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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 September 2006 (21.09.2006)

PCT

(10) International Publication Number
WO 2006/097537 A2

(51) International Patent Classification:
C07K 14/605 (2006.01) *A61K 47/48* (2006.01)
A61K 38/26 (2006.01)

(21) International Application Number:
PCT/EP2006/060855

(22) International Filing Date: 20 March 2006 (20.03.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
05102171.5 18 March 2005 (18.03.2005) EP

(71) Applicant *(for all designated States except US)*: **NOVO NORDISK A/S** [DK/DK]; Novo Allé, DK-2880 Bagsværd (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **LAU, Jesper** [DK/DK]; Rosenvænget 3, DK-3520 Farum (DK). **DÖRWALD, Florencio Zaragoza** [DE/DK]; Nøddelunden 22, DK-2765 Smørum (DK). **STEPHENSEN, Henrik** [DK/DK]; Brudelysvej 29, DK-3520 Farum (DK). **BLOCH, Paw** [DK/DK]; Solbærhegnet 2, DK-2630 Taastrup (DK). **HANSEN, Thomas, Kruse** [DK/DK]; Tibbevangen 78, DK-2730 Herlev (DK). **MADSEN, Kjeld** [DK/DK]; Nyvestergårdsvej 3, DK-3500 Værløse (DK).

(74) Common Representative: **NOVO NORDISK A/S**; Corporate Patents, Novo Allé, DK-2880 Bagsværd (DK).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ACYLATED GLP-1 COMPOUNDS

(57) Abstract: Protracted GLP-1 compounds and therapeutic uses thereof.

WO 2006/097537 A2

ACYLATED GLP-1 COMPOUNDS

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 alledgedly 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.

The present invention also provides pharmaceutical compositions comprising a compound according to the present 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^{α}-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 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 [Arg^{34}]GLP-1(7-37)Lys designates a GLP-1(7-37) analogue wherein the naturally occurring lysine at position 34 has been substituted with arginine and wherein a lysine has been added to the terminal amino acid residue, i.e. to the Gly^{37}. All amino acids 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 $N^{\varepsilon 26}$-((4S)-4-(hexadecanoylamino)-carboxy-butanoyl)[Arg^{34}, Lys^{26}]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 EC_{50} 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 Ultraturrax in buffer 1 (20 mM HEPES-Na, 10 mM EDTA, pH 7.4). The homogenate was centrifuged at 48,000 x 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 x 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 $MgCl_2$, pH 7.4) and with the following addiditions: 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, 20µg/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 experiment. The plate was incubated in the dark with slow agitation for three hours at room temperature followed by counting in the Fusion™ instrument (Perkin Elmer Life Sciences). Concentration-response curves were plotted for the individual compounds and EC_{50} values estimated using a four-parameter logistic model with Prism v. 4.0 (GraphPad, Carlsbad, CA).

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

DOCKET ALARM

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.