

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCHING AUTHORITY

PCT

To:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.
PCT/EP2018/071268

International filing date (day/month/year)
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International Patent Classification (IPC) or both national classification and IPC
INV. C07D487/04 C07K7/64 A61K47/62 C07K1/00 A61K38/12 A61P35/00

Applicant
HEIDELBERG PHARMA GMBH

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA:



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Date of completion of
this opinion

see form
PCT/ISA/210

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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:
 - ☒ the international application in the language in which it was filed.
 - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed:
 - ☐ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
5. Additional comments:

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	<u>1-13</u>
	No: Claims	
Inventive step (IS)	Yes: Claims	<u>1-7</u>
	No: Claims	<u>8-13</u>
Industrial applicability (IA)	Yes: Claims	<u>1-13</u>
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following documents; the numbering will be adhered to in the rest of the procedure.

- D1 MASAKO NAKAGAWA ET AL: "DYE-SENSITIZED PHOTO-OXYGENATION OF TRYPTOPHAN", TETRAHEDRON, vol. 41, no. 11, 1 January 1985 (1985-01-01), pages 2125-2132, XP002548899, ISSN: 0040-4020, DOI: 10.1016/S0040-4020(01)96583-4
- D2 US 2016/220687 A1 (ALHAMDAN OMAR ABDULAZIZ H [SA]) 4 August 2016
- D3 EP 2 497 499 A1 (HEIDELBERG PHARMA GMBH [DE]) 12 Sept. 2012
- D4 WO 2014/043403 A1 (AGENSYS INC [US]) 20 March 2014
- D5 WO 2012/041504 A1 (HEIDELBERG PHARMA GMBH [DE]; ANDERL JAN [DE]; SIMON WERNER [DE]; MUELL) 5 April 2012 (2012-04-05)

2. The claimed compounds of formula (I) according to current claim 1 differ from the compounds 18b, 18c and 16 of D1 on account of the R1CO-O- substituent.

None of D2-D5 disclose a R1COO- substituted 2-carboxy-3a-hydroxy-1,2,3,a,8,8a-hexahydropyrrolo[2,3-b]indole compound of formula (I) according to claim 1.

Thus, the subject-matter of claims 1-7 is novel over D1-D6.

D2 discloses in [0045]-[0046] a 6'-hydroxy-amanin derivative, whereas in present claim 8, the derivative has to have a 4'-hydroxy-, 5'-hydroxy-, 7'-hydroxy- substituent.

D3 is directed to a 6'-hydroxy-amanin or a 6'-alkoxy-amanin derivatives.

D4 and D5 are directed to a 6'-hydroxy-amanin or to a conjugate thereof linked via the 6'-hydroxy-group on the central tryptophan ring.

Thus, the subject-matter of claims 8-13 all related to amanintin derivative characterized by a 4'-, 5'- or 7'- hydroxylated tryptophan moiety is novel over D1-D5.

Thus, the present application meets the requirements of Art.33(2) PCT.

3. The present invention is directed to a novel method for the synthesis of amanitin derivatives, which are characterized by an hydroxylated substituent on the position 4', 5' or 7' of the central tryptophan moiety.

D5 is considered to be the closest prior art document.

The present invention differs from D5 on account of the use of a R1COO- substituted 2-carboxy-3a-hydroxy-1,2,3,a,8,8a-hexahydropyrrolo[2,3-b]indole compound of formula (I) according to claim 1 as starting material in the peptide synthesis of the amanitin derivative characterized by the presence of a hydroxylated tryptophan moiety.

Thus, the technical problem to be solved by the present application may be seen in the provision of an alternative method for synthesizing amanitins characterised by a hydroxylated tryptophan moiety.

It was shown in the examples of the present application that the hydroxylated substituted derivative of 2-carboxy-3a-hydroxy-1,2,3,a,8,8a-hexahydropyrrolo[2,3-b]indole HDP 30.2555, HDP30.2531 as precursor allows to prepare the 6'-acetylated amanin derivative HDP30.2569 and the 5'-acetylated amanin derivative HDP30.2544, which both could be conjugated as HDP30.2371 and HDP30.2602 that have been tested to analyse their cytotoxic activity.

Thus, the R1COO- substituted 2-carboxy-3a-hydroxy-1,2,3,a,8,8a-hexahydropyrrolo[2,3-b]indole compound of formula (I) according to claim 1 indeed allows to solve the above mentioned technical problem.

Starting from any prior art documents, the skilled person gets no incentive that would lead him to modify the peptide synthesis for the preparation of the 6'-hydroxylated amanitin derivatives of D5 in order to arrive at the presently claimed compound (I).

Thus, the proposed solution according to claim 1 is not obviously derivable from the prior art document. Therefore an inventive step according to Art.33(3) PCT could be recognized based on the R1COO-substituted derivative of 2-carboxy-3a-hydroxy-1,2,3,a,8,8a-hexahydropyrrolo[2,3-b]indole of formula (I) in the preparation of amanitins characterised by an hydroxylated substituent on the central tryptophan moiety according to claims 1-7.

As regards the subject-matter of claims 8-13, it differs from D5 only on account of the position of the hydroxy group on the central tryptophan moiety.

As this difference does not seem to result in an unexpected technical effect, the objective technical problem to be solved must be seen in the provision of an alternative to the 6'-hydroxylated amanitin derivatives of D5 for the same technical purpose, namely to be use in the treatment of cancer.

Claim 8 proposes as solution to this technical problem, the 4'-, 5'- and 7'-hydroxylated amanitin derivatives.

Starting from the teaching of D5, the skilled person would expect that the presently claimed hydroxylated amanitin derivatives according to claim 8 and the conjugate thereof according to claim 10 exhibit an anti-cancer activity.

Thus, the compounds of claim 8 are obvious alternatives to those disclosed in D5.

An inventive step according to Art.33(3) PCT could only be recognized for the subject-matter of claims 8-13 once it is shown that the claimed 4'-, 5'- and 7'-hydroxy-amanitin derivatives provide a surprising technical effect over the already 6'-hydroxylated amanitin derivatives disclosed in D5.

The present application does not contain any evidence for such surprising technical effect. The requirements of Art.33(3) PCT are therefore not considered to be met for the subject-matter of claims 8-13.

Re Item VIII

Certain observations on the international application

1. Claims 1 and 12 lack clarity under Art.6 PCT for the following reasons:

The expression "particularly" used in independent claim 1 has no limiting effect on the scope of said claim; that is to say, the feature following any such expression is to be regarded as entirely optional.

P1 and P2 are defined as protecting group. This definition renders the scope of claim 1 unclear, since it is not known to the skilled reader which structures are intended to be encompassed by "protecting group". In order to overcome this objection, P1 and P2 must be defined according to current claim 2, and thereby limited to Boc, $\text{PhCH}_2\text{OCO-}$, $\text{CH}_2=\text{CHCH}_2\text{OCO-}$ and trityl.

Claim 12 is directed to a "construct" whereas this term is not clearly defined in the specification.

2. Several documents have been acknowledged on [0037] by the expression "incorporated herein by reference". It is vague and ambiguous which technical features are referred to and this kind of reference contravenes also the basic requirements of each application that the invention must be comprehensible from the specification by itself. This paragraph [0037] should be deleted.