

blood glucose. GLP-1 is secreted in L-cells of the small intestine after food intake. It has a very short half-life of no longer than 2 minutes. It is reported that glucose increases secretion of GLP-1, which induces insulin secretion by pancreatic beta cells, ultimately controlling blood glucose level and improving beta cell functions. GLP-1 also suppresses secretion of glucagon, inhibits gastric emptying, and reduces food intake (Physiol Rev. 2007;87(4):1409-1439). Novo Nordisk's liraglutide is human GLP-1 derivative which has been developed to treat type 2 diabetes and obesity indications and is to be injected once per day. Liraglutide is a long-acting GLP-1 receptor agonist which binds to the same receptors as endogenous GLP-1, stimulating insulin secretion, thereby modulating blood glucose level and reducing appetite, thus inhibiting body weight gain and reducing triglycerides. Liraglutide has been marketed in the U.S. and Europe as Victoza for type II diabetes and Saxenda for obesity (Expert Rev Cardiovasc Ther. 2015;13(7):753-767). Exenatide, lixisenatide, albiglutide, and dulaglutide also have been developed for the treatment of diabetes. However, such GLP-1 receptor agonists are reported to cause side effects such as nausea, vomiting, appetite reduction, headache, constipation, and abdominal bloating (Korean J Med. 2014;87(1):9-13).

[9]

Oxyntomodulin is a peptide derived from proglucagon, a precursor of glucagon. Oxyntomodulin consists of 37 amino acid peptides, including the complete 29 amino acids of glucagon, and is known to be a dual agonist that binds both to GLP-1 and glucagon receptors. It produces body weight loss effect by reducing food intake and increasing energy metabolism. Oxyntomodulin is known to be more effective than selective GLP-1 receptor agonists at lowering body weight. It has been reported that the risk of hyperglycemia caused by a rise in glucose due to glucagon receptor activation may be offset by insulin secretion of GLP-1 receptors. Oxyntomodulin has been reported to reduce food intake and body weight, and to improve energy expenditure and glucose metabolism in non-clinical testing (Diabetes. 2009;58(10):2258-2266). In a clinical study, oxyntomodulin showed body weight loss effects of 2.3 kg on average when administered subcutaneously for 4 weeks, 3 times per day, to overweight and obese patients (Diabetes. 2005;54:2390-2395). It has been shown to produce significant insulin secretion and blood glucose lowering effects against placebo (Diabetes. 2013;62(Suppl. 1):A48). In another clinical study, oxyntomodulin reduced energy intake without side effects such as vomiting or appetite stimulation from continual use of oxyntomodulin (J Clin Endocrinol Metab. 2003;88:4696-4701). Oxyntomodulin's effectiveness at glycemic control, lowering of food intake, and satiety promotion have garnered interests in its potential as a new method of obesity treatment and glycemic control (Molecular metabolism. 2014;3:241-251). However, because oxyntomodulin, like GLP-1, can be cleaved by dipeptidyl peptidase-IV (DPP-IV), it is unstable *in vivo* and has a very short *in vivo* half-life (J Biol Chem. 2003 ;278: 22418-22423).

[10]

Therefore, studies are being conducted on DPP-IV resistant oxyntomodulin derivatives that make oxyntomodulin's pharmacological and treatment effects long-lasting by binding to GLP-1 and glucagon receptors in balanced and selective ways and overcome the side effects of each hormone peptide (Diabetes. 2009;58(10):2258-2266). Many pharmaceutical manufacturers including Merck, Zealand, MedImmune, and Hanmi Pharmaceutical are working on development of lead compounds.

[11]

[12]

Against this background, the present inventors have worked to solve the problem described

[266]

[Table 7]

Group	Drug and dose administered	Method of administration	
Experimental groups	Compound 3, 100 nmol/kg/QD	S.C	once a day x 4
	Compound 3, 300 nmol/kg/QD		

[267]

[268]

The group injected with Compound 3 showed significant difference in cumulative food intake against vehicle control group. Both dosages showed a body weight loss efficacy of approximately 12.5%. Lower body weight against vehicle control was maintained even after discontinuation.

[269]

[270]

<Experimental Example 5> Body weight loss efficacy evaluation by 10-day repeated injection of peptide of the present invention

[271]

This experiment aimed to compare the body weight loss efficacy of acylated oxyntomodulin peptide analog according to the present invention against commercially available diabetes treatments. Male laboratory mice (C57BL/6 mouse) were given diet containing high fat. The mice with high-fat-diet-induced obesity were separated into groups by body weight before the experiment began. Compound 3 of the present invention was prepared in distilled water containing 0.01% Tween80 to a dose of 100 nmol/kg or 300 nmol/kg. Semaglutide, a commercially available diabetes treatment, was prepared in the same vehicle to a dose of 100 nmol/kg. Then, they were subcutaneously injected once every 3 days for a total of 10 days as indicated in Table 8. Body weight and food intake was measured once per day at the same time each day to compare the body weight loss efficacy of the acylated oxyntomodulin peptide analog against Semaglutide. The results are shown in Figures 4a and 4b.

[272]

[273]

[Table 8]

Group	Drug and dose administered	Method of administration	
Comparison	Semaglutide, 100 nmol/kg/Q3D	S.C	Once every 3 days x 4
Experimental	Compound 3, 100 nmol/kg/Q3D		
	Compound 3, 300 nmol/kg/Q3D		

[274]

[275]

Compound 3 showed higher cumulative food intake against Semaglutide at the same dosage (100 nmol/kg) but still showed superior body weight loss efficacy against Semaglutide (Compound 3: 13.9%, Semaglutide: 9.7%). Also, Compound 3 at 300 nmol/kg showed body weight loss of approximately 16.9%. The acylated oxyntomodulin peptide analog according to the present invention showed superior dose-dependent body weight loss efficacy against Semaglutide.

[294]

At same dosage, Compounds 23 and 25 had higher cumulative food intake compared to Compound 3. Compounds 21 and 22 showed similar cumulative food intake as Compound 3. Compound 3 showed similar or better body weight loss compared to Compounds 21, 23 and 25. Compound 25 was confirmed to have better body weight loss efficacy than Compound 3. On the other hand, Compound 24, which showed somewhat weak action on glucagon receptor compared to other compounds in *in vitro* assay, showed significant body weight loss efficacy, at about 13.4% against initial body weight, showing inferior body weight loss efficacy compared to Compound 3. Compound 25 showed similar level of body weight loss efficacy to Compound 3 but had lower cumulative food intake. On the other hand, Compound 22 showed similar body weight loss efficacy to Compound 3 and similar cumulative food intake. Acylated oxyntomodulin cyclic peptide analogs showed different body weight loss efficacy patterns depending on their structures.

[295]

[296]

<Experimental Example 9> Body weight loss efficacy evaluation by 2-week repeated injection of peptide of present invention

[297]

Continuing from Experimental Example 8, this experiment was conducted to compare acylated oxyntomodulin peptide analogs of the present invention in low doses. Male laboratory mice (C57BL/6 mouse) were given diet containing high fat. The mice with high-fat-diet-induced obesity were separated into groups by body weight before beginning the experiment. Compounds 3 and 22 according to the present invention were prepared in distilled water containing 0.01% Tween80 to a dosage of 10 nmol/kg or 30 nmol/kg and as indicated in Table 12 injected subcutaneously once every 3 days for a total of 2 weeks. Body weight and food intake was measured once per day, at the same time each day. Body weight loss efficacy of Compound 3 and Compound 22 was compared. The results are shown in Figures 8a and 8b.

[298]

[299]

[Table 12]

Group	Drug and dose administered	Method of administration	
Experimental	Compound 3, 10 nmol/kg/Q3D	S.C	once every 3 days x 5
	Compound 3, 30 nmol/kg/Q3D		
	Compound 22, 10 nmol/kg/Q3D		
	Compound 22, 30 nmol/kg/Q3D		

[300]

[301]

At the same dosage, Compound 22 showed higher cumulative food intake compared to Compound 3. Compound 22 at 10 nmol/kg showed similar level of cumulative food intake as vehicle control group but body weight loss efficacy of 7.5%. Furthermore, Compound 22 at 30 nmol/kg had higher food intake than Compound 3 at the same dose but had similar levels of body weight loss efficacy. It can be inferred that Compound 22 is affected more by the efficacy resulting from glucagon receptor activation compared to Compound 3.

[302]

[303]

<Experimental Example 10> Body weight loss efficacy evaluation by 5-day repeated injection of peptide of present invention

[304]

This experiment was conducted to compare the body weight loss efficacy of acylated oxyntomodulin peptide analog of the present invention with commercially available diabetes/obesity treatment and an oxyntomodulin peptide analog lead compound currently in development. Male laboratory mice (C57BL/6 mouse) were given diet containing high fat. The mice with high-fat-diet-induced obesity were grouped by body weight before beginning the experiment. Compound 3 of the present invention was prepared in distilled water containing 0.01% Tween80 to dose of 30 nmol/kg. As comparative examples, Liraglutide (commercially available diabetes/obesity treatment) and MEDI0382 (in clinical trial) were prepared in the same vehicle to a dose of 30 nmol/kg. Then, they were subcutaneously injected once per day for 4 days total, as indicated in Table 13. Body weight and food intake was measured once per day, at the same time each day. Body weight loss efficacy of the analog was compared with Liraglutide and MEDI0382. The results are shown in Figures 9a and 9b.

[305]

[306]

[Table 13]

Group	Drug and dose administered	Method of administration	
Comparison	Liraglutide, 30 nmol/kg/QD	S.C	Once a day x 4
	MEDI0382, 30 nmol/kg/QD		
Experimental	Compound 3, 30 nmol/kg/ QD		

[307]

[308]

Among the three substances at same dose, the Compound 3 group showed most body weight loss from baseline (Compound 3: 18.6%, Liraglutide: 12.7%, MEDI0382: 8.0%).

[309]

[310]

<Experimental Example 11> Body weight loss efficacy evaluation by 4-week repeated injection of peptide according to present invention

[311]

This experiment was conducted to compare the body weight loss efficacy of acylated oxyntomodulin peptide analogs according to the present invention with commercially available diabetes treatment. Male obese and diabetic laboratory mice (FATZO mouse) were given diet containing high fat, and were grouped by body weight, body fat, non-fasting blood glucose, and glycated hemoglobin (HbA1c) before experiment. Compounds 3 and 22 according to the present invention were prepared in distilled water containing 0.01% Tween80 to a dose of 30 nmol/kg or 100 nmol/kg. Semaglutide, commercially available diabetes treatment, was prepared in the same vehicle to a dose of 100 nmol/kg. Then, they were given subcutaneously once every 3 days for a total of 4 weeks, as indicated in Table 14. Body weight and food intake was measured once per day, at the same time each day. Body fat was measured at 4 weeks before autopsy to compare the body weight and body fat reduction

efficacy of oxyntomodulin peptide analogs of the present invention against Semaglutide. The results are shown in Figures 10a through 10c.

[312]

[313]

[Table 14]

Group	Drug and dose administered	Method of administration	
Comparison	Semaglutide, 100 nmol/kg/Q3D	S.C	Once every 3 days x 10
Experimental	Compound 3, 30 nmol/kg/Q3D		
	Compound 3, 100 nmol/kg/Q3D		
	Compound 22, 30 nmol/kg/Q3D		
	Compound 22, 100 nmol/kg/Q3D		

[314]

[315]

At both same and lower dosage (100 and 30 nmol/kg respectively), Compounds 3 and 22 of the present invention shows highly superior body weight reduction efficacy against Semaglutide despite higher cumulative food intake. This is inferred to be a result of the mechanism of action of oxyntomodulin peptide analog according to the present invention being a dual agonist of GLP-1 and glucagon receptors, whereas Semaglutide is a GLP-1 receptor agonist.

[316]

[317]

<Experimental Example 12> Oral glucose tolerance test in mice of peptide according to present invention

[318]

In this experiment, glucose tolerance improvement effect in male laboratory mice (C57BL/6 mouse) of acylated oxyntomodulin peptide analogs according to the present invention was evaluated as improvement of postprandial glycemic control. Laboratory mice were fasted the day before the experiment. Then, Compound 3 or 22 or 25 according to the present invention was prepared in distilled water containing 0.01% Tween80 and injected subcutaneously 30 minutes before glucose loading. Glucose solution was orally administered 30 minutes after the injection of oxyntomodulin peptide analog. Whole blood glucose was measured via tail vein immediately before administering the drug and glucose, and for 2 hours after glucose loading at designated times. From the results, the area under the curve (AUC) of the blood glucose curve over time was produced to calculate the ratio of blood glucose AUC of the analog and comparison against glucose control as percentages to evaluate the efficacy of glucose tolerance improvement. Experiments were conducted separately for each compound. Compound 3 at 30 nmol/kg was used as comparison for Compound 22 or 25. The combined results are shown in Figure 11.

[319]

[320]

The peptide analogs showed significant, dose-dependent reduction of blood glucose AUC at and above 30 nmol/kg. In particular, Compound 25 showed significant glucose tolerance improvement efficacy and three times superior glucose tolerance improvement efficacy

[Claims]**[Claim 1]**

An oxyntomodulin peptide analog of the following formula I:

His-X₁-Gln-Gly-Thr-Phe-Thr-Ser-X₂-X₃-X₄-X₅-X₆-X₇-X₈-X₉-Arg-Arg-Ala-X₁₀-Asp-Phe-Val-Gln-Trp-Leu-X₁₁-X₁₂-X₁₃-X₁₄-X₁₅-X₁₆ (SEQ ID NO.49),

wherein:

X₁ is Aib (aminoisobutyric acid);

X₂ is Asp;

X₃ is Tyr or Z;

X₄ is Ser;

X₅ is Lys;

X₆ is Tyr;

X₇ is Leu;

X₈ is Asp;

X₉ is Ser, Aib (aminoisobutyric acid) or Z;

X₁₀ is Gln or Z;

X₁₁ is Met or Leu;

X₁₂ is Asn or Arg;

X₁₃ is Thr or Ala;

X₁₄ is Lys;

X₁₅ is absent;

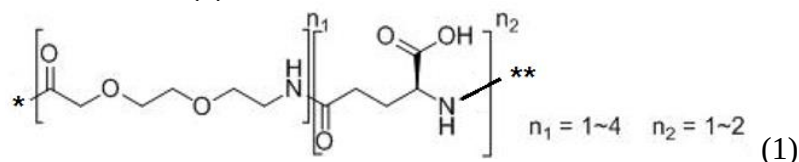
X₁₆ is absent;

C-terminal amino acid may optionally be amidated, wherein

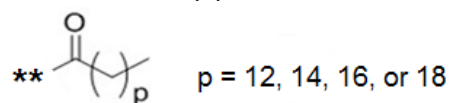
one of X₃, X₉ and X₁₀ is Z;

Z is modified Lys to which Z₁-Z₂ of the following formulas is attached;

Z₁ is Formula (1); and



Z₂ is Formula (3);



wherein * of Formula (1) is directly bound to a side chain of Lys, and

wherein ** of Formula (1) and ** of formula (3) are a bond between Z₁ and Z₂.

[Claim 2]

The oxyntomodulin peptide analog of claim 1, wherein X₁₁ is Leu, X₁₂ is Arg and X₁₃ is Ala.

[Claim 3]

The oxyntomodulin peptide analog of claim 1, wherein the said peptide analog is Compound 2(SEQ ID NO. 3), Compound 3(SEQ ID NO. 4), Compound 6(SEQ ID NO. 7), Compound 9(SEQ ID NO. 10), Compound 10(SEQ ID NO. 11), Compound 12(SEQ ID NO. 13), Compound 15(SEQ ID NO. 16), Compound 16(SEQ ID NO. 17), Compound 17(SEQ ID NO.

18), or Compound 18(SEQ ID NO. 19).

[Claim 4]

An oxyntomodulin peptide analog having an intramolecular cross-link at X₁₉ and X₂₀ of the amino acid sequence of the following Formula II: His-X₁₇-Gln-Gly-Thr-Phe-Thr-Ser-Asp-X₁₈-Ser-Lys-Tyr-Leu-Asp-X₁₉-Arg-Arg-Ala-X₂₀-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-Lys (SEQ ID NO.53),

wherein:

X₁₇ is Aib (Aminoisobutric acid);

X₁₈ is Z;

X₁₉ is Asp, Glu, Cys, Hcy (Homocysteine), Lys or Orn (Ornithine);

X₂₀ is Asp, Glu, Cys, Hcy (Homocysteine), Lys or Orn (Ornithine);

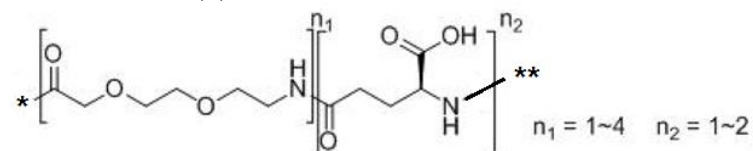
wherein the intramolecular cross-link at X₁₉ and X₂₀ forms a cyclic peptide via intramolecular bond or via a cross-linker;

said cyclic peptide has a lactam ring formed by amide bond between two residues at X₁₉ and X₂₀, or a cross-linked ring formed by a cross-linker between two residues at X₁₉ and X₂₀;

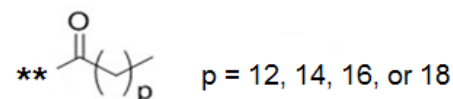
C-terminal amino acid may optionally be amidated; and

Z is modified Lys to which Z₁-Z₂ of the following formulas is attached;

Z₁ is Formula (1); and



Z₂ is Formula (3);



wherein * of Formula (1) is directly bound to a side chain of Lys, and

wherein ** of Formula (1) and ** of formula (3) are a bond between Z₁ and Z₂.

[Claim 5]

The oxyntomodulin peptide analog of claim 4, wherein:

the intramolecular bond between X₁₉ and X₂₀ is an intramolecular lactam ring forming bond when X₁₉ and X₂₀ are Asp or Glu and Lys or Orn respectively or Lys or Orn and Asp or Glu respectively.

[Claim 6]

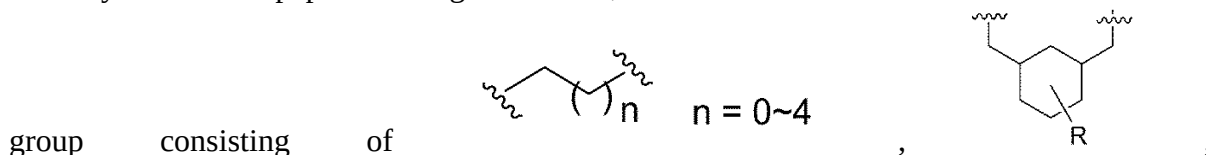
The oxyntomodulin peptide analog of claim 4, wherein:

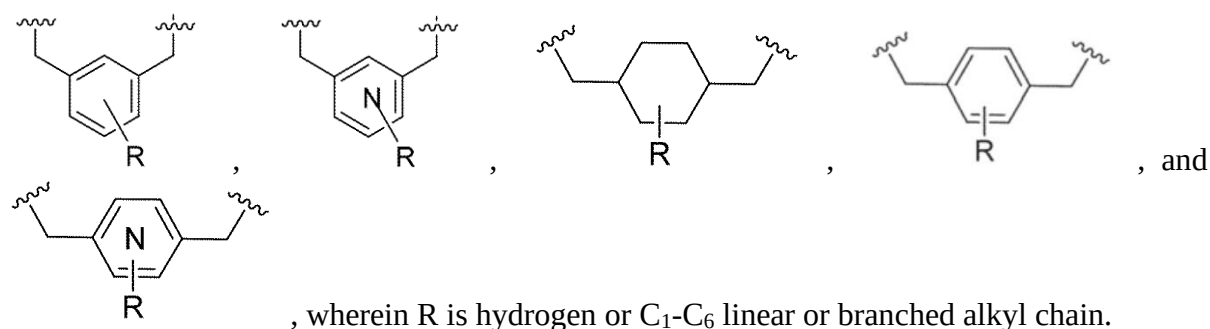
X₁₉ and X₂₀ are Cys or Hcy and Cys or Hcy respectively, and a cross-linker bonds to the thiol functional group at both Cys or Hcy side chains to form a ring; and

said cross-linker is C₁-C₆ linear or branched chain alkyl, C₃-C₈ saturated or unsaturated cycloalkyl, C₆-C₁₀ aryl, or C₅-C₁₂ heteroaryl, or C₅-C₁₂ fused heterocyclic aryl.

[Claim 7]

The oxyntomodulin peptide analog of claim 6, wherein the cross-linker is selected from the





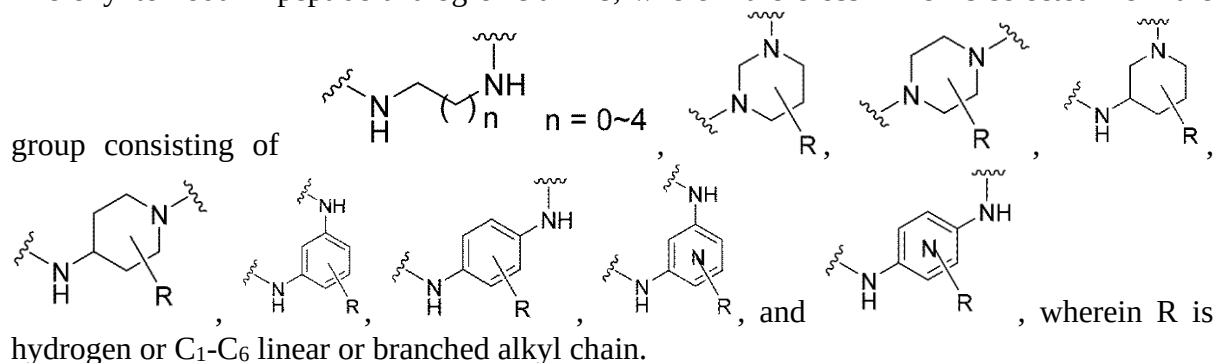
[Claim 8]

The oxyntomodulin peptide analog of claim 4, wherein:

X₁₉ and X₂₀ are Asp or Glu and Asp or Glu respectively, and a cross-linker forms an amide bond to the carboxylic group at both Asp or Glu side chains to form a ring; and said cross-linker is di-amino C₁-C₆ linear or branched alkyl chain, di-amino C₃-C₈ saturated or unsaturated cycloalkyl, aminopiperidine, piperazine, di-amino C₆-C₁₀ aryl, or di-amino C₅-C₁₂ heteroaryl, or di-amino C₅-C₁₂ fused heterocyclic aryl.

[Claim 9]

The oxyntomodulin peptide analog of claim 8, wherein the cross-linker is selected from the



[Claim 10]

The oxyntomodulin peptide analog of claim 4, wherein:

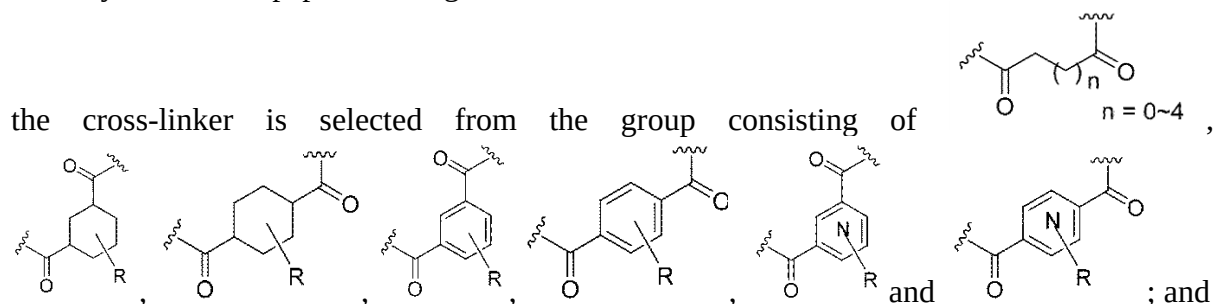
X₁₉ and X₂₀ are each Lys or Orn and Lys or Orn respectively;

a cross-linker forms an amide bond with the amine group at both Lys or Orn side chains to form a ring; and

said cross-linker is di-carbonyl C₁-C₆ linear or branched alkyl chain, di-carbonyl C₃-C₈ saturated or unsaturated cycloalkyl, di-carbonyl C₆-C₁₀ aryl or di-carbonyl C₅-C₁₂ heteroaryl, or di-carbonyl C₅-C₁₂ fused heterocyclic aryl.

[Claim 11]

The oxyntomodulin peptide analog of claim 10, wherein:



R is hydrogen or C₁-C₆ linear or branched alkyl chain.

[Claim 12]

The oxyntomodulin peptide analog of claim 4, wherein:

X₁₉ and X₂₀ are Asp or Glu and Lys or Orn respectively, or are Lys or Orn and Asp or Glu respectively; and

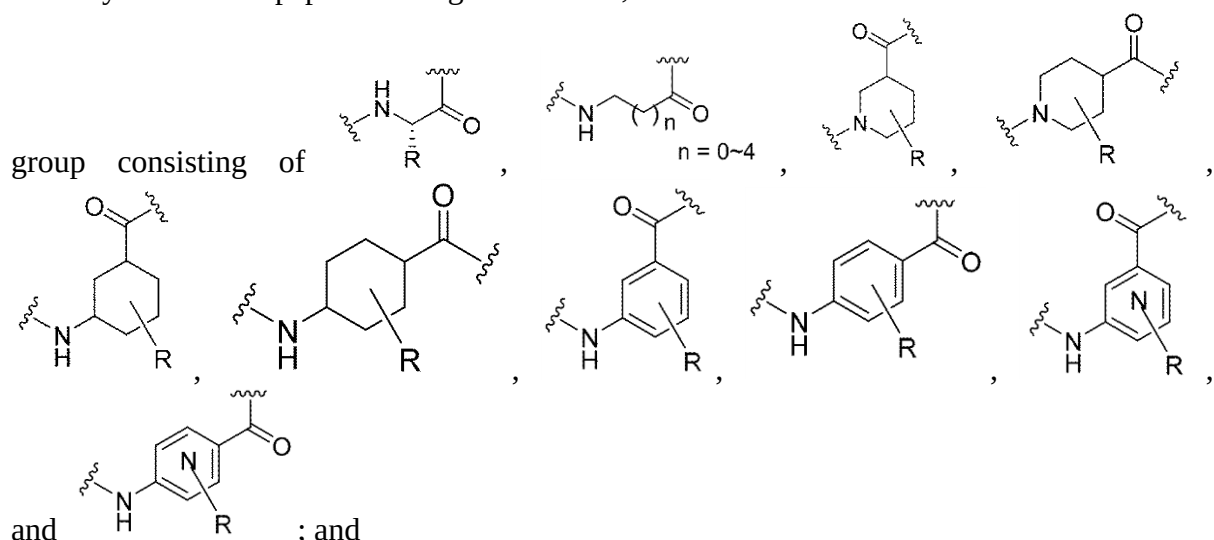
a cross-linker forms an amide bond between the carboxyl group of Asp or Glu side chain and the amine functional group of the cross-linker; and/or

the amine group at Lys or Orn side chain is connected via an amide bond to the carboxyl functional group of the cross-linker to form a ring; and

said cross-linker either an alpha amino acid selected from the group consisting of Gly, Val, Leu, and Ile; a beta amino acid; carbonyl C₁-C₆ linear or branched alkylamine; carbonyl C₃-C₈ saturated or unsaturated alkylamine; carbonyl piperidine; aminobenzoyl; carbonyl C₆-C₁₀ arylamine or carbonyl C₅-C₁₂ heteroarylamine, or carbonyl C₅-C₁₂ fused heterocyclic arylamine.

[Claim 13]

The oxyntomodulin peptide analog of claim 12, wherein the cross-linker is selected from the



R is hydrogen or C₁-C₆ linear or branched alkyl chain.

[Claim 14]

The oxyntomodulin peptide analog of claim 4, wherein said peptide analog is Compound 14(SEQ ID NO. 15), Compound 19(SEQ ID NO. 20), Compound 20(SEQ ID NO. 21), Compound 21(SEQ ID NO. 22), Compound 22(SEQ ID NO. 23), Compound 23(SEQ ID NO. 24), Compound 27(SEQ ID NO. 28), Compound 29(SEQ ID NO. 30), Compound 30(SEQ ID NO. 31), Compound 31(SEQ ID NO. 32), Compound 32(SEQ ID NO. 33), Compound 33(SEQ ID NO. 34), Compound 34(SEQ ID NO. 35), Compound 35(SEQ ID NO. 36), Compound 36(SEQ ID NO. 37), Compound 37(SEQ ID NO. 38), or Compound 38(SEQ ID NO. 39).

[Claim 15]

A pharmaceutical composition comprising the peptide analog of claim 1 and a pharmaceutically acceptable excipients for use in the prevention or treatment of conditions caused by or characterized by obesity or overweight.

[Claim 16]

A pharmaceutical composition comprising the peptide analog of claim 1 and a pharmaceutically acceptable excipients for use in the prevention or treatment of non-insulin-dependent diabetes accompanying obesity or overweight.

[Claim 17]

A pharmaceutical composition comprising the peptide analog of claim 4 and a pharmaceutically acceptable excipients for use in the prevention or treatment of conditions caused by or characterized by obesity or overweight.

[Claim 18]

A pharmaceutical composition comprising the peptide analog of claim 4 and a pharmaceutically acceptable excipients for use in the prevention or treatment of non-insulin-dependent diabetes accompanying obesity or overweight.