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USE OF NONA- AND DECAPEPTIDES IN THE PREPARATION OF A DRUG FOR THE TREATMENT OF AIDS

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(57) Claim

1. Peptides with an amino acid sequence according to general Formula I

Ac-D-Nal(2)-D-Phe(4-Cl)-xxx-A-
-B-yyy-zzz-Arg-C-D-Ala-NH_2

wherein:

xxx = D-Pal(3), D-Phe(4-Cl), D-Trp

yyy = D-Cit, D-Lys(R), D-Arg, D-Hci

R is selected from the group consisting of (C_1-C_4)-acyl and (C_1-C_{10})-alkyl.

zzz = L-Leu, Nle, Nva, t-Leu

A = Ser, Ser (sugar)

with the sugar is selected from the group consisting of glucose, galactose, allose, altrose, mannose, gulose, idose and talose.

B = Tyr, Lys (Nic), Mop

C = Pro, Ala

or pharmaceutically acceptable salts thereof such as for example hydrochloride, trifluoroacetate, acetate, sulfate, phosphate, mesylate or tosylate, with the exception of the following peptides

Ac-D-Nal(2)^1-D-Phe(4-Cl)^2-(D-Pal(3) or D-Trp)^3-Ser^4-Tyr^5-(D-Cit or D-Hci)^6-Leu^7-Arg^8-Pro^9-D-Ala^{10}-NH_2

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(54) Title: USE OF NONA- AND DECAPEPTIDES IN THE PREPARATION OF A DRUG FOR THE TREATMENT OF AIDS

(54) Bezeichnung: VERWENDUNG VON NONA- UND DEKAPEPTIDEN ZUR HERSTELLUNG EINES ARZNEIMITTELS ZUR BEKÄMPFUNG VON AIDS

(57) Abstract

Described are LHRH-antagonistic and bombesin-antagonistic nona- and decapeptides suitable for use in the preparation of a drug for the treatment of AIDS and ARC as well as for use in the preparation of an immunostimulation drug.

(57) Zusammenfassung

LHRH-antagonistische und Bombesin-antagonistische Nona- und Dekapeptide eignen sich zur Herstellung eines Medikaments zur Bekämpfung von Aids und ARC sowie zur Herstellung eines Arzneimittels zur Immunstimulation.

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USE OF NONA- AND DECAPEPTIDES IN THE PREPARATION OF A DRUG FOR THE TREATMENT OF AIDS

Description

Commencing in 1981, a growing incidence of hitherto rare, incurable infections was observed in homosexual men in New York and San Francisco. Severe defects in immune resistance were found in all patients. Soon afterwards, Luc Montagnier at the Institut Pasteur discovered a new virus, the human immunodeficiency virus, HIV).

Currently, only the didesoxynucleoside analog 3'-azido-3'-desoxythymidine (AZT) or Zidovudine (INN) is registered for the treatment of AIDS. It relieves the symptoms and prolongs the mean survival time of AIDS patients (EP-A 206 497). AZT therapy is associated with the development of resistant virus strains and serious bone marrow side effects. Further medicaments are currently undergoing clinical trials. Thus, for example, German published patent 39 35 580 describes the use of 1-octadecyl-2-methyl-glycero-3-phosphocholine for the preparation of medicaments for combating HIV infections. EP-A 493 378 describes the use of 2'-3'-dedeoxyguanosin or of mono or triphosphates of 2'-3'-dedeoxyguanosin for the same purpose. All these substances are still undergoing clinical trials and are not yet ready to be marketed.

Medicaments currently used as antiviral chemotherapy do not display the desired selectivity. There is consequently a great need for well tolerated, highly effective medicaments which not only delay the course of the illness, but also repress proliferation of the viruses and are, in addition, able to stabilise the weakened immune system of the patients.

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It has now surprisingly been found in an AIDS screening trial on CEM-IW cells that the LHRH-analog decapeptides of formulae II - VIII display an anti-HIV and a growth stimulating effect on cell cultures.

The object of the invention is the preparation of a medicament based on peptide LHRH antagonists ~~and on bombesin antagonists.~~ The compounds show little toxicity, even in the highest dosages used.

The amino acid sequence of LHRH is:

LHRH = p-Glu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8
-Pro^9-Gly^{10}-NH_2

General formula I describes the peptides of the invention:

Ac-D-Nal(2)-D-Phe(4Cl)-xxx-A-

-B-yyy-zzz-Arg-Pro-D-Ala-NH_2 Formula I

where:

xxx = D-Pal(3), D-Phe(4Cl)

yyy = D-Cit, D-Lys (R), D-Arg, D-Hci

R may have the meanings (C_1-C_4)-acyl or (C_1-C_{10})-alkyl.

zzz = L-Leu, NLe, Nva, t-Leu

A = Ser, Ser(sugar)

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sugar may have the meaning glucose, galactose, allose, altrose, mannose, gulose, idose or talose

B = Tyr, Lys(Nic), Mop

According to a first embodiment of this invention there is provided peptides with an amino acid sequence according to general Formula I

Ac-D-Nal(2)-D-Phe(4-Cl)-xxx-A-
-B-yyy-zzz-Arg-C-D-Ala-NH_2

wherein:

xxx = D-Pal(3), D-Phe(4-Cl), D-Trp

yyy = D-Cit, D-Lys(R), D-Arg, D-Hci

R is selected from the group consisting of (C_1-C_4)-acyl and (C_1-C_{10})-alkyl.

zzz = L-Leu, Nle, Nva, t-Leu

A = Ser, Ser (sugar)

with the sugar is selected from the group consisting of glucose, galactose, allose, altrose, mannose, gulose, idose and talose.

B = Tyr, Lys (Nic), Mop

C = Pro, Ala

or pharmaceutically acceptable salts thereof such as for example hydrochloride, trifluoroacetate, acetate, sulfate, phosphate, mesylate or tosylate, with the exception of the following peptides

Ac-D-Na1(2)^1-D-Phe(4-Cl)^2-(D-Pal(3) or D-Trp)^3-Ser^4-Tyr^5-(D-Cit or D-Hci)^6-Leu^7-Arg^8-Pro^9-D-Ala^{10}-NH_2

According to a second embodiment of this invention there is provided a medicament for treatment of a disease selected from the group consisting of viral infections, AIDS and AIDS-related complex (ARC), comprising a pharmaceutically acceptable amount of at least one peptide according to the first embodiment or a pharmaceutically acceptable salt thereof.

According to a third embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of at least one peptide with an amino acid sequence according to the general Formula I

Ac-D-Nal(2)-D-Phe(4-Cl)-xxx-A-
-B-yyy-zzz-Arg-C-D-Ala-NH_2

wherein:

xxx = D-Pal(3), D-Phe(4-Cl), D-Trp

yyy = D-Cit, D-Lys(R), D-Arg, D-Hci

R is selected from the group consisting of (C_1-C_4)-acyl and (C_1-C_{10})-alkyl.

zzz = L-Leu, Nle, Nva, t-Leu

A = Ser, Ser (sugar)

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sugar is selected from the group consisting of glucose, galactose, allose, altrose, mannose, gulose, idose and talose.

B = Tyr, Lys(Nic), Mop

C= Pro, Ala

or a pharmaceutically acceptable salt thereof such as for example hydrochloride, trifluoroacetate, acetate, sulfate, phosphate, mesylate or tosylate, or of a medicament according to the second embodiment or a peptide according to the first embodiment or a pharmaceutically acceptable salt thereof.

According to a fourth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula II

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Nle^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to a fifth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula III

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Nva^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to a sixth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula IV

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Trp^3, D-Cit^6, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to a seventh embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula V

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Pro^9,D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to an eighth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral

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infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VI

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Hci^6, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to a ninth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VII

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, t-Leu^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

According to a tenth embodiment of this invention there is provided a method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VIII

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6,Ala^9,D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

Throughout the specification and claims D-Cit is D-Citrulline and Hci is Homocitrulline.

The appropriate abbreviations for amino acids, peptides and their derivatives are recommended by the IUPAC-IUB Commission for Biochemical Nomenclature. (European J. Biochem, 1984, 138, 9-37).

Abbreviations for less common amino acids:

Dpa represents 2,3-diaminopropionic acid

Nal represents 3-(2-naphthyl)-alanine

Thi represents β-2'-thienylalanine

Tpi represents 2,3,4,9-tetrahydro-1H-pyrido-[3,4-b]-indol-3-carboxylic acid

Nic represents Nicotinoyl

Mop represents 4-(morpholinomethyl)-phenylalanine

Particularly preferred compounds according to general Formula I constitute the following amino acid sequences:

Formula II = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal(3)^3, D-Cit^6, Nle^7, D-Ala^{10}]-LHRH

Formula III = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal(3)^3, D-Cit^6, Nva^7, D-Ala^{10}]-LHRH

Formula IV = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Trp^3, D-Cit^6, D-Ala^{10}]-LHRH

Formula V = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal(3)^3, D-Cit^6, D-Ala^{10}]-LHRH

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Formula VI = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal(3)^3, D-HCi^6, D-Ala^{10}]-LHRH

Formula VII = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal^3, D-Cit^6, t-Leu^7, D-Ala^{10}]-LHRH

Formula VIII = [Ac-DNal(2)^1, D-Phe(pCl)^2, D-Pal(3)^3, D-Cit^6, Ala^9, D-Ala^{10}]-LHRH

The oligopeptides of the invention are synthesised according to conventional processes known from the literature. A summarising description of the appropriate process is, for example, contained in M. Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, Berlin, Heidelberg, New York 1984.

The principles of the solid phase synthesis of polypeptides are for example set out in the textbook by J.M. Stewart and J.D. Young, Solid Phase Peptide Synthesis, Pierce Chem. Co., Rockford, Il, 1984 (2nd edition) and in the survey by L- Barany et al., Int.J. Peptide Protein Res. 30, 705-739 (1987).

The syntheses of the peptides according to formulae II - VIII occur according to the block diagram on methyl-benzhydryl amine resin (hydrochloride commercial form) of Advanced Chem. Tech/Louisville (Kentucky), USA, which was converted into the free base in each case before binding of the C-terminal Boc-D-Alanin by 10% triethylamine in dichloromethane (V/V).

All the following Nα-Boc-protected amino acids were coupled in threefold molar excess in the presence of diisopropyl carbodiimide (DIC) and 1-hydroxybenzotriazol (HOBt) in CH_2Cl_2/DMF within 90 minutes, in dichloromethane/dimethyl-formamide mixtures of the composition 80/20 (V/V) and the Boc protecting group by reaction of 50 % trifluoroacetic acid (TFA) in dichloromethane for half an hour. Remains of free amino functions are blocked by acetylation in fivefold excess of acetylimidazole in dichloromethane. The

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sequence of the reaction steps in the resin-supported peptide synthesis is shown in the block diagram. To split the resin-bound peptides, the end product of the solid phase synthesis was in each case dried in a vacuum and treated in 500fold excess on HF/anisol 10 : 1 (V/V) for 45 to 60 minutes at 0°C.

After distillation of HF and anisol in a vacuum, all raw peptide amides accumulated as white solids by stirring out with anhydrous ethyl ether, the separation of also accumulating polymer carriers occurred by washing out with 50% (V/V) aqueous acetic acid. Cautious concentration of the acetate solutions in a vacuum yielded the corresponding peptides as highly viscous oils which gradually changed into white solids after the addition of absolute ether in the cold. The raw peptides were isolated, washed on a frit with absolute ether and dried in a vacuum.
Preparative cleaning was carried out using high pressure liquid chromatography (HPLC) under the conditions cited:

Parameters for preparative HPLC

Apparatus:	Shimadzu LC-8A pump
	Shimadzu SPD-6A detector
	Shimadzu C-R4A integrator
	Shimadzu SCL-6A controller
Reagents:	Acetonitrile Lichrosolv Merck Art. 30
	Trifluoroacetic acid Fluka No. 91700
	Purest water (Seralpur installation)
Mobile phase A:	970 ml water (VE water purest)
	+ 30 ml acetonitrile
	+ 1 ml trifluoroacetic acid

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Mobile phase B:	300 ml water (VE water, purest)
	+ 700 ml acetonitrile
	+ 1 ml trifluoroacetic acid

Flow rate: 40 ml/min

Pressure: 14 bar

Average time of flow of a preparative passage: approx. 30 to 40 min.

The correct identity of all peptides synthesized was demonstrated after HPLC purification by means of amino acid analysis, mass spectrometry and ^{1}H-NMR-spectroscopy.

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Block diagram for the solid phase synthesis of the LH-RH peptides: the repeated working steps of the solid phase synthesis are summarised in the following diagram; steps 1 to 17 describe the requisite sequence of operating steps for attaching one amino acid in each case:

Step	Reagents and Operations	Time [min]
1	Boc-amino acid, DIC, HOBt 1 : 1 : 1	90
2	Colour test for total reaction, if positive: repeat 1 and 2	
3	MeOH, washing	1
4	DIPEA, 10 % in CH_2Cl_2; neutralisation	2
5	MeOH; washing	1
6	CH_2Cl_2; washing, 3-times	2
7	$(CH_3CO)_2O$, imidazole, 1 : 1, acetylation	30
8	MeOH; washing, twice	1
9	CH_2Cl_2; washing, 3-times	2
10	TFA, 50% in CH_2Cl_2, splitting Boc	1
11	TFA, 50% in CH_2Cl_2, splitting Boc	30
12	Isopropanol; washing and decolorisation	2
13	DIPEA, 10% in CH_2Cl_2; neutralisation	3
14	MeOH; washing	1
15	DIPEA, 10% in CH_2Cl_2; neutralisation	3
16	MeOH; washing	1
17	CH_2Cl_2; washing, twice	2

Synthesis product according to the above block diagram:

Ac-D-Nal(2)-D-Phe(4Cl)-Xxx-A-B-Yyy-Zzz-Arg-Pro-D-Ala-NH_2
(according to Formula I)

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The completeness of the synthesis was checked using the chloranil test after Th. Christensen; Acta Chem. Scand, B 33, 763 (1979) and using Kaiser's ninhydrin test after Stewart, Young.

The invention relates to a process for the preparation of a medicament for the treatment of viral infections, preferably for the treatment of AIDS. Novel peptides and their syntheses are also described which may be used for antiviral treatment. The peptides are of low toxicity, even at the highest dosage used. Compared to the reference compound azidothymidine tested at the same time, the EC50 values of the peptides tested were between 5.9×10^{-7} mol/litre and 2.0×10^{-5} mol/litre in the NCI experiment.

The reference compound AZT (azidothymidine) tested at the same time had, for example, an EC50 value of 3.10×10^{-9} mol/l. All compounds were dosed in the range of 10^{-4} to 10^{-8} mol/l. The conventionally stated IC50 value (inhibitory concentration in which 50% of cells die in the non-infected culture) is therefore higher than the highest dosage.

The infected culture treated has an EC50 value of 4.5×10^{-5} mol/l. Since the values for the treated, non-infected culture do not fall to an IC50 value, only the value for the highest dosage can be given for the IC50 value, that is IC50 = $>3.3 \times 10^{-5}$ mol/l. The therapeutic index (TI 50 = IC50/EC50) is then greater than 7.30.

Description of the screening method for anti-HIV activity

The process is suitable for locating active substances which are effective in all phases of the virus proliferation cycle. The test principle is based on the destruction of the T4 lymphocytes by the HIV virus.

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Small amounts of HIV virus are added to the cell cultures. At least two complete viral reproduction cycles are needed to destroy the T4 lymphocytes and to be able to evaluate the results.

Active substances that react with virions (virus-like particles), cells or with products of the viral genes in order to interact with viral activities and thus block proliferation of the viruses will protect the cells from destruction and lysis.
The test system is automated to permit examination of a large number of cells infected with viruses. Compounds which degenerate, denaturise or that are rapidly metabolised cannot, however, be reliably discovered using the test procedure.

AZT (azidothymidine) and DDC serve as positive controls for the test.

Test procedure

1. T4 lymphocytes (CEM cell line) are mixed with virus in the ratio of virus : cell of ca. 1 : 0.05 in microtiter plates.

2. Unless otherwise stated, the substance to be tested is dissolved in dimethylsulfoxide (DMSO) and diluted in a ratio of 1 : 200 (parts by weight).
   Other dilutions are prepared in semilogarithmic steps with DMSO and then added to infected and non-infected cell cultures.

3. The cultures are incubated for 6-7 days at 37°C in a 5% CO_2 atmosphere.

4. The tetrazolium salt XTT is added to the cell cultures and the cell cultures are further incubated to run the formazan colour reaction by coupling with phenazine methosulfonate (PMS) through surviving cells.

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5. The individual cell cultures are analysed spectrophotometrically and examined microscopically to detect surviving cells in order to confirm the protective effect.

6. Treated, virus-infected cells are compared with treated, non-infected cells. Other comparisons (untreated, infected cells and untreated, uninfected cells, active substance-containing, cell-free depressions) are carried out on the same plate.

7. The activity of the tested compound is determined.

The remaining experimental details may be taken from Weislow, J.Nat. Canc. Inst. 81 (8), page 577 (1989).

The peptides according to the invention are thus suitable for the preparation of medicaments for combating AIDS and for combating diseases that are associated with infection with the immune deficiency virus (AIDS-related complex, ARC).

Dosage information:

The dosage of the medicament of the invention is between 0.01 mg and 10 mg in the case of daily administration.

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Example 1

Peptide according to formula II:

Mass spectrum: $[M+H^+]$= 1431

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:
8.65-7.1; many multiplets, aromatic and NH signals; 7.0 and 6.6, 2.1, 4H, aromatic H Tyr; 5.95 m, $NHCONH_2$ Cit; 4.8 - 4.1, several multiplets, C_dH; 3.75 and 3.5, 2 multiplets; aliphatic H;3.2-2.65, several multiplets, CβH aliphatic and aromatic amino acids; 2.1-1.3, several multiplets, residual aliphatic H; 1.780, d,3H, CH_3CO^-; 1.20, d,3H, CβHAlla; 0.80, m, Nle.

Example 2

Peptide according to formula III:

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:
8.65-7.1; many multiplets, aromatic and NH signals; 7.0 and 6.6 2d, 4H, aromatic H Tyr; 5.9 m, $NHCONH_2$, Cit; 4.8 - 4.1, several multiplets, C_2H; 3.75 and 3.5, 2 multiplets; aliphatic H; 3.2-2.65, several multiplets, CβH aliphatic and aromatic amino acids; 2.1-1.25, several multiplets, residual aliphatic H; 1,70,5, 3H, CH_3CO^-; 1.20, d,3 H, CβHA,Ala; 0.85,m, 3H, Nva.

Mass spectrum: $[M+H^+]$= 1417

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Example 3

Peptide according to formula IV:

Mass spectrum: $[M+H^+]$= 1469

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:
8.2-6.5, many multiplets, aromatic and NH signals; 5.8 and 5.4, 2 m, 3 H, NH-CO-NH_2 citrollia, 4.5, 4.3, 4.2 and 4.0, multiplets C_dH, 3.8 - 2.6, several multiplets; aliphatic and aromatic CβH; 2.0-1.9, several multiplets, residual aliphatic protons; 1,6,5,3 H, CH_3CO^-; 1.1, d,2H, CβH Ala; 0.7, d,6 H Cδ H leu

Example 4

Peptide according to formula V, INN: cetrorelix

Mass spectrum: $[M+H^+]$= 1431

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:

8.7-7.2, many multiplets, aromatic and NH signals; 7.05 and 6.65, 2d, 4H, aromatic H Tyr; 5.85, m $NHCONH_2$, Cit; 4.8-4.1, several multiplets; CαH; 3.8 and 3.55, 2 m aliphatic H; 3.3 - 2.7 CβH aliphatic and aromatic amino acids; 2.1-1.3, several multiplets, residual aliphatic signals; 1,7,5,3 H, CH_3CO^-; 1.2, d, 3 H, CβH Ala; 0.85 2d, 6H, Cδ H leu

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Example 5

Peptide according to formula VI:

Mass spectrum: $[M+H^+]$= 1444

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:
8.6-7.1, many multiplets, aromatic and NH signals; 7.0 and 6.6, 2 d, 4 H, aromatic H Tyr; 4.8-4.0, several multiplets; C H; 3.7 and 3.5, 2 m 4 H; 3.2-2.7, several multiplets, CβH aromatic and aliphatic amino acids; 2.0-1.0, several multiplets, residual aliphatic signals; 1,7, 5, 3H, CH_3CO; 1.2,d, 3H, CβH Ala; 0.8, dd, 6H, CδH leu

Example 6

Peptide according to formula VII:

Mass spectrum: $[M+H^+]$= 1431

^{1}H-NMR-spectrum (DMSO-d_6, 250 MHz) δ in ppm:

8.6-7.1, many multiplets, aromatic and NH signals; 7.0 and 6.6, 2d, 4H, aromatic H Tyr; 5.9 m $NHCONH_2$, Cit; 4.8-4.0, several multiplets; CαH; 3.85 and 3.5, 2 multiplets, aliphatic H; 3.1-2.7 several multiplets CβH aliphatic and aromatic amino acids; 2.1-1.3, several multiplets, residual aliphatic H; 1,7,5,3 H, CH_3CO^-; 1.20, d, 3H, CβH Ala; 0.85, 5, 9H, t-butyl, Tle

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Example 7

Peptide according to formula VIII:

Mass spectrum: $[M+H^+]$= 1405

^{1}H-NMR-spectrum (DMSO-d_6, 500 MHz) δ in ppm:
9.1-7.3, many multiplets, aromat. and NH signals; 7.21, dd,4H, aromatic H p-cl-Phe; 7.0 and 6.6, 2d, 4 H, aromatic H Tyr; 5.9 and 5,4,2m, 3H, $NHCONH_2$ Cit; 4.7-4.1, several multiplets, C H; 3.55-2.8, several multiplets, CβH aliphatic and aromatic amino acids; 1,70,5, 3H, CH_3CO^-; 1.55 and 1,45,2 m aliphatic signals Arg and Leu; 0.80, dd, 6H, CδH Leu

AUSTRALIAN PATENT OFFICE 13

The claims defining the invention are as follows:

1. Peptides with an amino acid sequence according to general Formula I

Ac-D-Nal(2)-D-Phe(4-Cl)-xxx-A-
-B-yyy-zzz-Arg-C-D-Ala-NH_2

wherein:

xxx = D-Pal(3), D-Phe(4-Cl), D-Trp

yyy = D-Cit, D-Lys(R), D-Arg, D-Hci

R is selected from the group consisting of (C_1-C_4)-acyl and (C_1-C_{10})-alkyl.

zzz = L-Leu, Nle, Nva, t-Leu

A = Ser, Ser (sugar)

with the sugar is selected from the group consisting of glucose, galactose, allose, altrose, mannose, gulose, idose and talose.

B = Tyr, Lys (Nic), Mop

C = Pro, Ala

or pharmaceutically acceptable salts thereof such as for example hydrochloride, trifluoroacetate, acetate, sulfate, phosphate, mesylate or tosylate, with the exception of the following peptides

Ac-D-Na1(2)^1-D-Phe(4-Cl)^2-(D-Pal(3) or D-Trp)^3-Ser^4-Tyr^5-(D-Cit or D-Hci)^6-Leu^7-Arg^8-Pro^9-D-Ala^{10}-NH_2

2. Peptide as set forth in claim 1 with the amino acid sequence according to Formula II

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Nle^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

3. Peptide as set forth in claim 1 with the amino acid sequence according to Formula III

[Ac-D-Nal(2)^1, D-Phe(4-Cl^2, D-Pal(3)^3, D-Cit^6, Nva^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

4. Peptide as set forth in claim 1 with the amino acid sequence according to Formula VII

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, t-Leu^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

5. Peptide as set forth in claim 1 with the amino acid sequence according to Formula VIII

[Ac-D-Nal(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Ala^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

6. A medicament for treatment of a disease selected from the group consisting of viral infections, AIDS and AIDS-related complex (ARC), comprising a pharmaceutically acceptable amount of at least one peptide according to any one of claims 1-6 or a pharmaceutically acceptable salt thereof.

AUSTRALIAN PATENT OFFICE 13

7. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of at least one peptide with an amino acid sequence according to the general Formula I

Ac-D-Nal(2)-D-Phe(4-Cl)-xxx-A-
-B-yyy-zzz-Arg-C-D-Ala-NH_2

wherein:

xxx = D-Pal(3), D-Phe(4-Cl), D-Trp

yyy = D-Cit, D-Lys(R), D-Arg, D-Hci

R is selected from the group consisting of (C_1-C_4)-acyl and (C_1-C_{10})-alkyl.

zzz = L-Leu, Nle, Nva, t-Leu

A = Ser, Ser (sugar)

sugar is selected from the group consisting of glucose, galactose, allose, altrose, mannose, gulose, idose and talose.

B = Tyr, Lys(Nic), Mop

C= Pro, Ala

or a pharmaceutically acceptable salt thereof such as for example hydrochloride, trifluoroacetate, acetate, sulfate, phosphate, mesylate or tosylate, or of a medicament according to claim 6 or a peptide according to any one of claims 1-5 or a pharmaceutically acceptable salt thereof.

8. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula II

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Nle^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

9. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula III

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Nva^7, Pro^9,D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

10. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula IV

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Trp^3, D-Cit^6, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

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11. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula V

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

12. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VI

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Hci^6, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

13. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VII

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, t-Leu^7, Pro^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

14. A method for the treatment or prophylaxis of a disease selected from the group consisting of viral infections, AIDS and ARC in a patient in need of said treatment or prophylaxis, comprising administering to said patient an effective amount of a peptide with an amino acid sequence according to Formula VIII

[Ac-D-Na1(2)^1, D-Phe(4-Cl)^2, D-Pal(3)^3, D-Cit^6, Ala^9, D-Ala^{10}]-LHRH

or a pharmaceutically acceptable salt thereof.

Dated 25 November, 1997
ASTA Medica Aktiengesellschaft
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

AUSTRALIAN PATENT OFFICE

INTERNATIONAL SEARCH REPORT

Interna' l Application No
PCT/EP 94/01037

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K37/43 A61K37/02

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)
IPC 5 C07K A61K

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DIALOG,File 157:AIDSLINE, No. 93333809 Int.Conf. AIDS (GERMANY),June 6-11,1993 9(1), p195 (abstr.no. PO-A18-0362) see the whole document ---	1-8, 10-12
A	EP,A,0 335 726 (IMMUNOBIOLOGY RESEARCH INSTITUTE) 4 October 1989 see the whole document ---	1-12
X	EP,A,0 299 402 (ASTA PHARMA AG) 18 January 1989 see the whole document ---	1-8
X	EP,A,0 192 492 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 27 August 1986 see the whole document ---	1-8

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

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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"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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 August 1994	31 -08- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Groenendijk, M

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INTERNATIONAL SEARCH REPORT

Internat l Applicaton No
PCT/EP 94/01037

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 09626 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 11 June 1992 see the whole document	1,9
X	WO,A,90 03980 (ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 19 April 1990 see the whole document; in particular page 37, line 26 - page 38, line 3	1,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern· ·al Application No
PCT/EP 94/01037

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0335726	04-10-89	WO-A-	8909229	05-10-89
EP-A-0299402	18-01-89	US-A-	4800191	24-01-89
		DE-A-	3823590	26-01-89
		DE-A-	3881592	15-07-93
		FI-C-	91075	10-05-94
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		WO-A-	9102746	07-03-91
		US-A-	5084555	28-01-92

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/EP 94/01037

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 5 A61K37/43 A61K37/02

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 5 C07K A61K

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	DIALOG,File 157:AIDSLINE, No. 93333809 Int.Conf. AIDS (GERMANY),June 6-11,1993 9(1), p195 (abstr.no. PO-A18-0362) siehe das ganze Dokument	1-8, 10-12
A	EP,A,0 335 726 (IMMUNOBIOLOGY RESEARCH INSTITUTE) 4. Oktober 1989 siehe das ganze Dokument	1-12
X	EP,A,0 299 402 (ASTA PHARMA AG) 18. Januar 1989 siehe das ganze Dokument	1-8
X	EP,A,0 192 492 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 27. August 1986 siehe das ganze Dokument	1-8

-/--

[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

* Besondere Kategorien von angegebenen Veröffentlichungen :
"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist
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"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
9. August 1994	31 -08- 1994

Name und Postanschrift der Internationale Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Groenendijk, M

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	WO,A,92 09626 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 11. Juni 1992 siehe das ganze Dokument	1,9
X	WO,A,90 03980 (ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 19. April 1990 siehe das ganze Dokument;speziell Seite 37, Zeile 26 - Seite 38, Zeile 3	1,9

INTERNATIONALER RECHERCHENBERICHT

Angaben zu Veröffentlichungen, die zur selben Patentfamilie gehören

Interne Aktenzeichen
PCT/EP 94/01037

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		CA-A-	2008454	02-09-90
		AU-B-	648037	14-04-94
		AU-A-	6294090	03-04-91
		CA-A-	2064896	22-02-91
		EP-A-	0489089	10-06-92
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