

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 March 2002 (14.03.2002)

PCT

(10) International Publication Number
WO 02/20596 A2

(51) International Patent Classification⁷: **C07K 14/435**,
A61K 38/17, A01N 63/02

(21) International Application Number: PCT/EP01/10173

(22) International Filing Date:
3 September 2001 (03.09.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0021639.0 4 September 2000 (04.09.2000) GB
0024538.1 6 October 2000 (06.10.2000) GB

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

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: MAUROTOXIN, PI1 AND HSTX1 DERIVATIVES

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(57) **Abstract:** Derivatives of Maurotoxin (MTX) in which the native disulfide bridge pattern (Cys3-Cys24, Cys9-Cys29, Cys19, Cys31-Cys34) has been disrupted are useful for the treatment of pathologies associated with dysfunctioning and/or activation of Ca²⁺-activated and/or voltage-gated K⁺ channel subtypes, such as IKCa1 or Kv1.2. One preferred group of derivatives is that in which one or more of the Cys residues have been replaced with ?-aminobutyrate (Abu) residues, thus breaking one or more of the four disulphide bridges. Within this group, the preferred derivative is that in which the Cys residues at position 9, 19, 29 and 34 have been replaced with a ?-aminobutyrate residues. However, the derivative in which the Cys residues at positions 19 and 34 have been replaced with (Abu) residues is excluded. Another preferred group of derivatives is that in which one or two of the amino acid residues of maurotoxin have been replaced by different amino acid residues resulting in the disulfide bridge pattern being changed to Cys3-Cys24, Cys9-Cys29, Cys13-Cys31, Cys19-Cys34. Within this group, the preferred compounds are that which the Arg residue at position 14 has been replaced by a Gln residue, that in which the Lys residue at position 15 has been replaced by a Gln residue, that in which the Arg residue at position 14 and Lys residue at position 15 have both been replaced by Gln residues, those in which neither of the residues at positions 32 and 33 is a Gly or Pro residue, and that in which the Gly residue at position 33 has been replaced by an Ala residue. Pi1 and HsTx1 derivatives with disrupted native disulfide bridge patterns are similarly useful.

Maurotoxin, Pi1 and HsTx1 derivatives5 **DESCRIPTION**

The invention relates to derivatives of maurotoxin and of particular toxins belonging to the same structural class of K^+ channel-acting short-chain scorpion toxins (less than 40 amino acid residues) that are cross-linked by four disulfide bridges, such as Pi1 and HsTx1. Among the toxin derivatives contemplated by the invention are truncated, modified, and mutated toxins (with either natural or non-natural amino acid residues, or non-natural peptide bonds or linkages) with four, or less than four, disulfide bridges. Mimetics of these compounds are also included. The invention also relates to the use of all these derivatives and mimetics for the treatment of neurological disorders, including immunological neurological disorders, associated with their action on modulation or blockade of specific K^+ channels, Ca^{2+} -activated and/or voltage-gated subtypes, and to pharmaceutical compositions containing them.

Maurotoxin (MTX), a toxin from the venom of the Tunisian chactidae scorpion *Scorpio maurus palmatus*, is a 34-mer peptide cross-linked by four disulfide bridges. The sequence of amino acid residues in MTX is VSCTGSKDCYAPCRKQTGCPNAKCINKSCKCYGC-NH₂ [SEQ. ID NO. 1]. MTX belongs to a distinct family of short-chain scorpion toxins with less than 40 residues, that are active onto several potassium channel subtypes (Kv and KCa channels). Contrary to most short-chain K^+ channel-acting scorpion toxins, this family can be distinguished by the presence of an additional disulfide bridge (four instead of the three commonly present in such toxins). This structural class also includes Pi1 [SEQ. ID NO. 2] and HsTx1 [SEQ. ID NO. 3] from the venoms of the scorpions *Pandinus imperator* and *Heterometrus spinifer*, respectively. These toxins share from 53 to 68% sequence identity with MTX but display different pharmacological selectivities. For instance, MTX and Pi1 are both active on some Ca^{2+} -activated K^+ channels, e.g. apamin-sensitive SK channels, whereas HsTx1 is reportedly inactive on these channel

types. Also, MTX was found to be active on rat Kv1.3 channels contrary to synthetic Pi1. Interestingly, MTX structurally differs from Pi1 and HsTx1, but also from other "classical" three disulfide-bridged scorpion toxins, by its unique disulfide bridge pattern. In three disulfide-bridged toxins, the half-cystine pairings are of the type C1-C4, C2-C5 and C3-C6 (e.g. charybdotoxin, P05, agitoxin 2, leiurotoxin 1). In short-chain four disulfide-bridged toxins, this pattern is altered by the insertion of two additional half-cystines within the amino acid sequence, one located after C3 and the other after C6. As a result, two novel patterns of the disulfide bridges are experimentally found depending on the toxin: (i) a pattern of the type C1-C5, C2-C6, C3-C7 and C4-C8 in both Pi1 and HsTx1 (which corresponds to an organization similar to that observed in three disulfide-bridged toxins), and (ii) an uncommon pattern of the type C1-C5, C2-C6, C3-C4 and C7-C8 in MTX. These two patterns possess in common the first two disulfide bridges but are differing by the two remaining disulfides. Though differences in pharmacological properties between short-chain four disulfide-bridged toxins obviously rely on their distinct amino acid sequences, it is also possible that changes in half-cystine pairings may contribute to either dramatic or discrete conformational alterations and repositioning of key residues that are involved in toxin selectivity. At the pharmacological level, MTX displays an uncommon enlarged specificity, being active - in the picomolar or nanomolar concentration range - on both Ca^{2+} -activated K^+ channels, e.g. small conductance apamin-sensitive Ca^{2+} -activated K^+ (SK) channels, and several voltage-gated (Kv) K^+ channel subtypes, including Kv1.2

In J. Biol. Chem., Vol. 275, No. 18, pp 13605-13612, 2000, Fajloun et al have described an MTX derivative with three instead of four disulfide bridges, formed by substituting α -aminobutyrate (Abu) residues for the Cys residues located at positions 19 and 34 (corresponding by numbers to positions C4 and C8). This derivative adopts the α/β scaffold with now conventional half-cystine pairings connecting C1-C5, C2-C6 and C3-C7 but remains lethal in mice by intracerebroventricular injection (LD_{50} : 0.25 $\mu\text{g}/\text{mouse}$).

The invention provides MTX, Pi1 and HsTx1 derivatives, in which specific residue

replacements, or a reorganization of half-cystine pairings has taken place, resulting in a novel, highly potent and more selective pharmacological profile.

5 In one preferred derivative, the substitution of the MTX half-cystine residues (by α -amino butyrate derivatives) located at positions 9, 19, 29, and 34 (corresponding by numbers to C2, C4, C6, and C8) results in a two disulfide-bridged MTX analog, i.e. VSCTGSKDAbuYAPCRKQTGAbuPNAKCINKSAbuKCYGAbu-NH₂, with novel, non-native arrangement of the half-cystine pairings (Cys3-Cys24 and Cys13-Cys31). Pharmacological assays of this structural analog, [Abu9,19,29,34]-MTX, reveal that
10 the blocking activity is potent (IC₅₀ = 42 nM) and highly selective for mammalian voltage-gated Kv1.2 channel subtype, although it remains active on Ca²⁺-activated K⁺ channels.

An alternative approach to replacement of Cys residues with Abu is the replacement
15 of one or two amino acid residues in natural MTX with other residues which prevent the folding of the molecule in such a way that the unconventional C1-C5, C2-C6, C3-C7 and C4-C8 disulfide bridge arrangement can occur. For instance, if neither of the residues at positions 32 and 33 is a Gly or Pro residue, the folding is altered in such a way that the MTX derivative adopts the conventional C1-C5, C2-C6, C3-C7 and C4-
20 C8 of Pi1 and HsTx1. In particular, replacement of the Gly residue at position 33 by an Ala residue substitution, forming [A33]-MTX [SEQ. ID NO. 4], is effective. Substitution of Arg14 and/or Lys15 by Gln to give [Q14]-MTX [SEQ. ID NO. 5], [Q15]-MTX [SEQ. ID NO. 6] or [Q14,Q15]-MTX [SEQ. ID NO. 7] also induces half-cystine pairings between Cys3-Cys24, Cys9-Cys29, Cys13-Cys31 and Cys19-Cys34.
25 [Q15]-MTX in particular was 1,000 times more potent than [Abu9,19,29,34]-MTX on mammalian voltage-gated Kv1.2 (IC₅₀ = 47 pM).

Contrary to natural MTX and Pi1 which are lethal in C57/BL6 mice (LD₅₀ = 4 mcg/kg of MTX or 10 mcg/kg of Pi1), MTX and Pi1 derivatives such as
30 [Abu9,19,29,34]-MTX or [Q15]-MTX are neither lethal nor toxic when injected intracerebroventricularly in these mice at active concentrations (up to 1.25 mg/kg in the case of [Abu9, 19, 29, 34]-MTX). The MTX derivatives, as well as the MTX

structurally homologous P11 or HsTx1 derivatives, of the invention are thus of potential therapeutic value for treating mammalian (including human) pathologies that are associated with a dysfunctioning of Ca^{2+} -activated and/or voltage-gated Kv channel subtypes. Such pathologies include immune and/or neurological diseases like

5 multiple sclerosis, Parkinson's and Alzheimer's diseases, both thought to be associated with a dysfunctioning of Ca^{2+} -activated and/or voltage-gated K^+ channel subtype(s). The axonal impulse propagation ensures a continued transmission of nervous signals to remote targets. In a number of neuropathies, the nervous conduction is either slowed down or locked. This could be linked to alteration or functional changes of

10 myelinated fibres (demyelination or alteration of the membrane properties at the Ranvier nodes). Among these neuropathies are multiple sclerosis (central nervous system; CNS) and Guillain-Barré syndrome (peripheral nervous system). It is now well-admitted that alteration of the axonal function is related to the presence of voltage-gated (Kv) K^+ channels (there is a widespread distribution of Kv1.1 and

15 Kv1.2 channel subtypes in nerve terminals throughout the brain, whereas Kv channels -independent of their subtypes- are considered to be ubiquitous, including in the peripheral nervous system), and possibly Ca^{2+} -activated K^+ channels. In the previously cited neuropathies, Ca^{2+} -activated K^+ channels and Kv channels are either abnormally exposed in demyelinated or inflammatory lesions, or over-expressed.

20 Therefore, selective K^+ -channel blockers, including Ca^{2+} -activated K^+ channel-acting and/or Kv-acting MTX/P11/HsTx1-derived peptides such as [Q15]-MTX, P11 amidated at C-terminus, [Abu9,19,29,34]-MTX, are of potential therapeutic value as symptomatic therapy of multiple sclerosis and related neuropathies, as well as for use as selective immuno-suppressant drugs by their blocking action on Ca^{2+} -activated

25 and/or Kv channels. Further, it can be speculated that such peptide blockers, in the CNS, could enhance transmitter release in pathways affected by progressive neurodegenerative diseases such as Alzheimer's disease.

Maurotoxin.ST25
SEQUENCE LISTING

<110> Cellpep S.A.

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<130> C139.007.02

<150> GB 0021639.0

<151> 2000-09-04

<150> GB 0024538.1

<151> 2000-10-06

<160> 7

<170> PatentIn version 3.1

<210> 1

<211> 34

<212> PRT

<213> Scorpio Maurus palmatus

<400> 1

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Thr	Gly	Cys	Pro	Asn	Ala	Lys	Cys	Ile	Asn	Lys	Ser	Cys	Lys	Cys	Tyr
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<400> 2

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1				5					10					15	

Gln	Thr	Gly	Cys	Pro	Asn	Ser	Lys	Cys	Ile	Asn	Arg	Met	Cys	Lys	Cys
			20					25					30		

6

Maurotoxin.ST25

Tyr Gly Cys
35

<210> 3
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<220>
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<400> 4

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1 5 10 15

Thr Gly Cys Pro Asn Ala Lys Cys Ile Asn Lys Ser Cys Lys Cys Tyr
20 25 30

Ala Cys

<210> 5
<211> 34
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7

Maurotoxin.ST25

<400> 5

Val	Ser	Cys	Thr	Gly	Ser	Lys	Asp	Cys	Tyr	Ala	Pro	Cys	Gln	Lys	Gln
1				5					10					15	

Thr	Gly	Cys	Pro	Asn	Ala	Lys	Cys	Ile	Asn	Lys	Ser	Cys	Lys	Cys	Tyr
			20					25					30		

Gly Cys

<210> 6

<211> 34

<212> PRT

<213> Artificial Sequence

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<400> 6

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1				5					10					15	

Thr	Gly	Cys	Pro	Asn	Ala	Lys	Cys	Ile	Asn	Lys	Ser	Cys	Lys	Cys	Tyr
			20					25					30		

Gly Cys

<210> 7

<211> 34

<212> PRT

<213> Artificial Sequence

<220>

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<400> 7

Val	Ser	Cys	Thr	Gly	Ser	Lys	Asp	Cys	Tyr	Ala	Pro	Cys	Gln	Gln	Gln
1				5					10					15	

Thr	Gly	Cys	Pro	Asn	Ala	Lys	Cys	Ile	Asn	Lys	Ser	Cys	Lys	Cys	Tyr
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8.

Maurotoxin.ST25
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Gly Cys

CLAIMS

1. A maurotoxin derivative in which the native disulfide bridge pattern (Cys3-Cys24, Cys9-Cys29, Cys13-Cys19, Cys31-Cys34) has been disrupted, but not including the derivative in which the Cys residues at positions 19 and 34 have been replaced with α -aminobutyrate (Abu) residues.
2. A maurotoxin derivative according to claim 1 in which the Cys residues at positions 9, 19, 29 and 34 have been replaced with Abu residues.
3. A maurotoxin derivative according to claim 1 in which one or two of the amino acid residues of maurotoxin have been replaced by different amino acid residues resulting in the disulfide bridge pattern being changed to Cys3-Cys24, Cys9-Cys29, Cys13-Cys31, Cys19-Cys34.
4. A maurotoxin derivative according to claim 3 in which the Arg residue at position 14 has been replaced by a Gln residue ([Q14]-MTX) [SEQ. ID NO. 5].
5. A maurotoxin derivative according to claim 3 in which the Lys residue at position 15 has been replaced by a Gln residue ([Q15]-MTX) [SEQ. ID NO. 6].
6. A maurotoxin derivative according to claim 3 in which the Arg residue at position 14 and Lys residue at position 15 have both been replaced by Gln residues ([Q14,Q15]-MTX) [SEQ. ID NO. 7].
7. A maurotoxin derivative according to claim 3 in which neither of the residues at positions 32 and 33 is a Gly or Pro residue.
8. A maurotoxin derivative according to claim 3 or claim 7 in which the Gly residue at position 33 has been replaced by an Ala residue ([A33]-MTX) [SEQ. ID NO. 4].

9. A P11 derivative in which the native disulfide bridge pattern (Cys4-Cys25, Cys10-Cys30, Cys14-Cys32, Cys20-Cys35) has been disrupted.
10. A P11 derivative in which the C-terminal extremity is amidated (Cys-NH₂) that confers an increased affinity for Ca²⁺-activated K⁺ channels.
11. A HsTx1 derivative in which the native disulfide bridge pattern (Cys3-Cys24, Cys9-Cys29, Cys13-Cys31, Cys19-Cys34) has been disrupted.
12. Use of a maurotoxin derivative, or a P11 derivative, or a HsTx1 derivative, according to any preceding claim for the treatment of a pathology, including a neurological disorder, that is associated with dysfunctioning and/or activation of Ca²⁺-activated and/or voltage-gated K⁺ channel subtypes, such as IKCa1 or Kv1.2.
13. Use of a maurotoxin derivative, or a P11 derivative, or a HsTx1 derivative, as an immuno-modulating drug, that is associated with an action (blockade, modulation and/or activation) on Ca²⁺-activated and/or voltage-gated K⁺ channel subtypes expressed on the surface of immune cells (e.g. T cells).
14. A pharmaceutical composition comprising a maurotoxin derivative, or a P11 derivative, or a HsTx1 derivative, according to any of claims 1 to 11 in admixture with a pharmaceutically acceptable diluent or carrier.