(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 October 2015 (15.10.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/155139 A1

(51) International Patent Classification:
C07K 14/435 (2006.01)

(21) International Application Number:
PCT/EP2015/057416

(22) International Filing Date:
2 April 2015 (02.04.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
14305501.0 7 April 2014 (07.04.2014) EP

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2015/155139 A1

(54) Title: EXENDIN-4 DERIVATIVES AS PEPTIDIC DUAL GLP-1 / GLUCAGON RECEPTOR AGONISTS

(57) Abstract: The present invention relates to dual GLP-1 / glucagon receptor agonists and their medical use, for example in the treatment of disorders of the metabolic syndrome, including diabetes and obesity, as well as for reduction of excess food intake.

Exendin-4 Derivatives as peptidic dual GLP-1 / Glucagon Receptor Agonists

Description

FIELD OF THE INVENTION

The present invention relates to dual GLP-1 / glucagon receptor agonists and their medical use, for example in the treatment of disorders of the metabolic syndrome, including diabetes and obesity, as well as for reduction of excess food intake. These dual GLP-1 / glucagon receptor agonists show reduced activity on the GIP receptor to reduce the risk of hypoglycemia and are structurally derived from exendin-4, a pure GLP-1 receptor agonist.

BACKGROUND OF THE INVENTION

Pocai et al (Obesity 2012;20:1566–1571; Diabetes 2009, 58, 2258) and Day et al. (Nat Chem Biol 2009;5:749) describe dual agonists of the glucagon-like peptide-1 (GLP-1) and glucagon receptors, e.g. by combining the actions of GLP-1 and glucagon in one molecule, which lead to a therapeutic principle with anti-diabetic action and a pronounced weight lowering effect superior to pure GLP-1 agonists, among others due to glucagon-receptor mediated increased satiety and energy expenditure.

Holst (Physiol. Rev. 2007, 87, 1409) and Meier (Nat. Rev. Endocrinol. 2012, 8, 728) describe that GLP-1 receptor agonists, such as GLP-1, liraglutide and exendin-4, have 3 major pharmacological activities to improve glycemic control in patients with T2DM by reducing fasting and postprandial glucose (FPG and PPG): (i) increased glucose-dependent insulin secretion (improved first- and second-phase), (ii) glucagon suppressing activity under hyperglycemic conditions, (iii) delay of gastric emptying rate resulting in retarded absorption of meal-derived glucose.

The amino acid sequence of GLP-1(7-36)-amide is shown as SEQ ID NO: 2.

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH_2

Liraglutide is a marketed chemically modified GLP-1 analog in which, among other modifications, a fatty acid is linked to a lysine in position 20 leading to a prolonged duration of action (Drucker DJ et al, Nature Drug Disc. Rev. 9, 267-268, 2010; Buse, J.B. et al., Lancet, 374:39-47, 2009).

The amino acid sequence of Liraglutide is shown as SEQ ID NO: 4.

HAEGTFTSDVSSYLEGQAAK((S)-4-Carboxy-4-hexadecanoylamino-butyryl-) EFIAWLVRGRG-OH

Glucagon is a 29-amino acid peptide which is released into the bloodstream when circulating glucose is low. Glucagon's amino acid sequence is shown as SEQ ID NO: 3.

HSQGTFTSDYSKYLDSRRAQDFVQWLMNT-OH

During hypoglycemia, when blood glucose levels drop below normal, glucagon signals the liver to break down glycogen and release glucose, causing an increase of blood glucose levels to reach a normal level. Recent publications suggest that glucagon has in addition beneficial effects on reduction of body fat mass, reduction of food intake, and increase of energy expenditure (KM Heppner, Physiology & Behavior 2010, 100, 545–548).

GIP (glucose-dependent insulinotropic polypeptide) is a 42 amino acid peptide that is released from intestinal K-cells following food intake. GIP and GLP-1 are the two gut enteroendocrine cell-derived hormones accounting for the incretin effect, which accounts for over 70% of the insulin response to an oral glucose challenge (Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology 2007; 132: 2131–2157).

GIP's amino acid sequence is shown as SEQ ID NO: 5.

YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ-OH

Peptides which are based on the structures of GLP-1 or glucagon, and bind and activate both the glucagon and the GLP-1 receptor (Hjort et al. Journal of Biological Chemistry, 269, 30121-30124,1994; Day JW et al, Nature Chem Biol, 5: 749-757, 2009) and suppress body weight gain and reduce food intake are described in patent applications WO 2008/071972, WO 2008/101017, WO 2009/155258, WO 2010/096052, WO 2010/096142, WO 2011/075393, WO 2008/152403, WO 2010/070251, WO 2010/070252, WO 2010/070253, WO 2010/070255, WO 2011/160630, WO 2011/006497, WO 2011/087671, WO 2011/087672, WO 2011/117415 ,WO 2011/117416, WO 2012/177443 WO 2012/177444, WO 2012/150503, WO 2013/004983, WO 2013/092703, WO 2014/041195 and WO 2014/041375, the contents of which are herein incorporated by reference. The body weight reduction was shown to be superior to pure GLP-1 agonists.

In addition, triple co-agonist peptides which not only activate the GLP-1 and the glucagon receptor, but also the GIP receptor are described in WO 2012/088116 and by VA Gault et al (Biochem Pharmacol, 85, 16655-16662, 2013; Diabetologia, 56, 1417-1424, 2013).

Exendin-4 is a 39 amino acid peptide which is produced by the salivary glands of the Gila monster (Heloderma suspectum) (Eng, J. et al., J. Biol. Chem., 267:7402-05,1992). Exendin-4 is an activator of the GLP-1 receptor, whereas it shows low activation of the GIP receptor and does not activate the glucagon receptor (see Table 1).

Table 1: Potencies of exendin-4 at human GLP-1, GIP and Glucagon receptors (indicated in pM) at increasing concentrations and measuring the formed cAMP as described in Methods.

SEQ ID NO:	peptide	EC50 hGLP-1 R [pM]	EC50 hGIP R [pM]	EC50 hGlucagon R [pM]
1	exendin-4	0.4	12500.0	>10000000

The amino acid sequence of exendin-4 is shown as SEQ ID NO: 1.

HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH2

Exendin-4 shares many of the glucoregulatory actions observed with GLP-1. Clinical and nonclinical studies have shown that exendin-4 has several beneficial antidiabetic properties including a glucose dependent enhancement in insulin synthesis and secretion, glucose dependent suppression of glucagon secretion, slowing down gastric emptying, reduction of food intake and body weight, and an increase in beta-cell mass and markers of beta cell function (Gentilella R et al., Diabetes Obes Metab.,11:544-56 , 2009; Norris SL et al, Diabet Med., 26:837-46, 2009; Bunck MC et al, Diabetes Care., 34:2041-7, 2011).

These effects are beneficial not only for diabetics but also for patients suffering from obesity. Patients with obesity have a higher risk of getting diabetes, hypertension, hyperlipidemia, cardiovascular and musculoskeletal diseases.

Relative to GLP-1, exendin-4 is resistant to cleavage by dipeptidyl peptidase-4 (DPP4) resulting in a longer half-life and duration of action in vivo (Eng J., Diabetes, 45 (Suppl 2):152A (abstract 554), 1996).

Exendin-4 was also shown to be much more stable towards degradation by neutral endopeptidase (NEP), when compared to GLP-1, glucagon or oxyntomodulin (Druce MR et al., Endocrinology, 150(4), 1712-1721, 2009). Nevertheless, exendin-4 is chemically labile due to methionine oxidation in position 14 (Hargrove DM et al., Regul. Pept., 141: 113-9, 2007) as well as deamidation and isomerization of asparagine in position 28 (WO 2004/035623).

Compounds of this invention are exendin-4 derivatives, which in addition to the agonistic activity at the GLP-1 receptor of native exendin-4 show agonistic activity at the glucagon receptor and which have – among others - the following modification: at position 14 an amino acid carrying an $-NH_2$ group in the side-chain, which is further substituted with a lipophilic residue (e.g. a fatty acid combined with a linker) and at

position 27 an Aib.

Bloom et al. (WO 2006/134340) disclose that peptides which bind and activate both the glucagon and the GLP-1 receptor can be constructed as hybrid molecules from glucagon and exendin-4, where the N-terminal part (e.g. residues 1-14 or 1-24) originates from glucagon and the C-terminal part (e.g. residues 15-39 or 25-39) originates from exendin-4. Such peptides comprise glucagon's amino acid motif YSKY in position 10-13. Krstenansky et al (Biochemistry, 25, 3833-3839, 1986) show the importance of these residues 10-13 of glucagon for its receptor interactions and activation of adenylate cyclase.

In the exendin-4 derivatives described in this invention, several of the underlying residues are different from glucagon and the peptides described in WO 2006/134340. In particular residues Tyr10 and Tyr13, which are known to contribute to the fibrillation of glucagon (DE Otzen, Biochemistry, 45, 14503-14512, 2006) are replaced by Leu in position 10 and Gln, a non-aromatic polar amino acid, in position 13. This replacement, especially in combination with isoleucine in position 23 and glutamate in position 24, leads to exendin-4 derivatives with potentially improved biophysical properties as solubility or aggregation behaviour in solution. The non-conservative replacement of an aromatic amino acid with a polar amino acid in position 13 of an exendin-4 analogue surprisingly leads to peptides with high activity on the glucagon receptor, keeping their activity on the GLP-1 receptor (see also WO2013/186240 .

Furthermore, we surprisingly found that compounds carrying an Aib amino acid in position 27 show reduced activity on the GIP receptor compared to the corresponding derivatives with Lys at position 27 as in native exendin-4, as shown in Example 5, Table 8. A reduced activation of the GIP receptor is potentially beneficial as there are reports in the literature that high levels of GIP in diabetics might in some cases lead to more frequent episodes of hypoglycemia (T McLaughlin et al., J Clin Endocrinol Metab, 95, 1851–1855, 2010; A Hadji-Georgopoulos, J Clin Endocrinol Metab, 56, 648-652, 1983).

Furthermore, compounds of this invention are exendin-4 derivatives with fatty acid

acylated residues in position 14. This fatty acid functionalization in position 14 resulted in exendin-4 derivatives with high activity not only at the GLP-1 receptor, but also at the glucagon receptor, when compared to the corresponding non-acylated exendin-4 derivatives, for example those shown in Example 5, Table 7. In addition, this modification results in an improved pharmacokinetic profile.

It is described in the literature (Murage EN et al., Bioorg. Med. Chem. 16 (2008), 10106-10112), that a GLP-1 analogue with an acetylated lysine at position14 showed significantly reduced potency on the GLP-1 receptor compared to natural GLP-1.

Compounds of this invention are more resistant to cleavage by neutral endopeptidase (NEP) and dipeptidyl peptidase-4 (DPP4), resulting in a longer half-life and duration of action in vivo, when compared with native GLP-1 and glucagon.

Compounds of this invention preferably are soluble not only at neutral pH, but also at pH 4.5. This property potentially allows co-formulation for a combination therapy with an insulin or insulin derivative and preferably with a basal insulin like insulin glargine /Lantus®.

BRIEF SUMMARY OF THE INVENTION

Native exendin-4 is a pure GLP-1 receptor agonist without activity on the glucagon receptor and low activity on the GIP receptor. Provided herein are exendin-4 derivatives based on the structure of native exendin-4 but differing at ten or more positions as compared to SEQ ID NO: 1 wherein the differences contribute to the enhancement of the agonistic activity at the glucagon receptor. Among other substitutions - methionine at position 14 is replaced by an amino acid carrying an $-NH_2$ group in the side-chain, which is further substituted by a lipophilic residue (e.g. a fatty acid combined with a linker). Furthermore, we surprisingly found that a replacement of the lysine at position 27 by Aib leads to reduced GIP receptor activity compared to the GLP-1 receptor activity. A reduced activation of the GIP receptor is potentially beneficial as there are reports in the literature that high levels of GIP in diabetics might in some cases lead to more frequent episodes of hypoglycemia (T McLaughlin et al., J Clin Endocrinol Metab, 95, 1851–1855, 2010; A Hadji-

Georgopoulos, J Clin Endocrinol Metab, 56, 648-652, 1983).

The invention provides a peptidic compound having the formula (I):

H_2N-His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-X14-Asp-Glu-Gln-Arg-Ala-Lys-Leu-Phe-Ile-Glu-Trp-Leu-Aib-X28-X29-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-R^1 (I)

X14 represents an amino acid residue with a functionalized -NH_2 side chain group, selected from the group consisting of Lys, Orn, Dab, or Dap, wherein the -NH_2 side chain group is functionalized by -Z-C(O)-R^5, wherein
Z represents a linker in all stereoisomeric forms and
R^5 is a moiety comprising up to 50 carbon atoms and heteroatoms selected from N and O,
X28 represents an amino acid residue selected from Ala, Lys and Ser,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 is NH_2 or OH,
or a salt or solvate thereof.

The compounds of the invention are GLP-1 and glucagon receptor agonists as determined by the observation that they are capable of stimulating intracellular cAMP formation in the assay system described in Methods.

According to another embodiment the compounds of the invention, particularly with a lysine at position 14 which is further substituted with a lipophilic residue, exhibit at least a relative activity of 0.1% (i.e. EC_{50} < 700 pM), more preferably of 1% (i.e. EC_{50} < 70 pM), more preferably of 5% (i.e. EC_{50} < 14 pM) and even more preferably of 10% (i.e. EC_{50} < 7 pM) compared to that of GLP-1(7-36)amide at the GLP-1 receptor. Furthermore, the compounds exhibit at least a relative activity of 0.09% (i.e. EC_{50} < 1111 pM), more preferably of 0.45% (i.e. EC_{50} < 222 pM) and even more preferably of 0.9% (i.e. EC_{50} < 111 pM) compared to that of natural glucagon at the glucagon receptor.

The term “activity” as used herein preferably refers to the capability of a compound to activate the human GLP-1 receptor and the human glucagon receptor. More preferably the term “activity” as used herein refers to the capability of a compound to stimulate intracellular cAMP formation. The term “relative activity” as used herein is understood to refer to the capability of a compound to activate a receptor in a certain ratio as compared to another receptor agonist or as compared to another receptor. The activation of the receptors by the agonists (e.g. by measuring the cAMP level) is determined as described herein, e.g. as described in the Example 4.

The compounds of the invention preferably have an EC_{50} for hGLP-1 receptor of 450 pmol or less, preferably of 200 pmol or less, more preferably of 100 pmol or less, more preferably of 50 pmol or less, more preferably of 25 pmol or less, more preferably of 10 pmol or less, more preferably of 8 pmol or less, and more preferably of 5 pmol or less and/or an EC_{50} for hGlucagon receptor of 500 pmol or less, preferably of 300 pmol or less, more preferably of 200 pmol or less, more preferably of 150 pmol or less and/or an EC_{50} for hGIP receptor of 750 pmol or more, preferably of 1500 pmol or more; more preferably of 2000 pmol or more. It is particularly preferred that the EC_{50} for both hGLP-1 and hGlucagon receptors is 250 pm or less, more preferably of 200 pmol or less, more preferably of 150 pmol or less. The EC_{50} for the hGLP-1 receptor, the hGlucagon receptor and the hGIP receptor may be determined as described in the Methods herein and as used to generate the results described in Example 4, Table 6.

The compounds of the invention have the ability to reduce the intestinal passage, increase the gastric content and/or to reduce the food intake of a patient. These activities of the compounds of the invention can be assessed in animal models known to the skilled person and also described herein in the Methods.
The compounds of the invention have the ability to reduce blood glucose level, and/or to reduce HbA1c levels of a patient. These activities of the compounds of the invention can be assessed in animal models known to the skilled person and also described herein in the Methods.

The compounds of the invention have the ability to reduce body weight of a patient. These activities of the compounds of the invention can be assessed in animal models

known to the skilled person and also described herein in the Methods and in Example 7.

It was found that peptidic compounds of the formula (I) particularly those with a lysine at position 14 which is further substituted with a lipophilic residue, showed increased glucagon receptor activation compared to derivatives having the original methionine (from exendin-4) or leucine at position 14 (see Table 7). Furthermore, oxidation (in vitro or in vivo) of methionine is not possible anymore.

It was also found that compounds carrying an Aib amino acid in position 27 show reduced activity on the GIP receptor compared to the corresponding derivatives with Lys at position 27 as in native exendin-4, as shown in Example 5, Table 8. A reduced activation of the GIP receptor is potentially beneficial as there are reports in the literature that high levels of GIP in diabetics might in some cases lead to more frequent episodes of hypoglycemia (T McLaughlin et al., J Clin Endocrinol Metab, 95, 1851–1855, 2010; A Hadji-Georgopoulos, J Clin Endocrinol Metab, 56, 648-652, 1983).

In one embodiment the compounds of the invention have a high solubility at acidic and/or physiological pH values, e.g., at pH 4.5 and/or at pH 7.4 at 25ºC, in another embodiment at least 1 mg/ml and in a particular embodiment at least 5 mg/ml.

Furthermore, the compounds of the invention preferably have a high stability when stored in solution. Preferred assay conditions for determining the stability is storage for 7 days at 40ºC in solution at pH 4.5 or pH 7.4. The remaining amount of peptide is determined by chromatographic analyses as described in the Examples. Preferably, after 7 days at 40ºC in solution at pH 4.5 or pH 7.4 the remaining peptide is at least 75%, more preferably at least 80%, even more preferably at least 85% and even more preferably at least 90%.

Preferably, the compounds of the present invention comprise a peptide moiety which is a linear sequence of 39 amino carboxylic acids, particularly α-amino carboxylic acids linked by peptide, i.e. carboxamide bonds.

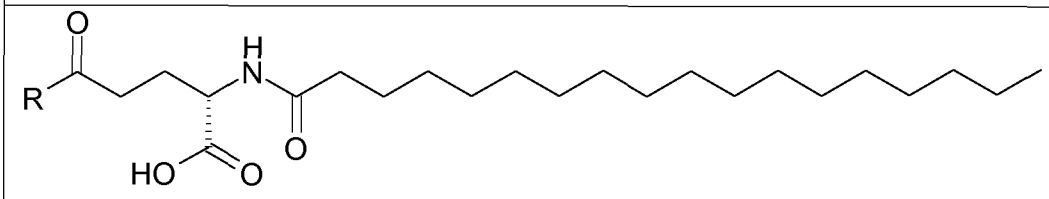
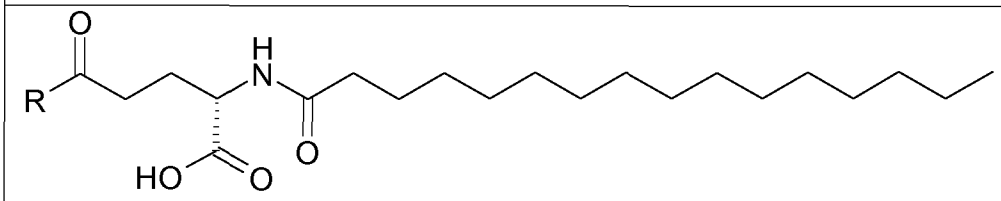
In one embodiment, R^1 is NH_2 and in a further embodiment R^1 is OH.

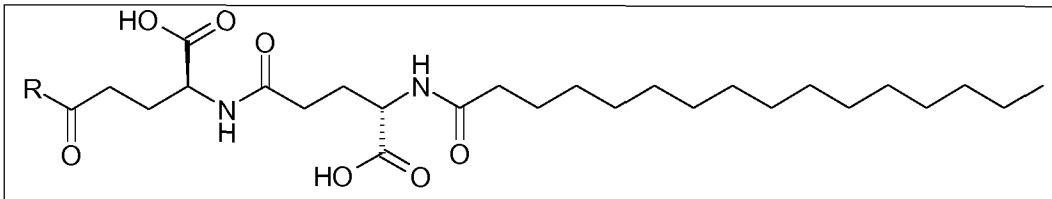
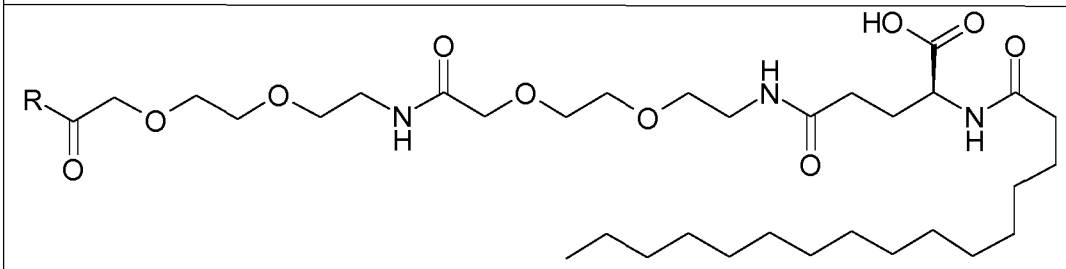
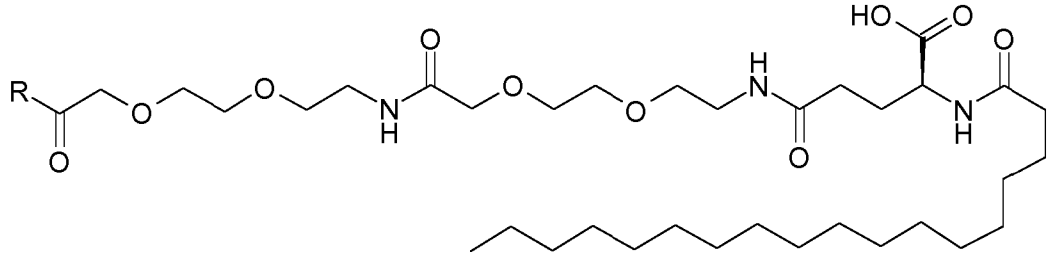
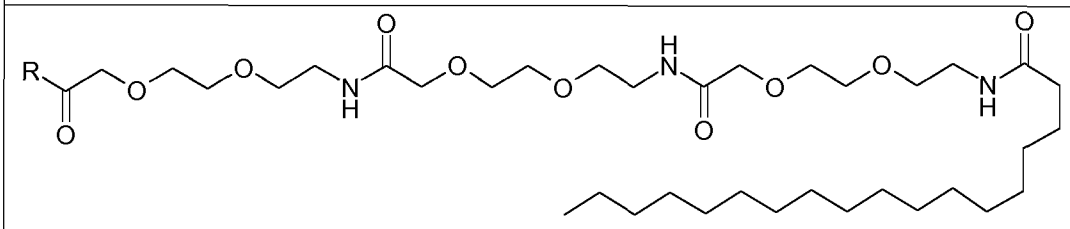
Specific preferred examples for -Z-C(O)-R^5 groups are listed in the following Table 2, which are selected from

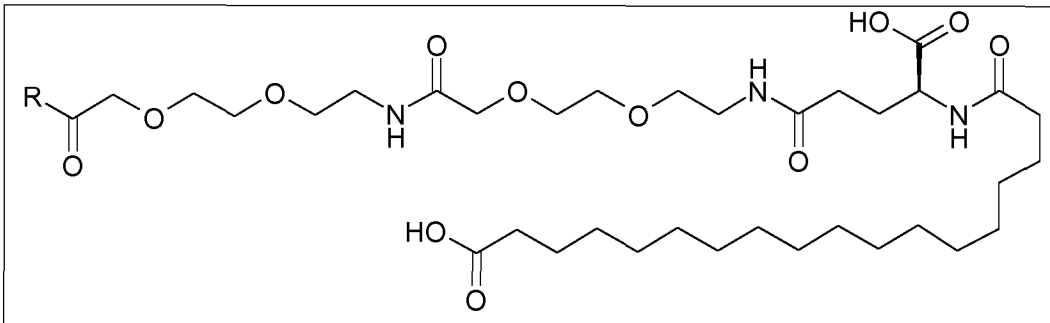
(S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-(17-carboxy-heptadecanoyl)amino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl.

Further preferred are stereoisomers, particularly enantiomers of these groups, either S- or R-enantiomers. The term "R" in Table 2 is intended to mean the attachment site of -Z-C(O)-R^5 at the peptide back bone, i.e. particularly the ε-amino group of Lys.

Table 2

Structure / IUPAC	name
 (S)-4-Carboxy-4-octadecanoylamino-butyryl-	γE-x70
 (S)-4-Carboxy-4-hexadecanoylamino-butyryl-	γE-x53

 (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-	γE-γE-x53
 (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl	AEEAc-AEEAc-γE-x53
 (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl	AEEAc-AEEAc-γE-x70
 [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-	AEEAc-AEEAc-AEEAc-x70

 (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-(17-carboxy-heptadecanoyl)amino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl	AEEAc-AEEAc-γE-x99

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized with a group -Z-C(O)R^5, wherein
Z represents a group selected from γE, γE-γE, AEEAc-AEEAc-γE and AEEAc-AEEAc-AEEAc and
R^5 represents a group selected from pentadecanyl, heptadecanyl or 16-carboxy-hexadecanyl.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized with a group -Z-C(O)R^5, wherein
Z represents a group selected from γE, γE-γE, AEEAc-AEEAc-γE and AEEAc-AEEAc-AEEAc and
R^5 represents a group selected from pentadecanyl or heptadecanyl.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-octadecanoylamino-butyryl-,
X28 represents Ala,
X29 represents an amino acid residue selected from Gly and D-Ala,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein

X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-,
X28 represents Ala,
X29 represents an amino acid residue selected from Gly and D-Ala,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents an amino acid residue selected from Ala, Ser and Lys,
X29 represents an amino acid residue selected from Gly and D-Ala,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-(17-carboxy-heptadecanoyl)amino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl,
X28 represents Ala,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-

butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-,
X28 represents Ala,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents Ser,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents Lys,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents an amino acid residue selected from Ala, Lys and Ser,
X29 represents D-Ala,

R^1 represents NH_2,

or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein

X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-(17-carboxy-heptadecanoyl)amino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl,

X28 represents an amino acid residue selected from Ala, Lys and Ser,

X29 represents Gly,

R^1 represents NH_2.

or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein

X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-,

X28 represents an amino acid residue selected from Ala, Lys and Ser,

X29 represents Gly,

R^1 represents NH_2.

or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein

X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,

X28 represents Ala,

X29 represents an amino acid residue selected from Gly and D-Ala,

R^1 represents NH_2.

or a salt or solvate thereof.

A further embodiment relates to a group of compounds, wherein

X14 represents Lys, wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-

or a salt or solvate thereof.

A still further embodiment relates to a group of compounds, wherein

X14 represents Lys, wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-.

or a salt or solvate thereof.

Specific examples of peptidic compounds of formula (I) are the compounds of SEQ ID NO: 6-19, as well as salts or solvates thereof.

Specific examples of peptidic compounds of formula (I) are the compounds of SEQ ID NO: 6-18, as well as salts or solvates thereof.

Specific examples of peptidic compounds of formula (I) are the compounds of SEQ ID NO: 7and 8 as well as salts or solvates thereof.

In certain embodiments, i.e. when the compound of formula (I) comprises genetically encoded amino acid residues, the invention further provides a nucleic acid (which may be DNA or RNA) encoding said compound, an expression vector comprising such a nucleic acid, and a host cell containing such a nucleic acid or expression

vector.

In a further aspect, the present invention provides a composition comprising a compound of the invention in admixture with a carrier. In preferred embodiments, the composition is a pharmaceutically acceptable composition and the carrier is a pharmaceutically acceptable carrier. The compound of the invention may be in the form of a salt, e.g. a pharmaceutically acceptable salt or a solvate, e.g. a hydrate. In still a further aspect, the present invention provides a composition for use in a method of medical treatment, particularly in human medicine.

In certain embodiments, the nucleic acid or the expression vector may be used as therapeutic agents, e.g. in gene therapy.

The compounds of formula (I) are suitable for therapeutic application without an additional therapeutically effective agent. In other embodiments, however, the compounds are used together with at least one additional therapeutically active agent, as described in “combination therapy”.

The compounds of formula (I) are particularly suitable for the treatment or prevention of diseases or disorders caused by, associated with and/or accompanied by disturbances in carbohydrate and/or lipid metabolism, e.g. for the treatment or prevention of hyperglycemia, type 2 diabetes, impaired glucose tolerance, type 1 diabetes, obesity and metabolic syndrome. Further, the compounds of the invention are particularly suitable for the treatment or prevention of degenerative diseases, particularly neurodegenerative diseases.

The compounds described find use, inter alia, in preventing weight gain or promoting weight loss. By "preventing" is meant inhibiting or reducing when compared to the absence of treatment, and is not necessarily meant to imply complete cessation of a disorder.

The compounds of the invention may cause a decrease in food intake and/or increase in energy expenditure, resulting in the observed effect on body weight.

Independently of their effect on body weight, the compounds of the invention may have a beneficial effect on circulating cholesterol levels, being capable of improving lipid levels, particularly LDL, as well as HDL levels (e.g. increasing HDL/LDL ratio).

Thus, the compounds of the invention can be used for direct or indirect therapy of any condition caused or characterised by excess body weight, such as the treatment and/or prevention of obesity, morbid obesity, obesity linked inflammation, obesity linked gallbladder disease, obesity induced sleep apnea. They may also be used for treatment and prevention of the metabolic syndrome, diabetes, hypertension, atherogenic dyslipidemia, atherosclerosis, arteriosclerosis, coronary heart disease, or stroke. Their effects in these conditions may be as a result of or associated with their effect on body weight, or may be independent thereof.

Preferred medical uses include delaying or preventing disease progression in type 2 diabetes, treating metabolic syndrome, treating obesity or preventing overweight, for decreasing food intake, increase energy expenditure, reducing body weight, delaying the progression from impaired glucose tolerance (IGT) to type 2 diabetes; delaying the progression from type 2 diabetes to insulin-requiring diabetes; regulating appetite; inducing satiety; preventing weight regain after successful weight loss; treating a disease or state related to overweight or obesity; treating bulimia; treating binge eating; treating atherosclerosis, hypertension, type 2 diabetes, IGT, dyslipidemia, coronary heart disease, hepatic steatosis, treatment of beta-blocker poisoning, use for inhibition of the motility of the gastrointestinal tract, useful in connection with investigations of the gastrointestinal tract using techniques such as X-ray, CT- and NMR-scanning.

Further preferred medical uses include treatment or prevention of degenerative disorders, particularly neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, ataxia, e.g spinocerebellar ataxia, Kennedy disease, myotonic dystrophy, Lewy body dementia, multi-systemic atrophy, amyotrophic lateral sclerosis, primary lateral sclerosis, spinal muscular atrophy, prion-associated diseases, e.g. Creutzfeldt-Jacob disease, multiple sclerosis, telangiectasia, Batten disease, corticobasal degeneration, subacute combined degeneration of spinal cord, Tabes dorsalis, Tay-Sachs disease, toxic

encephalopathy, infantile Refsum disease, Refsum disease, neuroacanthocytosis, Niemann-Pick disease, Lyme disease, Machado-Joseph disease, Sandhoff disease, Shy-Drager syndrome, wobbly hedgehog syndrome, proteopathy, cerebral β-amyloid angiopathy, retinal ganglion cell degeneration in glaucoma, synucleinopathies, tauopathies, frontotemporal lobar degeneration (FTLD), dementia, cadasil syndrome, hereditary cerebral hemorrhage with amyloidosis, Alexander disease, seipinopathies, familial amyloidotic neuropathy, senile systemic amyloidosis, serpinopathies, AL (light chain) amyloidosis (primary systemic amyloidosis), AH (heavy chain) amyloidosis, AA (secondary) amyloidosis, aortic medial amyloidosis, ApoAI amyloidosis, ApoAII amyloidosis, ApoAIV amyloidosis, familial amyloidosis of the Finnish type (FAF), Lysozyme amyloidosis, Fibrinogen amyloidosis, Dialysis amyloidosis, Inclusion body myositis/myopathy, Cataracts, Retinitis pigmentosa with rhodopsin mutations, medullary thyroid carcinoma, cardiac atrial amyloidosis, pituitary prolactinoma, Hereditary lattice corneal dystrophy, Cutaneous lichen amyloidosis, Mallory bodies, corneal lactoferrin amyloidosis, pulmonary alveolar proteinosis, odontogenic (Pindborg) tumor amyloid, cystic fibrosis, sickle cell disease or critical illness myopathy (CIM).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

The amino acid sequences of the present invention contain the conventional one letter and three letter codes for naturally occuring amino acids, as well as generally accepted three letter codes for other amino acids, such as Aib (α-aminoisobutyric acid).

The term „native exendin-4“ refers to native exendin-4 having the sequence HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH_2 (SEQ ID NO: 1).

The invention provides peptidic compounds as defined above.

The peptidic compounds of the present invention comprise a linear backbone of amino carboxylic acids linked by peptide, i.e. carboxamide bonds. Preferably, the

amino carboxylic acids are α-amino carboxylic acids and more preferably L-α-amino carboxylic acids, unless indicated otherwise. The peptidic compounds preferably comprise a backbone sequence of 39 amino carboxylic acids.

The peptidic compounds of the present invention may have unmodified side-chains, but carry at least one modification at one of the side chains.

For the avoidance of doubt, in the definitions provided herein, it is generally intended that the sequence of the peptidic moiety (I) differs from native exendin-4 at least at one of those positions which are stated to allow variation. Amino acids within the peptide moiety (I) can be considered to be numbered consecutively from 1 to 39 in the conventional N-terminal to C-terminal direction. Reference to a „position" within peptidic moiety (I) should be constructed accordingly, as should reference to positions within native exendin-4 and other molecules, e.g., in exendin-4, His is at position 1, Gly at position 2, ..., Met at position 14, ... and Ser at position 39.

An amino acid residue with an $-NH_2$ side chain group, e.g. Lys, Orn, Dab or Dap, is functionalized in that at least one H atom of the $-NH_2$ side chain group is replaced by $-Z-C(O)-R^5$, wherein R^5 comprises a lipophilic moiety, e.g. an acyclic linear or branched (C_8-C_{30}) saturated or unsaturated hydrocarbon group, which is unsubstituted or substituted e.g. by halogen, -OH and/or CO_2H and Z comprises a linker in all stereoisomeric forms, e.g. a linker comprising one or more, e.g. 1 to 5, preferably 1, 2 or 3 amino acid linker groups selected from the group γ-Glutamate (γE) and AEEAc. Preferred groups R^5 comprise a lipophilic moiety, e.g. an acyclic linear or branched (C_{12}-C_{20}) saturated or unsaturated hydrocarbon group, e.g. pentadecanyl, hexadecanyl or heptadecanyl, which is unsubstituted or substituted by CO_2H, more preferably pentadecanyl, heptadecanyl or 16-carboxy-hexadecanyl. In one embodiment amino acid linker groups are selected from γE, γE-γE, AEEAc-AEEAc-γE and AEEAc-AEEAc-AEEAc. In another embodiment the amino acid linker group is γE. In another embodiment the amino acid linker group is γE-γE. In another embodiment the amino acid linker group is AEEAc-AEEAc-γE. In another embodiment the amino acid linker group is AEEAc-AEEAc-AEEAc.

In a further aspect, the present invention provides a composition comprising a compound of the invention as described herein, or a salt or solvate thereof, in

admixture with a carrier.

The invention also provides the use of a compound of the present invention for use as a medicament, particularly for the treatment of a condition as described below.

The invention also provides a composition wherein the composition is a pharmaceutically acceptable composition, and the carrier is a pharmaceutically acceptable carrier.

Peptide synthesis

The skilled person is aware of a variety of different methods to prepare peptides that are described in this invention. These methods include but are not limited to synthetic approaches and recombinant gene expression. Thus, one way of preparing these peptides is the synthesis in solution or on a solid support and subsequent isolation and purification. A different way of preparing the peptides is gene expression in a host cell in which a DNA sequence encoding the peptide has been introduced. Alternatively, the gene expression can be achieved without utilizing a cell system. The methods described above may also be combined in any way.

A preferred way to prepare the peptides of the present invention is solid phase synthesis on a suitable resin. Solid phase peptide synthesis is a well-established methodology (see for example: Stewart and Young, Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, Ill., 1984; E. Atherton and R. C. Sheppard, Solid Phase Peptide Synthesis. A Practical Approach, Oxford-IRL Press, New York, 1989). Solid phase synthesis is initiated by attaching an N-terminally protected amino acid with its carboxy terminus to an inert solid support carrying a cleavable linker. This solid support can be any polymer that allows coupling of the initial amino acid , e.g. a trityl resin, a chlorotrityl resin, a Wang resin or a Rink resin in which the linkage of the carboxy group (or carboxamide for Rink resin) to the resin is sensitive to acid (when Fmoc strategy is used). The polymer support must be stable under the conditions used to deprotect the α-amino group during the peptide synthesis.

After the first amino acid has been coupled to the solid support, the α-amino

protecting group of this amino acid is removed. The remaining protected amino acids are then coupled one after the other in the order represented by the peptide sequence using appropriate amide coupling reagents, for example BOP, HBTU, HATU or DIC (N,N'-diisopropylcarbodiimide) / HOBt (1-hydroxybenzotriazole), wherein BOP, HBTU and HATU are used with tertiary amine bases. Alternatively, the liberated N-terminus can be functionalized with groups other than amino acids, for example carboxylic acids, etc.

Usually, reactive side-chain groups of the amino acids are protected with suitable blocking groups. These protecting groups are removed after the desired peptides have been assembled. They are removed concomitantly with the cleavage of the desired product from the resin under the same conditions. Protecting groups and the procedures to introduce protecting groups can be found in Protective Groups in Organic Synthesis, 3d ed., Greene, T. W. and Wuts, P. G. M., Wiley & Sons (New York: 1999).

In some cases it might be desirable to have side-chain protecting groups that can selectively be removed while other side-chain protecting groups remain intact. In this case the liberated functionality can be selectively functionalized. For example, a lysine may be protected with an ivDde ([1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl) protecting group (S.R. Chhabra et al., Tetrahedron Lett. 39, (1998), 1603) which is labile to a very nucleophilic base, for example 4% hydrazine in DMF (dimethyl formamide). Thus, if the N-terminal amino group and all side-chain functionalities are protected with acid labile protecting groups, the ivDde group can be selectively removed using 4% hydrazine in DMF and the corresponding free amino group can then be further modified, e.g. by acylation. The lysine can alternatively be coupled to a protected amino acid and the amino group of this amino acid can then be deprotected resulting in another free amino group which can be acylated or attached to further amino acids.

Finally the peptide is cleaved from the resin. This can be achieved by using King's cocktail (D. S. King, C. G. Fields, G. B. Fields, Int. J. Peptide Protein Res. 36, 1990, 255-266). The raw material can then be purified by chromatography, e.g. preparative RP-HPLC, if necessary.

Potency

As used herein, the term "potency" or "in vitro potency" is a measure for the ability of a compound to activate the receptors for GLP-1, glucagon or GIP in a cell-based assay. Numerically, it is expressed as the "EC50 value", which is the effective concentration of a compound that induces a half maximal increase of response (e.g. formation of intracellular cAMP) in a dose-response experiment.

Therapeutic uses

Metabolic syndrome is a combination of medical disorders that, when occurring together, increase the risk of developing type 2 diabetes, as well as atherosclerotic vascular disease, e.g. heart disease and stroke. Defining medical parameters for the metabolic syndrome include diabetes mellitus, impaired glucose tolerance, raised fasting glucose, insulin resistance, urinary albumin secretion, central obesity, hypertension, elevated triglycerides, elevated LDL cholesterol and reduced HDL cholesterol.

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health and life expectancy and due to its increasing prevalence in adults and children it has become one of the leading preventable causes of death in modern world. It increases the likelihood of various other diseases, including heart disease, type 2 diabetes, obstructive sleep apnoe, certain types of cancer, as well as osteoarthritis, and it is most commonly caused by a combination of excess food intake, reduced energy expenditure, as well as genetic susceptibility.

Diabetes mellitus, often simply called diabetes, is a group of metabolic diseases in which a person has high blood sugar levels, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. The most common types of diabetes are: (1) type 1 diabetes, where the body fails to produce insulin; (2) type 2 diabetes (T2DM), where the body fails to use insulin properly, combined with an increase in insulin deficiency over time, and (3)

gestational diabetes, where women develop diabetes due to their pregnancy. All forms of diabetes increase the risk of long-term complications, which typically develop after many years. Most of these long-term complications are based on damage to blood vessels and can be divided into the two categories “macrovascular” disease, arising from atherosclerosis of larger blood vessels and “microvascular” disease, arising from damage of small blood vessels. Examples for macrovascular disease conditions are ischemic heart disease, myocardial infarction, stroke and peripheral vascular disease. Examples for microvascular diseases are diabetic retinopathy, diabetic nephropathy, as well as diabetic neuropathy.

The receptors for GLP-1 and GIP as well as glucagon are members of the family of 7-transmembrane-spanning, heterotrimeric G-protein coupled receptors. They are structurally related to each other and share not only a significant level of sequence identity, but have also similar mechanisms of ligand recognition and intracellular signaling pathways.

Similarly, the peptides GLP-1, GIP and glucagon share regions of high sequence identity/similarity. GLP-1 and glucagon are produced from a common precursor, preproglucagon, which is differentially processed in a tissue-specific manner to yield e.g. GLP-1 in intestinal endocrine cells and glucagon in alpha cells of pancreatic islets. GIP is derived from a larger proGIP prohormone precursor and is synthesized and released from K-cells located in the small intestine.
The peptidic incretin hormones GLP-1 and GIP are secreted by intestinal endocrine cells in response to food and account for up to 70% of meal-stimulated insulin secretion. Evidence suggests that GLP-1 secretion is reduced in subjects with impaired glucose tolerance or type 2 diabetes, whereas responsiveness to GLP-1 is still preserved in these patients. Thus, targeting of the GLP-1 receptor with suitable agonists offers an attractive approach for treatment of metabolic disorders, including diabetes. The receptor for GLP-1 is distributed widely, being found mainly in pancreatic islets, brain, heart, kidney and the gastrointestinal tract. In the pancreas, GLP-1 acts in a strictly glucose-dependent manner by increasing secretion of insulin from beta cells. This glucose-dependency shows that activation of GLP-1 receptors is unlikely to cause hypoglycemia. Also the receptor for GIP is broadly expressed in peripheral tissues including pancreatic islets, adipose tissue, stomach, small

intestine, heart, bone, lung, kidney, testis, adrenal cortex, pituitary, endothelial cells, trachea, spleen, thymus, thyroid and brain. Consistent with its biological function as incretin hormone, the pancreatic ß-cell express the highest levels of the receptor for GIP in humans. There is some clinical evidence that the GIP-receptor mediated signaling could be impaired in patients with T2DM but GIP-action is shown to be reversible and can be restored with improvement of the diabetic status. While there are many reports that also GIP action on insulin secretion is glucose-dependent, there are also reports in the literature that high plasma levels of GIP might lead to more frequent episodes of hypoglycemia (T McLaughlin et al., J Clin Endocrinol Metab, 95, 1851–1855, 2010; A Hadji-Georgopoulos, J Clin Endocrinol Metab, 56, 648-652, 1983). In addition, plasma GIP levels in obese subjects were reported to be higher than normal, suggesting that GIP might induce obesity and insulin resistance (W Creutzfeldt et al. Diabetologia. 1978, 14,15-24). This is supported by reports that the ablation of the GIP receptor might prevent those conditions: GIP receptor knock-out mice fed on high-fat diet actually showed a suppression of body weight compared to wild-type mice (K Miyawaki et al. Nat Med. 2002, 8, 738-42), and long-term administration of the GIP receptor antagonist (Pro3)GIP also prevented obesity and insulin resistance in mice (VA Gault et al. Diabetologia. 2007, 50,1752-62). Therefore, goal of this invention was to provide dual GLP-1 /glucagon receptor agonists with reduced activity on the GIP receptor.

Glucagon is a 29 amino acid peptide hormone that is produced by pancreatic alpha cells and released into the bloodstream when circulating glucose is low. An important physiological role of glucagon is to stimulate glucose output in the liver, which is a process providing the major counterregulatory mechanism for insulin in maintaining glucose homeostasis in vivo.

Glucagon receptors are however also expressed in extra-hepatic tissues such as kidney, heart, adipocytes, lymphoblasts, brain, retina, adrenal gland and gastrointestinal tract, suggesting a broader physiological role beyond glucose homeostasis. Accordingly, recent studies have reported that glucagon has therapeutically positive effects on energy management, including stimulation of energy expenditure and thermogenesis, accompanied by reduction of food intake and body weight loss. Altogether, stimulation of glucagon receptors might be useful in

the treatment of obesity and the metabolic syndrome.

Oxyntomodulin is a peptide hormone consisting of glucagon with an eight amino acids encompassing C-terminal extension. Like GLP-1 and glucagon, it is pre-formed in preproglucagon and cleaved and secreted in a tissue-specific manner by endocrinal cells of the small bowel. Oxyntomodulin is known to stimulate both, the receptors for GLP-1 and glucagon and is therefore the prototype of a dual agonist.

As GLP-1 is known for its anti-diabetic effects, GLP-1 and glucagon are both known for their food intake-suppressing effects and glucagon is also a mediator of additional energy expenditure, it is conceivable that a combination of the activities of the two hormones in one molecule can yield a powerful medication for treatment of the metabolic syndrome and in particular its components diabetes and obesity.

Accordingly, the compounds of the invention may be used for treatment of glucose intolerance, insulin resistance, pre-diabetes, increased fasting glucose (hyperglycemia), type 2 diabetes, hypertension, dyslipidemia, arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or any combination of these individual disease components.

In addition, they may be used for control of appetite, feeding and calory intake, increase of energy expenditure, prevention of weight gain, promotion of weight loss, reduction of excess body weight and altogether treatment of obesity, including morbid obesity.

The compounds of the invention are agonists for the receptors for GLP-1 and for glucagon (e.g. “dual agonists”) with reduced activity on the GIP receptor and may provide therapeutic benefit to address a clinical need for targeting the metabolic syndrome by allowing simultaneous treatment of diabetes and obesity.

Further disease states and health conditions which could be treated with the compounds of the invention are obesity-linked inflammation, obesity-linked gallbladder disease and obesity-induced sleep apnea.

Although all these conditions could be associated directly or indirectly with obesity, the effects of the compounds of the invention may be mediated in whole or in part via an effect on body weight, or independent thereof.

Further, diseases to be treated are neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease, or other degenerative diseases as described above.

In one embodiment the compounds are useful in the treatment or prevention of hyperglycemia, type 2 diabetes, obesity.

Compared to GLP-1, glucagon and oxyntomodulin, exendin-4 has beneficial physicochemical properties, such as solubility and stability in solution and under physiological conditions (including enzymatic stability towards degradation by enzymes, such as DPP4 or NEP), which results in a longer duration of action in vivo. Therefore, the pure GLP-1 receptor agonist exendin-4 might serve as good starting scaffold to obtain exendin-4 analogues with dual GLP-1/glucagon receptor agonism.

Nevertheless, also exendin-4 has been shown to be chemically labile due to methionine oxdiation in position 14 as well as deamidation and isomerization of asparagine in position 28. Therefore, stability might be further improved by substitution of methionine at position 14 and the avoidance of sequences that are known to be prone to degradation via aspartimide formation, especially Asp-Gly or Asn-Gly at positions 28 and 29.

Pharmaceutical compositions

The term "pharmaceutical composition" indicates a mixture containing ingredients that are compatible when mixed and which may be administered. A pharmaceutical composition may include one or more medicinal drugs. Additionally, the pharmaceutical composition may include carriers, buffers, acidifying agents, alkalizing agents, solvents, adjuvants, tonicity adjusters, emollients, expanders, preservatives, physical and chemical stabilizers e.g. surfactants, antioxidants and other components, whether these are considered active or inactive ingredients. Guidance for the skilled in preparing pharmaceutical compositions may be found, for

example, in Remington: The Science and Practice of Pharmacy, (20th ed.) ed. A. R. Gennaro A. R., 2000, Lippencott Williams & Wilkins and in R.C.Rowe et al (Ed), Handbook of Pharmaceutical Excipients, PhP, May 2013 update.

The exendin-4 peptide derivatives of the present invention, or salts thereof, are administered in conjunction with an acceptable pharmaceutical carrier, diluent, or excipient as part of a pharmaceutical composition. A "pharmaceutically acceptable carrier" is a carrier which is physiologically acceptable (e.g. physiologically acceptable pH) while retaining the therapeutic properties of the substance with which it is administered. Standard acceptable pharmaceutical carriers and their formulations are known to one skilled in the art and described, for example, in Remington: The Science and Practice of Pharmacy, (20th ed.) ed. A. R. Gennaro A. R., 2000, Lippencott Williams & Wilkins and in R.C.Rowe et al (Ed), Handbook of Pharmaceutical excipients, PhP, May 2013 update. One exemplary pharmaceutically acceptable carrier is physiological saline solution.

In one embodiment carriers are selected from the group of buffers (e.g. citrate/citric acid), acidifying agents (e.g. hydrochloric acid), alkalizing agents (e.g. sodium hydroxide), preservatives (e.g. phenol), co-solvents (e.g. polyethylene glycol 400), tonicity adjusters (e.g. mannitol), stabilizers (e.g. surfactant, antioxidants, amino acids).

Concentrations used are in a range that is physiologically acceptable.

Acceptable pharmaceutical carriers or diluents include those used in formulations suitable for oral, rectal, nasal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and transdermal) administration. The compounds of the present invention will typically be administered parenterally.

The term “pharmaceutically acceptable salt” means salts of the compounds of the invention which are safe and effective for use in mammals. Pharmaceutically acceptable salts may include, but are not limited to, acid addition salts and basic salts. Examples of acid addition salts include chloride, sulfate, hydrogen sulfate, (hydrogen) phosphate, acetate, citrate, tosylate or mesylate salts. Examples of basic

salts include salts with inorganic cations, e.g. alkaline or alkaline earth metal salts such as sodium, potassium, magnesium or calcium salts and salts with organic cations such as amine salts. Further examples of pharmaceutically acceptable salts are described in Remington: The Science and Practice of Pharmacy, (20th ed.) ed. A. R. Gennaro A. R., 2000, Lippencott Williams & Wilkins or in Handbook of Pharmaceutical Salts, Properties, Selection and Use, e.d. P. H. Stahl, C. G. Wermuth, 2002, jointly published by Verlag Helvetica Chimica Acta, Zurich, Switzerland, and Wiley-VCH, Weinheim, Germany.

The term “solvate” means complexes of the compounds of the invention or salts thereof with solvent molecules, e.g. organic solvent molecules and/or water.

In the pharmaceutical composition, the exendin-4 derivative can be in monomeric or oligomeric form.

The term "therapeutically effective amount" of a compound refers to a nontoxic but sufficient amount of the compound to provide the desired effect. The amount of a compound of the formula (I) necessary to achieve the desired biological effect depends on a number of factors, for example the specific compound chosen, the intended use, the mode of administration and the clinical condition of the patient. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. For example the “therapeutically effective amount” of a compound of the formula (I) is about 0.01 to 50 mg/dose, preferably 0.1 to 10 mg/dose.

Pharmaceutical compositions of the invention are those suitable for parenteral (for example subcutaneous, intramuscular, intradermal or intravenous), oral, rectal, topical and peroral (for example sublingual) administration, although the most suitable mode of administration depends in each individual case on the nature and severity of the condition to be treated and on the nature of the compound of formula (I) used in each case.

Suitable pharmaceutical compositions may be in the form of separate units, for example capsules, tablets and powders in vials or ampoules, each of which contains

a defined amount of the compound; as powders or granules; as solution or suspension in an aqueous or nonaqueous liquid; or as an oil-in-water or water-in-oil emulsion. It may be provided in single or multiple dose injectable form, for example in the form of a pen. The compositions may, as already mentioned, be prepared by any suitable pharmaceutical method which includes a step in which the active ingredient and the carrier (which may consist of one or more additional ingredients) are brought into contact.

In certain embodiments the pharmaceutical composition may be provided together with a device for application, for example together with a syringe, an injection pen or an autoinjector. Such devices may be provided separate from a pharmaceutical composition or prefilled with the pharmaceutical composition.

Combination therapy

The compounds of the present invention, dual agonists for the GLP-1 and glucagon receptors, can be widely combined with other pharmacologically active compounds, such as all drugs mentioned in the Rote Liste 2014, e.g. with all weight-reducing agents or appetite suppressants mentioned in the Rote Liste 2014, chapter 1, all lipid-lowering agents mentioned in the Rote Liste 2014, chapter 58, all antihypertensives and nephroprotectives, mentioned in the Rote Liste 2014, or all diuretics mentioned in the Rote Liste 2014, chapter 36.

The active ingredient combinations can be used especially for a synergistic improvement in action. They can be applied either by separate administration of the active ingredients to the patient or in the form of combination products in which a plurality of active ingredients are present in one pharmaceutical preparation. When the active ingredients are administered by separate administration of the active ingredients, this can be done simultaneously or successively.

Most of the active ingredients mentioned hereinafter are disclosed in the USP Dictionary of USAN and International Drug Names, US Pharmacopeia, Rockville 2011.

Other active substances which are suitable for such combinations include in particular those which for example potentiate the therapeutic effect of one or more active substances with respect to one of the indications mentioned and/or which allow the dosage of one or more active substances to be reduced.

Therapeutic agents which are suitable for combinations include, for example, antidiabetic agents such as:

Insulin and Insulin derivatives, for example: Glargine / Lantus®, 270 - 330U/mL of insulin glargine (EP 2387989 A), 300U/mL of insulin glargine (EP 2387989 A), Glulisin / Apidra®, Detemir / Levemir®, Lispro / Humalog® / Liprolog®, Degludec / DegludecPlus, Aspart, basal insulin and analogues (e.g.LY-2605541, LY2963016, NN1436), PEGylated insulin Lispro, Humulin®, Linjeta, SuliXen®, NN1045, Insulin plus Symlin, PE0139, fast-acting and short-acting insulins (e.g. Linjeta, PH20, NN1218, HinsBet), (APC-002)hydrogel, oral, inhalable, transdermal and sublingual insulins (e.g. Exubera®, Nasulin®, Afrezza, Tregopil, TPM 02, Capsulin, Oral-lyn®, Cobalamin® oral insulin, ORMD-0801, NN1953, NN1954, NN1956, VIAtab, Oshadi oral insulin). Additionally included are also those insulin derivatives which are bonded to albumin or another protein by a bifunctional linker.

GLP-1, GLP-1 analogues and GLP-1 receptor agonists, for example: Lixisenatide / AVE0010 / ZP10 / Lyxumia, Exenatide / Exendin-4 / Byetta / Bydureon / ITCA 650 / AC-2993, Liraglutide / Victoza, Semaglutide, Taspoglutide, Syncria / Albiglutide, Dulaglutide, rExendin-4, CJC-1134-PC, PB-1023, TTP-054, Langlenatide / HM-11260C, CM-3, GLP-1 Eligen, ORMD-0901, NN-9924, NN-9926, NN-9927, Nodexen, Viador-GLP-1, CVX-096, ZYOG-1, ZYD-1, GSK-2374697, DA-3091, MAR-701, MAR709, ZP-2929, ZP-3022, TT-401, BHM-034. MOD-6030, CAM-2036, DA-15864, ARI-2651, ARI-2255, Exenatide-XTEN and Glucagon-Xten.

DPP4 inhibitors, for example: Alogliptin / Nesina, Trajenta / Linagliptin / BI-1356 / Ondero / Trajenta / Tradjenta / Trayenta / Tradzenta, Saxagliptin / Onglyza, Sitagliptin / Januvia / Xelevia / Tesave / Janumet / Velmetia, Galvus / Vildagliptin, Anagliptin, Gemigliptin, Teneligliptin, Melogliptin, Trelagliptin, DA-1229, Omarigliptin / MK-3102, KM-223, Evogliptin, ARI-2243, PBL-1427, Pinoxacin.

SGLT2 inhibitors, for example: Invokana / Canaglifozin, Forxiga / Dapagliflozin, Remoglifozin, Sergliflozin, Empagliflozin, Ipragliflozin, Tofogliflozin, Luseogliflozin, LX-4211, Ertuglifozin / PF-04971729, RO-4998452, EGT-0001442, KGA-3235 / DSP-3235, LIK066, SBM-TFC-039,

Biguanides (e.g. Metformin, Buformin, Phenformin), Thiazolidinediones (e.g. Pioglitazone, Rivoglitazone, Rosiglitazone, Troglitazone), dual PPAR agonists (e.g. Aleglitazar, Muraglitazar, Tesaglitazar), Sulfonylureas (e.g. Tolbutamide, Glibenclamide, Glimepiride/Amaryl, Glipizide), Meglitinides (e.g. Nateglinide, Repaglinide, Mitiglinide), Alpha-glucosidase inhibitors (e.g. Acarbose, Miglitol, Voglibose), Amylin and Amylin analogues (e.g. Pramlintide, Symlin).

GPR119 agonists (e.g. GSK-263A, PSN-821, MBX-2982, APD-597, ZYG-19, DS-8500), GPR40 agonists (e.g. Fasiglifam / TAK-875, TUG-424, P-1736, JTT-851, GW9508).

Other suitable combination partners are: Cycloset, inhibitors of 11-beta-HSD (e.g. LY2523199, BMS770767, RG-4929, BMS816336, AZD-8329, HSD-016, BI-135585), activators of glucokinase (e.g. TTP-399, AMG-151, TAK-329, GKM-001), inhibitors of DGAT (e.g. LCQ-908), inhibitors of protein tyrosinephosphatase 1 (e.g. Trodusquemine), inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrokinase, alpha2-antagonists, CCR-2 antagonists, SGLT-1 inhibitors (e.g. LX-2761), dual SGLT2 / SGLT1 inhibitors.

One or more lipid lowering agents are also suitable as combination partners, such as for example: HMG-CoA-reductase inhibitors (e.g. Simvastatin, Atorvastatin), fibrates (e.g. Bezafibrate, Fenofibrate), nicotinic acid and the derivatives thereof (e.g. Niacin), PPAR-(alpha, gamma or alpha/gamma) agonists or modulators (e.g. Aleglitazar), PPAR-delta agonists, ACAT inhibitors (e.g. Avasimibe), cholesterol absorption inhibitors (e.g. Ezetimibe), Bile acid-binding substances (e.g. Cholestyramine), ileal bile acid transport inhibitors, MTP inhibitors, or modulators of PCSK9.

HDL-raising compounds such as: CETP inhibitors (e.g. Torcetrapib, Anacetrapid, Dalcetrapid, Evacetrapid, JTT-302, DRL-17822, TA-8995) or ABC1 regulators.

Other suitable combination partners are one or more active substances for the treatment of obesity, such as for example: Sibutramine, Tesofensine, Orlistat, antagonists of the cannabinoid-1 receptor, MCH-1 receptor antagonists, MC4 receptor agonists, NPY5 or NPY2 antagonists (e.g. Velneperit), beta-3-agonists, leptin or leptin mimetics, agonists of the 5HT2c receptor (e.g. Lorcaserin), or the combinations of bupropione/naltrexone, bupropione/zonisamide, bupropione/phentermine or pramlintide/metreleptin.

Other suitable combination partners are:
Further gastrointestinal peptides such as Peptide YY 3-36 (PYY3-36) or analogues thereof, pancreatic polypeptide (PP) or analogues thereof.
Glucagon receptor agonists or antagonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists, Xenin and analogues thereof.

Moreover, combinations with drugs for influencing high blood pressure, chronic heart failure or atherosclerosis, such as e.g.: Angiotensin II receptor antagonists (e.g. telmisartan, candesartan, valsartan, losartan, eprosartan, irbesartan, olmesartan, tasosartan, azilsartan), ACE inhibitors, ECE inhibitors, diuretics, beta-blockers, calcium antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors and others or combinations thereof are suitable.

In another aspect, this invention relates to the use of a compound according to the invention or a physiologically acceptable salt thereof combined with at least one of the active substances described above as a combination partner, for preparing a medicament which is suitable for the treatment or prevention of diseases or conditions which can be affected by binding to the receptors for GLP-1 and glucagon and by modulating their activity. This is preferably a disease in the context of the metabolic syndrome, particularly one of the diseases or conditions listed above, most particularly diabetes or obesity or complications thereof.

The use of the compounds according to the invention, or a physiologically acceptable salt thereof, in combination with one or more active substances may take place simultaneously, separately or sequentially.

The use of the compound according to the invention, or a physiologically acceptable salt thereof, in combination with another active substance may take place simultaneously or at staggered times, but particularly within a short space of time. If they are administered simultaneously, the two active substances are given to the patient together; if they are used at staggered times, the two active substances are given to the patient within a period of less than or equal to 12 hours, but particularly less than or equal to 6 hours.

Consequently, in another aspect, this invention relates to a medicament which comprises a compound according to the invention or a physiologically acceptable salt of such a compound and at least one of the active substances described above as combination partners, optionally together with one or more inert carriers and/or diluents.

The compound according to the invention, or physiologically acceptable salt or solvate thereof, and the additional active substance to be combined therewith may both be present together in one formulation, for example a in a vial or a cartridge, or separately in two identical or different formulations, for example as so-called kit-of-parts.

LEGENDS TO THE FIGURES

Figure 1. Body weight development during 4 weeks of subcutaneous treatment with SEQ ID NO: 7 and SEQ ID NO: 8, 50µg/kg bid in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 2. Relative body weight change in % during 4 weeks of subcutaneous treatment with SEQ ID NO: 7 and SEQ ID NO: 8, 50µg/kg bid in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 3. Determination of total fat mass measured by nuclear magnetic resonance (NMR), two days before and after 4 weeks of treatment with SEQ ID NO: 7 and SEQ ID NO: 8, 50µg/kg bid in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 4. Acute effect of s.c. administration of compound SEQ ID NO: 7 and SEQ ID NO: 8 50µg/kg on blood glucose in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 5. Acute effect of s.c. administration of compound SEQ ID NO: 15, 50µg/kg qd on blood glucose in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 6. Relative body weight change in % during 4 weeks of subcutaneous treatment with SEQ ID NO: 15, 50µg/kg qd in female high-fat fed C57BL/6 mice. Data are mean+SEM.

Figure 7. Determination of total fat mass measured by nuclear magnetic resonance (NMR), two days before and after 4 weeks of treatment with SEQ ID NO: 15, 50µg/kg qd in female high-fat fed C57BL/6 mice. Data are mean+SEM.

METHODS

Abbreviations employed are as follows:

AA	amino acid
AEEAc	(2-(2-aminoethoxy)ethoxy)acetyl
cAMP	cyclic adenosine monophosphate
Boc	tert-butyloxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BSA	bovine serum albumin
tBu	tertiary butyl
DCM	dichloromethane

Dde	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-ethyl
ivDde	1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butyl
DIC	N,N'-diisopropylcarbodiimide
DIPEA	N,N-diisopropylethylamine
DMEM	Dulbecco's modified Eagle's medium
DMF	dimethyl formamide
DMS	dimethylsulfide
EDT	ethanedithiol
FA	formic acid
FBS	fetal bovine serum
Fmoc	fluorenylmethyloxycarbonyl
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HBSS	Hanks' Balanced Salt Solution
HBTU	2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HOBt	1-hydroxybenzotriazole
HOSu	N-hydroxysuccinimide
HPLC	High Performance Liquid Chromatography
HTRF	Homogenous Time Resolved Fluorescence
IBMX	3-isobutyl-1-methylxanthine
LC/MS	Liquid Chromatography/Mass Spectrometry
Mmt	monomethoxy-trityl
Palm	palmitoyl
PBS	phosphate buffered saline
PEG	polyethylene glycole
PK	pharmacokinetic
RP-HPLC	reversed-phase high performance liquid chromatography
Stea	stearyl
TFA	trifluoroacetic acid
Trt	trityl
UV	ultraviolet
γ-E	γ-Glutamate

General synthesis of peptidic compounds

Materials

Different Rink-Amide resins (4-(2',4'-Dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucylaminomethyl resin, Merck Biosciences; 4-[(2,4-Dimethoxyphenyl)(Fmoc-amino)methyl]phenoxy acetamido methyl resin, Agilent Technologies) were used for the synthesis of peptide amides with loadings in the range of 0.2-0.7 mmol/g.

Fmoc protected natural amino acids were purchased from Protein Technologies Inc., Senn Chemicals, Merck Biosciences, Novabiochem, Iris Biotech, Bachem, Chem-Impex International or MATRIX Innovation. The following standard amino acids were used throughout the syntheses: Fmoc-L-Ala-OH, Fmoc-Arg(Pbf)-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Asp(OtBu)-OH, Fmoc-L-Cys(Trt)-OH, Fmoc-L-Gln(Trt)-OH, Fmoc-L-Glu(OtBu)-OH, Fmoc-Gly-OH, Fmoc-L-His(Trt)-OH, Fmoc-L-Ile-OH, Fmoc-L-Leu-OH, Fmoc-L-Lys(Boc)-OH, Fmoc-L-Met-OH, Fmoc-L-Phe-OH, Fmoc-L-Pro-OH, Fmoc-L-Ser(tBu)-OH, Fmoc-L-Thr(tBu)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-L-Tyr(tBu)-OH, Fmoc-L-Val-OH.

In addition, the following special amino acids were purchased from the same suppliers as above: Fmoc-L-Lys(ivDde)-OH, Fmoc-L-Lys(Mmt)-OH, Fmoc-Aib-OH, Fmoc-D-Ser(tBu)-OH, Fmoc-D-Ala-OH, Boc-L-His(Boc)-OH (available as toluene solvate) and Boc-L-His(Trt)-OH.

The solid phase peptide syntheses were performed for example on a Prelude Peptide Synthesizer (Protein Technologies Inc) or similar automated synthesizer using standard Fmoc chemistry and HBTU/DIPEA activation. DMF was used as the solvent. Deprotection : 20% piperidine/DMF for 2 x 2.5 min. Washes: 7 x DMF. Coupling 2:5:10 200 mM AA / 500 mM HBTU / 2M DIPEA in DMF 2 x for 20 min. Washes: 5 x DMF.

In cases where a Lys-side-chain was modified, Fmoc-L-Lys(ivDde)-OH or Fmoc-L-Lys(Mmt)-OH was used in the corresponding position. After completion of the synthesis, the ivDde group was removed according to a modified literature procedure (S.R. Chhabra et al., Tetrahedron Lett. 39, (1998), 1603), using 4% hydrazine hydrate in DMF. The Mmt group was removed by repeated treatment with 1% TFA in dichloromethane. The following acylations were carried out by treating the resin with the N-hydroxy succinimide esters of the desired acid or using coupling reagents like HBTU/DIPEA or HOBt/DIC.

All the peptides that have been synthesized were cleaved from the resin with King's cleavage cocktail consisting of 82.5% TFA, 5% phenol, 5% water, 5% thioanisole, 2.5% EDT. The crude peptides were then precipitated in diethyl or diisopropyl ether, centrifuged, and lyophilized. Peptides were analyzed by analytical HPLC and checked by ESI mass spectrometry. Crude peptides were purified by a conventional preparative RP-HPLC purification procedure.

Alternatively, peptides were synthesized by a manual synthesis procedure:
0.3 g Desiccated Rink amide MBHA Resin (0.66 mmol/g) was placed in a polyethylene vessel equipped with a polypropylene filter. Resin was swelled in DCM (15 ml) for 1 h and DMF (15 ml) for 1h. The Fmoc group on the resin was de-protected by treating it twice with 20% (v/v) piperidine/DMF solution for 5 and 15 min. The resin was washed with DMF/DCM/DMF (6:6:6 time each). A Kaiser test (quantitative method) was used for the conformation of removal of Fmoc from solid support. The C-terminal Fmoc-amino acid (5 equiv. excess corresponding to resin loading) in dry DMF was added to the de-protected resin and coupling was initiated with 5 equivalent excess of DIC and HOBT in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF/DCM/DMF (6:6:6 time each). Kaiser test on peptide resin aliquot upon completion of coupling was negative (no colour on the resin). After the first amino acid attachment, the unreacted amino group, if any, in the resin was capped used acetic anhydride/pyridine/DCM (1:8:8) for 20 minutes to avoid any deletion of the sequence. After capping, resin was washed with DCM/DMF/DCM/DMF (6/6/6/6 time each). The Fmoc group on the C-terminal amino acid attached peptidyl resin was

deprotected by treating it twice with 20% (v/v) piperidine/DMF solution for 5 and 15 min. The resin was washed with DMF/DCM/DMF (6:6:6 time each). The Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive.

The remaining amino acids in target sequence on Rink amide MBHA Resin were sequentially coupled using Fmoc AA/DIC/HOBt method using 5 equivalent excess corresponding to resin loading in DMF. The concentration of each reactant in the reaction mixture was approximately 0.4 M. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF/DCM/DMF (6:6:6 time each). After each coupling step and Fmoc deprotection step, a Kaiser test was carried out to confirm the completeness of the reaction.

After the completion of the linear sequence, the ε-amino group of lysine used as branching point or modification point was deprotected by using 2.5% hydrazine hydrate in DMF for 15 min x 2 and washed with DMF/DCM/DMF (6:6:6 time each). The γ-carboxyl end of glutamic acid was attached to the ε-amino group of Lys using Fmoc-Glu(OH)-OtBu with DIC/HOBt method (5 equivalent excess with respect to resin loading) in DMF. The mixture was rotated on a rotor at room temperature for 2 h. The resin was filtered and washed with DMF/DCM/DMF (6x30 ml each). The Fmoc group on the glutamic acid was de-protected by treating it twice with 20% (v/v) piperidine/DMF solution for 5 and 15 min (25 ml each). The resin was washed with DMF/ DCM/DMF (6:6:6 time each). A Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive.

If the side-chain branching also contains one more γ-glutamic acid, a second Fmoc-Glu(OH)-OtBu used for the attachment to the free amino group of γ-glutamic acid with DIC/HOBt method (5 equivalent excess with respect to resin loading) in DMF. The mixture was rotated on a rotor at room temperature for 2 h. Resin was filtered and washed with DMF/ DCM/DMF (6x30 ml each). The Fmoc group on the γ-glutamic acid was de-protected by treating it twice with 20% (v/v) piperidine/DMF solution for 5 and 15 min (25 mL). The resin was washed with DMF/ DCM/DMF (6:6:6 time each). A Kaiser test on peptide resin aliquot upon completion of Fmoc-deprotection was positive.

Palmitic Acid & Stearic Acid attachment to side chains of Glutamic acid:

To the free amino group of γ-glutamic acid, palmitic acid or stearic acid (5 equiv.) dissolved in DMF was added and coupling was initiated by the addition of DIC (5

equiv.) and HOBt (5 equiv.) in DMF. The resin was washed with DMF/DCM/DMF (6:6:6 time each).

Final cleavage of peptide from the resin:

The peptidyl resin synthesized by manual synthesis was washed with DCM (6x10 ml), MeOH (6x10 ml) and ether (6x10 ml) and dried in vacuum desiccators overnight. The cleavage of the peptide from the solid support was achieved by treating the peptide-resin with reagent cocktail (80.0% TFA / 5% thioanisole/5% phenol/ 2.5 % EDT, 2.5 % DMS and 5 % DCM) at room temperature for 3 h. Cleavage mixture was collected by filtration and the resin was washed with TFA (2 ml) and DCM (2 x 5 ml). The excess TFA and DCM was concentrated to small volume under nitrogen and a small amount of DCM (5-10 ml) was added to the residue and evaporated under nitrogen. The process was repeated 3-4 times to remove most of the volatile impurities. The residue was cooled to 0°C and anhydrous ether was added to precipitate the peptide. The precipitated peptide was centrifuged and the supernatant ether was removed and fresh ether was added to the peptide and re-centrifuged. The crude sample was preparative HPLC purified and lyophilized. The identity of peptide was confirmed by LCMS.

Analytical HPLC / UPLC

Method A: detection at 210 - 225 nm

column: Waters ACQUITY UPLC® CSH™ C18 1.7 µm (150 x 2.1mm) at 50 °C

solvent: H_2O+0.5%TFA : ACN+0.35%TFA (flow 0.5 ml/min)

gradient: 80:20 (0 min) to 80:20 (3 min) to 25:75 (23 min) to 2:98 (23.5 min) to 2:98 (30.5 min) to 80:20 (31 min) to 80:20 (37 min)

optionally with mass analyser: LCT Premier, electrospray positive ion mode

Method B: detection at 210 - 225 nm

column: Aries prep XBC 18 (4.6x250 mm x 3.6 µm), Temp: 25 0C

solvent: H_2O+0.1%TFA : ACN+0.1%TFA (flow 1 ml/min)

gradient: Equilibration of the column with 2 % buffer B and elution by a gradient of 2% to 70% buffer B during 15 min.

General Preparative HPLC Purification Procedure

The crude peptides were purified either on an Äkta Purifier System, a Jasco semiprep HPLC System or a Agilent 1100 HPLC system. Preparative RP-C18-HPLC columns of different sizes and with different flow rates were used depending on the amount of crude peptide to be purified. Acetonitrile + 0.1% TFA (B) and water + 0.1% TFA (A) were employed as eluents. Product-containing fractions were collected and lyophilized to obtain the purified product, typically as TFA salt.

Solubility and Stability-Testing of exendin-4 derivatives

Prior to the testing of solubility and stability of a peptide batch, its purity (HPLC-UV) was determined.

For solubility testing, the target concentration was 10 mg pure compound/ml. Therefore, solutions from solid samples were prepared in different buffer systems with a concentration of 10 mg/mL compound based on the previously determined %purity. HPLC-UV was performed after 2 h of gentle agitation from the supernatant, which was obtained by 20 min of centrifugation at 4500 rpm.

The solubility was then determined by comparison of a 0.2µL-injection with the UV peak areas obtained with a stock solution of the peptide at a concentration of 1.2 mg/mL in DMSO (based on % purity), injecting various volumes ranging from 0.2 – 2µl. This analysis also served as starting point (t0) for the stability testing.

For stability testing, an aliquot of the supernatant obtained for solubility was stored for 7 days at 40°C. After that time course, the sample was centrifuged for 20 min at 4500 rpm and 0.2µL of the supernatant were analysed with HPLC-UV.

For determination of the amount of the remaining peptide, the peak areas of the target compound at t0 and t7 were compared, resulting in “% remaining peptide”, following the equation

% remaining peptide = [(peak area peptide t7) x 100]/peak area peptide t0.
The stability is expressed as “% remaining peptide”.

As HPLC/UPLC method Method B has been used, detecting at 214nM.

In vitro cellular assays for GLP-1, glucagon and GIP receptor efficacy

Agonism of compounds for the receptors was determined by functional assays measuring cAMP response of HEK-293 cell lines stably expressing human GLP-1, GIP or glucagon receptor.

cAMP content of cells was determined using a kit from Cisbio Corp. (cat. no. 62AM4PEC) based on HTRF (Homogenous Time Resolved Fluorescence). For preparation, cells were split into T175 culture flasks and grown overnight to near confluency in medium (DMEM / 10% FBS). Medium was then removed and cells washed with PBS lacking calcium and magnesium, followed by proteinase treatment with accutase (Sigma-Aldrich cat. no. A6964). Detached cells were washed and resuspended in assay buffer (1 x HBSS; 20 mM HEPES, 0.1% BSA, 2 mM IBMX) and cellular density determined. They were then diluted to 400000 cells/ml and 25 µl-aliquots dispensed into the wells of 96-well plates. For measurement, 25 µl of test compound in assay buffer was added to the wells, followed by incubation for 30 minutes at room temperature. After addition of HTRF reagents diluted in lysis buffer (kit components), the plates were incubated for 1 hr, followed by measurement of the fluorescence ratio at 665 / 620 nm. In vitro potency of agonists was quantified by determining the concentrations that caused 50% activation of maximal response (EC50).

Bioanalytical screening method for quantification of exendin-4 derivatives in mice and pigs

Mice were dosed 1 mg/kg subcutaneously (s.c.). The mice were sacrified and blood samples were collected after 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours post application. Plasma samples were analyzed after protein precipitation via liquid chromatography mass spectrometry (LC/MS). PK parameters and half-life were calculated using WinonLin Version 5.2.1 (non-compartment model).

Female Göttinger minipigs were dosed 0.1 mg/kg subcutaneously (s.c.). Blood samples were collected after 0.25, 0.5, 1, 2, 4, 8, 24, 32, 48, 56 and 72 hours post application. Plasma samples were analyzed after protein precipitation via liquid

chromatography mass spectrometry (LC/MS). PK parameters and half-life were calculated using WinonLin Version 5.2.1 (non-compartment model).

Gastric emptying and intestinal passage in mice

Female NMRI-mice of a body weight between 20 and 30 g are used. Mice are adapted to housing conditions for at least one week.

Mice are overnight fasted, while water remains available all the time. On the study day, mice are weighed, single-caged and allowed access to 500 mg of feed for 30 min, while water is removed. At the end of the 30 min feeding period, remaining feed is removed and weighed. 60 min later, a coloured, non-caloric bolus is instilled via gavage into the stomach. The test compound / reference compound or its vehicle in the control group is administered subcutaneously, to reach Cmax when coloured bolus is administered. After another 30 min, the animals are sacrificed and the stomach and the small intestine prepared. The filled stomach is weighed, emptied, carefully cleaned and dried and reweighed. The calculated stomach content indicates the degree of gastric emptying. The small intestine is straightened without force and measured in length. Then the distance from the gastric beginning of the gut to the tip of the farthest travelled intestinal content bolus is measured. The intestinal passage is given as relation in percent of the latter distance and the total length of the small intestine.
Comparable data can be obtained for both female and male mice.

Statistical analyses are performed with Everstat 6.0 by 1-way-ANOVA, followed by Dunnetts or Newman-Keuls as post-hoc test, respectively. Differences are considered statistically significant at the $p < 0.05$ level. As post hoc test Dunnet's Test is applied to compare versus vehicle control, only. Newman-Keul's Test is applied for all pairwise comparisons (i.e. versus vehicle and reference groups).

Automated assessment of feed intake in mice

Female NMRI-mice of a body weight between 20 and 30 g are used. Mice are adapted to housing conditions for at least one week and for at least one day single-caged in the assessment equipment, when basal data are recorded simultaneously. On the study day, test product is administered subcutaneously close to the lights-off

phase (12 h lights off) and assessment of feed consumption is directly started afterwards. Assessment included continued monitoring (every 30 min) over 22 hours. Repetition of this procedure over several days is possible. Restriction of assessment to 22 hours is for practical reasons to allow for reweighing of animals, refilling of feed and water and drug administration between procedures. Results can be assessed as cumulated data over 22 hours or differentiated to 30 min intervals.
Comparable data can be obtained for both female and male mice.

Statistical analyses are performed with Everstat 6.0 by two-way ANOVA on repeated measures and Dunnett's post-hoc analyses. Differences are considered statistically significant at the $p < 0.05$ level.

Acute and chronic effects after subcutaneous treatment on blood glucose and body weight in female diet-induced obese (DIO) C57BL/6 mice

C57BL/6 Harlan mice are housed in groups in a specific pathogen-free barrier facility on a 12h light/dark cycle with free access to water and standard or high-fat diet. After prefeeding on high-fat diet, mice are stratified to treatment groups (n = 8), so that each group has similar mean body weight. An age-matched group with ad-libitum access to standard chow is included as standard control group. Before the experiment, mice are subcutaneously (s.c.) injected with vehicle solution and weighed for 3 days to acclimate them to the procedures.

1) Acute effect on blood glucose in fed female DIO mice: initial blood samples are taken just before first administration (s.c.) of vehicle (phosphate buffer solution) or the exendin-4 derivatives (dissolved in phosphate buffer), respectively. The volume of administration is 5 mL/kg. The animals have access to water and their corresponding diet during the experiment. Blood glucose levels are measured at t = 0 h, t = 1 h, t = 2 h, t = 3 h, t = 4h, t = 6 h and t = 24 h (method: Accu-Check glucometer). Blood sampling is performed by tail incision without anaesthesia.

2) Chronic effect on body weight in female DIO mice: mice are treated once (s.c. in evening hours) or twice daily (s.c. in the morning and in the evening), respectively, at the beginning and the end of the light phase with either vehicle or exendin-4 derivatives for 4 weeks. Body weight is recorded daily. Two days before start of

treatment and on day 26, total fat mass is measured by nuclear magnetic resonance (NMR).

Statistical analyses are performed with Everstat 6.0 by repeated measures two-way ANOVA and Dunnetts post-hoc analyses (glucose profile) and 1-way-ANOVA, followed by Dunnetts post-hoc test (body weight, body fat). Differences versus vehicle-treated DIO control mice are considered statistically significant at the $p < 0.05$ level.

Effects of 4 weeks of treatment on glucose, HbA1c and oral glucose tolerance in female diabetic dbdb-mice

8 week old, female diabetic dbdb-mice of mean non-fasted glucose value of 14.5 mmol/l and a body weight of 37-40 g are used. Mice are individually marked and are adapted to housing conditions for at least one week.
7 days prior to study start, baseline values for non-fasted glucose and HbA1c are determined, 5 days prior to study start, mice are assigned to groups and cages (5 mice per cage, 10 per group) according to their HbA1c values to ensure even distribution of lower and higher values between groups (stratification).
Mice are treated for 4 weeks, by twice daily subcutaneous administration in the morning and the afternoon. Blood samples from the tail tip are obtained for HbA1c on study day 21 and oral glucose tolerance is assessed in the 4th week.
An oral glucose tolerance test is done in the morning without prior extra compound administration to majorly assess the effect of chronic treatment and less of acute compound administration. Mice are fasted for 4 hours prior to oral glucose administration (2 g/kg, t = 0 min). Blood samples are drawn prior to glucose administration and at 15, 30, 60, 90, 120, and 180 min. Feed is returned after the last blood sampling. Results are represented as change from baseline, glucose in mmol/l and HbA1c in %.
Statistical analyses are performed with Everstat Version 6.0 based on SAS by 1-way-ANOVA, followed by Dunnett's post-hoc test against vehicle-control. Differences are considered statistically significant at the $p < 0.05$ level.

Glucose lowering in non-fasted female diabetic dbdb-mice

Female diabetic dbdb-mice of mean non-fasted glucose value of 20-22 mmol/l and a body weight of 42 g +/- 0.6 g (SEM) are used. Mice are individually marked and are adapted to housing conditions for at least one week.

3-5 days prior to study start mice are assigned to groups and cages (4 mice per cage, 8 per group) according to their non-fasted glucose values to ensure even distribution of lower and higher values between groups (stratification). On the study day, mice are weighed and dosed (t = 0). Immediately prior to compound administration feed is removed while water remains available, and a first blood sample at a tail incision is drawn (baseline). Further blood samples are drawn at the tail incision at 30, 60, 90, 120, 240, 360, and 480 min.

Statistical analyses are performed with Everstat Version 6.0 based on SAS by 2-way-ANOVA on repeated measures, followed by Dunnett's post-hoc test against vehicle-control. Differences are considered statistically significant at the $p < 0.05$ level.

EXAMPLES

The invention is further illustrated by the following examples.

Example 1:

Synthesis of SEQ ID NO: 7

The manual synthesis procedure as described in Methods was carried out on a desiccated Rink amide MBHA Resin (0.66 mmol/g). The Fmoc-synthesis strategy was applied with DIC/HOBt -activation. In position 14 Fmoc-Lys(ivDde)-OH and in position 1 Boc-His(Boc)-OH were used. The ivDde-group was cleaved from the peptide on resin according to a modified literature procedure (S.R. Chhabra et al., Tetrahedron Lett. 39, (1998), 1603), using 4% hydrazine hydrate in DMF. The peptide was cleaved from the resin with King's cocktail (D. S. King, C. G. Fields, G. B. Fields, Int. J. Peptide Protein Res. 36, 1990, 255-266). The crude product was purified via preparative HPLC using an acetonitrile/water gradient (both buffers with 0.1% TFA). The purified peptide was analysed by LCMS (Method B).

Deconvolution of the mass signals found under the peak with retention time 14.29 min revealed the peptide mass 4649.20 which is in line with the expected value of 4649.29.

Example 2:

Synthesis of SEQ ID NO: 8

The manual synthesis procedure as described in Methods was carried out on a desiccated Rink amide MBHA Resin (0.66 mmol/g). The Fmoc-synthesis strategy was applied with DIC/HOBT -activation. In position 14 Fmoc-Lys(ivDde)-OH and in position 1 Boc-His(Boc)-OH were used. The ivDde-group was cleaved from the peptide on resin according to a modified literature procedure (S.R. Chhabra et al., Tetrahedron Lett. 39, (1998), 1603), using 4% hydrazine hydrate in DMF. The peptide was cleaved from the resin with King's cocktail (D. S. King, C. G. Fields, G. B. Fields, Int. J. Peptide Protein Res. 36, 1990, 255-266). The crude product was purified via preparative HPLC using an acetonitrile/water gradient (both buffers with 0.1% TFA). The purified peptide was analysed by LCMS (Method B).

Deconvolution of the mass signals found under the peak with retention time 14.05 min revealed the peptide mass 4634.80 which is in line with the expected value of 4635.27.

In an analogous way, the peptides listed in Table 3 were synthesized and characterized.

Table 3: list of synthesized peptides and comparison of calculated vs. found molecular weight

SEQ ID NO	calc. Mass	found mass	Monoisotopic or average mass
6	**4545.4**	**4546.0**	**monoisotopic**
7	**4649.3**	**4649.2**	**average**
8	**4635.3**	**4634.8**	**average**
9	**4534.2**	**4533.0**	**average**
10	**4506.1**	**4504.8**	**average**
11	**4520.2**	**4518.3**	**average**
12	**4662.4**	**4662.2**	**monoisotopic**
13	**4648.4**	**4648.4**	**monoisotopic**
14	**4703.5**	**4703.6**	**monoisotopic**
15	**4689.5**	**4689.5**	**monoisotopic**

16	**4793.5**	**4793.6**	**monoisotopic**
17	**4821.6**	**4821.6**	**monoisotopic**
18	**4837.6**	**4837.6**	**monoisotopic**
20	**4150.1**	**4150.2**	**monoisotopic**
21	**4675.5**	**4675.4**	**monoisotopic**
22	**4689.5**	**4689.5**	**monoisotopic**
23	**4138.6**	**4139.4**	**average**
24	**4123.6**	**4122.6**	**average**
25	**4164.1**	**4164.1**	**monoisotopic**

In an analogous way, the following peptides of Table 4 can be synthesized:

Table 4: List of peptides that can be synthesized in an analogous way.

SEQ ID NO
19

Example 3: Stability and solubility

Solubility and stability of peptidic compounds were assessed as described in Methods. The results are given in Table 5.

Table 5: Stability and solubility

SEQ ID NO	Stability [%]		solubility [mg/ml]	
	pH4.5	pH7.4	pH4.5	pH7.4
7	**89**	**100**	**>8**	**>8**
8	**98**	**100**	**>8**	**>8**
14	**93**	**100**	**>8**	**>8**
15	**96**	**96**	**>8**	**>8**

Example 4: In vitro data on GLP-1, glucagon and GIP receptor

Potencies of peptidic compounds at the GLP-1, glucagon and GIP receptors were

determined by exposing cells expressing human glucagon receptor (hGlucagon R), human GIP receptor (hGIP-R) or human GLP-1 receptor (hGLP-1 R) to the listed compounds at increasing concentrations and measuring the formed cAMP as described in Methods.

The results are shown in Table 6:

Table 6. EC50 values of exendin-4 derivatives at GLP-1, Glucagon and GIP receptors (indicated in pM)

SEQ ID NO	EC50 hGLP-1R	EC50 hGlucagon-R	EC50 hGIP-R
1	**0.4**	**>10000000**	**12500.0**
6	**14.1**	**136.0**	**2760.0**
7	**3.4**	**101.3**	**7617.5**
8	**2.3**	**28.1**	**2140.0**
9	**6.4**	**15.4**	**1160.0**
10	**3.5**	**52.4**	**890.0**
11	**10.3**	**261.0**	**13400.0**
12	**3.7**	**130.0**	**8290.0**
13	**1.8**	**22.8**	**2390.0**
14	**4.1**	**194.0**	**6530.0**
15	**2.4**	**42.6**	**1855.0**
16	**2.0**	**42.0**	**1870.0**
17	**2.8**	**16.2**	**906.0**
18	**8.3**	**8.6**	**2160.0**

Example 5: Comparison Testing

A selection of inventive exendin-4 derivatives comprising a functionalized amino acid in position 14 has been tested versus corresponding compounds having in this position 14 a 'non-functionalized' amino acid with otherwise identical amino acid sequence. The reference pair compounds and the corresponding EC50 values at GLP-1, Glucagon and GIP receptors (indicated in pM) are given in Table 7. As shown, the inventive exendin-4 derivatives show a superior activity in comparison to

the compounds with a 'non-functionalized' amino acid in position 14. Furthermore, a selection of inventive exendin-4 derivatives comprising an Aib in position 27 has been tested versus corresponding compounds having in this position a lysine residue as in native exendin-4 and otherwise identical amino acid sequence. The reference pair compounds and the corresponding EC50 values at GLP-1, Glucagon and GIP receptors (indicated in pM) are given in Table 8. As shown, the inventive exendin-4 derivatives show a reduced activity on the GIP receptor compared to the corresponding derivatives with Lys at position 27 as in native exendin-4.

Table 7. Comparison of exendin-4 derivatives comprising a non-functionalized amino acid in position 14 vs. exendin-4 derivatives comprising a functionalized amino acid in position 14 and otherwise identical amino acid sequence. EC50 values at GLP-1, Glucagon and GIP receptors are indicated in pM. (K=lysine, Nle=norleucine, L=leucine, γE-x53=(S)-4-Carboxy-4-hexadecanoylamino-butyryl-, γE-x70=(S)-4-Carboxy-4-octadecanoylamino-butyryl-,Ac=acetate, γE-γE-x53=(S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-)

SEQ ID NO	EC50 hGLP-1R	EC50 hGlucagon-R	EC50 hGIP-1	residue in position 14
24	**8.0**	**3090.0**	**114000.0**	**L**
23	**52.5**	**42900.0**	**177000.0**	**K**
8	**2.3**	**28.1**	**2140.0**	**K(γE-γE-x53)**
9	**6.4**	**15.4**	**1160.0**	**K(γE-x70)**
10	**3.5**	**52.4**	**890.0**	**K(γE-x53)**
15	**1.2**	**394.0**	**11900.0**	**L**
21	**1.2**	**3.2**	**193.0**	**K(γE-γE-x53)**

Table 8. Comparison of exendin-4 derivatives comprising an Aib in position 27 vs. exendin-4 derivatives comprising a Lys in position 27 and otherwise identical amino acid sequence. EC50 values at GLP-1, Glucagon and GIP receptors are indicated in pM.

SEQ ID NO	EC50 hGLP-1R	EC50 hGlucagon-R	EC50 hGIP-1	residue in position 27
21	**1.2**	**3.2**	**193.0**	**K**
8	**2.3**	**28.1**	**2140.0**	**Aib**
22	**1.8**	**5.9**	**148.0**	**K**
7	**3.4**	**101.3**	**7617.5**	**Aib**

Example 6: Pharmacokinetic testing

Pharmacokinetic profiles were determined as described in Methods. Calculated $T_{1/2}$ and Cmax values are shown in Table 9.

Table 9. Pharmacokinetic profiles of exendin-4 derivatives.

	Mice (1 mg/kg)		Mini pigs (0.1 mg/kg)	
SEQ ID NO	$T_{1/2}$ [h]	Cmax [ng/ml]	$T_{1/2}$ [h]	Cmax [ng/ml]
7	**3.1**	**5510.0**	**29**	**667**
8	**3.8**	**4360.0**	**18.1**	**476**

Example 7: Acute and chronic effects of SEQ ID NO: 7 and of SEQ ID NO: 8 after bid subcutaneous treatment on blood glucose and body weight in female diet-induced obese (DIO) C57BL/6 mice

1) Glucose profile

After blood sampling to determine the blood glucose baseline level, fed diet-induced obese female C57BL/6 mice were administered 50 µg/kg of SEQ ID NO: 7, 50 µg/kg of SEQ ID NO: 8 or phosphate buffered solution (vehicle control on standard or high-fat diet) subcutaneously. At predefined time points, more blood samples were taken to measure blood glucose and generate the blood glucose profile over 24 h.

SEQ ID NO: 7 and SEQ ID NO: 8 demonstrated a significant decrease in blood glucose compared to DIO control mice for time points t=1, 2, 3, 4, 6 and 24 h post compound dosing ($p < 0.0001$, 2-W-ANOVA-RM, post hoc Dunnett's Test; mean ± SEM; see Fig. 4).

2) Body weight

Female obese C57BL/6 mice were treated for 4 weeks twice daily subcutaneously with 50 µg/kg SEQ ID NO: 7, 50 µg/kg SEQ ID NO: 8 or vehicle. Body weight was recorded daily, and body fat content was determined before the start and after 4 weeks of treatment.

Treatment with 50 µg/kg SEQ ID NO: 7 showed a statistically significant decrease when compared to vehicle DIO control mice in daily body weight beginning on day 7 and continuing through the end of the study ($p>0.0001$ by the end of the study).

Treatment with 50 µg/kg SEQ ID NO: 8 reduced body weight significantly when compared to vehicle DIO control mice beginning on day 5 and continuing through the end of the study ($p>0.0001$ by the end of the study, Table 10, Fig. 1 and 2). These changes resulted from a decrease in body fat, as shown by the absolute changes in body fat content (Table 10, Fig. 3).

Table 10. Weight change in DIO mice over a 4-week treatment period (mean ± SEM)

Example (Dose)	Overall weight change (g)	Body fat change (g)
Control standard diet	+ 0.94 ± 0.4	+ 2.56 ± 0.4
Control high-fat diet	+ 3.83 ± 0.5	+ 5.00 ± 0.5
SEQ ID NO: 7 (50 µg/kg bid)	- 5.49 ± 0.9	- 3.73 ± 0.8
SEQ ID NO: 8 (50 µg/kg bid)	- 5.38 ± 0.5	- 3.81 ± 0.6

Example 8: Acute and chronic effects of SEQ ID NO: 15 after qd subcutaneous treatment on blood glucose and body weight in female diet-induced obese (DIO) C57BL/6 mice

1) Glucose profile

After blood sampling to determine the blood glucose baseline level, fed diet-induced obese female C57BL/6 mice were administered 50 µg/kg of SEQ ID NO: 15 or phosphate buffered solution (vehicle control on standard or high-fat diet)

subcutaneously once daily. At predefined time points, more blood samples were taken to measure blood glucose and generate the blood glucose profile over 24 h. SEQ ID NO: 15 demonstrated a significant decrease in blood glucose compared to DIO control mice for time points t=1, 2, 3, 4, 6 and 24 h post compound dosing ($p < 0.001$, 2-W-ANOVA-RM, post hoc Dunnett's Test; mean ± SEM) (see Fig. 5).

2) Body weight

Female obese C57BL/6 mice were treated for 4 weeks once daily subcutaneously with 50 µg/kg SEQ ID NO: 15 or vehicle. Body weight was recorded daily, and body fat content was determined before the start and after 4 weeks of treatment. Treatment with 50 µg/kg SEQ ID NO: 15 showed a statistically significant decrease when compared to vehicle DIO control mice in daily body weight beginning on day 4 and continuing through the end of the study ($p>0.001$ by the end of the study, Table 11, Fig. 6). These changes resulted from a decrease in body fat, as shown by the absolute changes in body fat content (Table 11, Fig. 7).

Table 11. Weight change in DIO mice over a 4-week treatment period (mean ± SEM)

Example (Dose)	Overall weight change (g)	Body fat change (g)
Control standard diet	+ 1.71 ± 0.3	+ 0.71 ± 0.4
Control high-fat diet	+ 5.41 ± 0.5	+ 3.26 ± 0.4
SEQ ID NO: 15 (50 µg/kg qd)	- 4.59 ± 1.1	- 4.01 ± 0.68

Table 12. Sequences

SEQ. ID	sequence
1	H-G-E-G-T-F-T-S-D-L-S-K-Q-M-E-E-E-A-V-R-L-F-I-E-W-L-K-N-G-G-P-S-S-G-A-P-P-P-S-NH2
2	H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-K-E-F-I-A-W-L-V-K-G-R-NH2
3	H-S-Q-G-T-F-T-S-D-Y-S-K-Y-L-D-S-R-R-A-Q-D-F-V-Q-W-L-M-N-T-OH
4	H-A-E-G-T-F-T-S-D-V-S-S-Y-L-E-G-Q-A-A-K(γE-x53)-E-F-I-A-W-L-V-R-G-R-G-OH
5	Y-A-E-G-T-F-I-S-D-Y-S-I-A-M-D-K-I-H-Q-Q-D-F-V-N-W-L-L-A-Q-K-G-K-K-N-D-W-K-H-N-I-T-Q-OH
6	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-x70)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-dAla-G-P-S-S-G-A-P-P-P-S-NH2
7	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-dAla-G-P-S-S-G-A-P-P-P-S-NH2
8	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
9	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-x70)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
10	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
11	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-dAla-G-P-S-S-G-A-P-P-P-S-NH2
12	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-S-dAla-G-P-S-S-G-A-P-P-P-S-NH2
13	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-S-G-G-P-S-S-G-A-P-P-P-S-NH2
14	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-K-dAla-G-P-S-S-G-A-P-P-P-S-NH2
15	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-K-G-G-P-S-S-G-A-P-P-P-S-NH2
16	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(AEEAc-AEEAc-γE-x53)-D-E-Q-R-

	A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
17	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(AEEAc-AEEAc-γE-x70)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
18	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(AEEAc-AEEAc-AEEAc-x70)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
19	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(AEEAc-AEEAc-γE-x99)-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
20	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-dAla-G-P-S-S-G-A-P-P-P-S-NH2
21	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-K-A-G-G-P-S-S-G-A-P-P-P-S-NH2
22	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K(γE-γE-x53)-D-E-Q-R-A-K-L-F-I-E-W-L-K-A-dAla-G-P-S-S-G-A-P-P-P-S-NH2
23	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-K-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
24	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-L-F-I-E-W-L-Aib-A-G-G-P-S-S-G-A-P-P-P-S-NH2
25	H-Aib-Q-G-T-F-T-S-D-L-S-K-Q-L-D-E-Q-R-A-K-L-F-I-E-W-L-K-A-G-G-P-S-S-G-A-P-P-P-S-NH2

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Claims

1. A peptidic compound having the formula (I):

H_2N-His-Aib-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-X14-Asp-Glu-Gln-Arg-Ala-Lys-Leu-Phe-Ile-Glu-Trp-Leu-Aib-X28-X29-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-R^1 (I)

X14 represents an amino acid residue with a functionalized -NH_2 side chain group, selected from the group consisting of Lys, Orn, Dab, or Dap, wherein the -NH_2 side chain group is functionalized by -Z-C(O)-R^5, wherein
Z represents a linker in all stereoisomeric forms and
R^5 is a moiety comprising up to 50 carbon atoms and heteroatoms selected from N and O,
X28 represents an amino acid residue selected from Ala, Lys and Ser,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 is NH_2 or OH,
or a salt or solvate thereof.

2. A compound of claim 1,
wherein R^1 is NH_2,
or a salt or solvate thereof.

3. A compound according to any one of claims 1 - 2,
wherein the peptidic compound has a relative activity of at least 0.09% compared to that of natural glucagon at the glucagon receptor.

4. A compound according to any one of claims 1 - 3, wherein the peptidic compound exhibits a relative activity of at least 0.1% compared to that of GLP-1(7-36)-amide at the GLP-1 receptor.

5. A compound of any one of claims 1 - 4, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized with a

group -Z-C(O)R^5 , wherein
Z represents a group selected from γE, γE-γE, AEEAc-AEEAc-γE and AEEAc-AEEAc-AEEAc and
R^5 represents a group selected from pentadecanyl, heptadecanyl or 16-carboxy-hexadecanyl .

6. A compound of any one of claims 1 - 5, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized with a group -Z-C(O)R^5 , wherein
Z represents a group selected from γE, γE-γE, AEEAc-AEEAc-γE and AEEAc-AEEAc-AEEAc and
R^5 represents a group selected from pentadecanyl or heptadecanyl.

7. A compound of any one of claims 1 - 6, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-,
X28 represents Ala,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

8. A compound of any one of claims 1 - 7, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents Ser,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

9. A compound of any one of claims 1 - 8, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents Lys,
X29 represents an amino acid residue selected from D-Ala and Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

10. A compound of any one of claims 1 - 9, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents an amino acid residue selected from Ala, Lys and Ser,
X29 represents D-Ala,
R^1 represents NH_2,
or a salt or solvate thereof.

11. A compound of any one of claims 1 - 10, wherein X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-hexadecanoylamino-butyryl-, (S)-4-Carboxy-4-octadecanoylamino-butyryl-, (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-hexadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, (2-{2-[2-(2-{2-[(4S)-4-Carboxy-4-octadecanoylamino-butyrylamino]-ethoxy}-ethoxy)-acetylamino]-ethoxy}-ethoxy)-acetyl, [2-(2-{2-[2-(2-{2-[2-(2-Octadecanoylamino-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetylamino}-ethoxy)-ethoxy]-acetyl-,
X28 represents an amino acid residue selected from Ala, Lys and Ser,
X29 represents Gly,
R^1 represents NH_2,
or a salt or solvate thereof.

12. A compound of any one of claims 1 - 11, wherein
X14 represents Lys wherein the -NH_2 side chain group is functionalized by (S)-4-Carboxy-4-((S)-4-carboxy-4-hexadecanoylamino-butyrylamino)-butyryl-,
X28 represents Ala,
X29 represents an amino acid residue selected from Gly and D-Ala,
R^1 represents NH_2,
or a salt or solvate thereof.

13. The compound of any one of claims 1 - 12, selected from the compounds of SEQ ID NO: 6-19, as well as salts or solvates thereof.

14. The compound of any one of claims 1 - 12, selected from the compounds of SEQ ID NO: 6-18, as well as salts or solvates thereof.

15. The compound of any one of claims 1 - 14 for use in medicine, particularly in human medicine.

16. The compound for use according to claim 15 which is present as an active agent in a pharmaceutical composition together with at least one pharmaceutically acceptable carrier.

17. The compound for use according to claim 15 or 16 together with at least one additional therapeutically active agent, wherein the additional therapeutically active agent is selected from the series of Insulin and Insulin derivatives, GLP-1, GLP-1 analogues and GLP-1 receptor agonists, polymer bound GLP-1 and GLP-1 analogues, dual GLP1/glucagon agonists, dual GLP1/GIP agonists, PYY3-36 or analogues thereof, pancreatic polypeptide or analogues thereof, Glucagon receptor agonists, GIP receptor agonists or antagonists, ghrelin antagonists or inverse agonists, Xenin and analogues thereof, DDP4 inhibitors, SGLT2 inhibitors, dual SGLT2 / SGLT1 inhibitors, Biguanides Thiazolidinediones, dual PPAR agonists, Sulfonylureas, Meglitinides, alpha-glucosidase inhibitors, Amylin and Amylin analogues, GPR119 agonists, GPR40 agonists, GPR120 agonists, GPR142 agonists, systemic or low-absorbable TGR5 agonists, Cycloset, inhibitors of 11-beta-HSD, activators of

glucokinase, inhibitors of DGAT, inhibitors of protein tyrosinephosphatase 1, inhibitors of glucose-6-phosphatase, inhibitors of fructose-1,6-bisphosphatase, inhibitors of glycogen phosphorylase, inhibitors of phosphoenol pyruvate carboxykinase, inhibitors of glycogen synthase kinase, inhibitors of pyruvate dehydrogenase kinase, alpha2-antagonists, CCR-2 antagonists, modulators of glucose transporter-4, Somatostatin receptor 3 agonists, HMG-CoA-reductase inhibitors, fibrates, nicotinic acid and the derivatives thereof, nicotinic acid receptor 1 agonists, PPAR-alpha, gamma or alpha/gamma) agonists or modulators, PPAR-delta agonists, ACAT inhibitors, cholesterol absorption inhibitors, bile acid-binding substances, IBAT inhibitors, MTP inhibitors, modulators of PCSK9, LDL receptor up-regulators by liver selective thyroid hormone receptor ß agonists, HDL-raising compounds, lipid metabolism modulators, PLA2 inhibitors , ApoA-I enhancers, thyroid hormone receptor agonists, cholesterol synthesis inhibitors, omega-3 fatty acids and derivatives thereof, active substances for the treatment of obesity, such as Sibutramine, Tesofensine, Orlistat, CB-1receptor antagonists, MCH-1 antagonists, MC4 receptor agonists and partial agonists, NPY5 or NPY2 antagonists, NPY4 agonists, beta-3-agonists, leptin or leptin mimetics, agonists of the 5HT2c receptor, or the combinations of bupropione/naltrexone (CONTRAVE), bupropione/zonisamide (EMPATIC), bupropione/phentermine or pramlintide/metreleptin, QNEXA (Phentermine+ topiramate), lipase inhibitors, angiogenesis inhibitors, H3 antagonists, AgRP inhibitors, triple monoamine uptake inhibitors (norepinephrine and acetylcholine), MetAP2 inhibitors, nasal formulation of the calcium channel blocker diltiazem, antisense against production of fibroblast growth factor receptor 4, prohibitin targeting peptide-1, drugs for influencing high blood pressure, chronic heart failure or atherosclerosis, such as angiotensin II receptor antagonists, ACE inhibitors, ECE inhibitors, diuretics, beta-blockers, calcium antagonists, centrally acting hypertensives, antagonists of the alpha-2-adrenergic receptor, inhibitors of neutral endopeptidase, thrombocyte aggregation inhibitors.

18. The compound for use according to any one of claims 15 - 17 for the treatment of glucose intolerance, insulin resistance, pre-diabetes, increased fasting glucose, hyperglycemia, type 2 diabetes, hypertension, dyslipidemia,

arteriosclerosis, coronary heart disease, peripheral artery disease, stroke or any combination of these individual disease components.

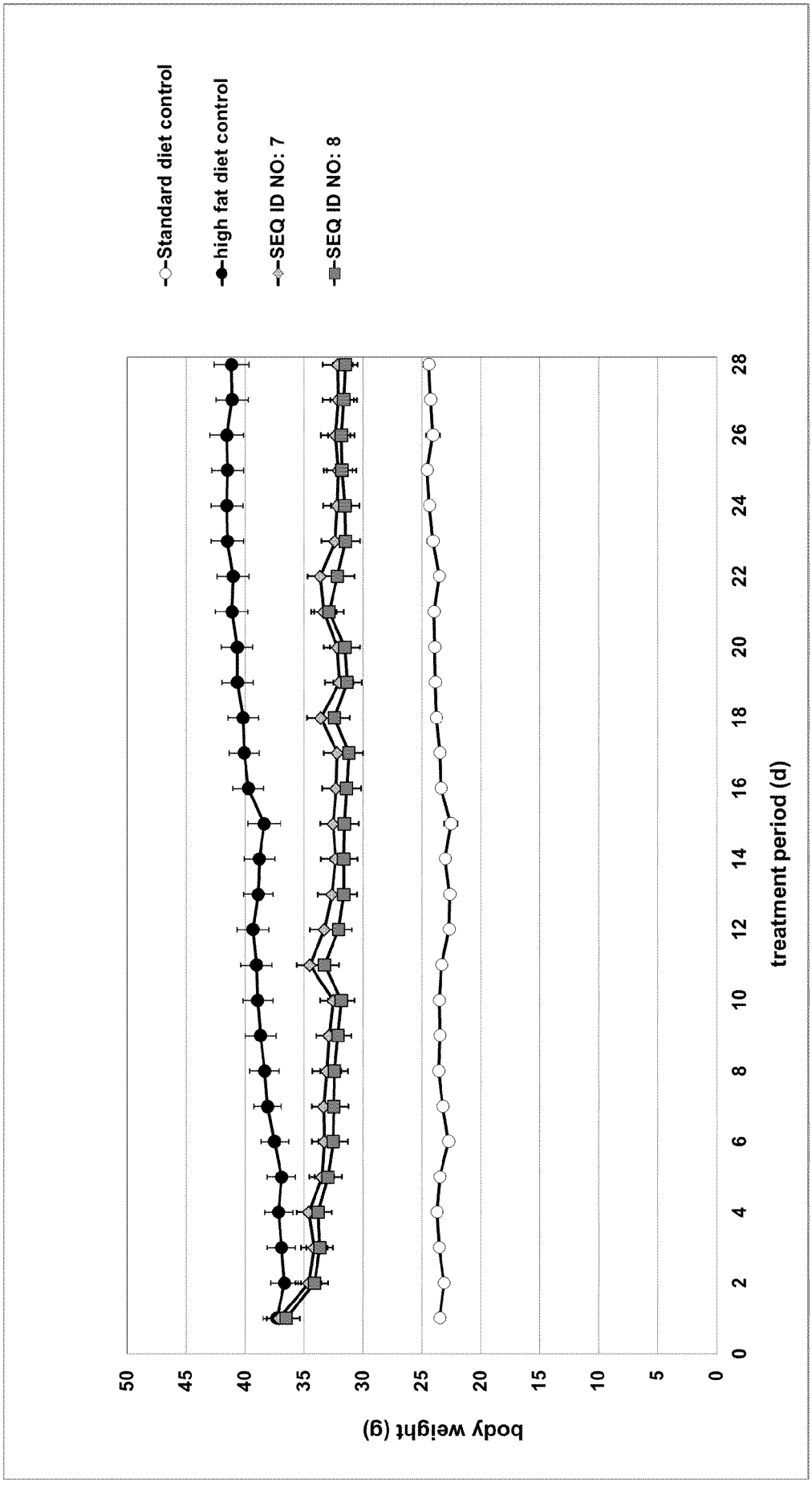
19. The compound for use according to any one of claims 15 - 17 for control of appetite, feeding and calory intake, increase of energy expenditure, prevention of weight gain, promotion of weight loss, reduction of excess body weight and altogether treatment of obesity, including morbid obesity.

20. The compound for use according to any one of claims 15 - 17 for the treatment or prevention of hyperglycemia, type 2 diabetes, obesity.

21. The compound for use according to any one of claims 15 - 17 for the simultaneous treatment of diabetes and obesity.

22. A pharmaceutical composition comprising at least one compound according to any one of claims 1 – 14 or a physiologically acceptable salt or a solvent of any of them.

Fig. 1:


Standard diet control
high fat diet control
SEQ ID NO: 7
SEQ ID NO: 8
body weight (g)
0
5
10
15
20
25
30
35
40
45
50
treatment period (d)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28

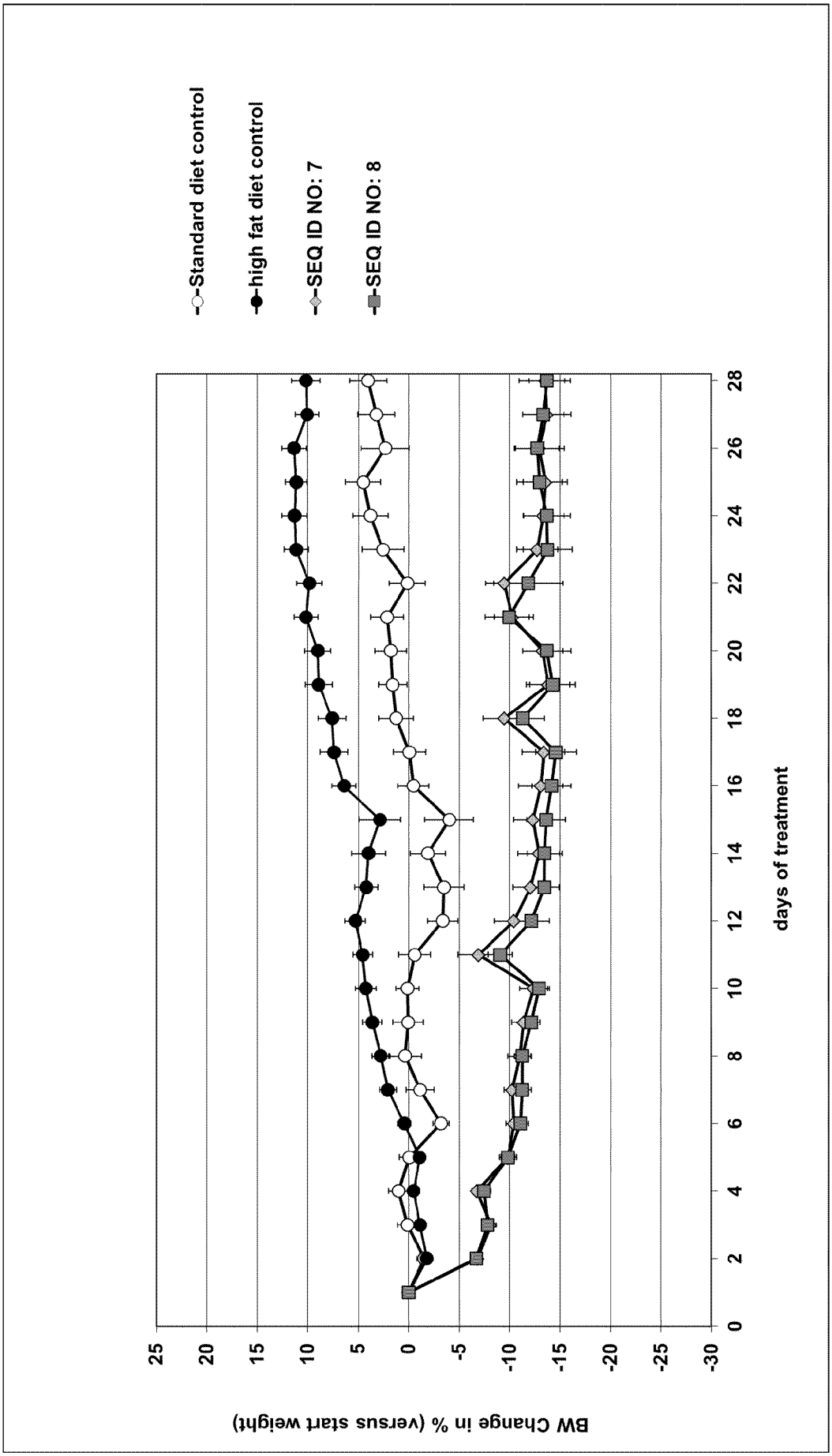

Standard diet control
high fat diet control
SEQ ID NO: 7
SEQ ID NO: 8
BW Change in % (versus start weight)
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
days of treatment

Fig. 2:

Fig. 3:

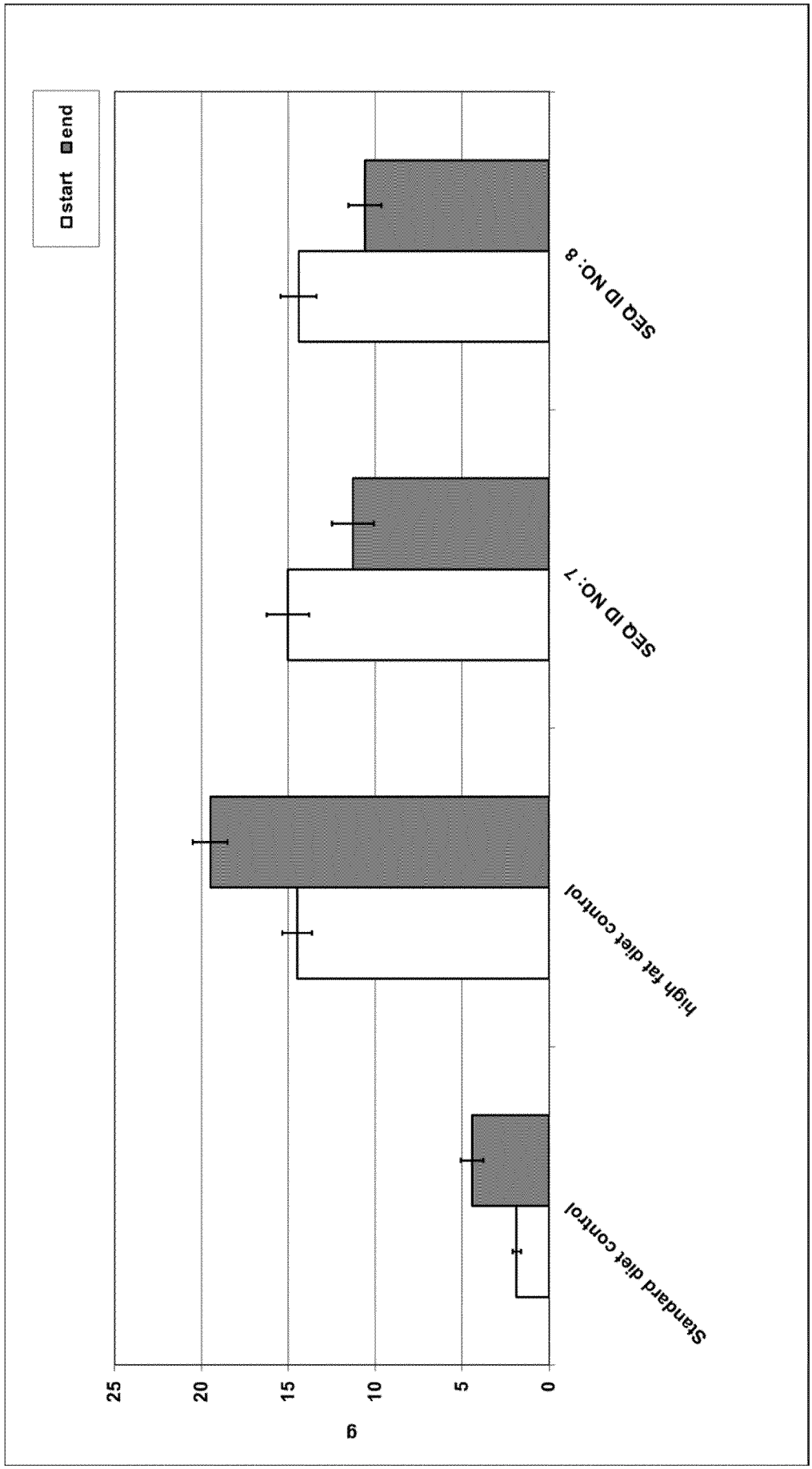

start
end
g
0
5
10
15
20
25
Standard diet control
high fat diet control
SEQ ID NO: 7
SEQ ID NO: 8

Fig. 4:

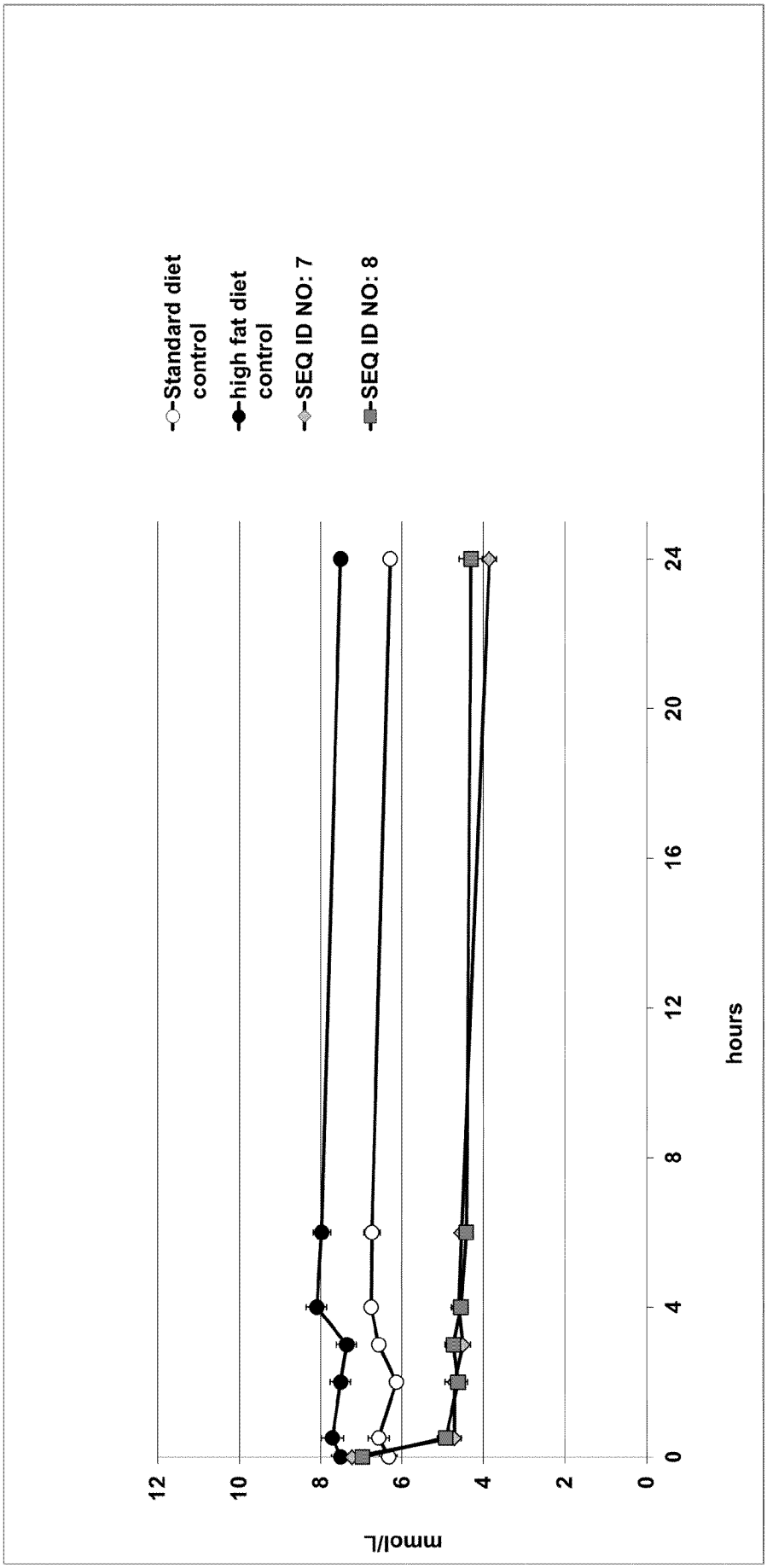

Standard diet control
high fat diet control
SEQ ID NO: 7
SEQ ID NO: 8
mmol/L
12
10
8
6
4
2
0
0
4
8
12
16
20
24
hours

Fig. 5:

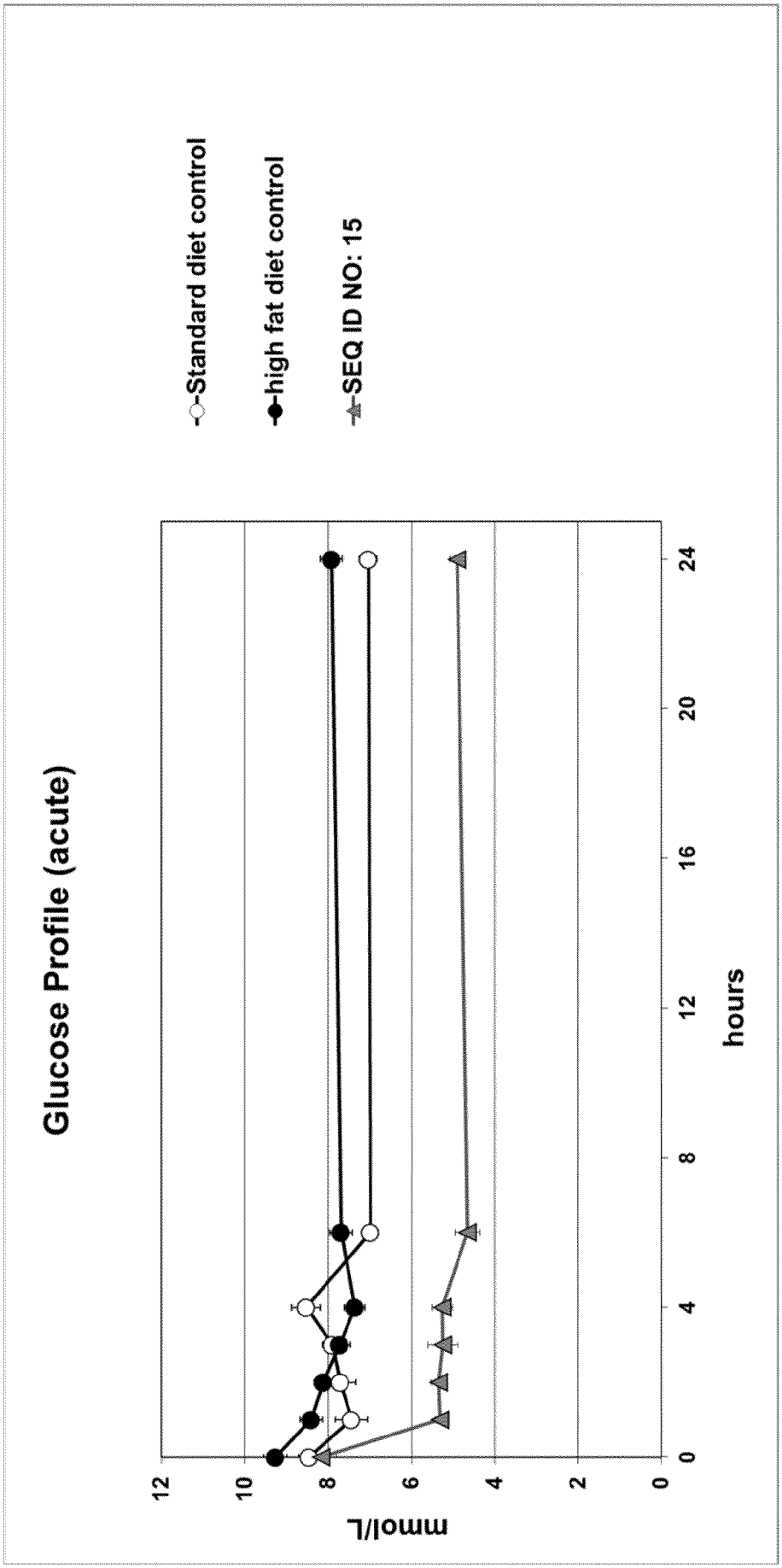

Glucose Profile (acute)
Standard diet control
high fat diet control
SEQ ID NO: 15
mmol/L
hours
0
2
4
6
8
10
12
0
4
8
12
16
20
24

Fig. 6:

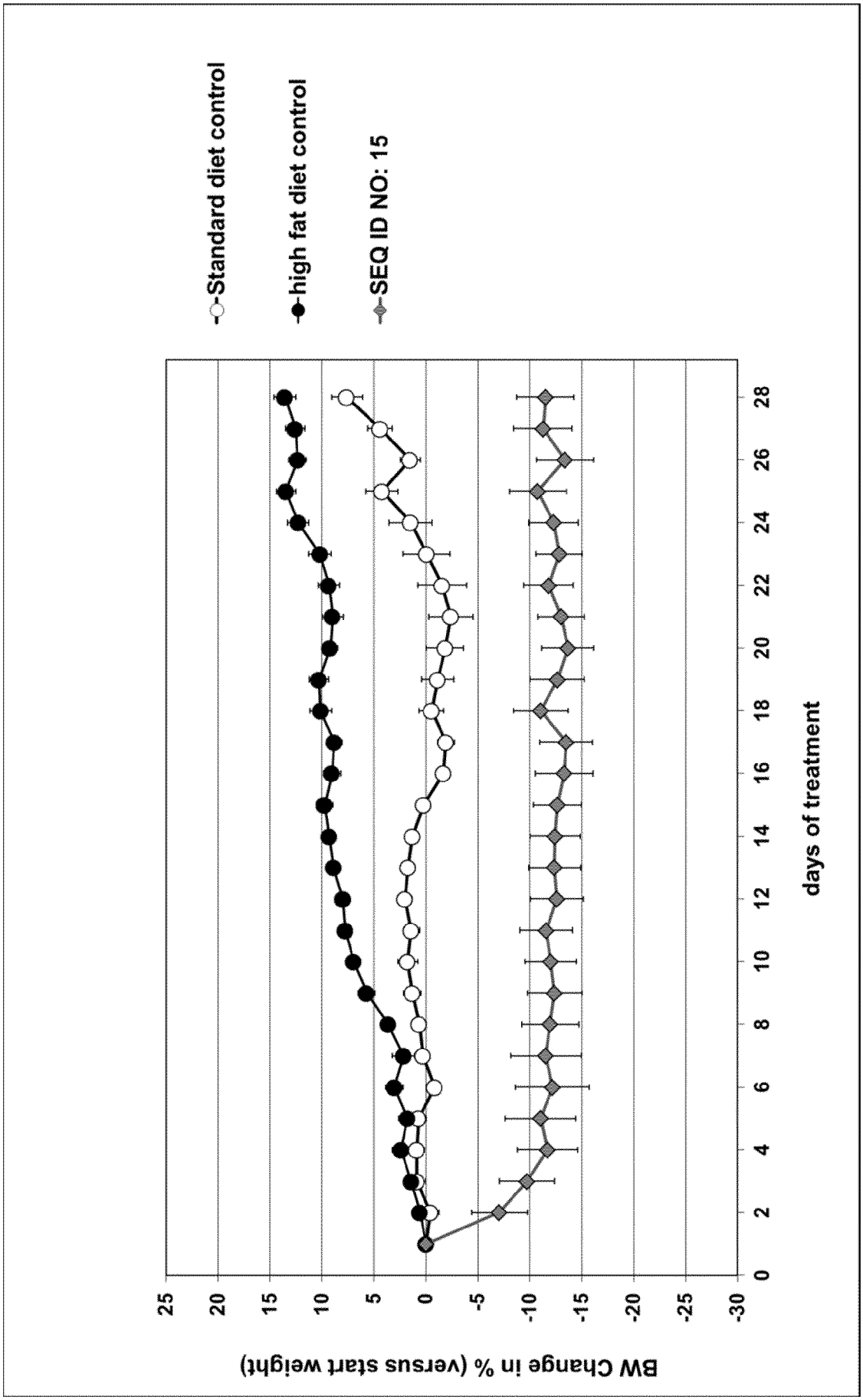
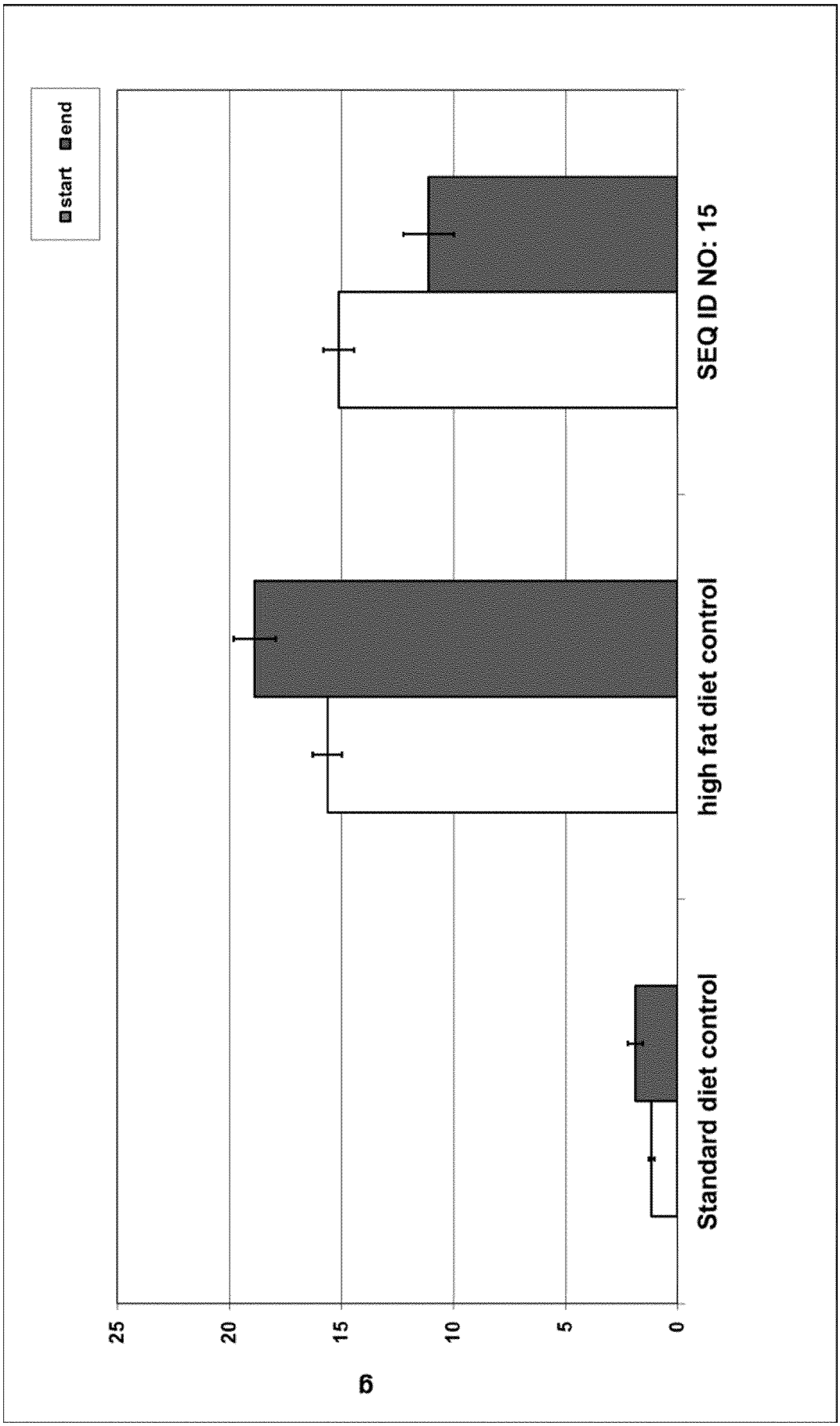

Standard diet control
high fat diet control
SEQ ID NO: 15
BW Change in % (versus start weight)
25
20
15
10
5
0
-5
-10
-15
-20
-25
-30
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
days of treatment

Fig. 7:


start
end
25
20
15
10
5
0
g
SEQ ID NO: 15
high fat diet control
Standard diet control

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/057416

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/435
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 2011/080103 A1 (NOVO NORDISK AS [DK]; GARIBAY PATRICK WILLIAM [DK]; SPETZLER JANE [DK]) 7 July 2011 (2011-07-07) claims 1-15	1-22
A	WO 2011/024110 A2 (RINAT NEUROSCIENCE CORP [US]; CHOU JOYCE CHING TSU [US]; LIN CHIA-YANG) 3 March 2011 (2011-03-03) claims 1-14	1-22

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 2015	22/06/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Stoyanov, Borislav

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/057416

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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