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[Continued on next page]

(54) Title: STABILIZED ANTI-MICROBIAL PEPTIDES

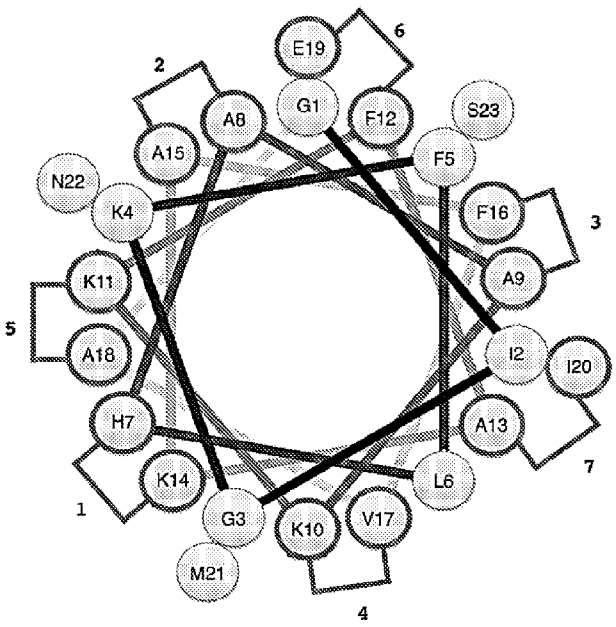

FIGURE 3

(57) Abstract: The present invention provides methods of designing and making structurally stabilized anti-microbial peptides for the prophylaxis and treatment of infection. Methods are also disclosed for designing stabilized anti-microbial peptides that are selectively lytic/cytotoxic to bacteria, allowing for internal use of anti-microbial peptides without mammalian membrane disruption and cytotoxicity.

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LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/40849

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/195, 14/35; A61K 39/04, 39/02; A61P 31/04 (2016.01)

CPC - C07K 14/195, 14/35; A61K 39/04, 39/02

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(8) Classifications: C07K 14/005, 14/195, 14/35; A61K 39/04, 39/02, 47/48, 9/06, 9/12; A61P 31/04, 31/00, 31/12 (2016.01)
CPC Classifications: C07K 14/005, 14/195, 14/35; G01N 33/68; A61K 39/04, 39/02, 47/48, 9/06, 9/12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; EBSCO; anti-microb*, antimicro*, mycobacterium*, tuberculosis*, bacter*, vaginosis*, HIV, human*, immunodef*, virus*, HIV-1, HIV-2, peptid*, stabil*, minimum*, inhibit*, concentrat*, MIC, negativ*, positiv*, microb*, topical*, vagin*, biofilm*, 'cystic fibrosis', lung*, intracell*

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 2014/0370042 A1 (WALENSKY, LD et al) 18 December 2014; paragraphs [0008], [0011], [0051], [0136], [0159], [0161]-[0167], [0169], [0171]-[0175], [0194], [0198], [0214], [0261], [0274], [0284], [0286], [0293].	1-11, 25-28, 52, 53/25, 53/52 — 12-16, 19-24, 29-51, 53/46
Y	US 2011/0288007 A1 (FOX, MA et al.) 24 November 2011; abstract; paragraphs [0048], [0059], [0096].	12-16, 48
Y	DE 19914817 A1 (DR PETRY GENMEDICS GMBH) 5 October 2000; abstract; page 6, sixth paragraph.	19 20
Y	WO 2013/039857 A1 (MODERNA THERAPEUTICS) 21 March 2013; paragraphs [0017], [0019], [0020], [00105], [00117], [00118], [00155], [00197], [00220], [00221].	21-23, 29-32, 39, 43-45
Y	US 2014/0296232 A1 (MASSACHUSETTS GENERAL HOSPITAL, et al.) 2 October 2014; paragraph [0012].	21, 24, 30, 33
Y	US 2011/0159091 A1 (STONE, et al.) 30 June 2011; abstract; paragraphs [0048],[0053], [0057]	34-38
Y	WO 2000/04915 A1 (INTRABIOTICS PHARMACEUTICALS, INC.) 3 February 2000; page 5, lines 3-7, 11-16.	41-42
Y	US 2001/0025048 A1 (CRABB, DM et al.) 27 September 2001; paragraphs [0006], [0009], [0018]-[0019].	46-51, 53/46
Y	WO 2014/100777 A2 (BHUSHAN, R et al.) 26 June 2014; abstract; paragraph [0024]	40

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 December 2016 (01.12.2016)	10 JAN 2017

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/40849

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ATASHILI, J et al. Bacterial Vaginosis And HIV Acquisition: A Meta-Analysis Of Published Studies. AIDS. Author Manuscript. 3 December 2009, Vol. 22, pages 1-16, doi: 0.1097/QAD.0b013e3283021a37; abstract.	36-38
Y	ACHARYA, T. Extracellular And Intracellular Bacteria And Their Preferred Growth Phase Within The Host. Bacteriology. 30 May 2013, pages 1-4; page 1, page 2.	47-48

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/40849

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Groups I+, Claims 1-11, 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 16 (in-part) and 19-53

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/40849

-***-Continued from Box No. III: Observations Where Unity Of Invention Is Lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-53 and SEQ ID NO: 1 are directed toward a compound having Formula (I); a pharmaceutical composition comprising said compound; a method of treating an infection caused by a microbe using said compound, and a method of inhibiting the growth of a microbe using said compound.

The compound, composition and methods will be searched to the extent the compound comprises an amino acid sequence encompassing SEQ ID NO: 1 (first exemplary amino acid sequence). Applicant is invited to elect additional peptide(s), with specified amino acid SEQ ID NO: for each, to be searched. Additional amino acid sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-11, 12 (in-part), 13 (in-part), 14 (in-part), 15 (in-part), 16 (in-part) and 19-53 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (amino acid sequence). Applicants must specify the claims that encompass any additionally elected amino acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an amino acid sequence encompassing SEQ ID NO: 2 (first exemplary elected amino acid sequence).

No technical features are shared between the amino acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a compound having Formula (I), or a pharmaceutically acceptable salt thereof, wherein the compound exhibits an antimicrobial effect against at least one microbe; a method of treating an infection caused by a microbe, the method comprising administering a therapeutically-effective amount of a compound having Formula (I), or a pharmaceutically acceptable salt thereof, to a subject having, or at risk of having, the infection caused by the microbe; a method of inhibiting the growth of a microbe comprising contacting the microbe with an effective amount of a compound having Formula (I), or a pharmaceutically acceptable salt thereof; and a pharmaceutical composition comprising a compound having Formula (I), or a pharmaceutically acceptable salt thereof, wherein the compound exhibits an antimicrobial effect against at least one microbe; wherein each R1 and R2 is independently H, alkyl, alkenyl, alkynyl, arylalkyl, cycloalkylalkyl, heteroarylalkyl, or heterocyclylalkyl, any of which is substituted or unsubstituted; each R3 is independently alkylene, alkenylene, or alkynylene, any of which is substituted or unsubstituted; each x is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; each w and y is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20; z is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10; and each Xaa is independently an amino acid.

However, these shared technical features are previously disclosed by WO 2014/159969 A1 to President and Fellows of Harvard College (hereinafter 'Harvard') in view of US 2011/0288007 A1 to Fox et al. (hereinafter 'Fox').

Harvard discloses a compound having Formula (I) (a compound of Formula (I) (Formula (I)); abstract, paragraphs [0006], [0081]); and a pharmaceutical composition comprising a compound having Formula (I) (a pharmaceutical composition comprising a compound having Formula (I)(Formula (i)); abstract, paragraph [0005]); wherein each R1 and R2 is substituted or unsubstituted alkyl (wherein each R2a and R2b (R1 and R2) is substituted or unsubstituted alkyl)0 paragraph [0081]); each R3 is independently alkylene, alkenylene, or alkynylene (each instance of ======= is a single, double or triple bond (each R3 is independently alkylene, alkenylene, or alkynylene); paragraph [0081]); each x is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (each m (x) is independently 2-6 (1-10); paragraph [0081]); each w and y is independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 (each of p and n (w and y) is from 1-100 (1-20); paragraph [0081]); z is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (q (z) is from 1-10; paragraph [0081]); and each Xaa is independently an amino acid (each Xaa is independently an amino acid; paragraph [0081]). Harvard further discloses wherein the peptides are alpha-helical peptides (wherein the peptides are alpha-helical peptides; paragraph [0005]), and wherein stapling the peptide stabilizes the alpha-helical conformation (wherein stapling the peptide stabilizes the alpha-helical conformation; paragraph [0005]).

Harvard does not specifically disclose wherein the compound exhibits an antimicrobial effect against at least one microbe; a method of treating an infection caused by a microbe, the method comprising administering a therapeutically-effective amount of a compound having Formula (I), or a pharmaceutically acceptable salt thereof, to a subject having, or at risk of having, the infection caused by the microbe; a method of inhibiting the growth of a microbe comprising contacting the microbe with an effective amount of a compound having Formula (I), or a pharmaceutically acceptable salt thereof.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from Box Previous Supplemental Box-***-

Fox discloses peptide compounds exhibiting an antimicrobial effect against at least one microbe (biocidal peptides (peptide compounds exhibiting an antimicrobial effect against at least one microbe); abstract); a method of treating an infection caused by a microbe (a method of treating an infection caused by a microbe); paragraph [0095]), the method comprising administering a therapeutically-effective amount of an antimicrobial peptide (the method comprising administering a therapeutically-effective amount of an antimicrobial peptide; paragraph [0095]), to a subject having, or at risk of having, the infection caused by the microbe (to a patient (subject) having, or at risk of having, the infection caused by the microbe; paragraphs [0092], [0095], [0102]); a method of inhibiting the growth of a microbe (a method of inhibiting the growth of a microbe; paragraph [0048]) comprising contacting the microbe with an effective amount of an antimicrobial peptide (comprising incubating the microbe (contacting the microbe) with an effective amount of an antimicrobial peptide; paragraph [0115]). Fox further discloses wherein the amino acid sequence of the peptide forms an alpha helical structure for the disruption of a microbial membrane (wherein the amino acid sequence of the peptide forms an alpha helical structure for the disruption of a microbial membrane; abstract, paragraph [0025]).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Harvard to have included the use of the methods disclosed thereby to stabilize the alpha helical structure of an alpha helical antimicrobial peptide, as disclosed by Fox, in order to improve the effectiveness and pharmacological properties, such as useful half-life, of the peptide compound.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Harvard and Fox references, unity of invention is lacking.