.96 ^{**}
P020 3 nmol/kg	9.6±1.32 ^{***}	268.28±24.64 ^{***#}
P020 10 nmol/kg	10.7±1.62	290.78±27.25 ^{**}
P020 30 nmol/kg	8.22±1.07 [*]	184.18±22.44 ^{****}
TZP 3 nmol/kg	17.35±1.21	419.85±26.42

TZP 10 nmol/kg	13.82±1.99	355.68±30.8*
TZP 30 nmol/kg	10.88±1.2	252.7±32.81**

T-Test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. vehicle group; #p<0.05, ##p<0.01, ###p<0.001 vs. Tirzepatide(TZP) group at the same dose. Results are expressed as Mean±SEM for 6 mice.

Table 11. Liver Weight and Serum TG in db/db Mice

Group	Liver Weight (g)	Serum TG(mmol/L)
Vehicle	2.61±0.19	1.87±0.13
P014 3 nmol/kg	2.26±0.18	1.39±0.07*
P014 10 nmol/kg	2.1±0.14	1.29±0.03**
P014 30 nmol/kg	1.89±0.31	1.14±0.18*
P016 3 nmol/kg	2.24±0.1 [#]	1.21±0.04**** [#]
P016 10 nmol/kg	1.98±0.12*	1.22±0.24*
P016 30 nmol/kg	1.78±0.08**	1.2±0.05***
P017 3 nmol/kg	2.14±0.08*** [#]	1.58±0.17
P017 10 nmol/kg	2.11±0.09*	1.19±0.05***
P017 30 nmol/kg	2.35±0.09	1.09±0.06***
P020 3 nmol/kg	2.03±0.09*** [#]	1.37±0.12*
P020 10 nmol/kg	2.09±0.1*	1.07±0.03**** [#]
P020 30 nmol/kg	1.83±0.16*	1.2±0.07**
TZP 3 nmol/kg	2.49±0.05	1.52±0.11
TZP 10 nmol/kg	2.31±0.17	1.21±0.04**
TZP 30 nmol/kg	2.07±0.15	1.18±0.12**

T-Test, *p<0.05, **p<0.01, ***p<0.001 vs. vehicle group; #p<0.05, ##p<0.01 vs. Tirzepatide(TZP) group at the same dose. Results are expressed as Mean±SEM for 6 mice.

Table 12. Serum ALT and TBIL in db/db Mice

Group	Serum ALT (U/L)	Serum TBIL (μmol/L)
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Vehicle	189.55±40.92	1.43±0.3
P014 3 nmol/kg	111.24±13.23	0.58±0.11*##
P014 10 nmol/kg	89.9±8.66*	0.58±0.15*
P014 30 nmol/kg	106.47±14.3	0.36±0.15*##
P016 3 nmol/kg	137.93±34.59	0.82±0.13
P016 10 nmol/kg	128.94±16.93	1.22±0.28
P016 30 nmol/kg	97.7±12.36*	0.73±0.16
P017 3 nmol/kg	105.16±7.1	0.88±0.26
P017 10 nmol/kg	141.65±12.72	1.59±0.07
P017 30 nmol/kg	176.48±51.6	1.11±0.1
P020 3 nmol/kg	214.56±43.83	1.54±0.25
P020 10 nmol/kg	113.48±24.18	0.91±0.05
P020 30 nmol/kg	112.47±16.69	0.95±0.28
TZP 3 nmol/kg	146.03±19.67	1.54±0.17
TZP 10 nmol/kg	138.66±31.26	1.2±0.3
TZP 30 nmol/kg	112.48±14.55	0.91±0.04

T-Test, * $p < 0.05$ vs. vehicle group; # $p < 0.05$, ## $p < 0.01$ vs. Tirzepatide(TZP)group at the same dose. Results are expressed as Mean±SEM for 6 mice.

To sum up, under this experimental system, the compounds according to the present invention can effectively reduce blood glucose in db/db mice by subcutaneous injection for 4 weeks. The Minimum effective dose (≤ 3 nmol/kg) for the compounds according to the present invention is one third of the effective dose for Tirzepatide (10 nmol/kg), and the maximum drug efficacy (30 nmol/kg) of some compounds according to the present invention such as P016 is superior to that of Tirzepatide. In addition, some compounds according to the present invention exhibit reduced liver weight and protective effects to the livers in db/db mice, while Tirzepatide had no similar effect.

(III) Repeated Administration on Male Rats for 2 Weeks

1. Experimental Schedule

77 SD male rats (SPF grade) are selected and randomly divided into 10 groups based on body weight. Group 1 is the vehicle control group, including 5 animals. Group 2 to Group 4 are the control groups administrated with commercially available Tirzepatide, each group including 8 animals, wherein 5 animals for primary test, and 3 animals for toxicokinetics test, and the

administration dose is 1, 3, and 10 mg/kg respectively. Group 5 to Group 7 are the groups administrated with the test substance 1; and Group 8 to Group 10 are the groups administrated with the test substance 2, each group including 8 animals, wherein 5 animals for primary test, and 3 animals for toxicokinetics test, and the administration dose is 1, 3 and 10 mg/kg respectively. Each of the above groups is continuously administered twice a week for 2 weeks (administrating at Day 1, Day 5, Day 8, Day 12 and Day 15).

During the experiment, the cage condition monitoring, detailed clinical observations, determination of the food consumption, determination of body weight, measurement of the blood biochemical and hematology indicator, investigation of the gross anatomy, weighing of the organ, studying on toxicokinetic are performed on all the groups of the animals.

2. Data Statistics

The tables below illustrates the combinations used for statistical comparison in detail. The tables are as follows:

Method for Statistical Analysis

Group as control	Groups used for comparison
1	2, 3, 4, 5, 6, 7, 8, 9, 10

Original data during each time period is tabulated. The average values and standard deviations and/or group change are calculated for each assay endpoint based on the group and sex. For each endpoint, the administrated group will be compared with the control group in the following aspect. A logarithmic conversion is performed to the endpoint data as desired prior to the start of the specific analyses.

Statistical Analysis

Item	Analytical Method
Food Consumption	comparison between groups
Weight	
Hematology (excluding white blood cell count)	
Coagulation	
Serum Biochemistry	

Organ Weight

Absolute Weight

Organ Coefficients relative to
body weight and brain

White Blood Cell Count	logarithmic conversion/group-group comparison
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Total White Blood Cells

White Blood Cell Differential Count
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3. Experimental Results

Body Weight/Weight Gain: After the first administration, the decrease of the body weight is observed in each of the following groups: Tirzepatide at a dose ≥ 3 mg/kg; P014 and P016 at a dose ≥ 1 mg/kg. With the continuation of the administration, the body weight is recovered, and by the end of the administration, all groups had a positive increase in body weight, but the weight gain is lower than that of the control group, showing an obvious dose-dependent relationship.

Food Intake: throughout the test procedure, a dose-dependent decrease in food intake is observed in each administration group (Tirzepatide, P014 and P016). **Blood Chemistry:** Amylase and triglyceride levels decrease. **Hematology:** reticulocyte level decreases slightly. **Gross Anatomy:** no abnormalities are found in each administration group (Tirzepatide, P014 and P016). **Organ Weight:** the weight of heart, liver and spleen and the like in each administration group (Tirzepatide, P014 and P016) decreased, which is not significantly different from that in the vehicle control group. This may be associated to the weight loss caused by pharmacological effects. **Toxicokinetic:** $C_{\max}$ and AUC in each administration group (Tirzepatide, P014 and P016) increase nearly proportionally to the dose. The administration groups (Tirzepatide, P014 and P016) show no substantial accumulation when continuously administered twice a week for 2 weeks.

Each administration group (Tirzepatide, P014 and P016) had a $MTD \geq 10$ mg/kg when continuously administered twice a week for 2 weeks. $C_{\max}$, AUC_{last} after the last dose and the safety window of the test are as follows:

Table 13. Safety Window for Each Administration Group

Test Article	Tirzepatide	P014	P016
MTD	2077 nmol/kg	1980 nmol/kg	1924 nmol/kg

	(MTD for 2 weeks)	(MTD for 2 weeks)	(MTD for 2 weeks)
Efficacy Dose	10 nmol/kg	3 nmol/kg	3 nmol/kg
Safety Window (based on dosage)	416	1319	1282
Safety Window (based on exposure AUC _{last})	130	596	920

To sum up, P016 and P014 have similar toxicity properties to Tirzepatide, but have a wider safety window (based on exposure, the safety window of P014 is more than 4 times that of Tirzepatide, and the safety window of P016 is more than 7 times of that of Tirzepatide).

In addition, the safety windows of the compounds according to the present invention, in particular P014 and P016, is 10 times larger, or 20 times larger, or 30 times larger, or 40 times larger, or 50 times larger, or 60 times larger, or 70 times larger, or 80 times larger, or 90 times larger than that of P001.

Meanwhile, under the same exposure, the compounds according to the present invention have a lower impact on heart rate (HR) than Tirepatide. The increase in HR for P016 is lower than that for Tirepatide. No sustained HR increase exceeding 30% is observed. At the same exposure, heart rate recovery for P016 is faster than TZP (the recover time for P016 is between 72 and 120 hours, and the recovery time for TZP >120 hours).

The peptide compounds provided by the present invention and the use thereof have been introduced in detail above.

Specific examples are applied herein to illustrate the principle and embodiments of the present invention. The illustration of the above examples are merely used to help understand the method and the central idea of the present invention. It should be indicated that those skilled in the art can make several improvements and modifications to the present invention without departing from the principles of the present invention, and these improvements and modifications also fall within the protection scope of the claims of the present invention.

Amino Acid Sequence:

Name	No.	Sequence
P001	SEQ ID NO:1	Y-X ₁ -E-G-T-X ₂ -T-S-D-Y-X ₃ -I-X ₄ -L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

			wherein X ₁ is Aib; X ₂ is αMePhe; X ₃ is Aib; X ₄ is Aib; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:1)
P007	SEQ NO:7	ID	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:7).
P008	SEQ NO:8	ID	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:8).
P013	SEQ NO:13	ID	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₈-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:13).
P014	SEQ NO:14	ID	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:14).
P015	SEQ NO:15	ID	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:15).

P016	SEQ ID NO:16	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:16).
P017	SEQ ID NO:17	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:17).
P018	SEQ ID NO:18	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:18).
P019	SEQ ID NO:19	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:19).
P020	SEQ ID NO:20	Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K; wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib;

		K at position 28 is chemically modified through conjugation to the ϵ-amino of the K side-chain with $[(2-(2\text{-amino-ethoxy})\text{-ethoxy})\text{-acetyl}]_3\text{-(}\gamma\text{-Glu)}_1\text{-CO-(CH}_2\text{)}_{20}\text{-CO}_2\text{H}$; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:20).
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What is claimed is:

1. A compound of the following Formula (AI), or a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-A11-A12-A13-L-D-K-A17-A-Q-A20-E-F-V-K-W-L-L-K-A29-G-P-S-S-G-A-P-P-P-S-K Formula (AI);

wherein X₁ is Aib; X₂ is α MePhe;

A11 is Aib or Ala;

A12 is Ala, Ile, Lys, Phe or Pya(4);

A13 is Aib, Cha, Leu, α MePhe or α MeTyr;

A17 is Gln or Ile;

A20 is Ala or Ser;

A29 is Gln or Gly,

1, 2, 3 or 4 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-Z, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 24, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates.

2. The compound according to claim 1, wherein

A11 is Aib; and/or

A12 is Ile; and/or

A13 is Aib; and/or

A17 is Gln; and/or

A20 is Ala; and/or

A29 is Gly; and/or

a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; preferably c is independently selected from 4, 16, 18 or 20.

3. A compound of the following Formula (I), or a pharmaceutically acceptable salt thereof:

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; 1, 2, 3 or 4 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z, wherein each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 24, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates; and the C-terminal amino acid is amidated as a C-terminal primary amide.

4. The compound according to claim 3, wherein the compound is

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I), or a pharmaceutically acceptable salt thereof,

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; 1 or 2 Ks, which are selected from the group consisting of K at position 16, K at position 24, K at position 28 and K at position 40, are chemically modified through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-Z, wherein Z is independently selected from the group consisting of -CH₃, carboxylic acids or carboxylic acid bioisosteres, phosphonates or sulfonates, preferably -CO₂H, each a is independently an integer from 0 to 5, each b is independently an integer from 0 to 5, and each c is independently an integer from 10 to 22; and the C-terminal amino acid is amidated as a C-terminal primary amide.

5. The compound according to claim 4, wherein

K at position 16 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 40 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H.

6. The compound according to claim 4, wherein

K at position 24 and K at position 28 are chemically modified with two modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 16 and K at position 24 are chemically modified with two modifying chains through conjugation to the ε-aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ-Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 16 and K at position 28 are chemically modified with two modifying chains through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 16 and K at position 40 are chemically modified with two modifying chains through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 24 and K at position 40 are chemically modified with two modifying chains through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H; or

K at position 28 and K at position 40 are chemically modified with two modifying chains through conjugation to the ϵ -aminos of the K side-chains with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H.

7. The compound according to any one of claims 3 to 6, wherein

a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; preferably c is independently selected from 4, 16, 18 or 20; and/or

Z is -CO₂H.

8. The compound according to claim 4, wherein the compound is

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

9. The compound according to claim 4, wherein the compound is

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K Formula (I);

wherein X₁ is Aib; X₂ is α MePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ϵ -amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)_a-(γ -Glu)_b-CO-(CH₂)_c-CO₂H, wherein a is independently selected from an integer of 0 to 5, b is independently selected from an integer of 0 to 5, and c is independently selected from an integer of 10 to 24; and the C-terminal amino acid is amidated as a C-terminal primary amide.

10. The compound according to any one of claims 8 to 9, wherein

a is independently selected from an integer of 1, 2, 3, 4 or 5, b is independently selected from an integer of 1, 2, 3, 4 or 5, and c is independently selected from an integer of 12 to 22; preferably c is independently selected from 4, 16, 18 or 20.

11. The compound according to claim 4, wherein the compound is

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:7); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₈-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:13); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:14); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:15); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 24 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:16); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:8); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:17); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₂-(γ-Glu)₃-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:18); or

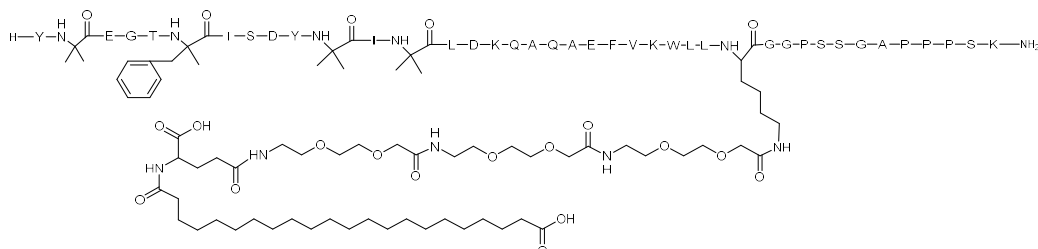
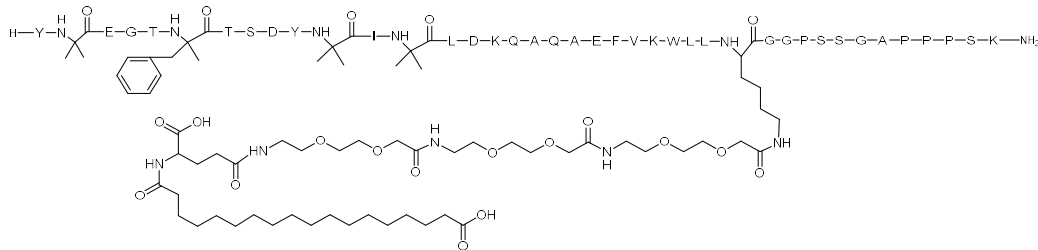
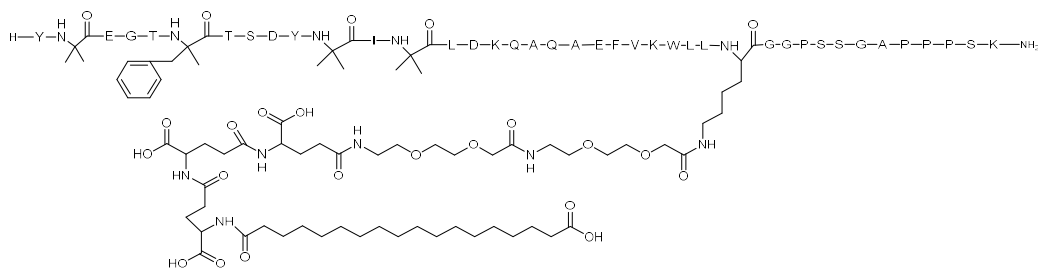
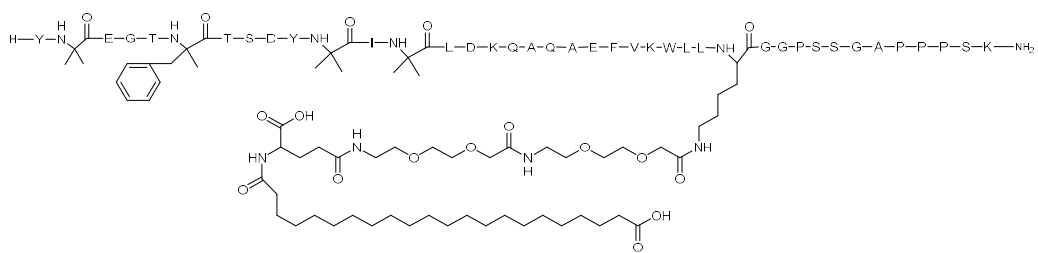
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wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₁₆-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:19); or

Y-X₁-E-G-T-X₂-T-S-D-Y-X₃-I-X₄-L-D-K-Q-A-Q-A-E-F-V-K-W-L-L-K-G-G-P-S-S-G-A-P-P-P-S-K;

wherein X₁ is Aib; X₂ is αMePhe; X₃ is Aib; X₄ is Aib; K at position 28 is chemically modified through conjugation to the ε-amino of the K side-chain with ([2-(2-amino-ethoxy)-ethoxy]-acetyl)₃-(γ-Glu)₁-CO-(CH₂)₂₀-CO₂H; and the C-terminal amino acid is amidated as a C-terminal primary amide (SEQ ID NO:20).

12. The compound according to claim 4, wherein the compound is



or a pharmaceutically acceptable salt thereof.

13. A pharmaceutical composition comprising the compound according to any one of claims 1 to 12 and a pharmaceutically acceptable carrier, diluent or excipient.

14. Use of the compound or the pharmaceutically acceptable salt thereof according to any one of claims 1 to 12, or the composition according to claim 13 in the manufacture of a medicament for treating or preventing a disease selected from hyperglycemia, impaired glucose tolerance, Type I diabetes mellitus, Type II diabetes mellitus, obesity, hypertension, Syndrome X, dyslipidemia, cognitive impairment, atherosclerosis, myocardial infarction, coronary heart disease and other cardiovascular diseases, stroke, inflammatory bowel syndrome, dyspepsia and gastric ulcers.

15. Use of the compound or the pharmaceutically acceptable salt thereof according to any one of claims 1 to 12, or the composition according to claim 13 in the manufacture of a medicament for delaying or preventing the disease development of Type II diabetes mellitus.
16. Use of the compound or the pharmaceutically acceptable salt thereof according to any one of claims 1 to 12, or the composition according to claim 13 in the manufacture of a medicament for reducing food intake, reducing β -cell apoptosis, increasing β -cell function and β -cell volume, and/or restoring glucose sensitivity of β -cell.
17. A method for treating Type II diabetes mellitus, comprising administering to a subject in need thereof, an effective amount of the compound according to any one of claims 1 to 12, or the composition according to claim 13.