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AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,  
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,  
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HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,  
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MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,  
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Declarations under Rule 4.17:

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Published:

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2 February 2017

(54) Title: COMPOSITIONS AND METHODS FOR TREATING BIOFILMS

(57) Abstract: The present invention relates to a novel composition for use in inhibiting or treating microbial biofilm formation, said composition comprising a plant defensin or a peptide derivative thereof and an antifungal compound, preferably caspofungin, anidulafungin, micafungin, or amphotericin B. Accordingly, the present invention relates to use of said composition for the inhibition or treatment of biofilms, particularly fungal biofilms, such as Candida biofilms, in a subject or on a surface or other medium susceptible to biofilm formation.



**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/BE2016/000028

A. CLASSIFICATION OF SUBJECT MATTER  
INV. A61K38/02      A61K38/10      C07K2/00      C07K7/00  
ADD.

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)  
A61K C07K

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)

EPO-Internal, BIOSIS, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	KIM VRIENS ET AL: "Synergistic Activity of the Plant Defensin HsAFP1 and Caspofungin against Candida albicans Biofilms and Planktonic Cultures", PLOS ONE, vol. 10, no. 8, 6 August 2015 (2015-08-06), page e0132701, XP055309561, DOI: 10.1371/journal.pone.0132701 the whole document  -----  -/--	1-25

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

\* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&amp;" document member of the same patent family</p>
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Date of the actual completion of the international search  12 October 2016	Date of mailing of the international search report  22/12/2016
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Roscoe, Richard
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## INTERNATIONAL SEARCH REPORT

International application No  
PCT/BE2016/000028

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	VRIENS KIM ET AL: "The radish defensins RsAFP1 and RsAFP2 act synergistically with caspofungin against Candida albicans biofilms", PEPTIDES, vol. 75, 28 November 2015 (2015-11-28), pages 71-79, XP029380968, ISSN: 0196-9781, DOI: 10.1016/J.PEPTIDES.2015.11.001 the whole document	1-25
Y	----- AN M. AERTS ET AL: "The Antifungal Plant Defensin HsAFP1 from Heuchera Sanguinea Induces Apoptosis in Candida Albicans", FRONTIERS IN MICROBIOLOGY, vol. 2, 1 January 2011 (2011-01-01), XP055309602, DOI: 10.3389/fmicb.2011.00047 Introduction; Fig.2; discussion.	1-25
Y	----- THEVISSSEN ET AL: "Therapeutic potential of antifungal plant and insect defensins", DRUG DISCOVERY TODAY, ELSEVIER, RAHWAY, NJ, US, vol. 12, no. 21-22, 1 November 2007 (2007-11-01), pages 966-971, XP022338227, ISSN: 1359-6446, DOI: 10.1016/J.DRUDIS.2007.07.016 Whole doc., espec. Table 1	1-25
Y	----- UMA SHANKAR SAGARAM ET AL: "Structure-Activity Determinants in Antifungal Plant Defensins MsDef1 and MtDef4 with Different Modes of Action against Fusarium graminearum", PLOS ONE, vol. 6, no. 4, 1 January 2011 (2011-01-01) , pages e18550-e18550, XP055063980, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0018550 Whole doc., esp. p.2, col.1	1-25
Y	----- ANA VALENTE ET AL: "Revealing the Properties of Plant Defensins through Dynamics", MOLECULES, vol. 18, no. 9, 13 September 2013 (2013-09-13), pages 11311-11326, XP055309768, DOI: 10.3390/molecules180911311 Whole doc., in particular top p.11315 ----- -/--	1-25

## INTERNATIONAL SEARCH REPORT

International application No

PCT/BE2016/000028

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	YAN JIANG ET AL: "Antimicrobial activities of recombinant mouse $\alpha$ -defensin 3 and its synergy with antibiotics", JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE, KLUWER ACADEMIC PUBLISHERS, BO, vol. 23, no. 7, 21 April 2012 (2012-04-21), pages 1723-1728, XP035076877, ISSN: 1573-4838, DOI: 10.1007/S10856-012-4645-Z abstract -----	1-25

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/BE2016/000028

## 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:

see additional sheet

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 an 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.:

1-6(completely); 7-25(partially)

### 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.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6(completely); 7-25(partially)

Truncated forms of HsAFP1 which display antifungal activity, in particular inhibition of biofilm formation and development and / or potentiation of the effect of an antifungal agent. Uses of these truncated forms, alone or in combination with other agents for treatment of various forms of fungal infection, in particular Candida infections with biofilm production.

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2. claims: 7-25(partially)

Use of HsAFP1 (according to Seq. ID No.1), alone or in combination with other agents for treatment of various forms of fungal infection, in particular Candida infections with biofilm production.

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