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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 September 2008 (25.09.2008)

PCT

(10) International Publication Number
WO 2008/115118 A1

(51) International Patent Classification:
A61K 31/47 (2006.01) *A61K 31/4709* (2006.01)
A01N 43/00 (2006.01) *A61P 31/04* (2006.01)
A01P 1/00 (2006.01)

(21) International Application Number:
PCT/SE2008/000199

(22) International Filing Date: 18 March 2008 (18.03.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0700695-0 21 March 2007 (21.03.2007) SE
0701509-2 21 June 2007 (21.06.2007) SE

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(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,

[Continued on next page]

(54) Title: METHOD AND MEANS FOR PREVENTING AND INHIBITING TYPE III SECRETION IN INFECTIONS CAUSED BY GRAM-NEGATIVE BACTERIA

(57) Abstract: A means for decreasing bacterial virulence in a mammal including man or in a plant by inhibition of the Type III secretion system at concentrations that do not prevent or substantially reduce bacterial growth comprises an N-substituted 7-quinolylmethyl amine, in particular one that is further substituted in 5- and 8-position on the quinoline ring. A corresponding therapeutic method and a pharmaceutical composition are also disclosed.

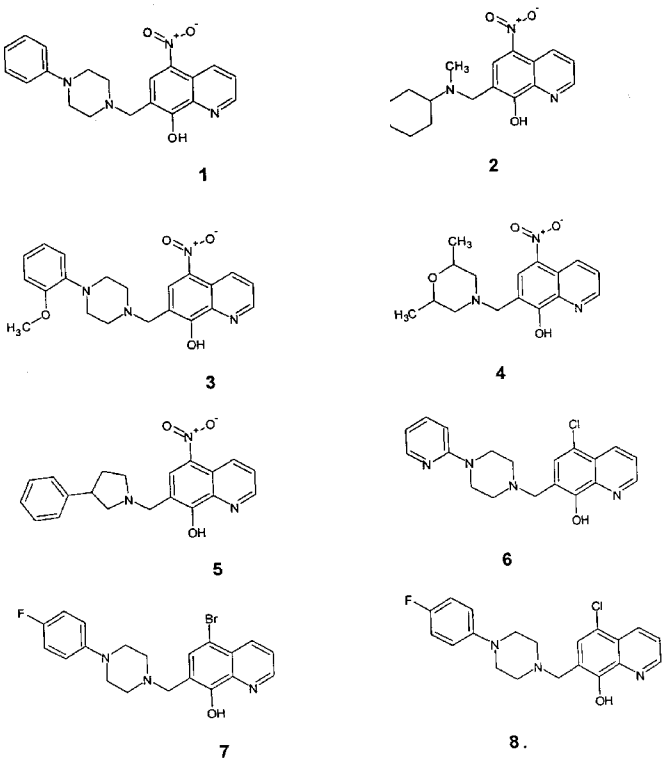


Fig. 3

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Published:
— *with international search report*

METHOD AND MEANS FOR PREVENTING AND INHIBITING TYPE III SECRETION IN INFECTIONS CAUSED BY GRAM-NEGATIVE BACTERIA

FIELD OF THE INVENTION

The invention relates to a method and a means for preventing and inhibiting Type III secretion in infections caused by Gram-negative bacteria.

BACKGROUND OF THE INVENTION

Antibiotic resistance among micro-organisms has dramatically escalated in the last ten years.[1-2] Such resistance proliferates readily in the bacterial kingdom through gene transfer, making the spread of resistance hard to control. A multitude of inefficient efforts to improve the situation have made it clear that development of novel anti-microbial drugs rapidly must take place. Recent advances in combinatorial chemistry,[3-5] genomics,[6,7] and screening technologies[8,9] have increased the capacity to identify novel bacterial targets and compounds that interfere with them. A novel mode of action is that of targeting microbial virulence rather than growth.[10,11] Virulence includes events that enable the bacterium to enter the host, disarm the host's defence, multiply, and finally spread within the host or to a new host. Agents that target virulence are potentially effective antimicrobials but also apply less selective pressure for resistance. Recent studies have revealed that various pathogenic bacteria use related virulence systems, findings that contradict the long held paradigm that each bacterium has a unique mode of action. The virulence system can be attacked by potentially effective antimicrobials derived by using a chemical genetics approach.

Regulation of the Type III secretion machinery for *Yersinia pseudotuberculosis* is relatively well understood.[12,13] Many of the genes required for the Type III secretion system are carried on a 70-kbp plasmid. Most of the genes have one of three designations; Ysc (*Yersinia* secretion proteins), Yops (*Yersinia* outer proteins) or Sycs (specific yop chaperones). The machinery can be compared to a syringe that injects effector proteins from the bacteria directly into the eukaryotic cell through pores in the membranes. The Type III secretion system (T3SS) is not constantly produced; it is only when the bacteria enter a host that genes from the plasmid are expressed. The regulation is not fully understood, but different parts of the machinery are produced in response to different signals, Fig 1. The core structure is assembled when bacteria enter a host and the temperature shifts to 37 °C, regulated by the temperature-triggered protein LcrF. Another signal is the contact with beta-integrin on the eukaryotic target cell that triggers the secretion of the inhibitor LcrQ. This secretion results in a strong Yop-production. Production and secretion of Yop has been found to be Ca^{2+} dependent *in vitro*. In the luciferase assay described in WO 2004/022775, a luxAB gene is under the control of a YopE-promoter, allowing identifying compounds that interfere with the type III secretion machinery in the absence of eukaryotic cells. Transcription of luxAB results in a luciferase that catalyses oxidation of flavine mononucleotides in the presence of an aldehyde, which is a light-emitting reaction. Secretion of Yops is the result of a fully functional T3SS and if any part of the complex T3SS is affected, a reduced transcription of *yopE/luxAB* will be seen. The assay includes a bacteria strain comprising a luxAB construct, grown in Ca^{2+} depleted medium, incubating the bacteria in Ca^{2+} depleted medium at 37 °C with an agent which antibacterial effect shall be determined, recording the light emitted by the bacteria upon addition of

decanal. This luciferase assay is well suited for high-throughput screening of large compound collections.[14] Compounds that interfere with the secretion system have potential both as drug candidates and molecular probes for functional studies of the T3SS in *Yersinia* and other Gram-negative.[15-17] Furthermore, a powerful feature of this assay is that it also can be used to identify activators of the system. Such compounds will be useful for investigations aimed at understanding the regulation of the system. Screening of a 17,500 compound library by this assay resulted in a number of hits, which inhibit the Type III secretion system at concentrations that do not prevent bacterial growth.

OBJECTS OF THE INVENTION

It is an object of the invention to provide a method and a means for preventing and inhibiting Type III secretion in infections caused by Gram-negative bacteria in a mammal or a plant.

It is another object of the invention to provide a means for preventing and/or inhibiting Type III secretion in infections caused by *Shigella subspecies*.

Further objects of the invention will become evident from the following summary of the invention, a number of preferred embodiments illustrated in figures, and the appended claims.

SUMMARY OF THE INVENTION

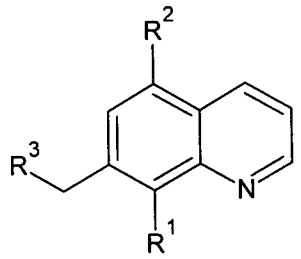
According to the present invention is disclosed a method and a means for decreasing bacterial virulence in a mammal including man or in a plant by inhibition of the Type III secretion system at concentrations that do not prevent bacterial growth. The means is a pharmacologically active

agent which is an N-substituted 7-quinolylmethyl amine, in particular one that is further substituted in 5- and 8-position on the quinoline ring.

According to a preferred aspect of the invention the methylene moiety of the N-substituted 7-quinolylmethyl amine is additionally substituted by any of methyl, ethyl, phenyl, chlorophenyl, bromophenyl, in particular 4-chlorophenyl.

According to a particularly preferred aspect of the invention the N-substituted 7-quinolylmethyl amine is N-substituted (8-hydroxy-7-quinolyl)methyl amine.

According to further preferred aspect of the invention is disclosed an agent capable of decreasing bacterial virulence in a mammal including man or in a plant by inhibition of the Type III secretion system, which is a quinoline of the general formula I



(I)

wherein

R^1 is OH, or C_1-C_4-alkoxy;

R^2 is H, nitro, Cl, Br, F or I;

R^3 is N-morpholinyl, N-piperazinyl, N-pyrrolidinyl or NR^4R^5, wherein N-morpholinyl is optionally mono- or disubstituted in 3- or/and 5-position by same or different C_1-C_4-alkyl and wherein N-piperazinyl is 4-R^6-1-N-piperazinyl, wherein R^6 is phenyl, methyl, methoxyphenyl, fluorophenyl, chlorophenyl, pyridyl; wherein N-pyrrolidinyl is optionally monosubstituted in 3-position, preferably by phenyl or phenyl monosubstituted by methoxy, fluoro, chloro, bromo;

R^4 and R^5 are both C_1-C_4-alkyl or one of them is C_1-C_4-alkyl and the other is cyclohexyl.

In the quinoline derivative of the general formula I it is more preferred for

R^1 to be OH.

In the quinoline derivative of the general formula I it is more preferred for

R^3 to be N-morpholinyl, N-piperazinyl or N-pyrrolidinyl, wherein N-morpholinyl is optionally disubstituted in 3- and 5-position by C_1-C_4-alkyl, and N-pyrrolidinyl is monosubstituted in 3-position, preferably by phenyl or phenyl monosubstituted by methoxy, fluoro, chloro, bromo.

Preferred Type III secretion blocker agents of the general formula I are shown in Fig. 3.

According to the invention is also disclosed a pharmaceutical composition comprising a pharmacologically effective amount of the agent of the invention and an pharmaceutically acceptable excipient. Any type of pharmaceutical composition capable of providing a pharmacologically effective Type III secretion inhibiting plasma or local concentration of the agent of the invention, in particular plasma concentrations ranging from 0.001 µg/ml to 10 µg/ml and more, is comprised by the invention. Oral and parenteral administration is preferred but does not exclude other ways of administration. The pharmaceutical composition of the invention may furthermore comprise an adjuvant enhancing the uptake of the Type III secretion blocker agent by infected cells or the attachment to such cells.

The method of treating infection by Gram-negative bacteria, in particular by a *Shigella subspecies,* in a person or an animal or a plant comprises the administration of the agent of the invention or the pharmaceutical composition of the invention to said person or animal or plant.

The invention will now be described in more detail by reference to preferred but not limiting embodiments illustrated in a number of figures.

SHORT DESCRIPTION OF THE FIGURES

Fig. 1 shows the current model for regulation of Yop and Syc expression; (A) At 37 °C LcrF is a positive regulator of Syc and Yop expression but at this stage Yop expression is suppressed by the negative regulator LcrQ; (B) Upon eukaryotic cell contact or Ca^{2+} depletion at 37 °C LcrQ is secreted resulting in Yop expression; (C) Schematic representation of reporter gene constructs for identification of inhibitors or activators of *Yersinia* Type III secretion system in absence of eukaryotic target cells;

Fig. 2 shows an *ex vivo test method* involving CalceinAM uptake by living cells with conversion to green fluorescent Calcein and Sytox Orange uptake by cells with damaged cell membrane with red fluorescence after bonding to the cell DNA;

Fig. 3 shows preferred Type III secretion blocker agents of the invention.

DESCRIPTION OF PREFERRED EMBODIMENTS

Bacterial strains. All strains used were *Y. psedotuberculosis* serotype III (YPIII) and in the following text strains are only labelled with the name of the virulence plasmid, deposited May 22, 2007 at the Polish Collection of Microorganisms (PCM), Accession Nos. B/00014, B/00015, and B/00016 (given by the International Depositary Authority).

Compounds. The screen was performed on a chemical library consisting of 17,500 unique compounds in 96-well plate format from ChemBridge Corporation (16981 Via Tazon, Suite G San Diego, CA 92127, USA). The compounds were dissolved in DMSO to give a stock solution of 5 mM. For compounds further characterised in the described experiments, additional 5 or 10 mg samples were purchased from ChemBridge Corp.

Luciferase Assay. The assay, described in WO 2004/022775, measures the effect of a substance in Yersinia strain pIB102EL where a *YopE* gene is transcriptionally fused to the *luxAB* gene. By growing the bacteria at 37 °C in a medium depleted for Ca^{2+}, *YopE* and *luxAB* expression are induced. The expression of luxAB can be measured as light emission by adding n-decanal to the solution. The blocking effect of a substance can thus be seen as a decreased light emission. The measurement is performed in white 96-well plates in a microplate reader. Strain with the luxAB construct was prepared from the *yadA* mutant pIB102 by constructing yopE-*luxAB operon* fusions essentially as described.[18] The resulting strain pIB102EL was struck and grown at room temperature on LB-plates containing chloramphenicol (Sigma) at a final concentration of 25 µg/mL. From plates not older than one week, bacteria for experiments were grown in liquid brain/heart infusion broth (Oxoid; Unipath Ltd., Basington, UK) containing 2.5 mM $CaCl_2$ or 20 mM $MgCl_2$ and 5 mM EGTA for Ca^{2+} depletion.

YopH Assay. The assay, described by F. Liang *et al*,[19] comprises detection of secretion of one of the effector molecules (YopH), in which the ability of YopH to function as a protein tyrosine phosphatase was utilised. *Yersinia*-strain pIB102 was induced for Type III secretion by depleting Ca^{2+} from the growth medium at 37 °C. The activity of YopH was

measured by investigating dephosphorylation by YopH of the chromogenic substrate pNPP (p-nitrophenyl phosphate) into the intensely yellow p-nitrophenol. Dephosphorylated pNPP, *i.e.* p-nitrophenol, was measured in a microplate reader at 405 nm. The blocking effect of a substance was seen as a decreased dephosphorylation and thus decreased colour emission of pNPP when different concentrations of the substances (0, 10, 20, 50 and 100 µM) were added to the growth medium to a final volume 100 µl.

Inhibition Test – ex vivo test. The test, described by Bailey *et al*,[14] comprises J774A cells (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH) seeded into a 96-well plate ($7x10^4$ cells/well) in DMEM (Dulbecco's Modified Eagle's Medium, Gibco) with 10 % FCS (Fetal Calf Serum) and gentamicin (3 µg/ml), grown for 12-24 h at 37 °C in 5 % CO_2. *Y. pseudotuberculosis* YPIII (pIB102)[20] and the translocation-deficient mutant, *yopB* (YPIII(pIB604)[21]) strains were grown over night at 26 °C in LB-broth supplemented with 25 µg/ml kanamycin, diluted 1/10 in DMEM and incubated on a rotary shaker at 26 °C for 1 h followed by 2 h at 37 °C prior to infection. The J774A cells were washed once with PBS and 50 µl fresh DMEM (without FCS and without gentamicin) containing the different compounds and 50 µl of T3SS induced *Y. pseudotuberculosis* (OD_{600} = 0.002) was added, giving a final multiplicity of infection (MOI) of 10. After 16 h of infection CalceinAM (Molecular Probes; Invitrogen) and Sytox Orange (Molecular Probes; Invitrogen) in 20 µl PBS were added to a final concentration of 1 µM and 0.05 µM respectively and the plate was incubated for 40 min at 37 °C in 5 % CO_2. CalceinAM is enzymatically transformed to green fluorescent Calcein in healthy cells (LIVE/DEAD® Viability/Cytotoxicity Kit *for mammalian cells*, Invitrogen). If the cell membrane of the eukaryotic cells was damaged, Sytox Orange was taken up and

bound to the DNA which increased the red florescence of Sytox Orange 400 times (Fig. 2). The fluorescence was then read in a microplate reader (TECAN GENios, Tecan Trading AG, Switzerland; Calcein at 485/535 nm and Sytox Orange at 535/595 nm) and the results were confirmed using fluorescent microscopy. Each compound was tested at least twice in triplicates in this assay. From the *ex vivo* experiments the toxicity of the substances was estimated; if a substance is toxic to the cells the relative amount of Calcein decreases and the relative amount of Sytox Orange increases without addition of pIB102. Substances toxic to eukaryotic cells were discarded and not tested further.

ETEC Negative Control. Enterotoxic *Escherichia coli* (ETEC) was used as a negative control in the *ex vivo test*, since this bacterium is a Gram-negative pathogen bacterium causing diarrhoea but while lacking the Type III secretion system. The perfect T3SS inhibiting substance should not have effect on ETEC in *ex vivo* infection studies. In reality, T3SS inhibiting substances at higher concentrations might affect ETEC even though they should be T3SS specific since they might be toxic at high concentration.

Table 1. Inhibition tests performed on *Y. pseudotuberculosis* with the Type III secretion blocker agents of the invention.

			Ex vivo	
Substance	**Lux Assay***	**YopH Assay***	**Y. pseudotb.†**	**ETEC (Neg Control)‡**
1	++	++	++	No data
2	+	-	-	-
3	++	+	+	-
4	+	+	+	+
5	++	++	+	++
6	+	+	+	+
7	+	+	++	++
8	+	+	++	+
Other analogues tested	-	No data	No data	No data

* + = < 50 % reduction of assay read-out at 50μM;
++ = < 50 % reduction of assay read-out at 20μM;
† + = > 70 % survival of the infected cells at 20μM; ++ = >70% survival of the infected cells at 10μM;
‡ + = > 70 % survival of the infected cells at 50μM; ++ = >70% survival of the infected cells at 20μM

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CLAIMS

1. An agent capable of decreasing bacterial virulence in a mammal including man or in a plant by inhibition of the Type III secretion system at concentrations that do not prevent or substantially reduce bacterial growth, which is an N-substituted 7-quinolylmethyl amine, in particular one that is further substituted in 5- and 8-position on the quinoline ring.

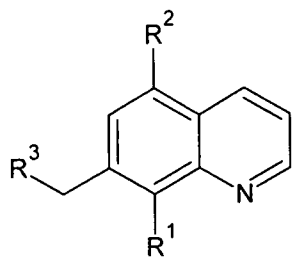
2. The agent of claim 1, wherein the methylene moiety of the N-substituted 7-quinolylmethyl amine is additionally substituted by any of methyl, ethyl, phenyl, chlorophenyl, bromophenyl, in particular 4-chlorophenyl.

3. The agent of claim 1 or 2, wherein the N-substituted 7-quinolylmethyl amine is N-substituted (8-hydroxy-7-quinolyl)methyl amine.

4. The agent of claim 1 to 3, wherein the agent is capable of decreasing bacterial virulence in a mammal including man.

5. The agent of claim 1 to 3, wherein the agent is capable of decreasing bacterial virulence in a plant.

6. An agent capable of decreasing bacterial virulence in a mammal including man or in a plant by inhibition of the Type III secretion system, which is a quinoline of the general formula I



(I)

wherein

R^1 is OH, C_1-C_4-alkoxy;

R^2 is H, nitro, Cl, Br, F or I;

R^3 is N-morpholinyl, N-piperazinyl, N-pyrrolidnyl or NR^4R^5, wherein N-morpholinyl is optionally mono- or disubstituted in 3- or/and 5-position by same or different C_1-C_4-alkyl; wherein N-piperazinyl is 4-R^6-1-N-piperazinyl, wherein R^6 is phenyl, methyl, methoxyphenyl, fluorophenyl, chlorophenyl, pyridyl and wherein N-pyrrolidinyl is optionally monosubstituted in 3-position, preferably by phenyl or phenyl monosubstituted by methoxy, fluoro, chloro, bromo;

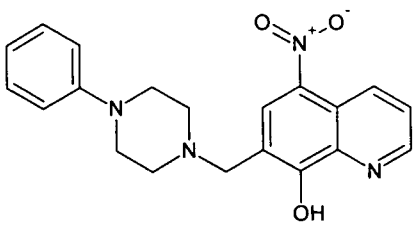
R^4 and R^5 are both C_1-C_4-alkyl or one of them is C_1-C_4-alkyl and the other is cyclohexyl.

7. The agent of claim 6, wherein R^1 is OH.

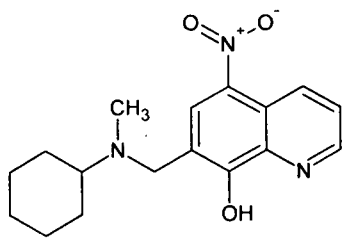
8. The agent of claim 6, wherein

R^3 is N-morpholinyl, N-piperazinyl or N-pyrrolidinyl, wherein N-morpholinyl is optionally disubstituted in 3- and 5-position by C_1-C_4-alkyl and wherein N-pyrrolidinyl is monosubstituted in 3-position, preferably by phenyl or phenyl monosubstituted by methoxy, fluoro, chloro, bromo.

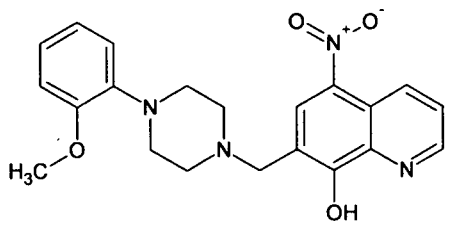
9. The agent of claim 1, wherein the Type III secretion blocker is chosen from



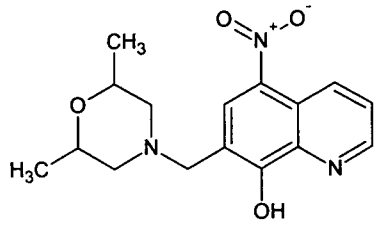
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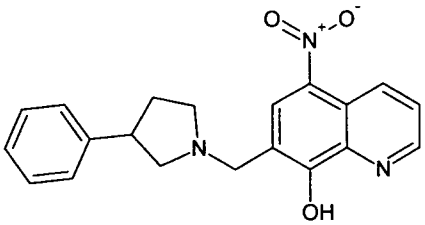
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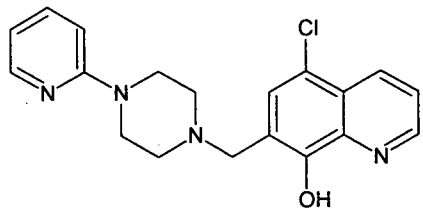
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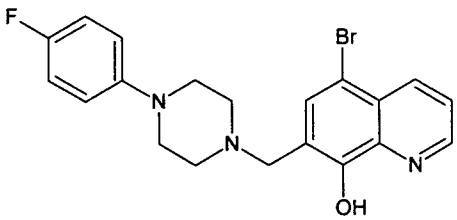
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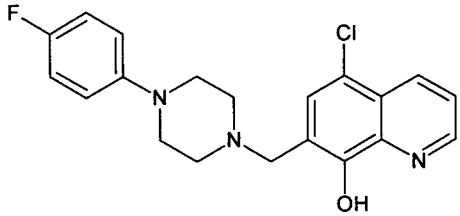
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6



7



8.

10. Use of the agent of any of claims 1-9 for the manufacture of a medicament for preventing and/or inhibiting an infection caused by Gram-negative bacteria having a Type III secretion system.

11. The use of claim 10, wherein the infection is caused by *Shigella subspecies*.

12. The use of claim 10, comprising repeated administration until said Gram-negative bacteria is no longer detectable in plasma or at site of infection.

13. The use of claim 10, wherein the administration extends over a period of from one day to two weeks or more.

14. A pharmaceutical composition for preventing and/or inhibiting Gram-negative bacteria, comprising a pharmacologically effective amount of the agent of any of claims 1-9 and a pharmaceutically acceptable carrier.

15. The composition of claim 14, in form of a single dose comprising from 0.001 µg/ml to 10 µg/ml.

16. The composition of claim 14, for oral administration.

17. The composition of claim 14, for parenteral administration.

18. The composition of claim 14, in a controlled-release form.

19. The agent of claim 1, wherein bacterial growth is not reduced by more than 20 % at a therapeutically effective plasma or local concentration.

20. A method of treating infection by Gram-negative bacteria in a person or an animal or a plant, comprising the administration of the agent of any of claims 1-9 or the pharmaceutical composition of any of claims 14-18.

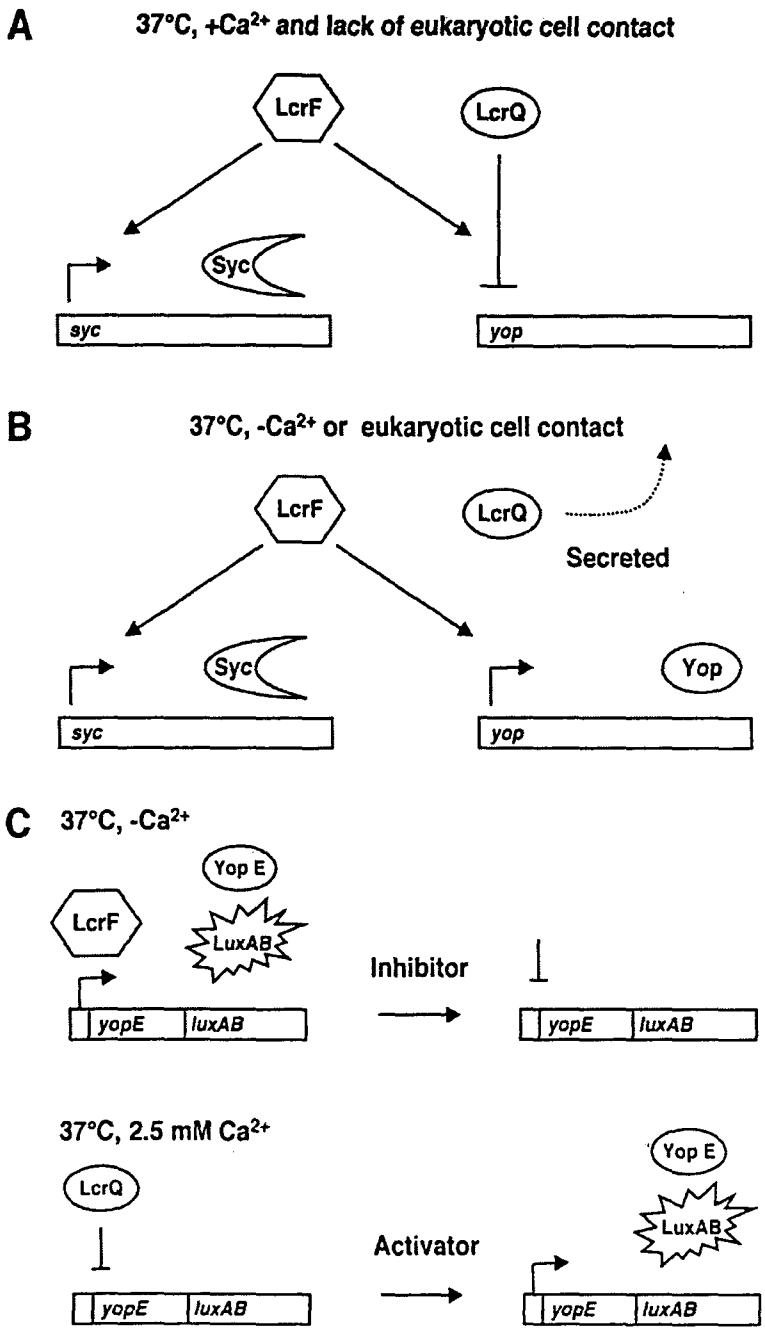

A
37°C, +Ca2+ and lack of eukaryotic cell contact
LcrF
LcrQ
Syc
syc
yop
B
37°C, -Ca2+ or eukaryotic cell contact
LcrF
LcrQ
Secreted
Syc
Yop
syc
yop
C
37°C, -Ca2+
Yop E
LcrF
LuxAB
Inhibitor
yopE
luxAB
yopE
luxAB
37°C, 2.5 mM Ca2+
LcrQ
Yop E
LuxAB
Activator
yopE
luxAB
yopE
luxAB

Fig. 1

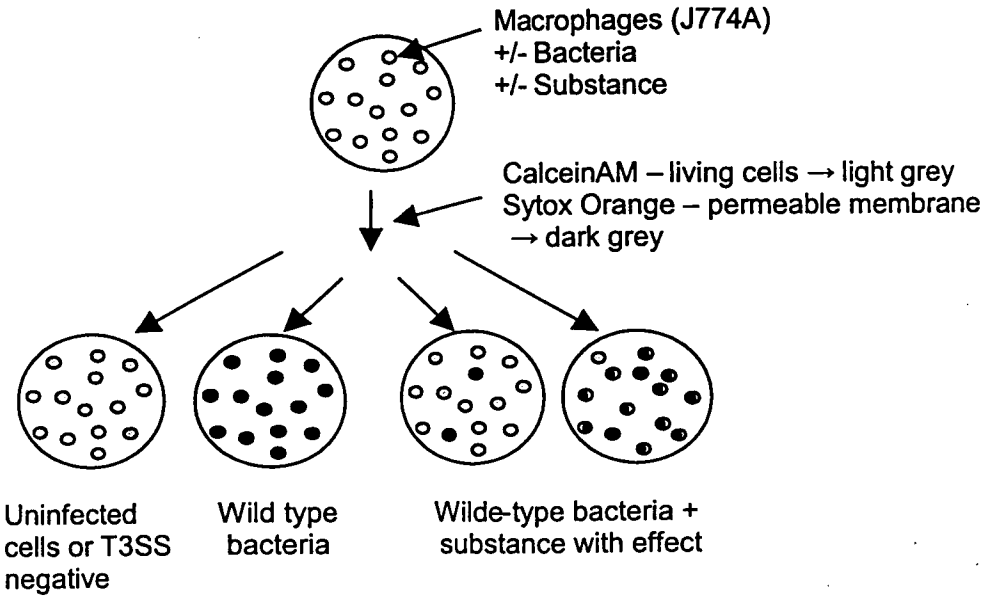
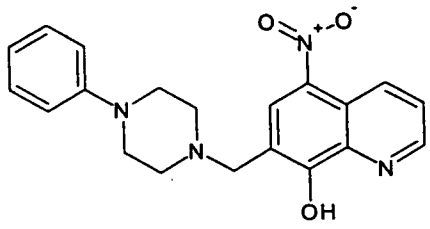
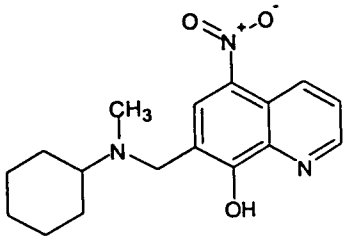

Macrophages (J774A)
+/- Bacteria
+/- Substance
CalceinAM – living cells → light grey
Sytox Orange – permeable membrane
→ dark grey
Uninfected cells or T3SS negative
Wild type bacteria
Wilde-type bacteria + substance with effect

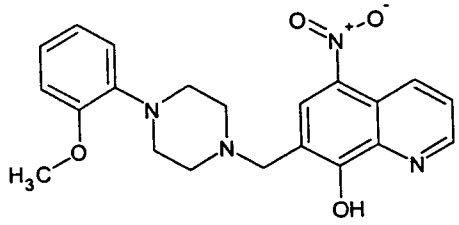
Fig. 2



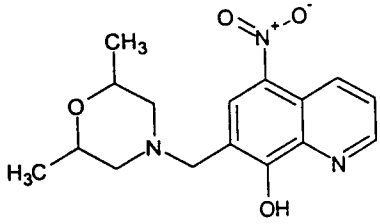
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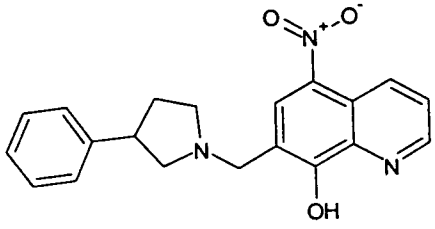
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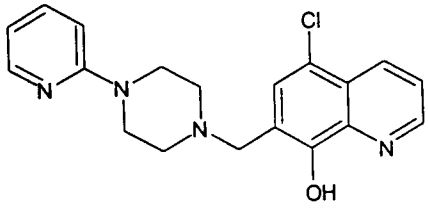
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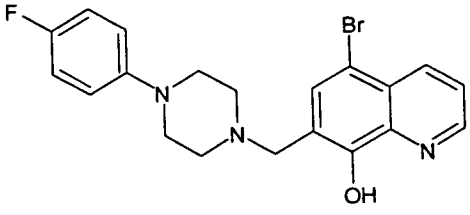
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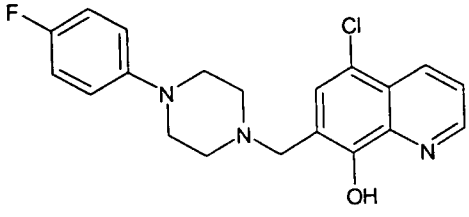
5



6



7



8.

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2008/000199

A. CLASSIFICATION OF SUBJECT MATTER

IPC: see extra sheet
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: A61K, C07D, A01N

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

SE,DK,FI,NO classes as above

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

EPO-INTERNAL, WPI DATA, PAJ, CHEM ABS DATA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2681910 A (BURCKHALTER, JOSEPH H.), 22 June 1954 (22.06.1954), column 2, line 9 - line 11, examples 1-7, 9-10, 13	1-20
	--	
X	STN International, File CAPLUS, CAPLUS accession no. 1964:41685, Document no. 60:41685, Ozawa, Tatsuo et al, "Interaction of additives on the antibacterial action of 8-quinolinol derivatives"; Shokuhin Eiseigaku Zasshi, (1963), 4(5). 283-7, RN: 91396-39-3	1-7,10-20
	--	

[X] Further documents are listed in the continuation of Box C. [X] 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
23 June 2008	25 -06- 2008
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson/PR Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2008/000199

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	THOMPSON, AU et al, "Antiamebic action of 5-chloro-7-diethylaminomethyl-8-quinolinol and of other substituted 8-quinolinols in vitro and in experimental animals"; American Journal of Tropical Medicine and Hygiene (1955), 4, 224-48 --	1-20
A	Movrin, M. et al, "Biologically active Mannich bases derived from nitroxoline"; Pharmazie (1980), 35(8),458-60, compound 9 --	1-20
A	STN Transcript from Registry; RN: 697241-30-8, 500119-62-0, 346596-69-8, 315698-36-3, 315698-35-2, 312536-49-5 -- --------	1-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE2008/000199

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.: 20
because they relate to subject matter not required to be searched by this Authority, namely:

Claim 20 relates to a method for treatment of the human or animal body by therapy, see PCT rule 39.1(iv). Nevertheless, a

.../...

2. ☒ Claims Nos.: 1-5, 10-19
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:

Present claims 1-5 and 10-19 relate to a compound defined by reference to a desirable characteristic or property, namely

.../...

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:

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 fee, this Authority did not invite payment of any additional fee.

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.:

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.

Box II.1

search has been made for this claim. The search has been directed to the technical content of the claim.

Box II.2

prevention and inhibition of type III secretion in infections caused by Gram-negative bacteria. The claims cover all N-substitute 7-quinolinylmethyl amine compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compounds by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The search has covered the general aspects of the invention to some extent, although it lacks the necessary precision in the definition of the subject-matter. Consequently, the search for the general concept of type III secretion system will retrieve a pertinent document only if this concept is described in general terms in a reference. Specific solutions previously known and falling under the general concept, but failing to mention this fact, are not likely to be revealed in such a search.

International patent classification (IPC)
A61K 31/47 (2006.01)
A01N 43/00 (2006.01)
A01P 1/00 (2006.01)
A61K 31/4709 (2006.01)
A61P 31/04 (2006.01)

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Use the application number as username.
The password is **KUMLREUQOK**.

Paper copies can be ordered at a cost of 50 SEK per copy from PRV InterPat (telephone number 08-782 28 85).

Cited literature, if any, will be enclosed in paper form.

US	2681910	A	22/06/1954	ES	201670	A	01/06/1952