

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 June 2006 (22.06.2006)

PCT

(10) International Publication Number
WO 2006/064530 A2

(51) International Patent Classification:
C07K 7/06 (2006.01)

(74) Agents: SANTI, Filippo et al.; Ing. Barzano' & Zanardo
Roma S.p.A., Via Piemonte 26, I-00187 Roma (IT).

(21) International Application Number:
PCT/IT2005/000733

(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.

(22) International Filing Date:
14 December 2005 (14.12.2005)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
RM2004A000607
15 December 2004 (15.12.2004) IT

(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).

(71) Applicant (*for all designated States except US*): BIOGEN S.r.l. [IT/IT]; Viale Battista Bardanzellu, 58/60, I-00155 Roma (IT).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): MELCHIORRI, Pietro [IT/IT]; Biogen S.r.l., Viale Battista Bardanzellu, 58/60, I-00155 Roma (IT). NEGRI, Lucia [IT/IT]; Biogen S.r.l., Viale Battista Bardanzellu, 58/60, I-00155 Roma (IT). ROCCHI, Raniero [IT/IT]; Biogen S.r.l., Viale Battista Bardanzellu, 58/60, I-00155 Roma (IT). CIOCCA, Luigi [IT/IT]; Biogen S.r.l., Viale Battista Bardanzellu, 58/60, I-00155 Roma (IT).

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: DERMORPHIN ANALOGS WITH ANALGESIC ACTIVITY

(57) Abstract: New opioid heptapeptide analogs of dermorphin characterised by the fact that, like dermorphin, they maintain a tyrosine residue in position 1 and a D- α -amino acid, preferably D-Ala, in position 2, and by the fact that in position 7 they have the amino acid of maximum basicity, i.e. arginine. The preferred peptide of the invention, Arg⁷-dermorphin, referred to as arginorphin, has a potent analgesic activity. This activity largely comes about at the peripheral opioid nociceptor level, without involving the more serious central side effects typical of opiates.



WO 2006/064530 A2

DERMORPHIN ANALOGS WITH ANALGESIC ACTIVITY

5 The present invention concerns dermorphin analogs with analgesic activity. More specifically, the invention concerns some new opioid heptapeptides having by a potent analgesic action that takes place substantially at the level of the receptors for peripheral opioid pain stimuli (nociceptors) without involving the more serious central side effects typical of opiates.

10 As is known, opiate alkaloids – of which morphine is the main exponent – are substances of plant origin that have powerful narcotic and analgesic properties long used in pain therapy, but subject to considerable limitations due to their many critical side effects. Morphine and its synthetic analogs may be administered as analgesics only for limited time periods due to the onset of gastrointestinal problems (constipation), or a growing tolerance to the
15 drug (requiring ever greater doses in order to obtain the desired analgesic effect), or even other adverse reactions such as respiratory depression and catalepsy. Opiate alkaloids are also notorious for their addictive property.

 It is also known that a large number of endogenic peptides, found in various regions of the central nervous system, show similar or correlated activities to those of opiates – bonding to or influencing in some other way the
20 opiate receptors and causing both agonistic and antagonistic effects to those exerted by opiates. Examples of such endogenic peptides are the enkephalins, two natural pentapeptides found in the brain, spinal chord and gastroenteric tract, that have opioid-like effects. Other examples are the dynorphins, opioid peptides present both in the central and peripheral nervous system,
25 some of which are involved in the regulation of pain, others in the hypothalamic regulation of hunger and thirst stimuli. Similar molecules of a peptidic type, both endogenous and derived from endogenous ones through synthetic modifications, that have activities similar to those of opiates, are generically
30 referred to as opioids.

 Various pharmacological studies carried out over time on this topic have led to the identification of several opioid receptors. Of these, the so-

called δ receptors show a selective affinity for enkephalins, the so-called μ receptors show greater selectivity for morphine and for other poly-cyclic alkaloids while the so-called κ receptors show a comparable affinity for both the aforesaid groups of ligands and a preferential affinity for dynorphins. In general, it has been established that the μ receptors are more often involved with analgesic effects, even if these effects may at times also be mediated by the δ or κ receptors.

In general, opioid peptides offer a model of drugs that are agonistic of the opioid receptors and unable to pass through the blood-brain barrier (hemato-encephalic barrier). This inability has been related to the poor capacity of the peptidic molecule to pass through the lipid membrane of cell walls. However, it is currently acknowledged that several categories of peptides can pass through the endothelial cell membranes of brain blood microvessels due to specific transfer systems. These systems actually enable the selective permeation of the hemato-encephalic barrier by some suitably modified peptides.

Some peptides pass through the hemato-encephalic barrier and can thus enter the central nervous system after peripheral administration because they are recognised by specific transportation systems located on blood vessel endothelia (Banks, W.A., Kastin, A.J., *Am. J. Physiol.* 259, E1-E10, 1990); others, glycosilated, use the sugar transfer systems (Negri, L. et al., *Br. J. Pharmacol.* 124, 1516-1522, 1998); others still, containing basic aminoacids, can pass through the barrier via the endothelial pinocytosis mechanism. As is known, the latter represents the mechanism through which cells engulf external fluid and its contents by forming invaginations in the cell membrane, which later close and open so as to form cytoplasmic vacuoles full of liquid.

In view of the above, research into opioid peptides has been geared to identifying the more suitable modifications to known or newly discovered endogenous peptides in order to obtain agents with opioid-like activity that could reach the opioid receptors of the central nervous system so as to produce pharmacological effects comparable to those of morphine. A further hindrance in this field is the fact that peptides are naturally subject to enzy-

- 3 -

matic degradation and are attacked by proteolytic enzymes which quickly hydrolyze them, drastically reducing their *in vivo* half-life.

For the above reasons, the activity of an opioid peptide as an analgesic is considerably different depending on the administration method used and the site of action. In order to obtain an analgesic effect at a central level, unlike what happens with opiate alkaloids, it is theoretically necessary to inject the drug intracerebroventricularly.

One opioid peptide that has received some attention over the last few years is dermorphin (Montecucchi, P.C. et al., *Int. J. Peptide Protein Res.*, 17, 275-283, 1981; Broccardo M. et al., *Br. J. Pharmacol.*, 73, 625-631, 1981), a heptapeptide isolated from methanol extracts from the skin of a South American frog species – *Phyllomedusa sauvagei* – characterised by the following aminoacid sequence:

H-Tyr-D-Ala- Phe-Gly-Tyr-Pro-Ser-NH₂ (A)

Dermorphin is the first example, in an animal organism, of a D-aminoacid, essential for biological activity, inserted in a peptide molecule. It has been hypothesised that the conversion of L-alanine into D-alanine is post-transcriptional.

When first discovered, dermorphin represented the most potent natural peptide described until then, acting selectively on the μ receptors. Either intracerebroventricularly or intratecally, its analgesic and catatonizing power was 2000-4000 times greater than that of morphine, 40 times greater than that of β -endorphine and 10,000 times greater than that of the enkephalins. However, also dermorphin entails a rapid development of addiction and the inevitable withdrawal symptoms when a chronic administration of the substance is interrupted.

Closely connected to the aforesaid two scientific publications of 1981 is the British patent GB 2166139 (Farmitalia Carlo Erba), filed on 25 October 1984, concerning numerous dermorphin analogs of both heptapeptide and pentapeptide type, and their pharmaceutically acceptable salts. With reference to the dermorphin sequence shown in (A), all the heptapeptides de-

- 4 -

scribed in the document are characterised by the fact that the last two amino-acids are maintained unchanged with respect to the natural peptide (Pro-Ser).

The peptides of the aforesaid British patent are described generically as biologically active and having interesting pharmacological properties as analgesics and antipsychotics. The analgesic activity of two of them is presented as assessed in rats according to the "tail pinch" pharmacological test (Bianchi, C. & Franceschini, J., *Br. J. Pharmacol.*, 9, 280, 1954), after subcutaneous administration, with no comparison with morphine as a reference drug and with no data on the duration of the analgesic action found.

In studies carried out after its discovery, it was found that the in-vitro degradation of dermorphin firstly produces hydrolysis in the Gly-Tyr bond of the peptide of formula (A), dermorphin(1-4) being the main metabolite. On the basis of these premises, the US patents US 5312899 and US 5602100, and European patent EP 350221 (designated inventor: Peter W. Schiller), all belonging to the same family, claim – as dermorphin analogs active in pain therapy and in the treatment of gastrointestinal disorders – numerous synthetic tetrapeptides constituting variants of dermorphin(1-4). Of these, the tetrapeptide DALDA (Tyr-D-Arg-Phe-Lys-NH₂) was later described as a potent and selective agonist of the μ opioid receptor (Schiller P.W. et al., *J. Med. Chem.*, 32, 698-703, 1989), particularly suitable as an anti-diarrhoeic. As with natural dermorphin, also in the case of the tetrapeptide analog to the C-end fragment (1-4) thereof it was found, in the cited literature, that the presence of a D- α -aminoacid in position 2 makes the claimed peptides particularly resistant to degradation by the proteases ubiquitously found in the gastrointestinal tract.

The present invention lies within the aforesaid research field, with a project (No. 10966 of 20 July 2000) funded by the Italian Ministry of Universities and of Scientific and Technical Research (MURST), dealing with the "pharmacological design, synthesis and evaluation of peptides resistant to proteolytic degradation and having a high penetration through the hematoencephalic barrier". Within said research project a total of 68 different peptides

- 5 -

were studied. These were obtained via various modifications starting from already known peptide molecules. These modifications were aimed at improving the activity of the known peptide agents in order to obtain the pharmacological effects of the reference analgesic product – morphine – but at the same time
5 avoiding the already mentioned side effects.

In view of the above, the present invention thus aims to provide new pharmacologically active opioid peptides having a good penetration capacity through the hemato-liquoral barrier, in order to enter the spinal chord fluid, but – above all – being devoid of the known effects on the central nervous system of the opiate drugs currently in use.
10

The demonstration of the existence of opioid receptors on the peripheral nociceptors has paved the way to research into drugs capable of producing analgesic effects by acting on localised opioid receptors at the level of peripheral nerve endings of the primary nociceptors. In the light of the foregoing literature, it is generally envisaged that the opioid peptides administered
15 through a peripheral channel do not reach – or only in small part reach – the opioid receptors of the central nervous system, but reach the peripheral receptors.

The synthesis of opioid peptides resistant to enzymatic degradation and having a sufficiently long half-life provides, according to the present invention, new analgesic drugs having peripheral activity that are devoid of the side effects on the central nervous system that are typical of the opiate drugs hitherto in use (constipation, respiratory depression, addiction, tolerance, catalepsy).
20

Therefore, the present invention specifically provides a peptide analog of dermorphin having the following general formula:
25



wherein:

B^2 represents a D- α -aminoacid,

30 A^3 , A^4 , A^5 and A^6 each represent an L- α -aminoacid, optionally substituted in the side chains with the substituent groups commonly found in natural amino-

acids, such as linear or branched alkyl groups, or phenyl or benzyl groups, in turn possibly substituted either with t-butoxycarbonyl (Boc) protective groups or fluorenyl-methyl-oxycarbonyl (Fmoc) groups, or a pharmaceutically acceptable salt thereof.

5 Preferably, in the proposed heptapeptide, **B²** is selected from among D-Ala, D-Arg, D-Val, D-Ile, D-Leu, D-Pro, D-Ser, D-Thr, D-Met, D-Lys or D-Orn, and more preferably it represents D-alanine.

 According to some specific options in relation to the aminoacids lying in positions 3-6, they can be the same as the ones of native dermorphin.
10 Therefore, independently of one another, **A³** preferably represents phenylalanine, **A⁴** preferably represents glycine, **A⁵** preferably represents tyrosine and **A⁶** preferably represents proline.

 The most deeply studied version of the peptide according to the present invention corresponds to Arg⁷-dermorphin and has the following general formula:
15



 This product has been called **arginorphin**. The present invention obviously also extends to the pharmaceutically acceptable salts of arginorphin.

20 As already noted, all the peptides according to the present invention are characterised by the fact that in position 1 they contain a link element made up of tyrosine, in position 2 an aminoacid in destrorotatory configuration, resistant to enzymatic degradation, and in position 7 the aminoacid presenting the maximum basicity, i.e. arginine.

25 According to a further aspect thereof, the present invention also concerns the use of a peptide analog of dermorphin as defined above, or one of its pharmaceutically acceptable salts, for the preparation of an opioid drug for pain therapy and/or for the treatment of gastrointestinal disorders, and/or having antipsychotic or anxiolytic activity. Preferably, as will be more evident
30 below, the proposed drug has a peripheral analgesic activity.

 In view of the above, the present invention further specifically provides, according to a preferred embodiment thereof, the use of Arg⁷-

- 7 -

dermorphin (arginorphin), having the following general formula:



or of a pharmaceutically acceptable salt thereof, for the preparation of a drug having a peripheral analgesic activity.

5 According to other specific embodiments thereof, the present invention also concerns a pharmaceutical composition for pain therapy and/or for the treatment of gastrointestinal disorders, and/or having antipsychotic or anxiolytic activity, containing a peptide analog of dermorphin, as defined above, or one of its pharmaceutically acceptable salts, along with any pharmaceutically acceptable carrier or excipient. In the case where the peptide of
10 the pharmaceutical formulation is arginorphin, the present invention specifically concerns a drug composition with peripheral analgesic activity and containing as active ingredient Arg⁷-dermorphin or a pharmaceutically acceptable salt thereof.

15 The specific features of the peptides of the present invention, as well as their advantages and synthesis methods, will be more evident with reference to the detailed description below, presented merely for exemplification purposes, together with the results of the experimentations carried out on the proposed peptides and some comparison data with alternative opioid peptides.
20

EXAMPLE

Solid phase synthesis of arginorphin (Arg⁷-dermorphin)

25 The synthesis of the peptide **H-Tyr-D-Ala- Phe-Gly-Tyr-Pro-Arg-NH₂**, called arginorphin, was carried out using the Advance Chemtech 348 Peptide Synthesizer, on a 0.06 mmol scale, and the resin Rink Amide MBHA ([4-(2'4'-dimethoxyphenyl-fluorenyl-methyl-aminomethyl-phenoxyacetamido-norleucyl-4-methyl-4-benzhydrylamino-polystyrene] – 0.085 g, substitution degree 0.73 mmol/g). The synthesis was carried out by the FastMoc method:
30 HBTU/HOBt/ DIEA (O-benzotriazolyl-N,N,N',N'-tetramethyluronio hexafluorophosphate/1-hydroxybenzotriazole/N-ethyl-diisopropylamine), solvent N-methylpyrrolidone (NMP), single acylation, coupling time of 45 min.

- 8 -

The residue of arginine was introduced as Fmoc-Arg(Pbf)-OH [N^G-Fmoc-N^G-(2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl)-L-arginine (Fmoc: fluorenyl-methyl-oxycarbonyl). To avoid a possible intra-chain aminolysis at the dipeptide level, and the consequent loss of the dipeptide in the form of diketopiperazine, in the acylation phase the dipeptide Fmoc-Tyr(But)-Pro-OH (But: *tert*-butile) was employed, containing the second and third aminoacids.

The final peptide-resin was unblocked in amine nitrogen by treatment with 20% piperidine in NMP, carefully washed with NMP and dried. The detachment of the resin and the simultaneous removal of the protective groups in the side chain was carried out by treatment with a mixture of trifluoroacetic acid (TFA), water and triethyl silane (95/2,5/ 2,5 in vol.) (4 ml/100 mg of peptide-resin, 2 hours at room temperature). The acidic solution was concentrated under vacuum and the peptide precipitated with an excess of *tert*-buthyl-methyl ether, collected by centrifugation, dissolved in a small volume of trifluoroacetic acid and reprecipitated twice with *tert*-buthyl-methyl ether, dried and further purified by semipreparatory HPLC (high performance liquid chromatography) and finally characterised by analytical HPLC, composition determination and molecular weight determination.

The final yield was in the region of 65-70%.

COMPARATIVE EXAMPLE

Synthesis of glycosilated derivates of endomorphins

H-Tyr-Pro-Trp-Phe-NH₂ and H-Tyr-Pro-Phe-Phe-NH₂

In order to compare the activity of the heptapeptide analogs of dermorphin with other peptide products obtained by suitably modified endogenous agents in order to increase their passage through the hemato-encephalic barrier, the study considered endomorphins, i.e. two endogenous tetrapeptides with the following formulas, respectively:

H-Tyr-Pro-Trp-Phe-NH₂ (Endomorphin 1)

H-Tyr-Pro-Phe-Phe-NH₂ (Endomorphin 2).

These were modified obtaining their respective glycosilated de-

rivates which, according to the literature and to what has been reported above, could present an enhanced capacity to pass through the hemato-encephalic barrier.

5 Peptide synthesis was carried out in the solid phase by means of an Advance Chemtech 348 Peptide Synthesizer, in the scale indicated and using the appropriate resin.

Purification of the raw products was carried out by semipreparatory HPLC (with a Vydac C18 column, 22x250 mm, 10 μ) using a Shimadzu LC-8A chromatograph with a SPD-6A spectrophotometric detector and Perkin Elmer
10 561 recording system. Elution was carried out with a concentration gradient selected each time on the basis of the chemical-physical properties of the product undergoing purification, using the following binary system:

A: 0.1% aqueous trifluoroacetic acid (TFA)

B: 0.1% TFA in a CH₃CN/H₂O mixture (9:1 v/v).

15 HPLC grade acetonitrile (CH₃CN) is a product manufactured by Carlo Erba, the milliQ water was obtained by purifying deionised water with the Reagent Grade Water System of Millipore.

The fractions containing the product were collected and dried to a constant weight. The purity of the single fractions was determined by analytical HPLC on a Vydac C18 column (4,6x250 mm, 10 μ), using a Perkin Elmer
20 series 410 chromatograph complete with solvent pressurisation box, mod. SEC-4, a LC-90 UV spectrophotometric detector and an LCI-100 integrator.

The exact aminoacid composition of the peptides was assessed by analysing the aminoacids after hydrolysis with 6N HCl (with 0.5% phenyl acting as a scavenger for the tyroxine), under vacuum, at 110°C for 24 hours.
25 The instrument used was the Carlo Erba automatic analyser mod. 3A30. The aminocacids were derivatised by reacting with ninhydrin and detected by recording the UV-visible absorption at λ = 570 nm. The proline content was determined by recording the absorption at λ = 440 nm. The tryptofan was degraded during acid hydrolysis.
30

The proton resonance spectra at 200 MHz were recorded with a

- 10 -

Bruker WP 200 SY spectrophotometer in the solvent indicated.

The mass spectra were obtained with the Perspective Biosystem Mariner 5220 instrument manufactured by Applied Biosystem, with the ionisation technique ESI-MS (electrospray ionisation mass spectrometry), and a TOF detector.

Preparation of H-Tyr-Pro-Trp-Phe-NH₂

a) **Boc-Tyr(But)-Pro-Trp(Boc)-Phe-OH**

The peptide Boc-Tyr(But)-Pro-Trp(Boc) (Boc: tert-butyl-oxycarbonyl) was synthesised in the solid phase according to the Fmoc protocol, and acid-labile protections were used for the side chain functions and for the N-terminal amine group. A solution of 0.5M HBTU/HOBt was used as a coupling reagent; an excess of 4 eq. of Fmoc-AA-OH and of coupling reagent was used, together with an excess of 8 eq. of DIEA.

The synthesis was carried out on Fmoc-Phe-SASRIN® resin (Bachem) (resin SASRIN: 2-methoxy-4-alkoxybenzyl alcohol) with 0.7 mmol/g loading, using 1 g of resin.

For peptide detachment from the resin, a 1% TFA solution in dichloromethane (DCM) was used; the resin underwent repeated short-term treatments (5-10 min) with the unblocking mixture and monitoring was carried out via TLC (thin-layer chromatography) of the filtrate composition.

The various fractions were recollected and concentrated in small volumes, the residue was taken again with ethyl acetate (AcOEt) and washed in H₂O up to pH 3. The organic phase was then dehydrated on Na₂SO₄ and concentrated to dryness.

496 mg of peptide was obtained (81% yield). ESI-MS: 868.46 (calculated as C₄₈H₆₁N₅O₁₀ = 864.44).

b) **Boc-Tyr(But)-Pro-Trp(Boc)-Phe-Glc(Ac₄)**

An amount of 0.5 mole of tetrapeptide was dissolved in 10 ml of DCM, to which was added HBTU (1.1 eq., equal to 208 mg), HOBt (1.1 eq., equal to 74 mg) and tetra-acetyl-glucosamine *HCl (1.1 eq., equal to 211 mg). The pH of the mixture was taken to 8.5 by adding DIEA.

The reaction progress was assessed by TLC; the reaction was

completed in 3 hours and the solvent was then concentrated in small volume and the residue was taken again with AcOEt. Washes were carried out on the organic phase, first with H₂O and later with a saturated solution of NaCl, and the solvent was then concentrated in order to obtain a solid residue.

5 The product obtained was characterised by HPLC (T_r = 19,9 min. with a binary gradient from 60 to 100% of B in 20 min.) and ESI-MS (m/z 0 1198.63, mass calculated for C₆₂H₈₀N₆O₁₈ = 1197.33).

 The protections to the functions in the side chain were removed by using a solution of 1:1 TFA in DCM in the presence of 2.5% TIS (tris-isopropyl-silane). After an hour, the solvent was then concentrated and the product was precipitated with Et₂O. This yielded 530 mg of solid product (88% yield).

c) H-Tyr-Pro-Trp-Phe-Glc

15 100 mg of H-Tyr-Pro-Trp-Phe-Glc(Ac₄) (Glc(Ac₄): 2,3,4,6-tetra-O-acetyl-D-glucopyranosyl) was dissolved in 3 ml of anhydrous MeOH and treated with sodium hydroxide to take the solution to a pH of 9; the progress of the reaction was followed via HPLC by using a binary gradient from 10 to 90% B in 30 min. The reaction was terminated by adding HCl up to pH 5.

 The product was purified through preparatory HPLC; the fractions containing the required compound were collected together and dried obtaining 18.8 mg of clean product.

 Analytical HPLC T_r = 18,7 min., binary gradient 10-90% B in 30 min.. ESI-MS: m/z 0 773.36, calculated for C₄₀H₄₈N₆O₁₀ = 772.46.

25 Study of the pharmacological properties of arginorphin (Arg⁷-dermorphin) in comparison with morphine and glycosilated endomorphins

 The analgesic activity of synthesised peptides has been studied in rats by using the tail flick test with a thermal stimulus (D'Amour F.E & Smith D.L., *J. Pharmacol. Exp. Ther.* 72, 74, 1941). A ray of light is concentrated on the animal's tail and an electronic device calculates the tail flick time to the nearest one-tenth of a second. The chosen test is universally accepted as the ideal analgesic test for drugs of analgesic activity at the central, spinal chord

- 12 -

or supra-spinal chord level, typical of opioid drugs.

Since natural peptides do not normally cross the blood brain barrier, they do not yield positive results in this test if they are administered peripherically (via subcutaneous, intraperitoneal or intravenous injection).

- 5 The intravenous administration of the peptides in the study confirmed their analgesic properties. The analgesic potency of the studied peptides, relative to morphine in rats, turned out to be the following:

TABLE 1

Tail flick test in rats – Intravenous administration

	EC ₅₀ relative dose	Potency
Morphine HCl	1 mg/kg	100
Arg ⁷ -dermorphin	2.5 mg/kg	25
H-Tyr-Pro-Trp-Phe-Glc	E ₃₀ = 30 mg/kg	0.5
H-Tyr-Pro-Trp-Phe-Glc(AC ₄)	E ₃₀ = 70 mg/kg	0.3

- 10 The duration of the analgesic effect varies depending on the derivative: for an E₅₀ dose, the mean durations relative to morphine turn out to be the following.

TABLE 2

Tail flick test in rats – Intravenous administration. Duration of the effect

	Duration (min.)	Relative duration
Morphine HCl	90	100
Arg ⁷ -dermorphin	130	145
H-Tyr-Pro-Trp-Phe-Glc	120	133
H-Tyr-Pro-Trp-Phe-Glc(AC ₄)	90	100

- 15 After intravenous administration the most potentl analgesic peptide turns out to be the agonist of the μ opioid receptors according to the present invention: Arg⁷-dermorphin, with an action duration that is approximately 50% higher than the one of morphine.

- 13 -

The glycosilate derivatives of endomorphins have very weak analgesic properties, never exceeding 1% of morphine activity. However, it must be noted that the action duration of H-Tyr-Pro-Trp-Phe-Glc is about 30% higher than the one of morphine.

- 5 Subcutaneous administration of the peptides under study provided power and analgesic duration data very similar to the ones obtained by intravenous administration.

Intrathecal administration yielded the following results.

TABLE 3

10 Tail flick test in rats – Intrathecal administration

	EC ₅₀ dose	Duration (min.)
Morphine HCl	1.2 µg/rat	120
Arg ⁷ -dermorphin	0.8 µg/rat	180
H-Tyr-Pro-Trp-Phe-Glc	E ₃₀ = 10 µg/rat	150
H-Tyr-Pro-Trp-Phe-Glc(AC ₄)	E ₃₀ = 30 µg/rat	100

- 15 The above data show that introducing a basic aminoacid (arginine) in the molecule enhances the penetration of these compounds through the hemato-encephalic barrier since the mean ratio between peripheral dose and intrathecal dose (spinal chord), which is about 1000, is similar to the ratio obtainable with morphine. The same intravenous/spinal ratio calculated for dermorphin and endomorphins is instead about 15,000: the modifications introduced have thus increased the penetration in the nervous system by about 15 fold.

- 20 As a comment to the above, it must be noted that the tail flick test (D'Amour & Smith) in rats is sensitive to the effects of powerful analgesics acting at the spinal chord level, but it does not measure the analgesic effects at a cerebral level or at the level of the peripheral nociceptive nerve endings. To measure the degree of penetration of the hemato-liquoral barrier at the spinal chord level, the test is carried out after peripheral administration (intra-

venous or subcutaneous) and spinal administration (intrathecal) of the drug. The ratio between intratecal ED₅₀ and peripheral ED₅₀ is a penetration index of the drug in the spinal chord through the spinal hemato-liquoral barrier.

5 In view of the above data, therefore, it may be concluded that Arg⁷-dermorphin enters the spinal liquor through the hemato-liquoral barrier, with a penetration index about three times lower than that of morphine. In rats it thus has a spinal analgesic action after peripheral administration (intravenous or subcutaneous), demonstrated with the aforesaid pharmacological test.

10 Study of the pharmacological properties of arginorphin (Arg⁷-dermorphin) as an analgesic with peripheral activity

As already noted, the opioid drugs capable of activating the receptors of the central nervous system (morphine, methadone, heroin, etc.) produce analgesia, catalepsy, respiratory depression, constipation, tolerance and addiction in rodents (rats, mice and guinea pigs). These effects are all considered an expression of the activation of the central opioid receptors, except for the constipation effect, which is partly due to action on the opioid receptors of the intestine.

20 These opioid effects have been studied in rats after administering the preferred peptide according to the present invention, Arg⁷-dermorphin, either directly to the central nervous system (by intracerebroventricular injection) or via subcutaneous administration. By comparing the effects obtained via the two different administration methods, it is easy to deduce the site of action of Arg⁷-dermorphin via activation of the opioid effects.

25 The analgesia was studied by using the paw pressure test in rats, by measuring the pressure necessary to produce a retraction of the paw (the Randal and Selitto test). The dose of Arg⁷-dermorphin that produced double the pressure necessary for paw retraction in 50% of the animals was defined as dose AD₅₀.

30 Catalepsy was studied by using the bar test, by measuring the time of maintaining an unusual posture obtained by placing both front legs of a rat on a bar situated 10 cm above the plane of the rat's hind legs. The rats which

maintained this posture for over a minute were considered to be cataleptic. A dose CD_{50} of Arg⁷-dermorphin was defined as the dose which determined catalepsy in 50% of the animals concerned.

5 The respiratory depression produced by the drug was assessed by measuring respiratory volume and frequency with the differential pletismographic method in rats, which were free to move about in the measurement chambers, before and after respiratory stimulation with a concentration of 5% of CO₂ in the air they breathed. A dose RD_{50} of Arg⁷-dermorphin was defined as the dose enabling a 50% reduction of the increase of respiratory volume
10 produced by inhaling CO₂ in 50% of the animals concerned.

Constipation was studied by measuring the transit time inside the intestines of coal particles ingested by the rats before and after Arg⁷-dermorphin administration. A dose SD_{50} of Arg⁷-dermorphin is the one producing double the ideal transit time of the ingested coal in 50% of the animals
15 concerned.

Tolerance of the analgesic effect of Arg⁷-dermorphin was studied by injecting the respective AD_{50} doses via the two administration routes twice a day for 30 days, at the same time measuring the time course of the analgesic reaction. The tolerance level is expressed as the number of administration
20 days necessary for extinguishing the analgesic response.

The addiction induced by the drug was measured with the place preference test by assessing the degree of preference of a rat for the cage compartment in which the animal had been injected with Arg⁷-dermorphin the day before, compared to the other compartment in which it was injected with a
25 physiological solution. The preference is expressed as a percentage of visits made by the rats to the drug compartment out of the total number of visits to the two compartments within an observation time of one hour.

The results obtained with the aforesaid tests, carried out via the two administration routes of Arg⁷-dermorphin (intracerebroventricular and subcutaneous), are reported in the following table.
30

TABLE 4
Paw pressure test with rats

EFFECT	Intracerebroventricular	Subcutaneous
ANALGESIA	AD ₅₀ = 0,0014 mg/kg	AD ₅₀ = 0,3 mg/kg
CATALEPSY	CD ₅₀ = 0,0035 mg/kg	CD ₅₀ > 100 mg/kg
RESPIRATORY DEPRESSION	RD ₅₀ = 0,0050 mg/kg	RD ₅₀ > 100 mg/kg
CONSTIPATION	SD ₅₀ = 0,0030 mg/kg	SD ₅₀ = 25 mg/kg
TOLERANCE	13 days	> 30 days
ADDICTION	85%	38%

As may be seen from the above data, the opioid effects of cata-
 5 lepsy, respiratory depression, tolerance and addiction are absent after peripheral administration of Arg⁷-dermorphin, while they are present if the drug is injected directly in the brain. The data show that, with subcutaneous administration, Arg⁷-dermorphin does not enter the brain and its powerful analgesic effect must be attributed to an action on the peripheral opioid receptors situ-
 10 ated on the nerve endings of the primary nociceptors.

Peripherally administered Arg⁷-dermorphin shows a constipation effect at a dose 80 times higher than the analgesic dosage. In comparison, morphine has a constipating effect at the usual analgesic dosages. This confirms what has also been observed with other opioid peptides: the opioid re-
 15 ceptors of the gastrointestinal tract are difficult to reach for opioids of a peptidic structure.

Preliminary results in rats show that the mean life of Arg⁷-dermorphin in the blood after intravenous administration in rats is about 50 minutes – a finding which is in agreement with the duration of an analgesic effect of about 180 minutes.
 20

As a comment to the above, it must be said that the analgesimetric

pressure test on the rat's paw, either normal or inflamed (the Randal and Selitto test), is sensitive to peripheral analgesic action, which is performed by pure analgesic drugs (some opioids) and anti-inflammatory analgesic drugs (e.g., COX inhibitors) at the level of the peripheral nociceptor nerve endings, after local or systemic administration (oral, subcutaneous or intravenous).

At subcutaneous doses ten times lower than those necessary for spinal analgesic effects, arginorphin has a powerful peripheral analgesic activity on skin peripheral nociceptors, like the one performed by COX inhibitor drugs, but exerted on the peripheral opioid receptors of the nociceptive nerve endings, as shown by the results obtained with the aforesaid pharmacological test.

Moreover, it should be noticed that the side effects of opioid analgesics – that are catalepsy, respiratory depression, constipation (with a slight participation of peripheral effects), tolerance and addiction in rats – are all attributable to cerebral effects, and are due to the fact that these drugs enter the brain. The ratio between central ED₅₀ doses and peripheral ED₅₀ doses of an analgesic necessary to obtain these effects is obviously an index of the degree of drug penetration in the brain.

The above results thus show that Arg⁷-dermorphin does not have any cerebral side effects after subcutaneous administration, in this way demonstrating that it entails little or no penetration through the cerebral hemato-liquoral barrier. Therefore, the proposed peptide represents a new opioid drug of peripheral activity, devoid of the more serious side effects of opiates, and is not classifiable among the drugs subjected to legal restrictions on the use of stupefacient substances.

The present invention has been disclosed with particular reference to some specific embodiments thereof, but it should be understood that modifications and changes may be made by the persons skilled in the art without departing from the scope of the invention as defined in the appended claims.

CLAIMS

1. A peptide analog of dermorphin having the following general formula:



5 wherein:

B^2 represents a D- α -aminoacid,

A^3 , A^4 , A^5 and A^6 each represent an L- α -aminoacid optionally substituted in the side chains,

or a pharmaceutically acceptable salt thereof.

10 2. A peptide according to claim 1, wherein B^2 is chosen from the group consisting of: D-Ala, D-Arg, D-Val, D-Ile, D-Leu, D-Pro, D-Ser, D-Thr, D-Met, D-Lys or D-Orn.

3. A peptide according to claim 2, wherein B^2 represents D-alanine.

15 4. A peptide according to any one of claims 1-3, wherein A^3 represents phenylalanine.

5. A peptide according to any one of claims 1-4, wherein A^4 represents glycine.

6. A peptide according to any one of claims 1-5, wherein A^5 represents tyrosine.

20 7. A peptide according to any one of claims 1-6, wherein A^6 represents proline.

8. The peptide according to claim 1, corresponding to Arg⁷-dermorphin and having the following general formula:



25 or a pharmaceutically acceptable salt thereof.

9. Use of a peptide analog of dermorphin as defined in claims 1-8, or of a pharmaceutically acceptable salt thereof, for the preparation of an opioid drug.

30 10. Use of a peptide according to claim 9 for the preparation of a drug for pain therapy and/or for the treatment of gastrointestinal disorders, and/or having an antipsychotic or anxiolytic activity.

11. Use of a peptide according to claim 9 for the preparation of a drug having a peripheral analgesic activity.

12. Use of Arg⁷-dermorphin, having the following general formula:



5 or of a pharmaceutically acceptable salt thereof, for the preparation of a drug having a peripheral analgesic activity.

13. A pharmaceutical composition for pain therapy and/or for the treatment of gastrointestinal disorders, and/or having an antipsychotic or anxiolytic activity, containing a peptide analog of dermorphin as defined in
10 claims 1-8, or a pharmaceutically acceptable salt thereof, together with any pharmaceutically acceptable carriers or excipients.

14. A pharmaceutical composition having a peripheral analgesic activity according to claim 13, containing Arg⁷-dermorphin as active ingredient and having the following general formula:



or a pharmaceutically acceptable salt thereof.