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(54) **Title:** BLOOD GLUCOSE LOWERING COMPOUND

(57) **Abstract:** The present invention relates to the field of medicine and the treatment of diabetes or hyperglycemia within that field. More particularly, the invention relates to a compound that lowers blood glucose, pharmaceutical compositions containing such a compound, and therapeutic uses of such a compound.



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BLOOD GLUCOSE LOWERING COMPOUND

The present invention relates to the field of medicine. More particularly, the invention is in the field of treatment of diabetes and hyperglycemia. The invention relates to a compound that lowers blood glucose, pharmaceutical compositions containing such a
5 compound and therapeutic uses of such a compound.

Insulin replacement therapy for diabetic patients ideally would parallel as closely as possible the pattern of endogenous insulin secretion in healthy individuals. The physiological demand for insulin may be separated into two phases: (a) a nutrient absorptive phase requiring a pulse of insulin to dispose of the meal-related blood glucose
10 surge, also known as “prandial” insulin, and (b) a post-absorptive phase requiring a sustained delivery of insulin to regulate hepatic glucose output for maintaining optimal fasting blood glucose, also known as a “basal” insulin.

Effective insulin therapy for people with diabetes generally may involve the combined use of two types of exogenous insulin formulations: a rapid-acting, mealtime
15 prandial insulin, and a longer-acting basal insulin administered once or twice daily to control blood glucose levels between meals. One goal of insulin therapy is to mimic the normal pattern of endogenous insulin secretion and activity as closely as possible without causing hypoglycemia. Characteristics of endogenous insulin that may be desirable to emulate include a binding affinity for the human insulin receptors, preferential binding to
20 the human insulin receptors over the human IGF-1 receptor, phosphorylation of the human insulin receptors, and glucose lowering in the blood. Another desirable property of exogenous insulin may be to mimic the activity of endogenous insulin in the liver and peripheral tissues.

A desirable exogenous basal insulin should also provide an extended and “flat”
25 time action—that is, it would control blood glucose levels for at least 12 hours, and preferably for 24 hours or longer, without significant risk of hypoglycemia. Some basal insulins have a duration of action of 24 hours or more, but insulins with an even longer duration of action may assist more patients in achieving better glycemic control. A compound with an extended duration of action, such as a period greater than 24 hours,
30 may lower the risk of nocturnal hypoglycemia and allow greater variability in daily dosing times without increasing a patient’s risk of hypoglycemia. WO13130683

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generally describes extending the half-life of peptides through the use of a protein conjugate.

The need exists for a new and improved basal insulin compound with an extended and flat duration of action profile to treat diabetes and hyperglycemia in patients in need thereof.

The present compound comprises an insulin compound which provides an extended and flat time action and which has been shown to control blood glucose levels for up to 72 hours. The insulin compound of the present invention is projected to have a flat pharmacokinetic profile with daily dosing, with low peak-to-trough ratios during its extended duration of action. In an embodiment, the present invention provides a compound that is useful in treating diabetes, reducing hemoglobin A1c, and reducing blood glucose levels in patients in need thereof. The compound of the present invention demonstrates glucose lowering of up to 89% and duration of action of up to 72 hours.

The present invention provides an insulin compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain. The compound of the present invention may be referred to herein as Compound 330.

The present invention also provides a pharmaceutical composition comprising a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain and one or more pharmaceutically acceptable excipients.

The present invention further provides a method of treating diabetes or hyperglycemia in a patient comprising administering to a patient in need thereof an

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effective amount of a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain. In a further embodiment, the present invention provides a method of treating diabetes or hyperglycemia in a patient comprising administering an effective amount to a patient in need thereof of a pharmaceutical composition comprising a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain, and one or more pharmaceutically acceptable excipients.

The present invention provides a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain for use in therapy. The present invention further provides a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain for use in the treatment of diabetes. In a further embodiment, the present invention provides a compound comprising an A chain and a B chain, wherein the amino

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acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a
5 disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain for use in the treatment of hyperglycemia.

The present invention also provides the use of a compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond
10 between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain in the manufacture of a medicament for the treatment of diabetes. The present invention provides the use of a
15 compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the B chain is SEQ ID NO: 2; and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond
20 between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain in the manufacture of a medicament for the treatment of hyperglycemia.

The term “treatment” or “treating” as used herein refers to the management and care of a patient having diabetes or hyperglycemia, or other condition for which insulin administration is indicated for the purpose of combating or alleviating symptoms and
25 complications of those conditions. Treating includes administering a compound or compositions containing a compound of the present invention to a patient in need thereof to prevent the onset of symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder. The patient to be treated is an animal, and preferably a human being.

30 As used herein, the term “effective amount” refers to the amount or dose of a compound of the present invention or a pharmaceutical composition containing a

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compound of the present invention, which upon single or multiple dose administration to the patient or subject, will elicit the biological or medical response of or desired therapeutic effect on a tissue, system, animal, mammal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. A dose can include a
5 higher initial loading dose, followed by a lower dose.

The terms "patient," "subject," and "individual," used interchangeable herein, refer to an animal, preferably the terms refer to humans. In certain embodiments, the patient, preferably a human, is further characterized with a disease or disorder or condition that would benefit from lowering glucose levels in the blood.

10 Pharmaceutical compositions comprising the compound of the present invention may be administered parenterally to patients in need of such treatment. Parenteral administration may be performed by subcutaneous, intramuscular or intravenous injection by means of a syringe, optionally a pen-like syringe, or mechanical driven injector. Alternatively, parenteral administration can be performed by means of an infusion pump.
15 Embodiments of the present invention provide pharmaceutical compositions suitable for administration to a patient comprising administering to a patient in need thereof a therapeutically effective amount of a compound of the present invention and one or more pharmaceutically acceptable excipients. Such pharmaceutical compositions may be prepared by any of a variety of techniques using conventional excipients for
20 pharmaceutical products which are well known in the art. (Remington's Pharmaceutical Sciences, 21st Edition, University of the Sciences in Philadelphia, Philadelphia, PA, USA (2006)).

The compound of the present invention may be prepared by a variety of techniques known to one of skill in the art. The compound may be prepared via a
25 precursor protein molecule using recombinant DNA techniques. The DNA, including cDNA and synthetic DNA, may be double-stranded or single-stranded. The coding sequences that encode the precursor protein molecule described herein may vary as a result of the redundancy or degeneracy of the genetic code. The DNA may be introduced into a host cell in order to produce the precursor protein of the present invention. The
30 host cells may be bacterial cells such as K12 or B strains of *Escherichia coli*, fungal cells such as yeast cells, or mammalian cells such as chinese hamster ovary ("CHO") cells.

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The compound of the present invention may be prepared via a precursor protein having the amino acid sequence of SEQ ID NO: 3. An appropriate host cell is either transiently or stably transfected or transformed with an expression system for producing the precursor protein of the present invention. The expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors will contain selection markers, e.g., tetracycline, neomycin, and dihydrofolate reductase, to permit selection of those cells transformed with the desired DNA sequences.

Plasmids based on a modified pBR322 plasmid may be used for expression and expression from the plasmids may be induced by a *PhoA* promoter. The precursor protein may include a secretion signal to enable it to be secreted into the cell periplasm. Accumulation of the precursor protein may occur in any of the cytoplasm, the periplasm, and the extracellular growth media. The precursor protein folds and disulfide bonds are formed during the folding process. After a two to four day fermentation, the precursor protein product may be harvested from the medium, from the cell periplasm and/or from a total cell lysate.

The compound of the present invention may be prepared by a variety of procedures known in the art, as well as those methods described below. The specific synthetic steps for each of the routes described may be combined in different ways to prepare the compound of the invention.

Example 1

Expression and Purification of Compound 330

A precursor protein for Compound 330 is expressed in B strain *E. coli* bacteria, strain BL21 from New England Biolabs, product #C2530H. The precursor protein is produced from a modified pBR322 plasmid, whose gene is regulated by a *phoA* promoter. Typical fermentations are run from 36 – 72 hours during which the protein is secreted into the media.

Medium is conditioned by lowering the pH with glacial acetic acid. At a pH of 4, many contaminating proteins, lipids, cellular debris, and other matter precipitate and are removed by centrifugation. This conditioned media is put through a depth filter prior to an ultrafiltration/diafiltration (“UFDF”) step designed to remove salt and exchange the

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protein into 5 mM acetate, 10 mM NaCl, pH 4. The precursor protein is captured at low pH on Big Bead Q resin, an anion exchanger with large pore sizes to allow the passage of fines and other debris from the fermentation. The charged resin is washed and the precursor protein is eluted by salt gradient.

5 The pool from the anion exchange is treated with trypsin and carboxipeptidase B to remove regions of the precursor protein, generating a two-chain insulin. The cleavage process is performed at pH 7.5, and 15 °C, for 24 hours. The reaction is quenched by lowering the pH to 4 via acid addition. The resulting solution is concentrated and diafiltered by TFF into 5 mM Acetate, pH 4.

10 The compound is captured by a cation exchanger (Fast Flow S Sepharose) selected for its ability to bind the positively charged portion of the compound. The charged resin is washed and the compound is eluted by salt gradient. The pool from the cation exchange step is further purified by reversed phase chromatography. The compound is bound to a YMC basic C8 resin with 10 µm bead size in the presence of
15 0.16% phosphoric acid, and is eluted by an acetonitrile gradient. The final pool is prepared for storage by an overnight dialysis step, followed by concentration, and buffer exchange using spin concentrators to place it in a final buffer of 20 mM Tris, 135 mM NaCl, pH 7.5.

In Vitro Receptor Affinity

20 Binding affinities of proteins are determined in receptor binding assays performed on membranes prepared from stably-transfected 293EBNA cells (293HEK human embryonic kidney cells expressing EBNA-1) over-expressing human insulin receptor isoform A (hIR-A), stably transfected 293HEK cells over-expressing human insulin receptor isoform B (hIR-B) containing a C9 epitope tag at the C-terminus, or stably
25 transfected 293HEK cells over-expressing the human IGF-1 receptor (hIGF-1R).

 Receptor binding affinities (K_i) are determined from a competitive radioligand binding assay using either human recombinant (3-[125 I]-iodotyrosyl-A14)-insulin (2200 Ci/mmol, for hIR-A and hIR-B assays) or human recombinant [125 I]-insulin-like growth factor-1 (1800 to 2600 Ci/mmol, for the hIGF-1R assay), (Perkin Elmer Life and
30 Analytical Sciences). The assays are performed with a scintillation proximity assay (SPA) method using polyvinyltoluene (PVT) wheat germ agglutinin-coupled SPA beads

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(Perkin Elmer Life and Analytical Sciences). SPA Assay Buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% w/v fatty-acid free BSA) is used for all reagent preparations.

Serial dilutions of test samples are prepared in Assay Buffer using a Freedom/Evo robot (Tecan) and 50 μ L of dilution added to 96-well white, clear-bottom microplates (Corning/Costar) with a TeMO robot (Tecan). Radioligand (50 μ L), membranes (50 μ L), and SPA beads (50 μ L) are added using a Multi-drop (Thermo Scientific) instrument. Following 10-hour incubation at room temperature, radioactivity is determined using a Microbeta Trilux scintillation counter (Perkin Elmer Life and Analytical Sciences).

Values for test samples are calculated as percent relative to the activity of unlabeled human insulin or IGF-1 after correcting for non-specific binding. IC_{50} values are determined from 4-parameter logistic non-linear regression analysis (XLFit version 4.0, IDBS). If necessary, curve top or bottom parameters are set to 100 or 0, respectively. The affinity constant (K_i) is calculated from the IC_{50} value based upon the equation, $K_i = IC_{50} / (1 + D / K_d)$, where D equals the concentration of radio-ligand used in the experiment and K_d equals the equilibrium binding affinity constant of the radioligand determined from saturation binding analysis (hIR-A = 0.251 nM; hIR-B = 0.205 nM; hIGF-1R = 0.233 nM). Reported values for K_i are shown as geometric mean $\pm$ the standard error of the mean (SEM), with the number of replicate determinations indicated by "n" (Table 1). A qualifier (>) indicates that the data does not reach 50% inhibition, compared to maximum binding, whereby the K_i is calculated using the highest concentration of the compound tested in the assay and no standard error is calculated.

Compound 330 has binding affinity at both hIR-A and hIR-B and selectivity over hIGF-1R (Table 1).

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Table 1: Human Insulin Receptor Isoform A or B (hIR-A or hIR-B) and Human Insulin-Like Growth Factor-1 Receptor (hIGF-1R) Binding Affinity

Sample	Receptor Binding Affinity, K _i , nM (SEM, n)		
	hIR-A	hIR-B	hIGF-1R
Compound 330	4.53 (0.63, n = 10)	6.07 (0.60, n = 10)	>3530 (n = 5)
Human insulin	0.189 (0.006, n = 45)	0.233 (0.008, n = 45)	80.1 (5.8, n = 35)
IGF-1	4.10 (0.26, n = 45)	50.8 (2.7, n = 45)	0.111 (0.008, n = 33)

5

Receptor Functional Activation

Functional activity is determined by ELISA quantitation of hIR-A, hIR-B, or hIGF-1R auto-phosphorylation. Stably-transfected human 293HEK cells that over express hIR-A, hIR-B, or hIGF-1R, each containing a C-terminal C9 epitope tag (TETSQVAPA), are treated at 37 °C for 1 hour with 3-fold serially diluted test compounds in serum-free medium (DMEM, high glucose with glutamine, 10 mM HEPES, pH 7.4, 1 mM sodium pyruvate, 0.8 mg/mL geneticin, 1% penicillin/streptomycin) supplemented with 0.1% fraction V-fatty acid-free BSA (Sigma-Aldrich (St. Louis, MO, USA)). Cells are rinsed with ice cold PBS and lysed with ice cold NP40 buffer [1% NP-40 (IGEPAL CA-630), 150 mM NaCl, 50 mM TRIS, pH 7.4, 2 mM vanadate, and cOmplete™ protease inhibitors].

Tyrosine phosphorylation is determined using a sandwich ELISA by capture with anti-C9 monoclonal antibody (RHO 1D4 Antibody, University of British Columbia) and detection with anti-phosphotyrosine monoclonal 4G10®-horseradish peroxidase (4G10®-HRP) conjugate (EMD Millipore, Billerica, MA, USA) and 3,3',5,5'-tetramethylbenzidine (TMB) Pierce HRP substrate (Thermo Scientific, Rockford, IL, USA). The absorbance is recorded at 450 nm using a PerkinElmer Envision plate reader. The absorbance values are normalized to the maximal response of control cells treated with human insulin (100 nM, for the hIR-A and hIR-B assays) or 10 nM IGF-1 (for the hIGF-1R assay). The data

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are analyzed using a 4-parameter (curve maximum, curve minimum, EC50, Hill slope) logistic (sigmoidal) nonlinear regression routine (XLFit version 4.0: Activity Base, IDBS). Functional potency is reported as the concentration eliciting a half-maximal response (EC50), with values shown as the geometric mean $\pm$ the standard error of the mean (SEM), with the number of replicate determinations indicated by “n”.

Compound 330 is an agonist for hIR-A and hIR-B and is selective for insulin receptors compared to hIGF-1R (Table 2).

Table 2: Human Insulin Receptor (hIR-A and hIR-B) and Human Insulin-like Growth Factor-1 Receptor (hIGF-1R) Phosphorylation in 293 Cells

Sample	Receptor Phosphorylation EC50, nM (SEM, n)		
	hIR-A	hIR-B	hIGF-1R
Compound 330	39.2 (5.8, n = 12)	34.8 (4.9, n = 13)	4270 (611, n = 3)
Human insulin	2.65 (0.31, n = 12)	2.28 (0.28, n = 13)	254 (29, n = 6)
IGF-1	91.5 (12.1, n = 11)	253 (36, n = 4)	2.15 (0.22, n = 6)

Evaluation of In Vivo Potency in a Rat Model of Type 1 Diabetes

The effects of Compound 330 are investigated in a streptozotocin (STZ)-treated rat diabetes model. Male Sprague-Dawley rats, 400-425 gram body weight, are obtained from Harlan Labs, Indianapolis, IN. After acclimation for approximately one week, the rats are anesthetized with isoflurane and given a single injection of streptozotocin (Zanosar®, item #89256, Teva Parenteral Medicines, 40 mg/kg IV). The rats are used in studies three days after injection of the streptozotocin; only animals with non-fasted blood glucose between 400-550mg/dl are used in these studies.

Rats are distributed into groups to provide comparable variance in blood glucose and body weight; rats are randomized using Block Randomized Allocation Tool. Blood glucose is measured using Accucheck Aviva glucometer (Roche). STZ-treated rats are given a single subcutaneous (SC) injection of Compound 330 or vehicle, Sterile Normal Saline (0.9% w/v sodium chloride solution). Blood samples for glucose measurements are collected by tail bleed. The animals have free access to food and water throughout the experiment. Plasma samples from these studies are sent for analysis of compound levels.

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Blood glucose levels for Compound 330 are measured at three dosage levels, 30, 100, 300 nmol/kg in (STZ)-treated rats. Blood glucose is measured 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 36, 48, 72, and 96 hours after injection. Data shown are mean $\pm$ (standard error mean) SEM (n=5). Statistical analysis was performed using JMP software, and treatment groups are compared to a vehicle control group (* P < 0.05).

Compound 330 shows glucose lowering over the control group of up to 76% with a 100 nmol/kg dose at 8 hours and up to 89% with a 300 nmol/kg dose at 24 hours (Table 3).

Table 3: Blood Glucose Levels over Time after Administration of Compound 330 (mg/dL)

Time (hours)	Compound 330 100 nmol/kg (SEM)	Compound 330 300 nmol/kg (SEM)	Vehicle (SEM)
0	484 (28)	532 (13)	552 (6)
0.5	471 (52)	539 (18)	541 (7)
1	470 (50)	519 (8)	550 (14)
2	373 (55)	423 (30)	498 (12)
4	301 (53)	139 (24)	512 (30)
6	196 (46)	111 (41)	456 (13)
8	111 (17)	90 (15)	466 (23)
10	136 (18)	119 (12)	488 (31)
12	280 (61)	141 (25)	567 (20)
24	156 (48)	59 (6)	528 (8)
36	386 (76)	98 (7)	590 (10)
48	330 (66)	121 (26)	572 (13)
72	409 (58)	451 (22)	553 (13)
96	480 (51)	503 (9)	562 (10)

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Sequences

Polypeptide Sequence (SEQ ID NO: 1)

GIVEQCCTSI₁CSLYQLENYCGSGPAGGTSESATPESGPGSE₂PATSGSETPGTSESAT
PESGPGSE₃PATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEEGTSES
5 ATPESGPGSE₄PATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTSTEEGTS
TEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSE₅PATSGSETPG
SEPATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPATSGSET
PGTSESATPESGPGTSTEPSEGSAPG

10 Polypeptide Sequence (SEQ ID NO: 2)

FVNQHL₁CGSHLVEALYLVCGERGFFYTKPT

Polypeptide Sequence (SEQ ID NO: 3)

MKKNIAFL₁LLASMFVFSIATNAYAGSPGTSTEPSEGSAPGTSESATPESGPGTSESAT
15 PESGPGGAPGSGSGSGGGGGIEGRFVNQHL₂CGSHLVEALYLVCGERGFFYTKPTR
GGGGRGIVEQCCTSI₃CSLYQLENYCGSGPAGGTSESATPESGPGSE₄PATSGSETPG
TSESATPESGPGSE₅PATSGSETPGTSESATPESGPGTSTEPSEGSAPGSPAGSPTSTEE
EGTSESATPESGPGSE₆PATSGSETPGTSESATPESGPGSPAGSPTSTEEGSPAGSPTS
TEEGTSTEPSEGSAPGTSESATPESGPGTSESATPESGPGTSESATPESGPGSE₇PATSG
20 GSETPGSE₈PATSGSETPGSPAGSPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGSEPA
TSGSETPGTSESATPESGPGTSTEPSEGSAPG

WE CLAIM:

1. A compound comprising an A chain and a B chain, wherein the amino acid sequence of the A chain is SEQ ID NO: 1 and the amino acid sequence of the B chain is SEQ ID NO: 2, and wherein the A and B chains contain a disulfide bond between the cysteine at position 7 of the A chain and the cysteine at position 7 of the B chain, a disulfide bond between the cysteine at position 20 of the A chain and the cysteine at position 19 of the B chain, and a disulfide bond between the cysteine at position 6 of the A chain and the cysteine at position 11 of the A chain.
2. A pharmaceutical composition comprising the compound of Claim 1 and one or more pharmaceutically acceptable excipients.
3. A method of treating diabetes in a patient comprising administering to a patient in need thereof an effective amount of the compound of Claim 1 or the composition of Claim 2.
4. A method of treating hyperglycemia in a patient comprising administering to a patient in need thereof an effective amount of the compound of Claim 1 or the composition of Claim 2.
5. The compound of claim 1 for use in therapy.
6. The compound of claim 1 for use in the treatment of diabetes.
7. The compound of claim 1 for use in the treatment of hyperglycemia.
8. The use of a compound of claim 1, in the manufacture of a medicament for the treatment of diabetes.
9. The use of a compound of claim 1, in the manufacture of a medicament for the treatment of hyperglycemia.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/018085A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/28 A61P3/10
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)
A61K C07K A61P

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, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/130684 A1 (AMUNIX OPERATING INC [US]; SCHELLENBERGER VOLKER [US]; PODUST VLADIMIR) 6 September 2013 (2013-09-06) cited in the application claims 1-48	1-9
A	----- VOLKER SCHELLENBERGER ET AL: "A recombinant polypeptide extends the in vivo half-life of peptides and proteins in a tunable manner", NATURE BIOTECHNOLOGY, GALE GROUP INC, US, vol. 27, no. 12, 1 December 2009 (2009-12-01), pages 1186-1190, XP002665891, ISSN: 1087-0156, DOI: 10.1038/NBT.1588 [retrieved on 2009-11-15] the whole document ----- -/-	1-9



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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

3 April 2017

Date of mailing of the international search report

26/04/2017

Name and mailing address of the ISA/

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Authorized officer

Schnack, Anne

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/018085

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a. ☒ forming part of the international application as filed:
- ☐ in the form of an Annex C/ST.25 text file.
- ☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13~~ter~~.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
- ☐ in the form of an Annex C/ST.25 text file (Rule 13~~ter~~.1(a)).
- ☐ on paper or in the form of an image file (Rule 13~~ter~~.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/018085

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WILLIAM R. STROHL: "Fusion Proteins for Half-Life Extension of Biologics as a Strategy to Make Biobetters", BIODRUGS, vol. 29, no. 4, 16 July 2015 (2015-07-16), pages 215-239, XP55304636, NZ ISSN: 1173-8804, DOI: 10.1007/s40259-015-0133-6 the whole document -----	1-9
A	WO 2013/169547 A1 (LILLY CO ELI [US]) 14 November 2013 (2013-11-14) claims 1-85 -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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