**EUROPEAN PATENT SPECIFICATION**

**ANALOGUES OF GLP-1**

**GLP-1 ANALOGE**

**ANALOGUES DU GLP-1**

**References cited:**


- BIOLOGICAL ABSTRACTS, 2002, Philadelphia, PA, US; abstract no. PC9, DONG J.Z. ET AL: 'Human glucagon-like peptide-1 amide (hGLP1(7-36)NH2) is cleaved by plasma enzymes not only at the N-terminus but also at the C-terminus; development of novel GLP-1 analogs which are resistant to enzymatic degradation.' page S148;

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Description

Background of the Invention

[0001] The present invention is directed to a peptide analogue of glucagon-like peptide-1, the pharmaceutically-acceptable salts thereof, to methods of using such analogue to treat mammals and to pharmaceutical compositions useful therefor comprising said analogue.

[0002] Glucagon-like peptide-1 (7-36) amide (GLP-1) is synthesized in the intestinal L-cells by tissue-specific post-translational processing of the glucagon precursor proglucagon (Varndell, J.M., et al., J. Histochem Cytochem, 1985:33:1080-6) and is released into the circulation in response to a meal. GLP-1 (7-36) NH2 has the amino acid sequence: His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-Ser-Tyr-Leu-Gly-Glu-Ala-Ala-Lys-Glu-Phe-Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-NH2.

The plasma concentration of GLP-1 rises from a fasting level of approximately 15 pmol/L to a peak postprandial level of 40 pmol/L. It has been demonstrated that, for a given rise in plasma glucose concentration, the increase in plasma insulin is approximately threefold greater when glucose is administered orally compared with intravenously (Kreymann, B., et al., Lancet 1987:2, 1300-4). This alimentary enhancement of insulin release, known as the incretin effect, is primarily hormonal and GLP-1 is now thought to be the most potent physiological incretin in humans. In addition to the insulino-tropic effect, GLP-1 suppresses glucagon secretion, delays gastric emptying (Wettergren A., et al., Dig Dis Sci 1993:38:665-73) and may enhance peripheral glucose disposal (D’Alessio, D.A. et al., J. Clin Invest 1994:93:2293-6).

[0003] In 1994, the therapeutic potential of GLP-1 was suggested following the observation that a single subcutaneous (s/c) dose of GLP-1 could completely normalize postprandial glucose levels in patients with non-insulin-dependent diabetes mellitus (NIDDM) (Gutniak, M.K., et al., Diabetes Care 1994:17:1039-44). This effect was thought to be mediated both by increased insulin release and by a reduction in glucagon secretion. Furthermore, an intravenous infusion of GLP-1 has been shown to delay postprandial gastric emptying in patients with NIDDM (Williams, B., et al., J. Clin Endo Metab 1996:81:327-32). Unlike sulphonylureas, the insulinotropic action of GLP-1 is dependent on plasma glucose concentration (Holz, G.G. 4th, et al., Nature 1993:361:362-5). Thus, the loss of GLP-1-mediated insulin release at low plasma glucose concentration protects against severe hypoglycaemia. This combination of actions gives GLP-1 unique potential therapeutic advantages over other agents currently used to treat NIDDM.


[0005] GLP-1 is, however, metabolically unstable, having a plasma half-life (t1/2) of only 1-2 min in vivo. Exogenously administered GLP-1 is also rapidly degraded (Deacon, C.F., et al., Diabetes 44:1126-1131, 1995). This metabolic instability limits the therapeutic potential of native GLP-1. Hence, there is a need for GLP-1 analogues that are more active or are more metabolically stable than native GLP-1.

Summary of the Invention

[0006] In one aspect, the present invention is directed to a compound of formula:

\[ (\text{Aib}^{8,35})\text{hGLP-1(7-36)}\text{NH}_2, \]  

(1)

or a pharmaceutically acceptable salt thereof.


[0008] In another aspect, the present invention provides a pharmaceutical composition comprising an effective amount of a compound of formula (I) as defined hereinabove or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier or diluent.

[0009] In yet another aspect, the present invention provides the use of a compound of formula (1) or a pharmaceutically acceptable salt thereof for the manufacture of a medicament capable of eliciting an agonist effect from a GLP-1 receptor in a subject in need thereof.

[0010] In a further aspect, the present invention provides use of a compound of formula (1) or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of a disease selected from the group consisting of Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system disease, restenosis, neurodegenerative disease, renal failure, congestive heart failure, ne-
with the efficacy of some of the analogues is where the disease being treated is Type I diabetes or Type II diabetes.

The term "(C\textsubscript{1}-C\textsubscript{30}) hydrocarbon moiety" encompasses alkenyl and alkynyl, and in the case of alkenyl, there are C\textsubscript{2}-C\textsubscript{30}.

The term "halo" encompasses fluoro, chloro, bromo and iodo.

The full names for the abbreviations used herein are as follows: Boc for \(\text{t-butyloxycarbonyl}\), HF for hydrogen fluoride, Fm for formyl, Xan for xanthyl, BzI for benzyl, Tos for tosyl, DNP for 2,4-dinitrophenyl, DMF for dimethylformamide, DCM for dichloromethane, HBTU for 2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate, DIEA for diisopropylethylamine, HOAc for acetic acid, TFA for trifluoroacetic acid, DMAP for 4-dimethylaminopyridine, HOBt for N-hydroxybenzotriazolyl, and PAM resin for 4-hydroxymethylphenylaceticamidomethyl resin.

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The protected amino acid 1-(N-tert-butoxycarbonyl)alanine was added to the remaining aqueous solution. The peptide mixture was cooled in an ice-water bath. The pH of the aqueous layer was adjusted to about 3 by adding 4N HCl. The organic layer was separated. The aqueous layer was extracted with ethyl acetate (1 x 100 ml). The two organic layers were combined and washed with water (2 x 150 ml), dried over anhydrous MgSO\textsubscript{4}, filtered, and concentrated to dryness under reduced pressure. The residue was recrystallized in ethyl acetate/hexanes. 9.2 g of the pure product was obtained. 29% yield.

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In the synthesis of a GLP-1 analogue of this invention containing Aib, the coupling time is 2 hrs. for these residues and the residue immediately following them.

A compound of the present invention can be tested for activity as a GLP-1 binding compound according to the following procedure.

**Cell Culture:**

**Radioligand Binding:**

The peptides of this invention can be prepared by standard solid phase peptide synthesis. See, e.g., Stewart, J.M., et al., Solid Phase Synthesis (Pierce Chemical Co., 2d ed. 1984).

The protected amino acid 1-(N-tert-butyloxycarbonyl-alanine)-1-cyclohexane-carboxylic acid (Boc-A6c-OH) was synthesized as follows. 19.1 g (0.133 mol) of 1-amino-1-cyclohexane-carboxylic acid (Acros Organics, Fisher Scientific, Pittsburgh, PA) was dissolved in 200 ml of dioxane and 100 ml of water. To it was added 67 ml of 2N NaOH. The solution was cooled in an ice-water bath. 32.0 g (0.147 mol) of di-tert-butyl dicarbonate was added to this solution. The reaction mixture was stirred overnight at room temperature. Dioxane was then removed under reduced pressure. 200 ml of ethyl acetate was added to the remaining aqueous solution. The mixture was cooled in an ice-water bath. The pH of the aqueous layer was adjusted to about 3 by adding 4N HCl. The organic layer was separated. The aqueous layer was extracted with ethyl acetate (1 x 100 ml). The two organic layers were combined and washed with water (2 x 150 ml), dried over anhydrous MgSO\textsubscript{4}, filtered, and concentrated to dryness under reduced pressure. The residue was recrystallized in ethyl acetate/hexanes. 9.2 g of the pure product was obtained. 29% yield.

**Radioligand Binding:**

Membranes were prepared for radioligand binding studies by homogenization of the RIN cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000 g / 10 min), and the final pellets were resuspended in 50 mM Tris-HCl, containing 2.5 mM MgCl\textsubscript{2}, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% BSA. For assay, aliquots (0.4 ml) were incubated with 0.05 mM \((\text{GLP-1})\text{(7-36)}\) (2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing test peptides. After a 100 min incubation (25 °C), the bound (\((\text{GLP-1})\text{(7-36)}\)) was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac...
Accordingly, the present invention includes compositions wherein the compound of formula (I) is in association with a pharmaceutically acceptable carrier, as an active ingredient, a compound of formula (I) in association with a pharmaceutically acceptable carrier, as an active ingredient, a compound of formula (I) within its scope pharmaceutical compositions comprising:

- The peptide of this invention can be provided in the form of pharmaceutically acceptable salts. Examples of such salts include, but are not limited to, those formed with organic acids (e.g., acetic, lactic, maleic, citric, malic, ascorbic, succinic, benzoic, methanesulfonic, toluenesulfonic, or pamoic acid), inorganic acids (e.g., hydrochloric acid, sulfuric acid, or phosphoric acid), and polymeric acids (e.g., tannic acid, carboxymethyl cellulose, polylactic, polyglycolic, or copolymers of polylactic-glycolic acids). A typical method of making a salt of a peptide of the present invention is well known in the art and can be accomplished by standard methods of salt exchange. Accordingly, the TFA salt of a peptide of the present invention (the TFA salt results from the purification of the peptide by using preparative HPLC, eluting with TFA containing buffer solutions) can be converted into another salt, such as an acetate salt by dissolving the peptide in a small amount of 0.25 N acetic acid aqueous solution. The resulting solution is applied to a semi-prep HPLC column (Zorbax, 300 SB, C-8). The column is eluted with (1) 0.1N ammonium acetate aqueous solution for 0.5 hrs., (2) 0.25N acetic acid aqueous solution for 0.5 hrs. and (3) a linear gradient (20% to 100% of solution B over 30 min.) at a flow rate of 4 ml/min (solution A is 0.25N acetic acid aqueous solution; solution B is 0.25N acetic acid in acetonitrile/water, 80:20). The fractions containing the peptide are collected and lyophilized to dryness.

As is well known to those skilled in the art, the known and potential uses of GLP-1 is varied and multidinous (See, Todd, J.F., et al., Clinical Science, 1998, 95, pp. 325-329; and Todd, J.F. et al., European Journal of Clinical Investigation, 1997, 27, pp.533-536). Thus, the administration of the compounds of this invention for purposes of eliciting an agonist effect can have the same effects and uses as GLP-1 itself. These varied uses of GLP-1 may be summarized as follows, treatment of: Type I diabetes, Type II diabetes, obesity, glucagonomas, secretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system diseases, restenosis, neurodegenerative diseases, renal failure, congestive heart failure, nephrotic syndrome, cirrhosis, pulmonary edema, hypertension, and disorders wherein the reduction of food intake is desired. GLP-1 analogues of the present invention that elicit an antagonist effect from a subject can be used for treating the following: hypoglycemia and malabsorption syndrome associated with gastroectomy or small bowel resection.

Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, a compound of formula (I) in association with a pharmaceutically acceptable carrier.

The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. In general, an effective dosage for the activities of this invention is in the range of 1x10⁻⁷ to 200 mg/kg/day, preferably 1x10⁻⁴ to 100 mg/kg/day, which can be administered as a single dose or divided into multiple doses.

The compound of this invention can be administered by oral, parenteral (e.g., intramuscular, intraperitoneal, intravenous or subcutaneous injection, or implant), nasal, vaginal, rectal, sublingual or topical routes of administration and can be formulated with pharmaceutically acceptable carriers to provide dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than such inert diluents, e.g., lubricating agents such as magnesium stearate. The case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as coca butter or a suppository wax.

Compositions for nasal or sublingual administration.
tivation are also prepared with standard excipients well known in the art.


Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The following examples describe synthetic methods for making a peptide of this invention, which are well-known to those skilled in the art. The examples are provided for the purpose of illustration and is not meant to limit the scope of the present invention in any manner.

Boc-[Ala-OH, Boc-D-Arg(Tos)-OH and Boc-D-Asp(OcHex)] were purchased from Nova Biochem, San Diego, California. Boc-Aun-OH was purchased from Bachem, King of Prussia, PA. Boc-Ava-OH and Boc-Ado-OH were purchased from Chem-Impex International, Wood Dale, IL. Boc-Nal-OH was purchased from Synthetech, Inc. Albany, OR.

Example 1

(Aib8,35)hGLP-1(7-36)NH2

The title peptide was synthesized on an Applied Biosystems (Foster City, CA) model 430A peptide synthesizer which was modified to do accelerated Boc-chemistry solid phase peptide synthesis. See Schnolzer, et al., Int. J. Peptide Protein Res., 90:180 (1992). 4-methylbenzhydrolamine (MBHA) resin (Peninsula, Belmont, CA) with the substitution of 0.91 mmol/g was used. The Boc amino acids (Bachem, CA, Torrance, CA; Nova Biochem., LaJolla, CA) were used with the following side chain protection: Boc-Ala-OH, Boc-Arg(Tos)-OH, Boc-Asp(OcHex)-OH, Boc-Tyr(2BrZ)-OH, Boc-His(DNP)-OH, Boc-Val-OH, Boc-Leu-OH, Boc-Gly-OH, Boc-Gln-OH, Boc-Ile-OH, Boc-Lys(2ClZ)-OH, Boc-Thr(Bzl)-OH, Boc-Ser(Bzl)-OH, Boc-Phe-OH, Boc-Ala-OH, Boc-Glu(OcHex)-OH and Boc-Trp(Fm)-OH. The synthesis was carried out on a 0.20 mmol scale. The Boc groups were removed by treatment with 100% TFA for 2 x 1 min. Boc amino acids (2.5 mmol) were preactivated with HBTA (2.0 mmol) and DIEA (1.0 mL) in 4 mL of DMF and were coupled without prior neutralization of the peptide-resin TFA salt. Coupling times were 5 min. except for the Boc-Ala-OH residues and the following residues, Boc-Lys(2ClZ)-OH and Boc-His(DNP)-OH wherein the coupling times were 2 hours.

At the end of the assembly of the peptide chain, the resin was treated with a solution of 20% mercaptoethanol/10% DIEA in DMF for 2 x 30 min. to remove the DNP group on the His side chain. The N-terminal Boc group was then removed by treatment with 100% TFA for 2 x 2 min. After neutralization of the peptide-resin with 10% DIEA in DMF (1 x 1 min), the formyl group on the side chain of Trp was removed by treatment with a solution of 15% ethanolamine/15% water/70% DMF for 2 x 30 min. The peptide-resin was washed with DMF and DCM and dried under reduced pressure. The final cleavage was done by stirring the peptide-resin in 10 mL of HF containing 1 mL of anisole and dithiothreitol (24 mg) at 0°C for 75 min. HF was removed by a flow of nitrogen. The residue was washed with ether (6 x 10 mL) and extracted with 4N HOAc (6 x 10 mL).

The peptide mixture in the aqueous extract was purified on reverse-phase preparative high pressure liquid chromatography (HPLC) using a reverse phase VYDAC® C18 column (Nest Group, Southborough, MA). The column was eluted with a linear gradient (20% to 50% of solution B over 105 min.) at a flow rate of 10 mL/min (Solution A = water containing 0.1% TFA; Solution B = acetonitrile containing 0.1% of TFA). Fractions were collected and checked on analytical HPLC. Those containing pure product were combined and lyophilized to dryness. 135 mg of a white solid was obtained. Purity was 98.6% based on analytical HPLC analysis.

The molecular weight at 3339.7 (in agreement with the calculated molecular weight of 3339.7).

(Aib8,35)hGLP-1(7-36)NH2

Claim 1

1. A compound of formula:

(Aib8,35)hGLP-1(7-36)NH2

or a pharmaceutically acceptable salt thereof.
2. A compound as claimed in claim 1 or a pharmaceutically acceptable salt thereof for use as a medica-
ment for the treatment of disease.

3. A compound according to claim 2 or a pharmaceutically acceptable salt thereof for use in a phar-
aceutical composition comprising the compound and a pharmaceutically acceptable carrier or diluent.

4. A compound as claimed in claim 2 or a pharmaceutically acceptable salt thereof, in which the disease
is selected from the group consisting of Type I diabetes, Type II diabetes, obesity, glucagonomas, se-
cretory disorders of the airway, metabolic disorder, arthritis, osteoporosis, central nervous system dis-
ease, restenosis and neurodegenerative disease.

5. A compound as claimed in claim 4 or a pharmaceutically acceptable salt thereof, wherein said disease
is Type I diabetes or Type II diabetes.

6. Use of a compound according to claim 1 for the manufacture of a medicament capable of eliciting
an agonist effect from a GLP-1 receptor in a subject in need thereof.

Revendications

1. Composé de formule :

\[(\text{Aib}^{8, 35})\text{hGLP-1(7-36)NH}_2\] (I)

ou un sel pharmaceutiquement acceptable de celui-ci.

2. Composé selon la revendication 1 ou un sel pharmaceutiquement acceptable de celui-ci en vue de
l'utilisation comme médicament pour le traitement de maladies.

3. Composé selon la revendication 2 ou un sel pharmaceutiquement acceptable de celui-ci en vue de
l'utilisation dans une composition pharmaceutique comprenant le composé et un support ou diluant
pharmaceutiquement acceptable.

4. Composé selon la revendication 2 ou un sel pharmaceutiquement acceptable de celui-ci, dans le-
quel la maladie est choisie dans le groupe constitué par le diabète de type I, le diabète de type II, l'obé-
sité, les glucagonomes, les troubles sécrétoires des voies aériennes, les troubles métaboliques, l'arthri-
te, l'ostéoporose, les maladies du système nerveux central, la resténose et les maladies neurodégéné-
ratives.

5. Composé selon la revendication 4 ou un sel pharmaceutiquement acceptable de celui-ci, dans le-
quel ladite maladie est le diabète de type I ou le dia-
bète de type II.

6. Utilisation d'un composé tel que défini à la revendica-
tion 1 pour la fabrication d'un médicament capa-
bles de déclencher un effet agoniste à partir d'un ré-
cepteur du GLP-1 dans un sujet en ayant besoin.