



US008536122B2

(12) **United States Patent**
Lau et al.

(10) **Patent No.:** **US 8,536,122 B2**
(45) **Date of Patent:** ***Sep. 17, 2013**

(54) **ACYLATED GLP-1 COMPOUNDS**

(75) Inventors: **Jesper Lau**, Farum (DK); **Florencio Zaragoza Doerwald**, Smorum (DK); **Paw Bloch**, Taastrup (DK); **Thomas Kruse Hansen**, Herlev (DK)

(73) Assignee: **Novo Nordisk A/S**, Bagsvaerd (DK)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **13/412,283**

(22) Filed: **Mar. 5, 2012**

(65) **Prior Publication Data**

US 2012/0295847 A1 Nov. 22, 2012

Related U.S. Application Data

(63) Continuation of application No. 11/908,834, filed as application No. PCT/EP2006/060855 on Mar. 20, 2006, now Pat. No. 8,129,343.

(60) Provisional application No. 60/664,497, filed on Mar. 23, 2005.

(30) **Foreign Application Priority Data**

Mar. 18, 2005 (EP) 05102171

(51) **Int. Cl.**

A61K 38/26 (2006.01)
A61K 38/28 (2006.01)
A61P 3/10 (2006.01)
A61P 7/12 (2006.01)
C07K 14/605 (2006.01)
C07K 5/00 (2006.01)
C07K 7/00 (2006.01)
C07K 16/00 (2006.01)
C07K 17/00 (2006.01)

(52) **U.S. Cl.**

USPC **514/7.2**; 514/11.7; 530/308

(58) **Field of Classification Search**

None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,545,618 A 8/1996 Buckley et al.
6,268,343 B1 7/2001 Knudsen et al.
6,528,486 B1 3/2003 Larsen et al.
2001/0011071 A1 8/2001 Knudsen et al.
2004/0001827 A1 1/2004 Dennis
2004/0053370 A1 3/2004 Glaesner et al.

FOREIGN PATENT DOCUMENTS

EP 1329458 A2 7/2003
EP 05102171.5 3/2005
EP 1704165 A1 9/2006
JP 2000-500505 A 1/2000
JP 2002-504527 A 2/2002
JP 2002-508162 A 3/2002
JP 2003-505347 2/2003
JP 2004-528014 A 9/2004
JP 2004-535442 A 11/2004
JP 2010-116407 A 5/2010
RU 2006107600 A 10/2007
WO 90/11296 10/1990
WO 91/11457 A1 8/1991
WO 96/29342 9/1996
WO 98/08871 3/1998
WO 98/08872 A1 3/1998
WO 99/43341 9/1999
WO 99/43361 A1 9/1999
WO 99/43705 A1 9/1999
WO 99/43708 9/1999
WO 9943707 9/1999
WO 00/34331 6/2000
WO 00/69911 11/2000
WO 01/04156 1/2001
WO 0151071 7/2001
WO 0258725 1/2002
WO 02/46227 A2 6/2002
WO 02098446 A1 12/2002
WO 03/002136 1/2003
WO 03/013573 A1 2/2003
WO 03/040309 A2 5/2003
WO 03/058203 A2 7/2003
WO 03/087139 A2 10/2003
WO 2004/065621 A1 8/2004
WO 2004/074315 A2 9/2004
WO 2004/093823 A2 11/2004
WO 2004/099246 A2 11/2004
WO 2005/014049 A2 2/2005
WO 2005/027978 3/2005
WO 2005/028516 A2 3/2005
WO 2005/058958 A2 6/2005
WO 2006/005667 A2 1/2006
WO 2006/037810 A2 4/2006
WO 2006/097536 A2 9/2006
WO 2006/097537 9/2006
WO 2006/097537 A2 9/2006
WO 2006/097538 A1 9/2006

OTHER PUBLICATIONS

Declaration of Per Franklin Nielsen, 2012.
Annual Report 2003 Novo Nordisk A/S.
Curry, Stephen, Plasma Albumin as a Fatty Acid Carrier, *Advances in Molecular and Cell Biology*, 2004, vol. 33, pp. 29-46.
Annual Report 2004, Novo Nordisk A/S.
Nauck et al., The Once-Weekly Human GLP-1 Analogue . . . , EASD, 2012, 49th Annual Meeting.
International Non-Roprietary Names . . . , 2003, vol. 17(2), pp. 115, 125.
Table of S.C. Half-Life(Mining) and Potency Data 2011.
Berendsen, 1998, "A Glimpse of the Holy Grail?" *Science* 282:642-643.
Bradley et al., 2002, "Limits of Cooperativity in a Structurally Modular Protein: Response of the Notch Ankyrin Domain to Analogous Alanine Substitutions in Each Repeat," *Journal of Molecular Biology* 324:373-386.

(Continued)

Primary Examiner — Marcela M Cordero Garcia
(74) *Attorney, Agent, or Firm* — Richard W. Bork

(57) **ABSTRACT**

Protracted GLP-1 compounds and therapeutic uses thereof

(56)

References Cited

OTHER PUBLICATIONS

- Chuang et al., 2002, "Pharmaceutical Strategies Utilizing Recombinant Human Serum Albumin," *Pharmaceutical Research* 19(5):569-577.
- Han, 2002, "Targeted Prodrug Design to Optimize Drug Delivery," *AAPS Pharmsci* 2(1):1-11.
- Hodgson et al., 2004, "The Synthesis of Peptides and Proteins Containing Non-Natural Amino Acids," *Chemical Reviews* 33(7):422-430.
- Holz et al., 2003, "Glucagon-Like Peptide-1 Synthetic Analogs: New Therapeutic Agents for Use in the Treatment of Diabetes Mellitus," *Current Medicinal Chemistry* 10(22):2471-2483.
- Kim et al., 2003, "Development and Characterization of a Glucagon-Like Peptide 1-Albumin Conjugate," *Diabetes* 52:751-759.
- Makino et al., 2005, "Semisynthesis of Human Ghrelin: Condensation of a Boc-Protected Recombinant Peptide With a Synthetic O-Acylated Fragment," *Biopolymers* 79(5):238-247.
- Okada, 2001, "Synthesis of Peptides by Solution Methods," *Current Organic Chemistry* 5(1):1-43.
- Ostrovsky, 1975, "Comparative Characteristics of the Hydrophobic Nature of Certain Proteins by Their Interaction With 2-P Toluindino," *Ukrayins'kyi Biokhimichnyi Zhurnal* 47(6):701-707.
- Picó, 1990, "Use of 1-Anilino-8-Naphthalene Sulfonate as a Reporter Molecule to Study the Bile Salts-Bovine Serum Albumin Binding," *Studia Biophysica* 136(1):21-26, Abstract XP-008039734.
- Rudinger, 1976, "Characteristics of the Amino Acids as Components of a Peptide Hormone Sequence," *Peptides Hormones*, JA Parsons Edition, University Park Press, Jun. 1976, pp. 1-7.
- Schinzel et al., 1991, "The Phosphate Recognition Site of *Escherichia coli* Maltodextrin Phosphorylase," *Federation of European Biochemical Society Jul. 1991*, 286(1, 2):125-128.
- Sheffield, 2001, "Modification of Clearance of Therapeutic and Potentially Therapeutic Proteins," *Current Drug Targets Cardiovascular & Haematological Disorders* 1(1):1-22.
- SIGMA Genosys (Web Site), *Designing Custom Peptides*, pp. 1-2, Accessed Aug. 16, 2004.
- Voet et al., 1995, *Biochemistry 2nd ed.*, John Wiley & Sons, Inc., pp. 235-241.
- Wallace, 1995, "Peptide Ligation and Semisynthesis," *Current Opinion in Biotechnology* 6(4):403-410.
- Zobel et al., 2003, "Phosphate Ester Serum Albumin Affinity Tags Greatly Improve Peptide Half-Life In Vivo," *Bioorganic & Medicinal Chemistry Letters* 13:1513-1515.
- Knudsen, L.B. et al., "Potent Derivatives of Glucagon-Like Peptide-1 With Pharmacokinetic Properties Suitable for Once Daily Administration", *Journal of Medicinal Chemistry*, 2000 vol. 43, pp. 1664-1669.
- Deacon, C.F. et al., "Dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1 which have extended metabolic stability and improved biological activity," 1998, *Diabetologia*, vol. 41, pp. 271-278.
- Kurtzhals, P, et al., "Albumin Binding of Insulins Acylated With Fatty Acids: Characterization of the Ligand-Protein Interaction and Correlation Between Binding Affinity and Timing of the Insulin Effect In Vivo," *Biochem J*, 1995, vol. 312, pp. 725-731.
- Soltero et al., "The Oral Delivery of Protein and Peptide Drugs," *Innovations in Pharmaceutical Technology*, 2001, vol. 1, No. 9, pp. 106-110.
- Watanabe et al., "Structure-Activity Relationships of Glucagon-Like Peptide-1 (7-36) Amide: Insulinotropic Activities in Perfused Rat Pancreases, and Receptor Binding and Cyclic AMP Production in RINm5F Cells," *Journal of Endocrinology*, 1994, vol. 140, pp. 45-52.
- Inflammatory Bowel Disease from e-Medicine*, pp. 1.24, Accessed Sep. 24, 2008.
- Ngo JT et al., "Computational Complexity, Protein Structure Prediction, and the Levinthal Paradox," *The Protein Folding Problem and Tertiary Structure Prediction*, K. Mere Jr. and S. LeGrand Edition, 1994, pp. 491-495.
- Residue definition from www.dictionary.com, pp. 1-6, Accessed May 5, 2009.
- Small Bowel Syndrome from e-Medicine*, pp. 1-21, Accessed Sep. 24, 2008.
- Green, Brian D. et al *Biological Chemistry. Degradation, Receptor Binding, Insulin . . .* 2004 385 2 169-177.
- Greenwald Journal of the Controlled Release Peg Drugs: An Overview* 2001 74-159-171.
- Ji, J. et al. *Biomaterials Stearyl Poly (Ethylene Oxide) Grafted Surfaces for Preferential Adsorption of Albumin*. 2001 22-3015-3023.
- Knudsen, L.B. *Journal of Medicinal Chemistry Glucagon-Like Peptide-1 . . .* 2004 47-4128-4134.
- Simonovsky et al. *Journal of Biomaterials Science, Polymer Edition Poly(Ether Urethane)S Incorporating Long Alkyl Side-Chains With Terminal Carboxyl Groups as Fatty Acid Mimics: Synthesis, Structural Characterization and Protein Adsorption* 2005 16 12 1463-1483.
- Soltero and Ekwuribe *Innovations in Pharmaceutical Technology the Oral Delivery of Protein and Peptide Drugs*. 2001 1-106-110.
- Still, J. Gordon, *Diabetes/Metabolism Research Reviews, Development of Oral Insulin: Progress and Current Status*, 2002, vol. 18, Suppl 1, pp. S29-S37.
- Veronese F. M *Biomaterials Peptide and Protein Pegylation: A Review of Problems and Solutions* 2001 22 5 405-417.
- English abstract of JP 2004535442, Sep. 16, 2004.
- English abstract of RU 2006107600, Oct. 27, 2007.
- English abstract of JP 2010116407, May 27, 2010.
- English abstract of JP 2004528014, Sep. 16, 2004.

1

ACYLATED GLP-1 COMPOUNDS**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application is a Continuation of copending U.S. application Ser. No. 11/908,834, filed Sep. 17, 2007, which is a 35 U.S.C. §371 national stage application of International Patent Application PCT/EP2006/060855 (published as WO 2006/097537), filed Mar. 20, 2006, which claimed priority of European Patent Application 05102171.5, filed Mar. 18, 2005; this application further claims priority under 35 U.S.C. §119 of U.S. Provisional Application 60/664,497, filed Mar. 23, 2005.

FIELD OF THE INVENTION

This invention relates to the field of therapeutic peptides, i.e. to new protracted GLP-1 compounds.

BACKGROUND OF THE INVENTION

A range of different approaches have been used for modifying the structure of glucagon-like peptide 1 (GLP-1) compounds in order to provide a longer duration of action in vivo. WO 96/29342 discloses peptide hormone derivatives wherein the parent peptide hormone has been modified by introducing a lipophilic substituent in the C-terminal amino acid residue or in the N-terminal amino acid residue.

WO 98/08871 discloses GLP-1 derivatives wherein at least one amino acid residue of the parent peptide has a lipophilic substituent attached.

WO 99/43708 discloses GLP-1(7-35) and GLP-1(7-36) derivatives which have a lipophilic substituent attached to the C-terminal amino acid residue.

WO 00/34331 discloses acylated GLP-1 analogs.

WO 00/69911 discloses activated insulinotropic peptides to be injected into patients where they are supposed to react with blood components to form conjugates and thereby allegedly providing longer duration of action in vivo.

WO 02/46227 discloses GLP-1 and exendin-4 analogs fused to human serum albumin in order to extend in vivo half-life.

Many diabetes patients particularly in the type 2 diabetes segment are subject to so-called "needle-phobia", i.e. a substantial fear of injecting themselves. In the type 2 diabetes segment most patients are treated with oral hypoglycaemic agents, and since GLP-1 compounds are expected to be the first injectable product these patients will be administered, the fear of injections may become a serious obstacle for the widespread use of the clinically very promising GLP-1 compounds. Thus, there is a need to develop new GLP-1 compounds which can be administered less than once daily, e.g. once every second or third day preferably once weekly, while retaining an acceptable clinical profile.

SUMMARY OF THE INVENTION

The invention provides a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

2

invention and the use of compounds according to the present invention for preparing medicaments for treating disease.

The invention provides a method for increasing the time of action in a patient of a GLP-1 analog, characterised in acylating said GLP-1 analog with a moiety B—U' as disclosed in any of the preceding claims, on the lysine residue in position 26 of said GLP-1 analog.

DESCRIPTION OF THE INVENTION

In the present specification, the following terms have the indicated meaning:

The term "polypeptide" and "peptide" as used herein means a compound composed of at least five constituent amino acids connected by peptide bonds. The constituent amino acids may be from the group of the amino acids encoded by the genetic code and they may be natural amino acids which are not encoded by the genetic code, as well as synthetic amino acids. Natural amino acids which are not encoded by the genetic code are e.g., γ -carboxyglutamate, ornithine, phosphoserine, D-alanine and D-glutamine. Synthetic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α -aminoisobutyric acid), Abu (α -aminobutyric acid), Tle (tert-butylglycine), β -alanine, 3-aminomethyl benzoic acid, anthranilic acid.

The 22 proteogenic amino acids are:

Alanine, Arginine, Asparagine, Aspartic acid, Cysteine, Cystine, Glutamine, Glutamic acid, Glycine, Histidine, Hydroxyproline, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tryptophan, Tyrosine, Valine.

Thus a non-proteogenic amino acid is a moiety which can be incorporated into a peptide via peptide bonds but is not a proteogenic amino acid. Examples are γ -carboxyglutamate, ornithine, phosphoserine, the D-amino acids such as D-alanine and D-glutamine, Synthetic non-proteogenic amino acids comprise amino acids manufactured by chemical synthesis, i.e. D-isomers of the amino acids encoded by the genetic code such as D-alanine and D-leucine, Aib (α -aminoisobutyric acid), Abu (α -aminobutyric acid), Tle (tert-butylglycine), 3-aminomethyl benzoic acid, anthranilic acid, des-amino-Histidine, the beta analogs of amino acids such as β -alanine etc. D-histidine, desamino-histidine, 2-amino-histidine, β -hydroxy-histidine, homohistidine, N^c-acetyl-histidine, α -fluoromethyl-histidine, α -methyl-histidine, 3-pyridylalanine, 2-pyridylalanine or 4-pyridylalanine, (1-aminocyclopropyl) carboxylic acid, (1-aminocyclobutyl) carboxylic acid, (1-aminocyclopentyl) carboxylic acid, (1-aminocyclohexyl) carboxylic acid, (1-aminocycloheptyl) carboxylic acid, or (1-aminocyclooctyl) carboxylic acid;

The term "analogue" as used herein referring to a polypeptide means a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues and/or wherein one or more amino acid residues have been deleted from the peptide and/or wherein one or more amino acid residues have been added to the peptide. Such addition or deletion of amino acid residues can take place at the N-terminal of the peptide and/or at the C-terminal of the peptide. A simple system is often used to describe analogues: For example [Arg³⁴]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted

3

for which the optical isomer is not stated is to be understood to mean the L-isomer. In embodiments of the invention a maximum of 17 amino acids have been modified. In embodiments of the invention a maximum of 15 amino acids have been modified. In embodiments of the invention a maximum of 10 amino acids have been modified. In embodiments of the invention a maximum of 8 amino acids have been modified. In embodiments of the invention a maximum of 7 amino acids have been modified. In embodiments of the invention a maximum of 6 amino acids have been modified. In embodiments of the invention a maximum of 5 amino acids have been modified. In embodiments of the invention a maximum of 4 amino acids have been modified. In embodiments of the invention a maximum of 3 amino acids have been modified. In embodiments of the invention a maximum of 2 amino acids have been modified. In embodiments of the invention 1 amino acid has been modified.

The term "derivative" as used herein in relation to a peptide means a chemically modified peptide or an analogue thereof, wherein at least one substituent is not present in the unmodified peptide or an analogue thereof, i.e. a peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters and the like. An example of a derivative of GLP-1(7-37) is N^{ε26}-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg³⁴, Lys²⁶] GLP-1-(7-37).

The term "GLP-1 peptide" as used herein means GLP-1(7-37) (SEQ ID No 1), a GLP-1(7-37) analogue, a GLP-1(7-37) derivative or a derivative of a GLP-1(7-37) analogue. In one embodiment the GLP-1 peptide is an insulinotropic agent.

The term "insulinotropic agent" as used herein means a compound which is an agonist of the human GLP-1 receptor, i.e. a compound which stimulates the formation of cAMP in a suitable medium containing the human GLP-1 receptor (one such medium disclosed below). The potency of an insulinotropic agent is determined by calculating the EC₅₀ value from the dose-response curve as described below.

Baby hamster kidney (BHK) cells expressing the cloned human GLP-1 receptor (BHK-467-12A) were grown in DMEM media with the addition of 100 IU/mL penicillin, 100 µg/mL streptomycin, 5% fetal calf serum and 0.5 mg/mL Geneticin G-418 (Life Technologies). The cells were washed twice in phosphate buffered saline and harvested with Versene. Plasma membranes were prepared from the cells by homogenisation with an Ultraturax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000×g for 15 min at 4° C. The pellet was suspended by homogenization in buffer 2 (20 mM HEPES-Na, 0.1 mM EDTA, pH 7.4), then centrifuged at 48,000×g for 15 min at 4° C. The washing procedure was repeated one more time. The final pellet was suspended in buffer 2 and used immediately for assays or stored at -80° C.

The functional receptor assay was carried out by measuring cyclic AMP (cAMP) as a response to stimulation by the insulinotropic agent. cAMP formed was quantified by the AlphaScreen™ cAMP Kit (Perkin Elmer Life Sciences). Incubations were carried out in half-area 96-well microtiter plates in a total volume of 50 µL buffer 3 (50 mM Tris-HCl, 5 mM HEPES, 10 mM MgCl₂, pH 7.4) and with the following additions: 1 mM ATP, 1 µM GTP, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 0.01% Tween-20, 0.1% BSA, 6 µg membrane preparation, 15 µg/mL acceptor beads, 20 µg/mL donor beads preincubated with 6 nM biotinyl-cAMP. Compounds to be tested for agonist activity were dissolved and diluted in buffer 3. GTP was freshly prepared for each experi-

4

the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC₅₀ values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, Calif.).

The term "DPP-IV protected" as used herein referring to a polypeptide means a polypeptide which has been chemically modified in order to render said compound resistant to the plasma peptidase dipeptidyl aminopeptidase-4 (DPP-IV). The DPP-IV enzyme in plasma is known to be involved in the degradation of several peptide hormones, e.g. GLP-1, GLP-2, Exendin-4 etc. Thus, a considerable effort is being made to develop analogues and derivatives of the polypeptides susceptible to DPP-IV mediated hydrolysis in order to reduce the rate of degradation by DPP-IV. In one embodiment a DPP-IV protected peptide is more resistant to DPP-IV than GLP-1(7-37) or Exendin-4(1-39).

Resistance of a peptide to degradation by dipeptidyl aminopeptidase IV is determined by the following degradation assay:

Aliquots of the peptide (5 nmol) are incubated at 37° C. with 1 µL of purified dipeptidyl aminopeptidase IV corresponding to an enzymatic activity of 5 mU for 10-180 minutes in 100 µL of 0.1 M triethylamine-HCl buffer, pH 7.4. Enzymatic reactions are terminated by the addition of 5 µL of 10% trifluoroacetic acid, and the peptide degradation products are separated and quantified using HPLC analysis. One method for performing this analysis is: The mixtures are applied onto a Vydac C18 widepore (30 nm pores, 5 µm particles) 250×4.6 mm column and eluted at a flow rate of 1 ml/min with linear stepwise gradients of acetonitrile in 0.1% trifluoroacetic acid (0% acetonitrile for 3 min, 0-24% acetonitrile for 17 min, 24-48% acetonitrile for 1 min) according to Siegel et al., Regul. Pept. 1999; 79:93-102 and Mentlein et al. Eur. J. Biochem. 1993; 214:829-35. Peptides and their degradation products may be monitored by their absorbance at 220 nm (peptide bonds) or 280 nm (aromatic amino acids), and are quantified by integration of their peak areas related to those of standards. The rate of hydrolysis of a peptide by dipeptidyl aminopeptidase IV is estimated at incubation times which result in less than 10% of the peptide being hydrolysed.

The term "C₁₋₆-alkyl" as used herein means a saturated, branched, straight or cyclic hydrocarbon group having from 1 to 6 carbon atoms. Representative examples include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, tert-pentyl, n-hexyl, isohexyl, cyclohexane and the like. The term "pharmaceutically acceptable" as used herein means suited for normal pharmaceutical applications, i.e. giving rise to no adverse events in patients etc.

The term "excipient" as used herein means the chemical compounds which are normally added to pharmaceutical compositions, e.g. buffers, tonicity agents, preservatives and the like.

The term "effective amount" as used herein means a dosage which is sufficient to be effective for the treatment of the patient compared with no treatment.

The term "pharmaceutical composition" as used herein means a product comprising an active compound or a salt thereof together with pharmaceutical excipients such as buffer, preservative, and optionally a tonicity modifier and/or a stabilizer. Thus a pharmaceutical composition is also known in the art as a pharmaceutical formulation.

The term "treatment of a disease" as used herein means the management and care of a patient having developed the dis-

5

the administration of the active compounds to eliminate or control the disease, condition or disorder as well as to alleviate the symptoms or complications associated with the disease, condition or disorder.

In another aspect the present invention relates to an acylated GLP-1 analogue that can bind to albumin and the GLP-1 receptor simultaneously.

In another aspect the present invention relates to an acylated GLP-1 analogue that bind to the GLP-1 receptor with an affinity below 100 nM, preferable below 30 nM in the presence of 2% albumin.

In another aspect the present invention relates to an acylated GLP-1 analogue which affinity to the GLP-1 receptor is only partly decreased when comparing the affinity in the presence of very low concentration (e.g. 0.005% to 0.2%) of human albumin to the affinity in the presence of 2% human albumin. The shift in binding affinity under these conditions is less than 50 fold, preferable below 30 fold and more preferable below 10 fold.

The term "albumin binding moiety" as used herein means a residue which binds non-covalently to human serum albumin. The albumin binding residue attached to the therapeutic polypeptide typically has an affinity below 10 μ M to human serum albumin and preferably below 1 μ M. A range of albumin binding residues are known among linear and branched lipophilic moieties containing 4-40 carbon atoms having a distal acidic group.

The term "hydrophilic linker" as used herein means a spacer that separates a peptide and an albumin binding residue with a chemical moiety which comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

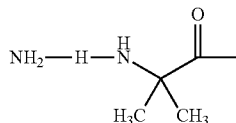
The term "acidic groups" as used herein means organic chemical groups which are fully or partly negatively charged at physiological pH. The pKa value of such groups is below 7, preferable below 5. This includes but is not limited to carboxylic acids, sulphonic acids, phosphoric acids or heterocyclic ring systems which are fully or partly negatively charged at physiological pH.

In the below structural formula II the moiety U is a di-radical may be attached to the terminal groups B and the aminogroup of the lysine amino acid in the peptide in two different ways. In embodiments of the invention the U in formula II is attached with the group B attached at the end of the alkyl chain and the peptide at the other end.

In the formulas below the terminal bonds from the attached groups are to be regarded as attachment bonds and not ending in methylene groups unless stated.

6

In the formulas below



means the H₂N-His-Aib-N-terminal of the GLP-1 analogue.

In an embodiment the invention provides a GLP-1 analog acylated with a lipophilic albumin binding moiety containing at least two free acidic chemical groups attached via a non natural amino acid linker to the lysine residue in position 26.

In an embodiment, the term free acidic chemical groups is to be understood as having the same meaning as "acidic groups" as used herein.

In an embodiment the invention provides an acylated GLP-1 analog where said GLP-1 analog is stabilised against DPP-IV by modification of at least one amino acid residue in positions 7 and 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), and where said acylation is a diacid attached to the lysine residue in position 26 optionally via a non natural amino acid hydrophilic linker.

In an embodiment of the invention a GLP-1 analog having a modification of at least one non-proteogenic amino acid residue in positions 7 and/or 8 relative to the sequence GLP-1(7-37) (SEQ ID No 1), which is acylated with a moiety to the lysine residue in position 26, and where said moiety comprises at least two acidic groups, wherein one acidic group is attached terminally.

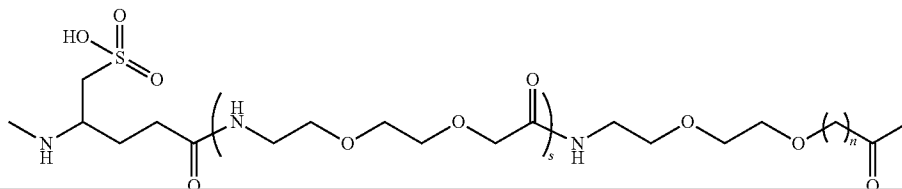
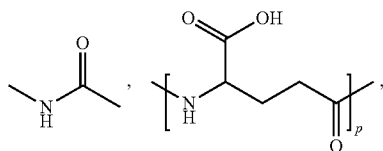
An embodiment provides a GLP-1 analog according to the above embodiment, wherein the moiety attached in position 26 comprises a hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiments, wherein the hydrophilic linker comprises at least 5 non-hydrogen atoms where 30-50% of these are either N or O.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the moiety attached in position 26 comprises an albumin binding moiety separated from the peptide by the hydrophilic linker.

An embodiment provides a GLP-1 analog according to the above embodiment, wherein the albumin binding moiety is a linear or branched lipophilic moiety containing 4-40 carbon atoms having a distal acidic group.

An embodiment provides a GLP-1 analog according to any of the above embodiments, wherein the acylated moiety is B-U', where U' is selected from



Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts



Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research



With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips



Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS

Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS

Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS

Sync your system to PACER to automate legal marketing.