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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 May 2011 (26.05.2011)

PCT

(10) International Publication Number
WO 2011/060976 A1

(51) International Patent Classification:
A61K 31/4045 (2006.01) *A61P 31/04* (2006.01)
C07D 209/16 (2006.01)

(21) International Application Number:
PCT/EP2010/063405

(22) International Filing Date:
13 September 2010 (13.09.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/272,937 20 November 2009 (20.11.2009) US

(71) Applicants *(for all designated States except US)*: **UNIVERSITE DE LIEGE** [BE/BE]; Interface Entreprises-Université, ANTHEUNIS Nicole, avenue Pré-Aily, 4, B-4031 Angleur (BE). **UNIVERSITEIT UTRECHT HOLDING BV** [NL/NL]; Yalelaan, 40, NL-3583 CM Utrecht (NL). **UNIVERSITY OF LJUBLJANA** [SI/SI]; Kongresni trg 12, 1000 Ljubljana (SI). **UNIVERSITY OF LEEDS** [GB/GB]; 101 Clarendon Road, Leeds Yorkshire LS2 9JT (GB). **COMMISSARIAT A L'ENERGIE ATOMIQUE** [FR/FR]; Batiment le ponant D, 25 rue Leblanc, F-75015 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **TERRAK, Mohammed** [BE/BE]; rue Champs des Alouettes, 14, B-4557 Fraiture-en-condroz (BE). **BREUKINK, Evert Johan** [NL/NL]; Lindelaar, 20, NL-3984 AK Odijk (NL). **GOBEC, Stanislas** [SI/SI]; Poklukarjeva,22, 1000 Ljubljana (SI). **CHOPRA, Ian** [GB/GB]; 8 Swallow Close, Pool in Wharfedale, Otley Yorkshire LS21 1RR (GB). **VERNET, Thierry** [FR/FR]; 16 rue de Strasbourg, F-38000 Grenoble (FR).

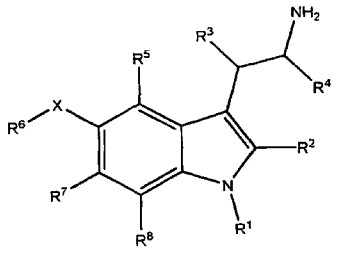
(74) Common Representative: **UNIVERSITE DE LIEGE**; Interface Entreprises-Université, ANTHEUNIS Nicole, avenue Pré-Aily, 4, B-4031 Angleur (BE).

(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, CL, 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,

[Continued on next page]

(54) Title: TRYPTAMINE-DERIVED COMPOUNDS AS ANTIBACTERIAL AGENTS

(57) Abstract: The present invention relates to a compound according to formula I, a pharmaceutically acceptable salt and/or N-oxide thereof for use as an antibacterial agent.



(I)

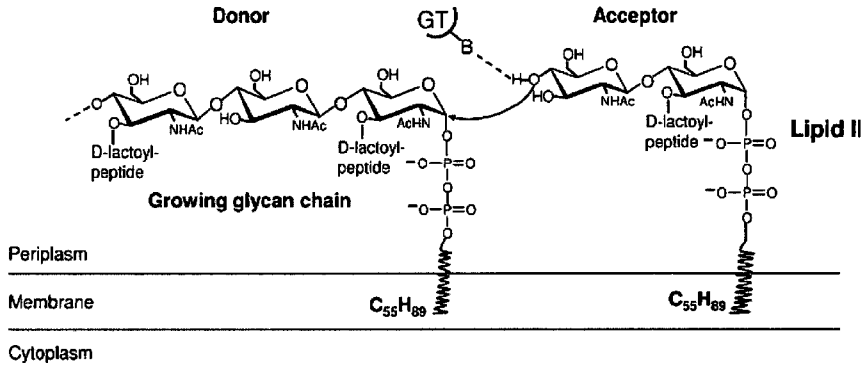


Figure 1

WO 2011/060976 A1

ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

TRYPTAMINE-DERIVED COMPOUNDS AS ANTIBACTERIAL AGENTS

The present invention relates to compounds that have a demonstrated antibacterial activity, pharmaceutical compositions containing them as the active ingredient, to their use as medicaments, and to their use in the manufacture of medicaments for use in the treatment of bacterial infections, more specifically of gram positive sensitive and resistant bacteria.

Background of the Invention

Peptidoglycan is an essential component of the bacterial cell wall and its biosynthesis is required for bacterial survival and thus, constitute an important antibacterial target. The last two steps, the transglycosylation (GT) and transpeptidation (TP) respectively, occur on the external surface of the plasma membrane which dispenses an inhibitory drug from crossing the plasma membrane in order to reach its target and kill the bacteria. The TP activity was extensively explored as the target of β-lactams (penicillins, cephalosporins, carbapenems), the most frequently used class of antibiotics. However, resistance to β-lactams is growing, resulting in multidrug-resistant bacterial strains such as methicillin-resistant *Staphylococcus aureus* (MRSA).

Presently glycopeptide antibiotics such as vancomycin have been used clinically with success against otherwise resistant bacteria. Vancomycin blocks the polymerization of peptidoglycan by binding to the terminal D-Ala-D-Ala dipeptide of a so-called lipid II precursor and nascent peptidoglycan. However, recently MRSA strains resistant to even these glycopeptide antibiotics have been reported. The threat of bacterial resistance spread is exacerbated by a decrease in new drugs discovery in the last decades, and the low number of antibiotics under development, which could result in a major world-wide public health problem.

Since there is an urgent need for the development of novel classes of antibiotics with new modes of action and effective against resistant pathogens.

WO2008/110690 discloses a family of tryptamine-derived antibiotics, some of which were found of limited activity against sensitive and ciprofloxacine resistant *S.*

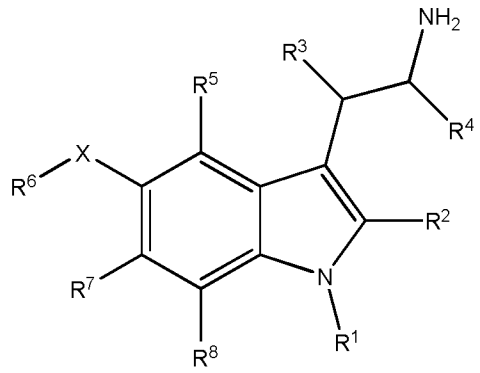
aureus. These active derivatives however were not demonstrated to inhibit other bacteria that may cause infections, specifically in mammalian subjects.

Some antibiological activities of simple indole derivatives have been disclosed in "Effect of lipophilic substituents on some biological properties of indoles", Calvert W. Whitehead, and Celia A. Whitesitt, J. Med. Chem., 1974, 17 (12), 1298-1304. However, the article only shows few compounds that exhibit antibacterial activity in the range of clinically important antibiotics, let alone against sensitive *S. aureus*. Furthermore, the article does not disclose patient subjects.

Consequently, in order to overcome the threat of widespread multi-drug resistant organisms, there is an on-going need to develop new antibiotic classes able to cure infections due to resistant pathogens.

Summary of the invention

Accordingly, the present invention relates to a compound according to formula (I):



(I),

a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof, wherein

R^1 represents an optionally substituted benzyl group, which is optionally substituted at the aromatic ring by one ore more alkyl, alkoxy , hydroxyl, thioalkyl, halogen, nitro, cyano or amino groups,

R^2 represents a lower alkyl group comprising from 1 to 4 carbon atoms, a lower alkyl group substituted by halogen, a cycloalkyl group, an aryl group , a lower alkoxy

group , a lower thioalkyl group, a cycloalkyloxy group, an aralkyloxy group or an alkanoyl group;

R^3, R^4, R^5, R^7 and R^8 may independently be the same or different, and represent a hydrogen atom, a halogen atom, a hydroxyl group, a nitro group, a formyl group, an amino group which may be protected or substituted, a lower alkyl, cycloalkyl, aryl, lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, ureido or monocyclic heterocyclic group;

X represents an oxygen atom or a sulphur atom;

R^6 represents a hydrogen atom, a lower alkyl group, a cycloalkyl group , an aryl group, a lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, alkanesulfonyl or monocyclic heterocyclic group, all of which may optionally be substituted; for use in treating pathologies associated with bacterial infections in a subject in need of treatment.

The present invention further refers to use of the compounds as antibacterial agents, and to a method for the treatment of pathologies associated with bacterial infections in a subject in need of treatment, more specifically compounds acting as peptidoglycan GT inhibitors, and to methods for the treatment of pathologies associated with bacterial infections comprising the administration, to a patient, whether an animal or a human, in need thereof, of at least one compound of formula (I) in a form and amount suitable for achieving the therapeutic effect.

The present invention also relates to antibacterial agents and pharmaceutical compositions comprising a compounds, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof, and to the use of the compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof for the manufacture of a medicament for the treatment and prevention of pathologies associated with bacterial infections in a subject in need of treatment.

Short description of the Figures

Fig. 1 depicts a GT catalyzed glycan chain elongation step in peptidoglycan polymerization.

Fig. 2A depicts structures of preferred compounds Example 1 (2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl]) and 2 ((2-(1-(3,4-

dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine) according to the invention, as well as comparative Example 1 (NSC 17384)

Fig. 2B shows the the predicted binding model of a preferred compound according to the invention, 2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl] *to S. aureus* PBP2 active site.

Fig.3 depicts the effect of compound Example 2 on bacterial viability.

Fig.4 shows the Interaction of a preferred compound according to the invention with lipid vesicles and lipid II.

Fig. 5 depicts the dose response curve of the inhibition of *E. coli* PBP1b by preferred compound Ex.1 according to the invention.

Fig.6 shows the effect of preferred compounds Ex.1 and Ex.2 according to the invention and control agents on lipid II cycle.

Fig.7 Shows the synthesis of a library of *N*-benzyltryptamines analogues according to the invention and comparative examples.

Detailed Description of the Invention

The present invention relates to a family of compounds which have been discovered to act as a new class of antibiotics. More particularly, it relates to GT inhibitors, as illustrated by the compounds of examples 1 and 2, which have been discovered and shown to be highly active against several purified GT enzymes in the micromolar range. More importantly, the compounds according to the invention were also shown to possess antibacterial activity not only against sensitive, but also against resistant Gram-positive bacteria.

The compounds according to the invention have a unique scaffold according to formula (I) as defined herein above, which is completely different from known GT inhibitors, and thus are exemplary for a new class of antibiotics.

At present, the only well-characterized inhibitor targeting the GTs is the natural product moenomycin, a potent antibacterial phosphoglycolipid antibiotic active at nanomolar concentrations. However, despite intense studies of its structure-function properties it has not been developed for use in human chemotherapy because of poor pharmacokinetics properties related to its C25 lipid chain. A delipidated

moenomycin derivative is inactive and only the sugar moiety can be reduced to three saccharide units while retaining good antibacterial activity.

As used herein, the term alkyl refers to a saturated, straight-chain or branched hydrocarbon group having from 1 to 20 carbon atoms, preferably from 1 to 12 carbon atoms, especially from 1 to 6 carbon atoms, for example the methyl, ethyl, propyl, isopropyl, isobutyl, tert-butyl, n-hexyl, 2,2-dimethylbutyl or n-octyl group. The term "lower alkyl" denotes a saturated straight- or branched-chain group containing from 1 to 7 carbon atoms, for example, methyl, ethyl, propyl, isopropyl, n-butyl, i-butyl, 2-butyl, t-butyl and the like. Preferred lower alkyl groups are groups with 1-4 carbon atoms.

As used herein, the term "lower alkoxy" denotes an alkyl group as defined above, which is attached via an oxygen atom.

As used herein, the term "lower alkyl substituted by halogen" denotes an alkyl group as defined above, wherein at least one hydrogen atom is replaced by halogen, for example CF_3, CHF_2, CH_2F, CH_2CF_3, $CH_2CH_2CF_3$, $CH_2CF_2CF_3$ and the like. The term "halogen" denotes chlorine, iodine, fluorine and bromine.

The term "cycloalkyl" denotes a saturated carbocyclic ring, containing from 3 to 6 carbon atoms, for example, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

The term "aryl" as used herein is a carbocyclic ring system, containing from 6 to 10 carbon atoms forming one or more rings, and wherein at least one ring is aromatic in nature, for example phenyl, naphthyl or 5,6,7,8-tetrahydronaphthalen-1-yl. The most preferred aryl group is phenyl.

The terms alkenyl and alkynyl refer to at least partially unsaturated, straight-chain or branched hydrocarbon groups having from 2 to 20 carbon atoms, preferably from 2 to 12 carbon atoms, especially from 2 to 6 carbon atoms, for example the ethenyl, allyl, acetylenyl, propargyl, iso-prenyl and hex-2-enyl groups. Preferably, alkenyl groups have one or two double bonds, and alkynyl groups have one or two, triple bonds.

The term “heterocyclic” embraces both “heteroaryl“ and “heterocycloalkyl” groups. The term “heteroaryl“as used herein is an aromatic ring system, containing

from 5 to 10 ring atoms forming one or more rings, wherein at least one ring atom is a heteroatom selected from the group consisting of O, N and S, and wherein at least one ring is aromatic in nature, for example oxazolyl, pyridyl, thiophenyl, quinolinyl, pyrrolyl, furyl, benzoimidazolyl, imidazolyl and the like. The most preferred group is pyridyl. .

The term "heterocycloalkyl " denotes a fully saturated ring system, wherein one or two ring atoms are N, O or S, for example piperazinyl, pyrrolidinyl, morpholinyl or piperidinyl.

The term "heteroalkyl" refers to an alkyl, alkenyl or alkynyl group in which one or more, preferably 1, 2 or 3 carbon atoms have been replaced by an oxygen, nitrogen, phosphorus, boron, selenium, silicon or sulphur atom (preferably oxygen, sulphur or nitrogen). The term heteroalkyl refers also to a carboxylic acid or a group derived from a carboxylic acid, such as, for example, acyl, acyl-alkyl, alkoxycarbonyl, acyloxy, acyloxyalkyl, carboxy-alkylamide or alkoxycarbonyloxy. Specific examples of heteroalkyl groups are methoxy, trifluoromethoxy, ethoxy, n-propyloxy, isopropyloxy, tert-butoxy, methoxymethyl, ethoxymethyl, methoxyethyl, methylamino, ethylamino, dimethylamino, diethylamino, isopropylethylamino, methylaminomethyl, ethylaminomethyl, diisopropylaminoethyl, enol ether, dimethylaminomethyl, dimethylaminoethyl, acetyl, propionyl, butyryloxy, acetyloxy, methoxycarbonyl, ethoxycarbonyl, N-ethyl-N-methylcarbamoyl and N-methylcarbamoyl. Further examples of heteroalkyl groups are nitrile, isonitrile, cyanate, thiocyanate, isocyanate, isothiocyanate and alkylnitrile groups.

"Pharmaceutically acceptable" such as pharmaceutically acceptable salt, carrier, excipient, etc., means pharmacologically acceptable and substantially non-toxic to the subject to which the particular compound is administered.

The term "pharmaceutically acceptable salt" embraces salts with inorganic and organic acids, such as hydrochloric acid, nitric acid, sulfuric acid, phosphoric acid, citric acid, formic acid, fumaric acid, maleic acid, acetic acid, succinic acid, tartaric acid, methane-sulfonic acid, p-toluenesulfonic acid and the like.

The term “prodrug” refers to a precursor form of the compound that is metabolized to form the active ingredient (see for example R. B. Silverman, Medizinische Chemie, V C H Weinheim, 1995, Chapter 8, page 361 ff), to which the present invention also relates, preferably consist of at least one compound of the formula (I) and at least one pharmacologically acceptable protecting group that is removed under physiological conditions, for example a hydroxy, alkoxy, aralkyloxy, acyl or acyloxy group, such as, for example, a methoxy, ethoxy, benzyloxy, acetyl or acetyloxy group.

The term "Pharmaceutically acceptable N-oxide" refers to N-oxides of tertiary nitrogen atoms in a molecule, which may be more potent than their corresponding tertiary amine, or less. N-oxides may or may not be reduced to their corresponding tertiary amines after indigestion. When N-oxides are converted to their corresponding tertiary amines, the conversion may be in mere trace amounts or nearly quantitative. Further, once formed, N-oxides may be more active than their corresponding tertiary amines, less active or even completely inactive.

"Therapeutically effective amount" means an amount that is effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated. The term “subject” herein refers to a living organism, including a human being, pet and farm animal, such as mammal, bird, amphibian, fish, molluse or arthropod.

The term “in need of treatment” refers to the inhibition, effective treatment or prevention of infection by a bacterium. Preferably, the subject in need of treatment is a mammal, more preferably a human being, or a pet or farm animal.

“In need of treatment “more preferably refers to indications in accordance with the present invention are those, which include bacterial infections by Gram-positive bacteria, both sensitive and resistant to β-lactam and/or glycopeptide antibiotics.

The treatment preferably includes inhibition, effective treatment or prevention of infection of bacterial infections wherein preferably the bacteria use transglycosylation (GT) reaction in the formation of their cell walls, which preferably is inhibited by the active compounds according to the invention. The term “treating”

thus includes the treatment of an acute bacterial infection, or the prevention of such invention, e.g. by preventing colonisation.

The increase of bacterial resistance to antibiotics results in a decline of the effective antibacterial treatments available. Therefore, the discovery and development of new antibiotic classes able to cure infections due to resistant pathogens are urgently needed.

Without wishing to be bound to any particular theory, it is believed that Peptidoglycan is an essential polymer and the main constituent of the bacterial cell wall. Its biosynthesis pathway requires several steps and offers still many unexplored antibacterial targets for the development of new antibacterial drugs. The last two steps in peptidoglycan biosynthesis typically involve the assembly of the cell wall polymer from the monomeric intermediate. This takes normally place outside the plasma membrane and relies on the activity of the glycosyltransferases (GTs) which use the Lipid II precursor to synthesize glycan chains (Fig. 1) and the transpeptidases which catalyze the cross-linking of two glycan chains via the peptide side chains. Inhibition of either of these two reactions leads to bacterial cell death.

Penicillins target the transpeptidation reaction but antibiotic therapy based on the inhibition of the GTs has not yet been developed. However, since recently the 3D structures of GTs and their complexes with moenomycin A have been determined (Heaslet et al., 2009; Lovering et al., 2007; Sung et al., 2009; Yuan et al., 2008), the structures confirmed the catalytic mechanism of glycan chains elongation and the mode of action of moenomycin. The GT domain contains an extended enzymatic cleft which can accommodate six sugar units. It is divided into two sub-sites, a donor site for the elongating chain and acceptor site for the Lipid II substrate. Between the sub-sites a flexible region is proposed to play an important role in the translation of the product from the acceptor site to the donor site through a folding and unfolding process (Lovering et al., 2008). The enzymatic cavity is flanked by several conserved residues and harbours the two essential glutamate residues which offer many possibilities for ligand binding *(Terrak et al., 2008)*. Moenomycin occupies the donor site, mimicking the elongating glycan chain and completely blocking peptidoglycan

polymerisation. Using a high-throughput method, based on competition of small molecules with fluorescent moenomycin has been recently used to screen for GT donor site binder using fluorescence anisotropy assay (Cheng et al., 2008).

In the compound of formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof for use as an antibacterial agent according to the invention, X preferably represents an oxygen atom or a sulphur atom, since compounds with this substitution have strong antibacterial activity.

R^1 preferably represents a mono-, di- or tri-substituted benzyl group, more preferably a 2-chlorobenzyl or a 3,4-dichlorobenzyl group. R^2 preferably is an alkyl group comprising from 1 to 4 carbon atoms, preferably methyl. Preferably, R^3 and R^4 represent a hydrogen atom.

Again more preferably, X represents sulphur atom, since compounds with sulphur atoms at this position have shown a surprisingly stronger effect in bacterial growth inhibition than those with oxygen atoms,

Preferably, R^1 represents a mono-, di- or tri-substituted benzyl group. More preferred are *2*-chlorobenzyl or a *3,4*-dichlorobenzyl group, the latter being the most preferred. Preferably, R^5, R^7 and R^8 represent a hydrogen atom.

Particularly active compounds , pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof are 2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl], (2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, or combinations thereof. These compounds according to the invention are preferably used in cases wherein the pathologies associated with the bacterial infection are caused by sensitive, or single and/or multiple resistant Gram - positive bacteria.

The present invention further relates to (2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof, and its use as a medicament, preferably its use as an antibacterial agent for treating pathologies associated with bacterial infections in a subject in need of treatment.

The compounds according to the invention are preferably employed such that the compounds inhibit the cell wall synthesis in the metabolic pathway of the bacteria. This advantageously inhibits the Glycosyltransferase 51 in the metabolism of the bacteria.

The present invention further relates to an antibacterial agent and/or a pharmaceutical composition comprising one or more compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof.

The present invention further relates to a process for preparing an antibacterial composition and/or pharmaceutical composition, the process comprising formulating one or more compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof, and optionally a utilizable carrier or otherwise suitable additive.

The present invention thus also provides pharmaceutical antibacterial compositions containing compounds of the invention, for example compounds of formula (I) and their pharmaceutically acceptable acid addition salts, and a pharmaceutically acceptable carrier. Such pharmaceutical compositions can be in the form of tablets, coated tablets, dragees, hard and soft gelatin capsules, solutions, emulsions or suspensions. The antibacterial compositions also can be in the form of suppositories or injectable solutions. The dosage at which compounds of the invention can be administered can vary within wide limits and will, of course, have to be adjusted to the individual requirements in each particular case.

The present invention also relates to a method for the treatment of pathologies associated with bacterial infections or preventing a bacterial infection in a subject, comprising the administration, to a subject in need thereof, of one or more compounds of the formula (I)as defined herein above, a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof.

The method for treatment according to the invention is advantageously applied where the subject is a higher animal, preferably a mammalian subject. This may advantageously be a human being, but also applies to wild, farm or pet animals.

The method for treatment of pathologies is advantageously associated with bacterial infections by bacteria employing Glycosyltranferase 51 in their metabolic pathway; wherein comprising one or more compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof, when administered in an appropriate way and amount, inhibit the pathway such that the cell membranes of the bacteria are at least in part damaged,

The bacteria may be sensitive, but also single- or multiple resistant bacteria may be treated. The one or more compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof may advantageously also be used in combination with other antibiotic or otherwise useful compounds. Preferably, the subject compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof are used to treat pathologies for gram positive bacteria.

The subject invention also relates to the use of one or more compounds of the formula (I), a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof according to the invention for the manufacture of a medicament for the treatment or prevention of a bacterial infection in a subject in need of such treatment, preferably as defined herein above.

One or more compounds of the formula (I) according to the invention , a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof can advantageously be prepared by methods known in the art, which processes comprise, if desired, converting the compounds obtained into pharmaceutically acceptable salts. The starting materials are either commercially available, are otherwise known in the chemical literature, or can be prepared in accordance with methods well known in the art.

The present invention relates also to the therapeutic use of the compounds of the formula (I), their pharmacologically acceptable salts and solvates and hydrates and N-oxides as well as formulations and pharmaceutical compositions. The present invention relates also to the use of those active ingredients in the production of

medicaments for preventing and/or treating diseases, especially those diseases that are the result of a bacterial infection.

The pharmaceutical compounds of the invention, in addition to one or more compounds of the invention, may preferably contain a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include pharmaceutically inert, inorganic and organic carriers

One or more compounds of the formula (I) according to the invention , a pharmaceutically acceptable salt, N-oxide and/or prodrug thereof are preferably generally administered using the known and acceptable methods, either on their own or in combination with any desired other therapeutic agent. Administration can preferably be effected, for example, by one of the following methods: orally, for example in the form of dragées, coated tablets, pills, semi-solid preparations, soft or hard capsules, solutions, emulsions or suspensions; parenterally, for example in the form of an injectable solution; rectally in the form of suppositories; by inhalation, for example in the form of a powder formulation or spray; transdermally or intranasally. For the production of such tablets, pills, semi-solid preparations, coated tablets, dragées and hard gelatin capsules, the therapeutically acceptable product may preferably be mixed with pharmacologically inert, inorganic or organic pharmaceutical carrier substances, for example with lactose, sucrose, glucose, gelatin, malt, silica gel, starch or derivatives thereof, talcum, stearic acid or salts thereof, skimmed milk powder and the like. For the production of soft capsules, pharmaceutical carriers such as, for example, vegetable oils, petroleum, animal or synthetic oils, wax, fat and polyols can be used. For the production of liquid solutions and syrups, pharmaceutical carriers such as, for example, water, alcohols, aqueous saline solution, aqueous dextrose, polyols, glycerol, vegetable oils, petroleum and animal or synthetic oils can be used. For suppositories, pharmaceutical carriers such as, for example, vegetable oils, petroleum, animal or synthetic oils, wax, fat and polyols can be used. For aerosol formulations, compressed gases suitable for that purpose can be used, such as, for example, oxygen, nitrogen and carbon dioxide

The pharmaceutical compositions may further preferably contain preservatives, solubilisers, stabilizers, wetting agents, emulsifiers, sweeteners, colorants, flavourings, salts for varying the osmotic pressure, buffers, masking agents or antioxidants. They can also contain still other therapeutically valuable substances.

The present invention also provides a method for preparing compositions of the invention which comprises bringing one or more compounds of formula (I) and/or pharmaceutically acceptable salts, prodrugs and/or N-oxides thereof and, if desired, one or more other therapeutically valuable substances into a galenical administration form together with one or more therapeutically inert carriers.

The above-described components for orally administered or injectable compositions are merely representative. Further materials as well as processing techniques and the like are set out in Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton, Pa. (1990).

Detailed description of the Figures

Figure 2 shows structures of the GT inhibitors compounds Examples 1, 2 and 3 and the predicted binding model of compound 1 to *S. aureus* PBP2 active site.

Figure 2 A shows the structures of the GT inhibitors Example 1 (2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl]) and 2, (2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, and Example 3 1-Benzyl-2-methyl-5-methoxytryptamine.

Figure 2 B depicts a superimposition of the computer model of compound Example 1 on the X-ray structure of moenomycin (moenomycin rings are labeled from A to F) bound to PBP2 (pdb code: 2OLV). Amino acids that form interactions with inhibitor Ex.1 are presented as sticks. Hydrophobic residues are shown as semi-transparent yellow surface. (Hydrogen bonds between compound Example 1 free amino group and Asp156 side chain are presented as dotted lines).

Figure 3 depicts the effect of compound Ex.2 on bacterial viability.

Figure 3A cell viability curves after exposure of *S. aureus* to variable concentration of compound Example 2. Figure 3 B shows the cell density of the same cell cultures.

Figure 4 depicts the Interaction of compound Example 2 with lipid vesicles and lipid II:

A. Interaction of **5b** with DOPC vesicles in the presence and absence of lipid II.

B. Effect of **5b** on nisin pore formation in DOPC LUVs containing 0,1 mol % lipid II.

C. Disruption of membrane integrity by **5b** in DOPC/DOPG (1:1 mol/mol) vesicles. (The results from a representative experiment are shown.)

D. Effect of **5b** on membrane potential. Effect of nisin on *Micrococcus flavus* is shown for comparison. Dye release was monitored at excitation and emission wavelengths of 622 and 670 nm, respectively.

E. Competition of undecaprenylpyrophosphate (11-PP), undecaprenylphosphate (11-P) and DOPG with lipid II (20 fold molar access over lipid II) for **5b** (2.5 μM) binding. The averages of two independent experiments are shown with the error bars indicating the spread between values.

F and G. ^{31}P and ^{1}H NMR, respectively, of undecaprenylpyrophosphate in Triton X-100 micelles in the presence or absence of 1 mM **5b**.

A.Interaction of Example 2 with DOPC vesicles in the presence and absence of lipid II.

B. Example 2 interference with nisin pore formation in DOPC LUVs containing 0,1 mol % Lipid II.

C. Disruption of membrane integrity by Ex.2 in DOPC/DOPG (1:1 mol/mol) vesicles. (The results from a representative experiment are shown.)

D. Effect of Example 2 on membrane potential. Effect of nisin in Micrococcus flavus is shown for comparison. Dye release was monitored at excitation and emission wavelengths of 622 and 670 nm, respectively.

E. Competition of undecaprenylpyrophosphate (11-PP), undecaprenylphosphate (11-P) and DOPG with lipid II for Ex.2 binding (20-fold molar excess over lipid II). The averages of two independent experiments are shown with the error bars indicating the spread between values.

F and G. ^{31}P and ^{1}H NMR, respectively, of undecaprenylpyrophosphate in Triton X-100 micelles in the presence or absence of 1 mM of compound EX.2.

Figure 5 shows the dose response curve of the inhibition of E. coli PBP1b by compound Ex.1. An IC50 value of 58.9 μM (± 3.3) was calculated from this curve.

Figure 6 shows the effect of compounds Ex.1 and Ex.2 and control agents on lipid II cycle.

Experimental Part

Experimental procedures and Chemicals

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DOPG), 1,2-dioleoyl-sn-glycero-3-[phospho-rac-(3-lysyl(1-glycerol))] (lysyl-DOPG) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) were purchased from Avanti Polar Lipids Inc. Nisin A was produced, isolated, and purified as described (Kuipers et al., 1992). Moenomycin A, is a gift from Aventis (Romainville, France). The fluorescent dye 3,3'-diethylthiodicarbocyanine iodide (DiSC2(5)) is from Molecular Probes Inc. Lipid I, Lipid II and Dansyl-Lipid II were synthesized and purified as described elsewhere (Breukink et al., 2003). Undecaprenylphosphate and undecaprenylpyrophosphate were obtained by phosphorylation of undecaprenol (Danilov et al., 1989) that was isolated from Laurus nobilis as described (Swiezewska et al., 1994). Radiolabeled [14C]meso-diaminopimelic acid (A2pm)-labelled Lipid II was prepared essentially as previously described (Terrak et al., 1999).

The small molecule compounds tested in this study were obtained from the National Cancer Institute and solubilised in DMSO at a concentration of 100 mM or 10 mM depending on the solubility of the compound. The identities of the active

compounds Ex.1 and Ex.2 were verified by mass spectrometry and NMR. The data (not shown) were consistent with the expected mass and structure for both compounds.

NMR data

NMR measurements were performed using undecaprenylpyrophosphate in detergent micelles in the presence and absence of Compound Ex.2. ^{31}P NMR revealed a change in chemical shift ($\Delta\delta P$=-0.4 ppm) for one of the phosphate groups in the presence of Compound Ex.2 (Fig. 4F, top). ^{1}H-NMR also showed changes in chemical shift for the protons adjacent to the phosphate groups (Fig. 4F, bottom). No significant shifts were observed when Compound Ex.2 was added to undecaprenylphosphate-containing Triton-X100 micelles.

GT51 family as target of the antibacterial compounds according to the invention

In order to prove the activity of the novel antibacterial agents, five model proteins of the GT51 family were prepared and employed *Escherichia coli* PBP1b, *Staphylococcus aureus* PBP2 and MtgA, *Enterococcus hirae* PBP2 and *Thermotoga maritima* PBP1a. *E. coli* PBP1b was produced and purified as described in Terrak *et al.*, 1999. *S. aureus* MtgA and PBP2 were produced and purified as described in Terrak *et al.*, 2006 and Lovering et al, 2007, respectively. The gene encoding (A68-Q723) PBP2 of *Enterococcus hirae* was cloned into pET28a (+) expression plasmid (Novagen) and the His Tag PBP2 was expressed in *E. coli* BL21 (DE3). The cells were grown at 37°C to an optical density of 0.8 at 600 nm, protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and incubation was continued overnight at 18°C. The cells were resuspended in 25 mM HEPES pH 7.5, 500 mM NaCl and the PBP2 was purified in one step on HisTrap column (GE, Heathcare). *T. maritima* PBP1a was produced and purified as described in Offant *et* al., Febs J. (in press)

Lipid II preparation and GT activity assay optimization

Lipid II is the natural substrate of the GT. A dansylated form of lipid II (DNS-LII) was synthesized and purified as described elsewhere (Breukink et al., 2003). in vitro using the MraY/MurG coupled assay (Bertsche et al., 2005 with modifications). This analogue is used by GTs to polymerize uncross-linked peptidoglycan, a process that can be directly monitored by following the decrease of fluorescence during the reaction (Schwartz *et al.*, 2001). Activity assays used 50 µl of optimal reaction mixture for each of the four GTs tested : *E. coli* PBP1b was used at 100 nM in the presence of 10 µM DNS-LII, 20% DMSO, 10 mM $CaCl_2$, 50 mM Hepes pH 7.5, 200 mM NaCl, 0.024% decylPEG, 1 unit of muramidase. *S. aureus* MtgA was used at 2 µM in the presence of 20 µM DNS-LII, 10% DMSO, 10 mM $MnCl_2$, 50 mM Hepes pH 7.5, 200 mM NaCl, 1 unit of muramidase. *E. hirea* PBP2 was used at 300 nM in the presence of 10 µM DNS-LII, 20% DMSO, 10 mM $CaCl_2$, 50 mM Hepes pH 7.5, 0.024% decylPEG, 1 unit of muramidase. *T. maritima* PBP1a was used at 150 nM in the presence of 10 µM DNS-LII, 20% DMSO, 10 mM $CaCl_2$, 50 mM Hepes pH 7.5, 200 mM NaCl, 0.024% decylPEG, 1 unit of muramidase.

Radioactive assay.

Radiolabeled [^{14}C]*meso*-diaminopimelic acid (A_2pm)-labelled Lipid II was prepared essentially as previously described (Terrak et al., 1999).

The reaction for the radioactive assay was performed in the same condition as for the fluorescence assay using [^{14}C]lipid II instead of the fluorescent substrate and by omitting the muramidase. The reaction products were separated by TLC isopropanol- ammonium hydroxide-H_2O (5:3:1; V/V) and analyzed using Typhoon Trio+ Imager and QuantityOne sofware (GE Healthcare).

Search for new GT inhibitors via structure-based virtual Screening.

The aim of structure-based virtual screening is to reduce a large number of compounds to a smaller subset, which is more likely to contain biologically active compounds. This is particularly helpful because of the difficulty of having high amount of lipid II substrate.

A virtual screening of small molecules library from the NCI Diversity set (2000 compounds) was carried out on the complex S. aureus PBP2-moenomycin

(PDB code 2olv, Lovering et al. 2007, Science 315, 1402-5) using eHiTS 6.1 software (SimBioSys, Inc., Toronto, Canada, 2006). This search resulted in a short list of potential hits.

In vitro GT inhibition assay of virtual Hits

In the first round of screening, the thirty highest ranked compounds were selected based on the information of their structures, score etc. Only 21 of them were solubilised and tested for E. coli PBP1b GT activity inhibition at 0.2 and 1 mM final concentrations. Compounds which inhibit E. coli PBP1b or interfere with the fluorescent test were tested with radioactive lipid II (Terrak et al., 1999). One compound, Ex.2 according to the invention inhibited the GT activity of E. coli PBP1b.

2D similarity search of the entire NCI database (250000) aimed at finding structural analogues of Example 1 led to the selection of 13 compounds, of which 12 were tested for biological activity, while one was found to be insoluble. Example 2 with a structure very similar to Example 1 was found to be even more active on E. coli PBP1b.

The compound Example 2 was also able to inhibit the activity of four other GTs, *S. aureus* MtgA and PBP2, *T. maritima* PBP1a and *E. hirae* PBP2 (Table 1).

IC50 values of the active compounds Ex.1 and 2 were determined with dansyl-lipid II fluorescent assay. The initial velocity of the reaction was determined in the presence of variable concentrations of inhibitor (50-1500 μM) and plotted versus the inhibitor concentration. The IC50 value was determined as the concentration of inhibitor that decreases the initial velocity by a factor of 2 (Fig.2, Table 1), and were all in the micromolar range.

Antibacterial activity of in vitro hits (determination of MIC).

Minimum inhibitory concentration (MIC) determinations were carried out using the Clinical and Laboratory Standard Institute (CLSI, 2009) broth microdilution method. Compounds were solubilised in 100% DMSO at a concentration of 10 mg/ml, and twenty-fold diluted in Mueller-Hinton broth (MHB), just before utilization. Inoculums of each strain (approximately 5.10^5 CFU/ml) were prepared in MHB.

The two compounds (Ex.1 and Ex.2) inhibited growth of all Gram positive bacteria and some resistant strains (Table 2), while they showed limited activity against Gram-negative bacteria. The MIC values of compounds Ex.1 and Ex.2 against *E. coli* 1411 were 64 and 16 μg/ml respectively which fell further with polymyxin nonapeptide (PMBN) treatment, or in an *acr*AB efflux mutant to the values observed in Gram-positive (Table 2).

The specificity of the structure according to the invention was confirmed by testing of 17 structural analogues, none of which contained the primary amine, for inhibition of GTs. None of the selected compounds was active. Furthermore, a pharmacophore model was established which shows the spatial arrangement of pharmacophores Ex.1 and 2 (Fig. 5).

Accordingly, the subject novel compounds for use as antibacterial agents, more specifically compounds acting as peptidoglycan GT inhibitors were determined through the use of structure-based virtual screening of small molecules from the National Cancer Institute library.

Selected hits were evaluated using the enzymatic activity of E. coli PBP1b and other GTs enzymes in the presence of Lipid II substrate followed by the determination of their effect on bacterial growth. The active compounds were then submitted to several assays to evaluate their specificity and mode of action.

Proteins expression and purification

Five model proteins of the GT51 family were prepared and used in this study. *E. coli* PBP1b, *S. aureus* MtgA and PBP2, were produced and purified as previously described (Lovering et al., 2007; Terrak et al., 1999; Terrak and Nguyen-Distèche, 2006). The PBP1a (Glu34-Gly643) *from Thermotoga maritima* MSB8 was expressed in BL21RIL *E. coli* cells to limit the effect of rare codons. The cells were grown in LB at 37°C to an optical density of 0.8 at 600 nm, protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and incubation was continued overnight at 15°C. The cells were resuspended in the 25 mM HEPES pH 7.5, 500 mM NaCl, 0.74% CYMAL-4 and a pill of Complete™ EDTA-free protease inhibitors (Roche) and lysed by sonication. After centrifugation (20,000 *g*, 30 min, 4°C), the protein was purified on Ni-NTA Superflow column (Qiagen) using buffer A (25 mM HEPES pH 7.5, 500 mM NaCl, 0.37% CYMAL-4) and eluted with two steps of 60 mM and 250 mM imidazole in buffer A. the protein was further purified by a gel filtration and a second Ni-affinity step following TEV cleavage of the poly His tag. The residual contaminants are removed by an ion exchange chromatography which delivered a pure protein as analyzed by SDS-PAGE stained with Coomassie (Offant et al., FEBS J., in press). The gene encoding (A68-Q723) PBP2 of *Enterococcus hirae* was cloned into pET28a (+) expression plasmid (Novagen) and the His Tag PBP2 was expressed in *E. coli* BL21 (DE3). The cells were grown at 37°C to an optical density of 0.8 at 600 nm, protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and incubation was continued overnight at 18°C. The cells were resuspended in 25 mM HEPES pH 7.5, 500 mM NaCl and the PBP2 was purified in one step on HisTrap column (GE, Heathcare),

Virtual screening

The virtual screening was carried out on a suitably equipped workstation,. using the programme eHiTS 6.0 from SimBioSys Inc. (Zsoldos et al., 2006; Zsoldos et al., 2007) for the active site detection and docking. Open Babel (http://openbabel.org) was used for manipulating the ligands with various chemical

formats. PyMol from DeLano Scientific was used for visual inspection of the results and the graphical representations.

The 3D structures of the compounds from the NCI Diversity Set were obtained from the NCI webpage (http://dtp.nci.nih.gov/dw/testmasters/chem3d.html). The 1990 highly diverse compounds of the NCI Diversity Set represented a broad chemical spectrum of whole NCI database. However, no special preparation of the 3D structures was applied since eHiTS automatically evaluates all of the possible protonation states for ligands and enzymes. The crystal structure of *S. aureus* PBP2 as a complex with moenomycin (PDB entry 2OLV) (Lovering et al., 2007) was used for virtual screening. PBP2 crystal structure was initially prepared with eHiTS. The active site detection was carried out using the 'complex' parameter with high accuracy. The program automatically detected the ligand in the complex and selected the part of the enzyme within a 7Å margin around the ligand as the active site. The NCI Diversity Set was then docked to the active site. The scoring was according to the eHiTS_Score that is included in the eHiTS software package.

In vitro glycosyltransferase activity and GT inhibition assays

Fluorescence assay. GT activity was monitored using the continuous fluorescence assay method (Schwartz et al., 2002). For the screening of numerous conditions simultaneously this technique was adapted to 96-well plate format (Greiner Bio-One). The standard reaction was carried out in 50 µl at 30°C and contained 50 mM Hepes pH 7.5, 200 mM NaCl, 0.024% decyl PEG, 10 mM CaCl2, 20% DMSO, 10 µM dansyl-Lipid II, 1 unit of muramidase and 100 nM PBP1b. The data were collected for 30 min using a Victor 3 fluorimeter (Perkin Elmer) with excitation at 355 nm and emission at 536 nm.

For the other GTs enzymes the reaction conditions were adapted for optimal activity as follows, with only variable conditions given: S. aureus MtgA was used at 2 µM in the presence of 20 µM dansyl-Lipid II, 10% DMSO and 10 mM MnCl2. S. aureus PBP2 was used at 2.5 µM in the presence of 20 µM dansyl-Lipid II and 50 mM sodium acetate pH 5. E. hirae PBP2 and T. maritima PBP1a were used at 300 nM and 150 nM respectively.

Radioactive assay. The reaction, containing a final volume of 30 µl, for the radioactive assay was performed in the same condition as for the fluorescence assay using 4 µM [14C]Lipid II (0.126 µCi nmol-1) instead of the fluorescent substrate and by omitting the muramidase. The reaction was stopped with moenomycin (10 µM) and the products were separated by TLC in isopropanol-ammonium hydroxide-H2O (5:3:1; V/V) and analyzed using Typhoon Trio+ Imager and ImageQuant TL software (GE Healthcare).

GT inhibition assay. IC50 values of compounds Ex.1 and Ex.2 were determined using a fluorescence based assay. The initial velocity of the reaction was determined in the presence of variable concentrations of inhibitor (20-1500 µM) and plotted versus the inhibitor concentration using Sigma plot program (Systas Software). The IC50 value was determined as the concentration of inhibitor that decreases the initial velocity by a factor of 2 using three independents experiments.

Table 1 shows the . IC50 values of compounds Example 1 and 2 for different GTs:

Table 1. IC50 values of compounds Example 1 and 2 for different GTs.

		IC50 (µM)				
Compd.	NSC no.	EcoPBP1b	SauPBP2	TmPBP1a	EhiPBP2	SauMtgA
Ex.1	17383	58.9 ± 3.3	nd	nd	nd	nd
Ex.2	17382	29.2 ± 12	20.7 ± 1	56.3 ± 7	32.6 ± 6	26.9 ± 9

EcoPBP1b, E. coli PBP1b; SauPBP2 and SauMtgA, S. aureus PBP2 and MtgA; EhiPBP2, E. hirae PBP2; TmaPBP1a, T. maritima PBP1a. For comparison, the IC50 value of moenomycin for S. aureus PBP2 is 1.3 µM (±0.3) nd, not determined

MIC determination

Minimum inhibitory concentration (MIC) determinations were carried out using the Clinical and Laboratory Standard Institute (CLSI, 2009) broth microdilution method. Compounds were solubilised in 100% DMSO at a concentration of 10 mg/ml, and twenty-fold diluted in Mueller-Hinton broth (MHB), just before utilization. Inoculums

of each strain (approximately 5.10^5 CFU/ml) were prepared in MHB. *M. hominis* MICs were determined as previously reported (Waites et al., 2001).

Table 2 shows the results of the antibacterial effects of compounds according to the invention.

Table 2. Susceptibility of panel of bacteria to compound Ex.1 and Ex.2 and moenomycin.

	MIC (µg/ml)		
	Ex.1	**Ex.2**	moenomycin
E. faecalis ATCC 29212	16	4 (10µM)	4
L. innocua ATCC 33090	8	4	4
S. aureus ATCC 25923 (MSSA)	8	4	0.5
S. aureus ATCC 43300 (MRSA)	8	4	0.5
S. aureus PL1 (MRSA)	8	4	0.5
M. luteus ATCC 9341	8	4	> 64
S. epidermidis ATCC 12228	8	4	4
S. pneumoniae D39	16	nd.	nd
E.coli 1411	64	16	nd
E.coli 1411 + PMBN (4ug/ml)	16	8	nd
P. aeruginosa	> 250	nd	nd
M. hominis	*> 64*	*> 64*	*>64*

S. aureus means Staphylococcus aureus; L. innocua, Listeria innocua ; M. luteus, Micrococcus luteus ; S. epidermis, Streptococcus epidermis; S. pneumoniae, Streptococcus pneumoniae ; E. coli, Escherichia coli ; P. aeruginosa, Pseudomonas aeruginosa. Mycoplasma hominis; nd, not determined.

*MIC (moenomycin) for E. coli K12 = 8µg/ml (van Heijenoort et al., Journal of General Microbiology (1 987), **133,** 667-674)*

Effect on bacterial viability

S. aureus ATCC 25923 colonies grown overnight on Mueller-Hinton agar (MHA) plates were suspended in MHB (≅ 1.10^8 CFU/mL) and aliquoted. Compound 5b was added to reach 0.5, 1, 2 or 4 times the MIC and mixed. Samples were taken at

different time intervals of incubation at 37°C, serially diluted and plated on MHA in triplicate. The colonies were counted after incubation at 37°C for 18 to 24h. (See panel A in Fig. 3)

Pathway reporter assays in Bacillus subtilis

To characterise the mode of action of Ex.2, Bacillus subtilis reporter system was employed which differentiates inhibitors of cell envelope, DNA, RNA, protein and fatty acid biosynthesis by quantifying the upregulation of a specific luciferase reporter construct in each case as described (Fischer et al., 2004; Urban et al., 2007). Table 4 shows the outcome of assays:

Table 4: Induction of B. subtilis antibiotic biosensors in response to a panel of antimicrobial agents

Antimicrobial Agent	Upregulated biosynthetic pathway				
	Cell Wall	Protein	RNA	DNA	Fatty Acid
Vancomycin	+	-	-	-	-
Flucloxacillin	+	-	-	-	-
Ex.1 or Ex.2	+	-	-	-	-
Nisin	-	-	-	-	-
Cetyltrimethylammonium bromide	-	-	-	-	-
Tetracycline	-	+	-	-	-
Anhydrotetracycline	-	+	-	-	-
Fusidic Acid	-	+	-	-	-
Rifampicin	-	-	+	-	-
Rifamycin SV	-	-	+	-	-
Ciprofloxacin	-	-	-	+	-
Trimethoprim	-	-	-	+	-
Triclosan	-	-	-	-	+

+/- Induced/uninduced biosensor in response to a range of concentrations of antimicrobial agent.

The assay based on LiaRS two-component system in Bacillus subtilis sensing stress on cell wall caused by compounds interfering with Lipid II cycle was used according to Burkard and Stein, 2008; Mascher et al., 2004. The Bacillus subtilis strain BFS2470 carries a β-galactosidase reporter gene under the control of liaI promoter. The effect of antibiotics or compounds spotted on a Whatman disc is revealed by

blue/white selection method after overnight incubation at 37°C on agar-plate containing X-Gal.(This section is related to fig 6)

Preparation of large unilamellar vesicles (LUVs)

Large unilamellar vesicles were prepared essentially as described (Breukink et al., 1997). Desired amounts of lipid solutions in chloroform were mixed and evaporated under a gentle stream of nitrogen. The lipid film was subsequently dried for 20 min under vacuum. The film was hydrated by the addition of the buffer of choice under mechanical agitation and submitted to ten freeze-thaw cycles using liquid nitrogen and a water bath. The lipid suspension was then extruded ten times through a polycarbonate membrane filter with a pore size of 200 nm (Whatman International) (Hope et al., 1985). The final phospholipid concentration was determined by phosphate analysis according to Rouse et al. (Rouser et al., 1970).

Vesicles binding assay

DOPC with or without 1 mol% Lipid II vesicles were prepared as described above in 10 mM MES-KOH, 15 mM K2SO4 at pH 7. Vesicles (1 mM lipid Pi) were incubated with 5 μM and 20 μM Ex.2, respectively, for 15 min at room temperature. The mixture was centrifuged in a TLA 120.2 rotor using a Beckman Ultracentrifuge (TL-100) for 1.5 h at 100 krpm and 20 °C. The amount of Ex.2 before centrifugation and in the supernatant and pellet was determined by fluorescence on a Cary Eclipse fluorescence spectrophotometer (Varian Inc.). The percentage of Ex.2 in the supernatant and pellet was determined by comparing the maximal value (350 nm) of fluorescence emission intensity.

Carboxyfluorescein leakage assay in large unilamellar vesicles

Carboxyfluorescein (CF)-loaded LUVs were prepared in 50 mM MES-KOH, 100 mM K2SO4, pH 6.5 (K+-buffer) as described above with the addition of 50 mM CF. Following the extrusion step, the vesicle suspension was applied to Sephadex G-50 spin columns equilibrated with K+-buffer to remove free CF. The final phospholipid concentration was determined by phosphate analysis according to Rouser et al. (Rouser et al., 1970). Vesicles were resuspended in K+-buffer to a concentration of 25 μM and Ex.2 was added at the desired concentration 1 min prior

to the addition of nisin A (50 nM, final conc.). High-salt experiments were conducted in K+-buffer supplemented with 0.5M NaCl. The nisin-induced CF leakage from the vesicles was monitored with excitation and emission wavelengths set at 430 nm and 513 nm, respectively. Triton X-100 was added 1 min after the addition of nisin, to a final concentration of 0,2% (w/v) to fully disrupt the lipid vesicles and the corresponding fluorescence was taken as 100% leakage. The Ex.2-induced reduction of leakage is expressed as a percentage of the leakage observed in the absence of Ex.2 relative to the total release of carboxyfluorescein after addition of Triton X-100.

Membrane permeabilization assay

The cytoplasmic membrane depolarization activity of Ex.2 was determined with the membrane potential-sensitive dye DiSC2(5) using Staphylococcus simulans and Micrococcus flavus grown in LB broth at 37°C and 30°C, respectively. Bacterial cells in the mid-logarithmic phase were centrifuged, washed in 5 mM HEPES (pH 7.8), and resuspended in the same buffer to an optical density at 600 nm of 0.05 in a 1 cm cuvette. A stock solution of DiSC2(5) was added to a final concentration of 0.4 μM and quenching was allowed to occur at room temperature for approximately 1 min. The desired concentration of Ex.2 was added and changes in fluorescence due to the disruption of the membrane potential gradient across the cytoplasmic membrane were continuously recorded with a Cary Eclipse spectrofluorometer at an excitation and emission wavelengths of 622 and 670 nm, respectively(. The results are shown in Fig 4D.

Membrane damage assay

The fast effect of Ex.2 on cell growth and its activity *on S. aureus* L-form strains was confirmed by the investigation into its effect on cell membranes using the LIVE/DEAD BacLight™ (backlight is a trademark of Invitrogen, Carlsbad, CA, USA.) bacterial viability assay as previously reported (Hilliard et al., 1999). The membrane damage inflicted by 10 minutes exposure to a panel of agents on *S. aureus* SH1000 was analysed. Compound Ex. 2 and several control antimicrobial agents were tested at 4x MIC in comparison to a drug free control (Table 5). After 10 minutes, cells treated with the test agent Ex.2 maintained 33% membrane integrity (67%

damage), which does suggest that this agent causes some damage to staphylococcal membranes and may explain the fast drop in cell viability and cell turbidity when cells were exposed to the compound.

Table 5 depicts the results of the membrane assay:

Table 5. Effect of Ex.2 on staphylococcal membrane integrity

Antimicrobial agent	*S. aureus* SH1000 MIC (μg/ml)	BacLight result (% membrane integrity)
Drug-free control	-	100
5% SDS (w/v)	-	0
CTAB	2	0
Nisin	2	14.9 ± 1.0
Tetracycline	0.5	99.9 ± 4.6
Vancomycin	2	83.5 ± 4.4
Ex. 2	8	32.9 ± 1.1

CTAB, Cetyltrimethylammonium bromide

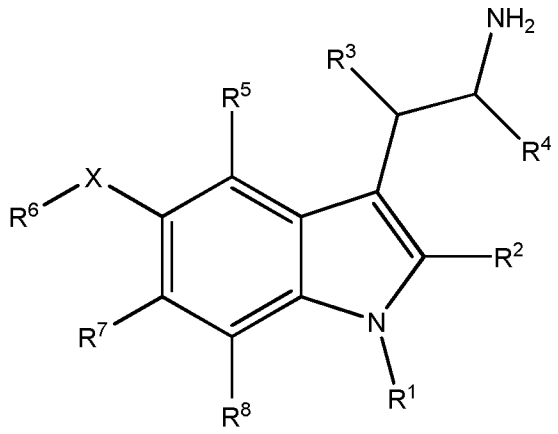
The above experiments clearly show the effectiveness of then novel class of antibiotic compounds, and their novel effect on cell growth through inhibition of a glycosyltransferase enzyme complex.

While the present invention has been described with reference to specific preferred embodiments, it should be appreciated that variations are possible without departing from the scope of the invention. Therefore, the invention is not intended to be limited by the description in the specification but only by the language of the claims and equivalents thereof.

Claims

1. A compound according to formula (I),

(I)



a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof for the treatment of pathologies associated with bacterial infections in a subject in need thereof, wherein R^1 represents an optionally substituted benzyl group, which is optionally substituted at the aromatic ring by one ore more alkyl, alkoxy , hydroxyl, thioalkyl, halogen, nitro, cyano or amino groups,

R^2 represents a lower alkyl group comprising from 1 to 4 carbon atoms, a cycloalkyl group, an aryl group , a lower alkoxy group , a lower thioakyl group, a cycloalkyloxy group, an aralkyloxy group or an alkanoyl group;

R^3, R^4, R^5, R^7 and R^8 may independently be the same or different, and represent a hydrogen atom, a halogen atom, a hydroxyl group, a nitro group, a formyl group, an amino group which may be protected or substituted, a lower alkyl, cycloalkyl, aryl, lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, ureido or monocyclic heterocyclic group;

X represents an oxygen atom or a sulphur atom;

R^6 represents a hydrogen atom, a lower alkyl group, a cycloalkyl group , an aryl group, a lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, alkanesulfonyl or monocyclic

heterocyclic group, all of which may optionally be substituted; for use in treating pathologies associated with bacterial infections in a subject in need of treatment.

2. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to claim 1, wherein X represents sulphur atom.

3. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to claim 1 or 2, wherein R^1 represents a mono-, di- or tri-substituted benzyl group.

4. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any of the previous claims, wherein R^1 represents a *2*-chlorobenzyl or a *3,4*-dichlorobenzyl group.

5. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any of the previous claims, wherein R^2 is an alkyl group comprising from 1 to 4 carbon atoms, preferably methyl.

6. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any of the previous claims, wherein R^3 and R^4 represent a hydrogen atom.

7. A compound a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any of the previous claims, wherein R^5, R^7 and R^8 represent a hydrogen atom.

8. A compound a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any of the previous claims, wherein the compound is 2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl], (2-(1-(3,4-

dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, or combinations thereof.

9. (2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof.

10. 2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof for use as a medicament.

11. 2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof for use in treating pathologies associated with bacterial infections in a subject in need of treatment.

12. A compound, a pharmaceutically acceptable salt, hydrate, solvate, N-oxide and/or prodrug thereof according to any one of claims 1 to 11, wherein the pathologies are associated with the a bacterial infection by sensitive, or single and/or multiple resistant Gram -positive bacteria.

13. A compound for use according to any one of claims 1 to 11, wherein the compound inhibits the transglycosylation reaction in the cell wall metabolic pathway of the bacteria.

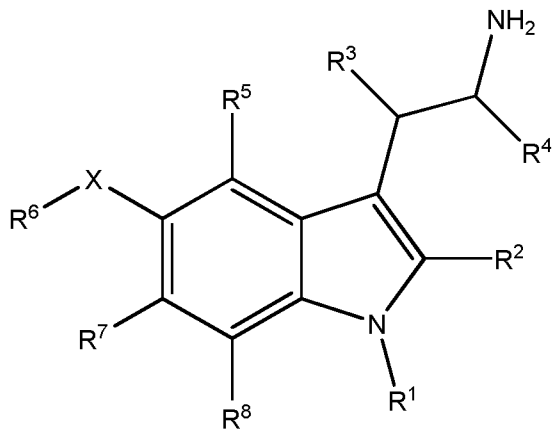
14. A compound for use according to claim 13, wherein the compound inhibits the Glycosyltransferase 51 in the metabolism of the bacteria.

15. An antibacterial agent comprising a compound of the formula (I) according to any one of claims 1 to 14.

16. A pharmaceutical composition comprising a compound of the formula (I) according to any one of claims 1 to 15.

17. A process for preparing an antibacterial composition comprising formulating the compound of the formula (I) according to any one of claims 1 to 14, and optionally a utilizable carrier.

18. A method for the treatment of pathologies associated with bacterial infections or preventing a bacterial infection in a subject, comprising the administration, to a subject in need thereof, of at least one compound of formula (I)



a pharmaceutically acceptable salt, prodrug and/or N-oxide thereof, wherein

R^1 represents an optionally substituted benzyl group, which is optionally substituted at the aromatic ring by one ore more alkyl, alkoxy , hydroxyl, thioalkyl, halogen, nitro, cyano or amino groups,

R^2 represents a lower alkyl group comprising from 1 to 4 carbon atoms, a cycloalkyl group, an aryl group , a lower alkoxy group , a lower thioakyl group, a cycloalkyloxy group, an aralkyloxy group or an alkanoyl group;

R^3, R^4, R^5, R^7 and R^8 may independently be the same or different, and represent a hydrogen atom, a halogen atom, a hydroxyl group, a nitro group, a formyl group, an amino group which may be protected or substituted, a lower alkyl, cycloalkyl, aryl, lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, ureido or monocyclic heterocyclic group;

X represents an oygen atom or a sulphur atom;

R^6 represents a hydrogen atom, a lower alkyl group, a cycloalkyl group , an aryl group, a lower alkoxy, cycloalkyloxy, aralkyloxy, alkanoyl, alkanesulfonyl or monocyclic heterocyclic group, all of which may optionally be substituted.

19. A method according to claim 18, wherein X represents sulphur atom.

20. A method according to claim 18 or 19, wherein R^1 represents a mono-, di- or tri-substituted benzyl group.

21. A method according to any one of claims 18 to 20, wherein R^1 represents a *2*-chlorobenzyl or a *3,4*-dichlorobenzyl group.

22. A method for treatment according to any one of claims 18 to 21, wherein the subject is a higher animal.

23. A method for treatment according to any one of claims 18 to 22, wherein the subject is a mammalian subject.

24. A method for treatment according to claim 23, wherein the subject is a human being.

25. A method according to any one of claims 18 to 24, wherein R^2 is an alkyl group comprising from 1 to 4 carbon atoms, preferably methyl.

26. A method according to any one of claims 18 to 25, wherein R^3 and R^4 represent a hydrogen atom.

27. A method according to any one of claims 18 to 26, wherein R^5, R^7 and R^8 represent a hydrogen atom.

28. A method according to any one of claims 18 to 27, wherein the compound is 2-[1-[(2-chlorophenyl)methyl]-2-methyl-5-methylsulfanylindol-3-yl], (2-(1-(3,4-dichlorobenzyl)-2-methyl-5-(methylthio)-1H-indol-3-yl)ethanamine, or combinations thereof

29. A method according to any one of claims 18 to 28, wherein the pathologies are associated with bacterial infections by bacteria employing Glycosyltranferase, preferably Glycosyltranferase 51 in their metabolic pathway; and wherein the compound, when administered in an appropriate way and amount, inhibits the pathway such that the cell membranes of the bacteria are at least in part damaged.

30. Use of at least one compound according to any one of claims 1 to 14 for the manufacture of a medicament for the treatment or prevention of a bacterial infection in a subject in need of such treatment.

Figure 1

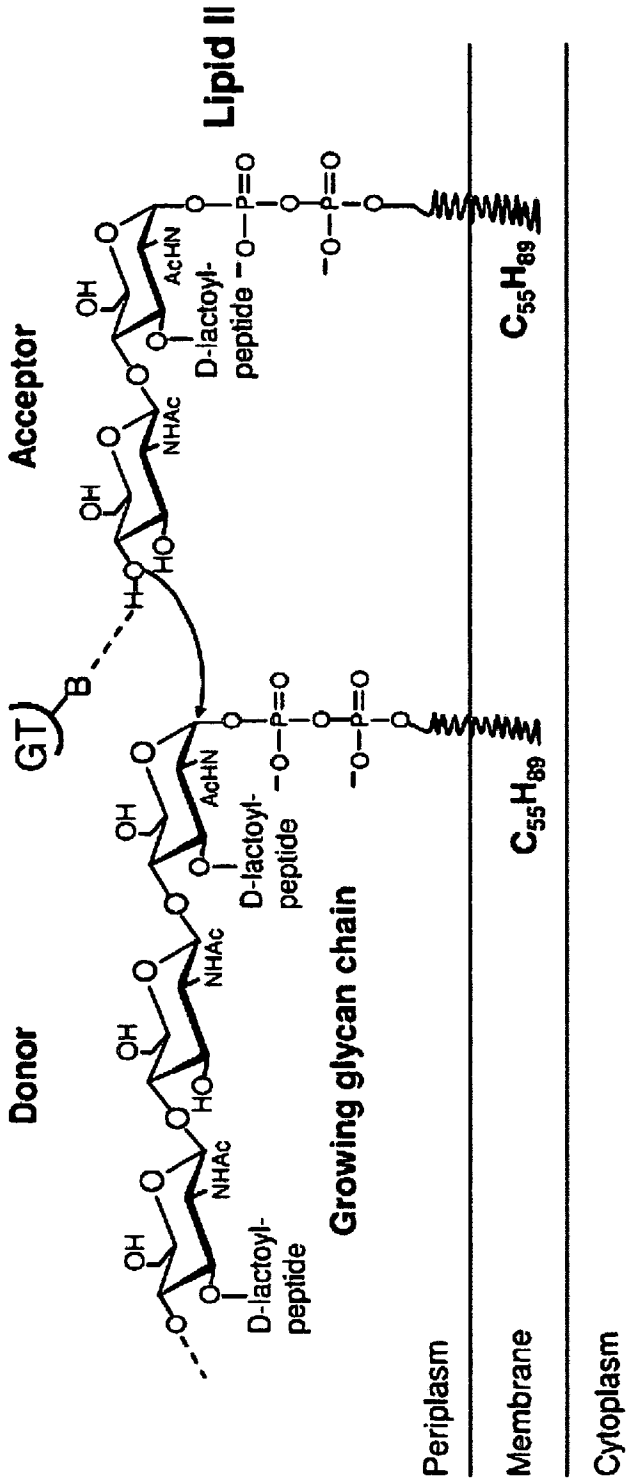
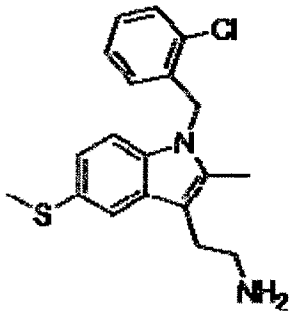
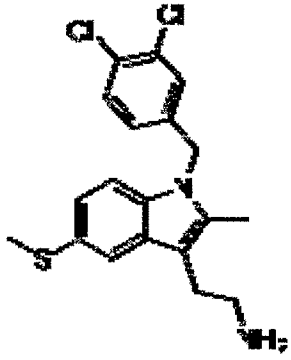

Lipid II
Acceptor
Donor
Growing glycan chain
GT
B
AcHN
NHAc
OH
HO
D-lactoyl-peptide
$C_{55}H_{89}$
Periplasm
Membrane
Cytoplasm

Figure 2

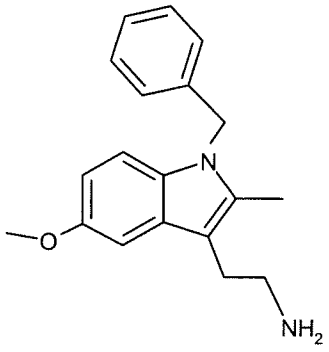
A



Ex.1



Ex.2



Ex.3

B

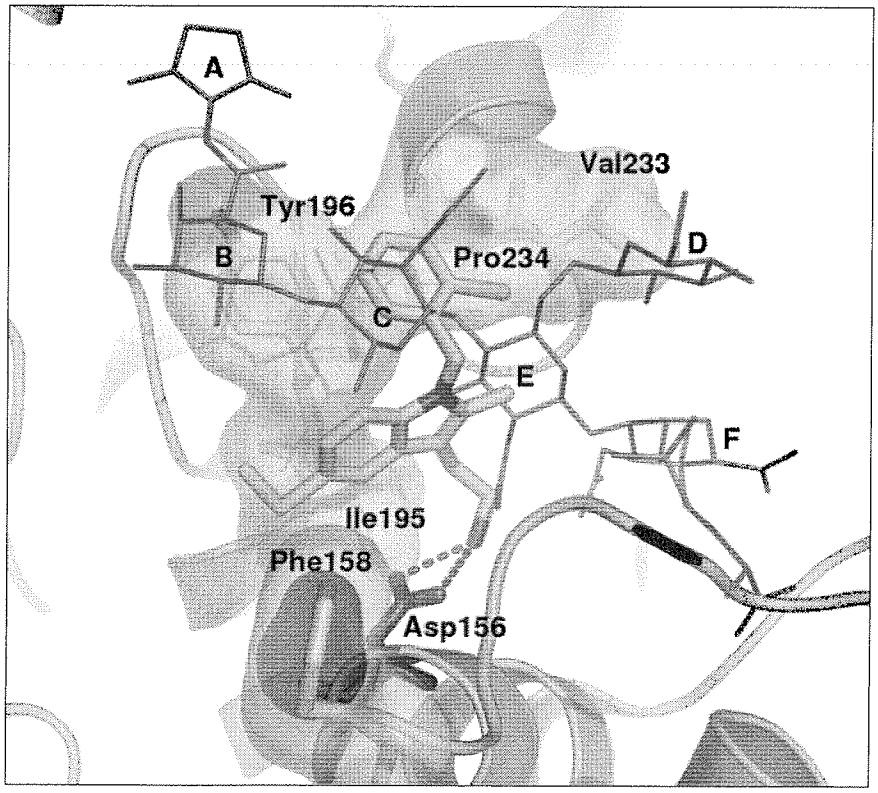
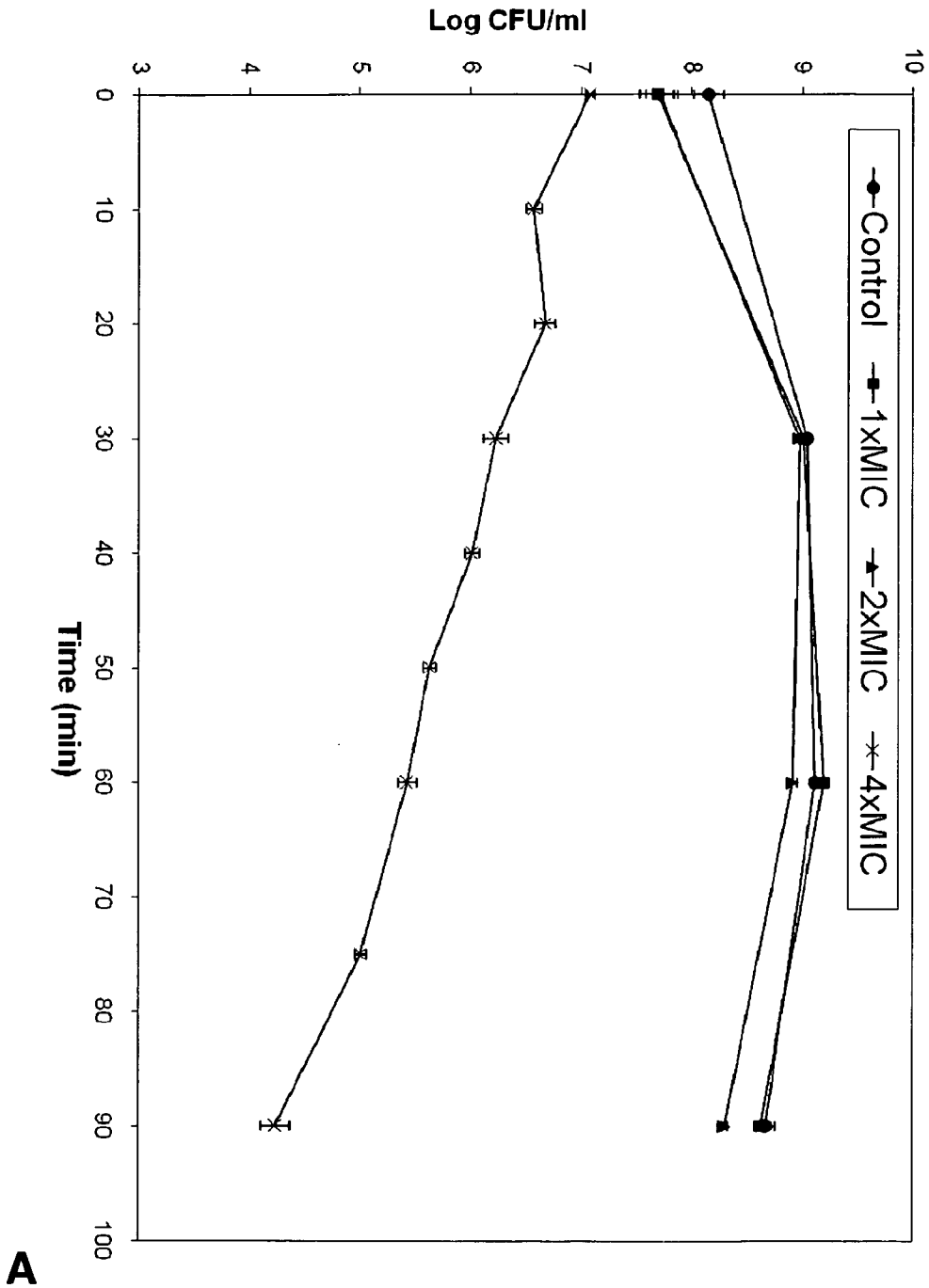


Figure 3


Log CFU/ml
3
4
5
6
7
8
9
10
Time (min)
0
10
20
30
40
50
60
70
80
90
100
Control
1xMIC
2xMIC
4xMIC

A

Figure 3

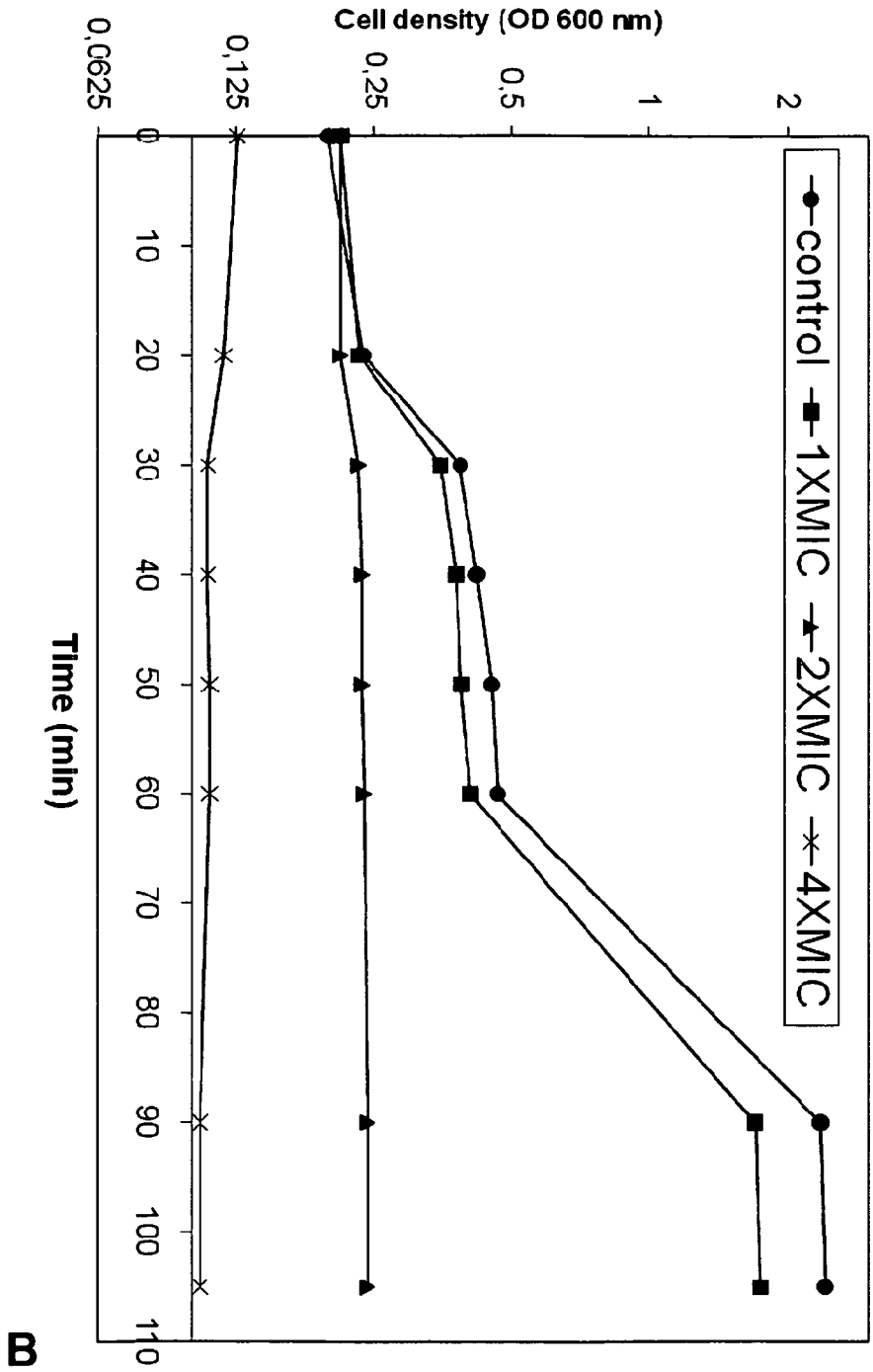

Cell density (OD 600 nm)
0,0625
0,125
0,25
0,5
1
2
Time (min)
0
10
20
30
40
50
60
70
80
90
100
110
control
1XMIC
2XMIC
4XMIC
B

Figure 4

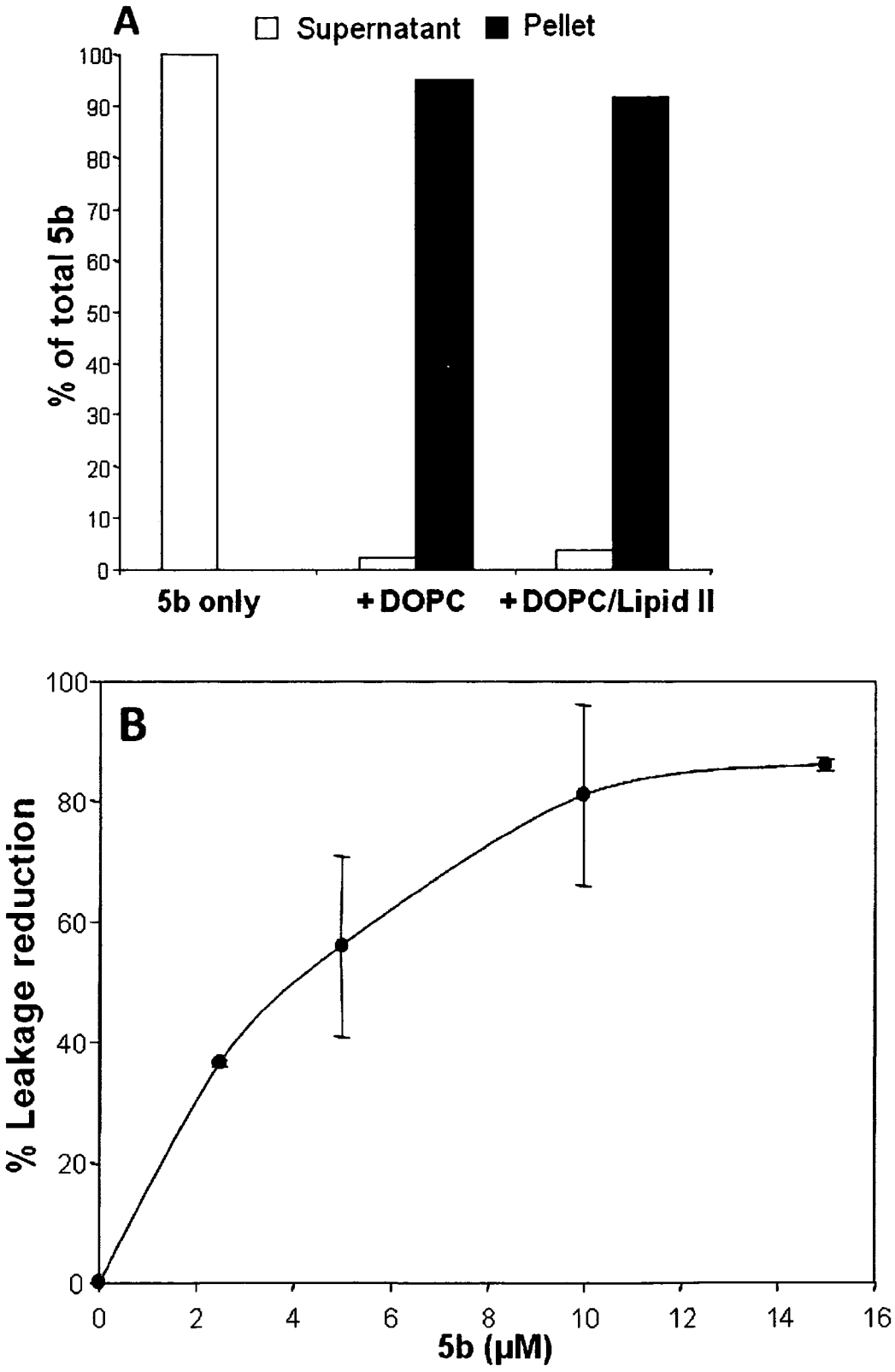
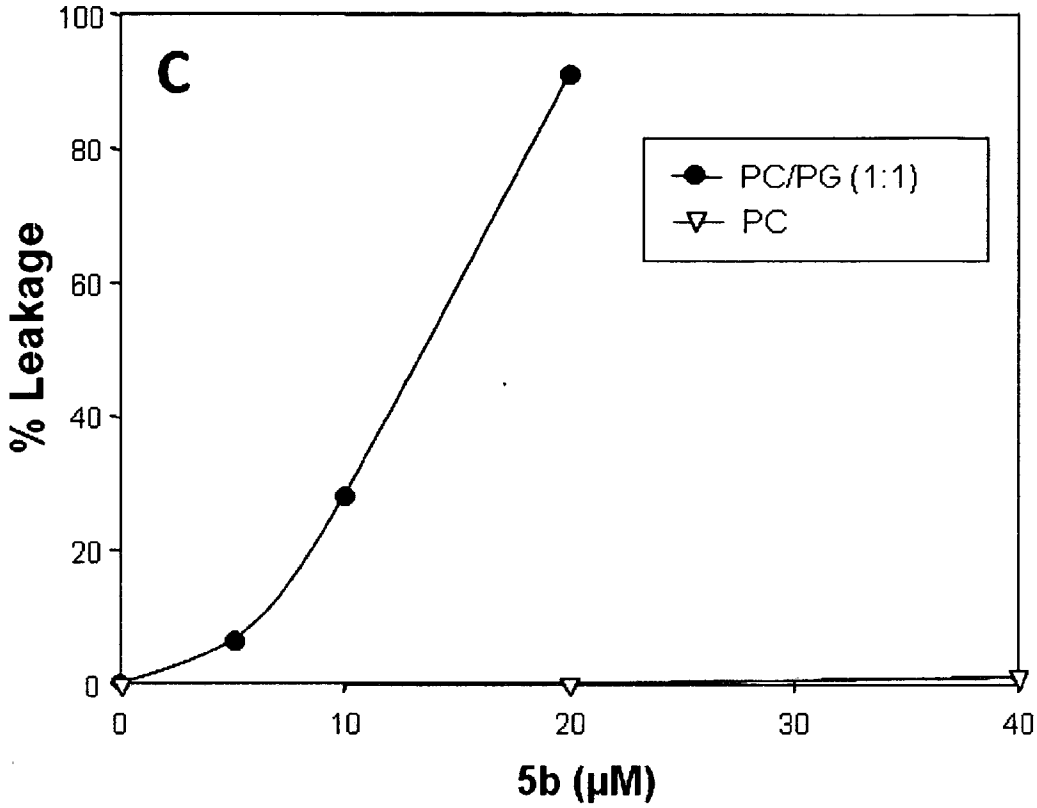

A
Supernatant
Pellet
% of total 5b
100
90
80
70
60
50
40
30
20
10
0
5b only
+DOPC
+DOPC/Lipid II
B
% Leakage reduction
100
80
60
40
20
0
0
2
4
6
8
10
12
14
16
5b (μM)

Figure 4


C
% Leakage
100
80
60
40
20
0
0
10
20
30
40
5b (µM)
PC/PG (1:1)
PC

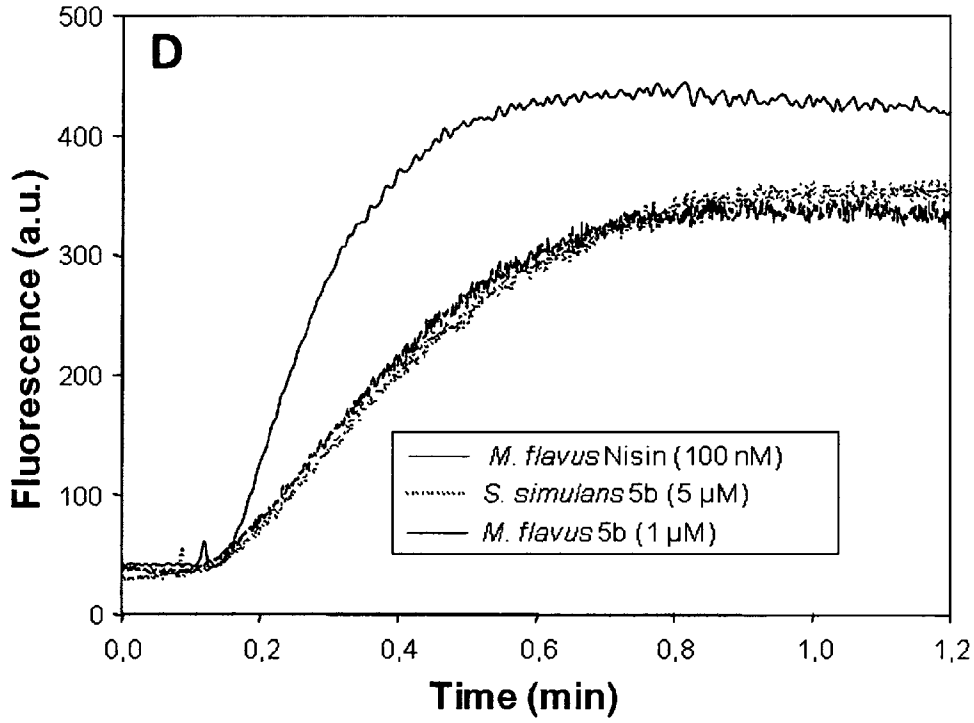

D
Fluorescence (a.u.)
500
400
300
200
100
0
0,0
0,2
0,4
0,6
0,8
1,0
1,2
Time (min)
M. flavus Nisin (100 nM)
S. simulans 5b (5 µM)
M. flavus 5b (1 µM)

Figure 4

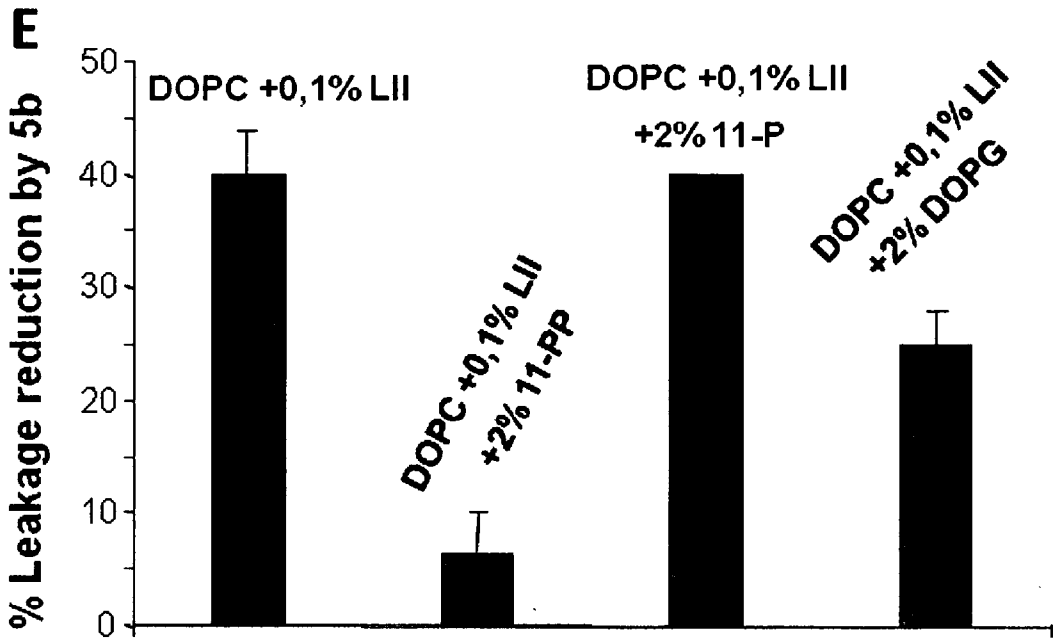
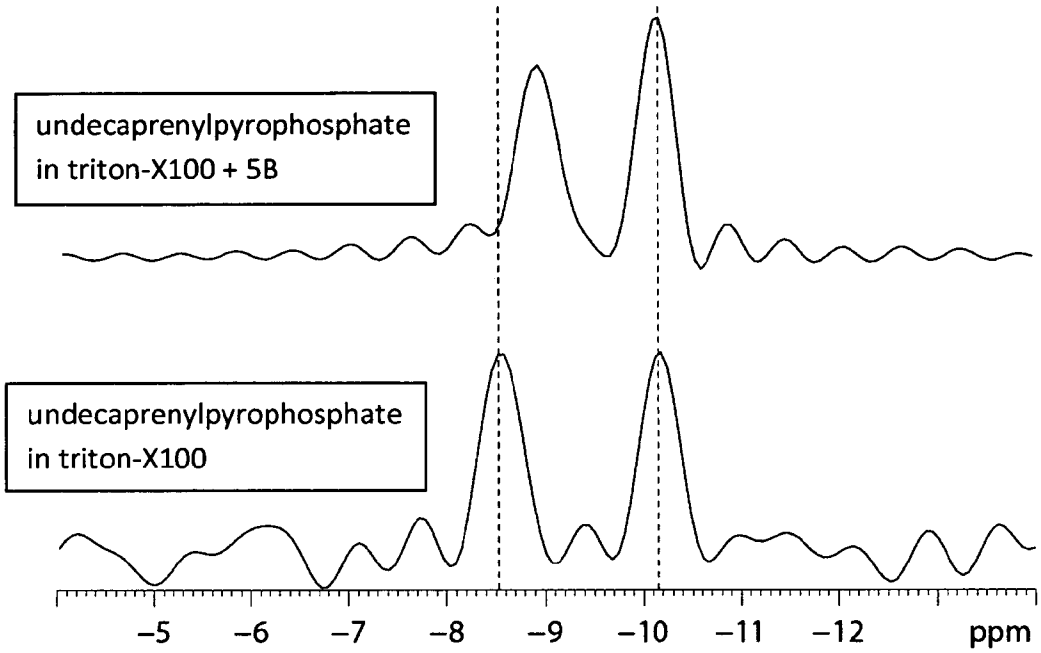

E
% Leakage reduction by 5b
50
40
30
20
10
0
DOPC +0,1% LII
DOPC +0,1% LII
+2% 11-PP
DOPC +0,1% LII
+2% 11-P
DOPC +0,1% LII
+2% DOPG

Figure 4

F


undecaprenylpyrophosphate
in triton-X100 + 5B
undecaprenylpyrophosphate
in triton-X100
−5
−6
−7
−8
−9
−10
−11
−12
ppm

G

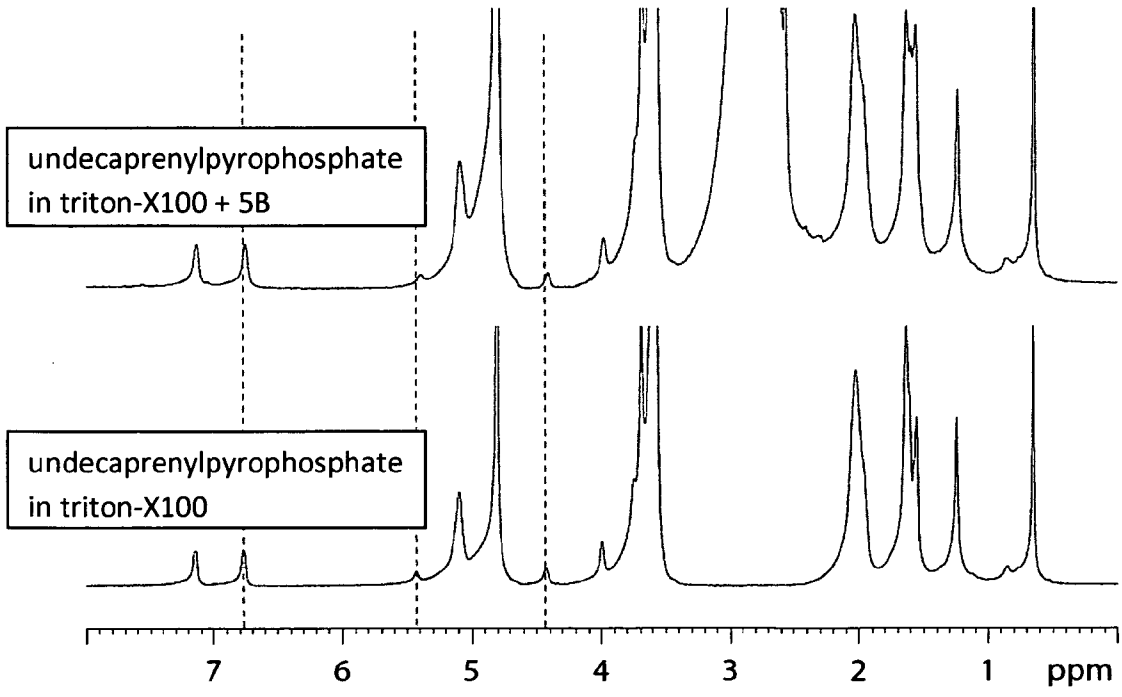

undecaprenylpyrophosphate
in triton-X100 + 5B
undecaprenylpyrophosphate
in triton-X100
7
6
5
4
3
2
1
ppm

Figure 5

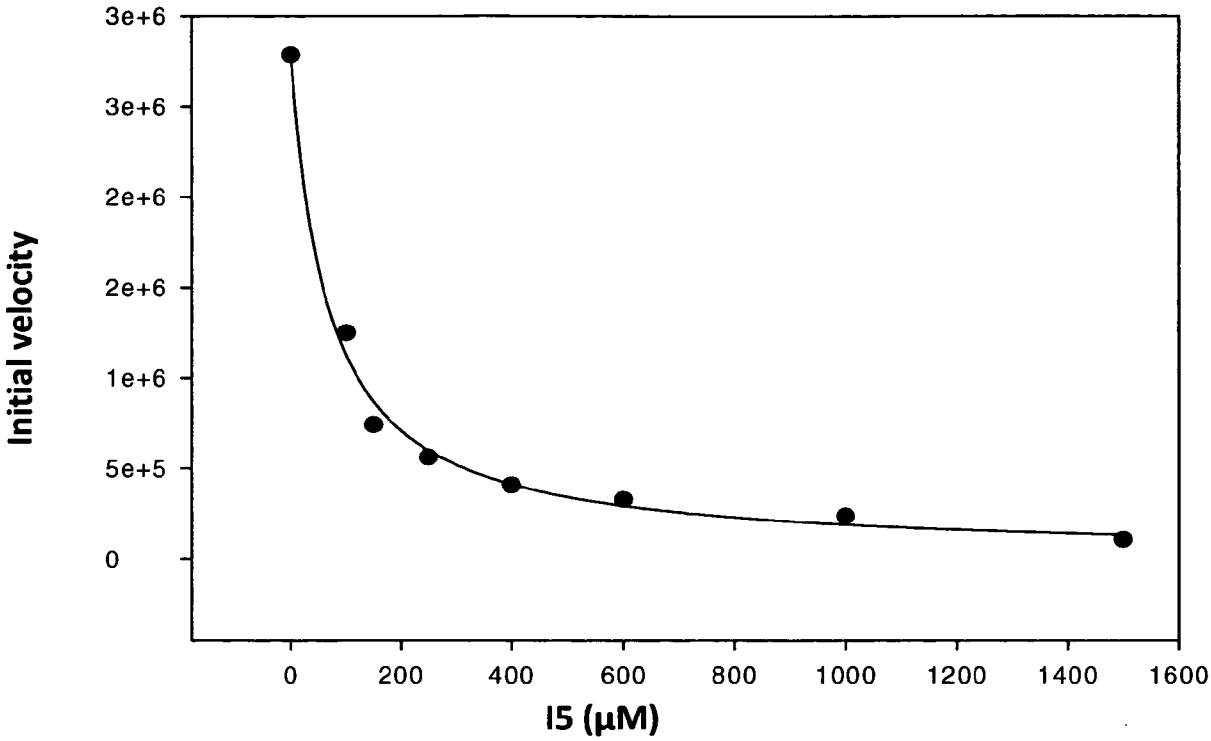
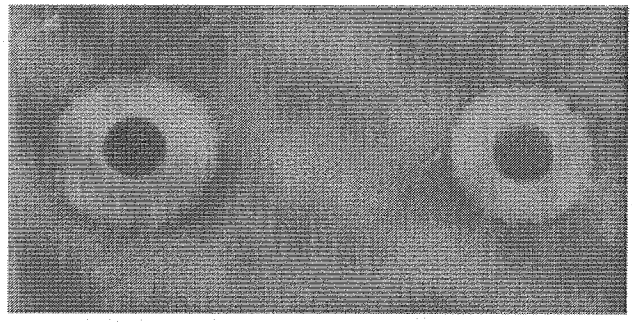
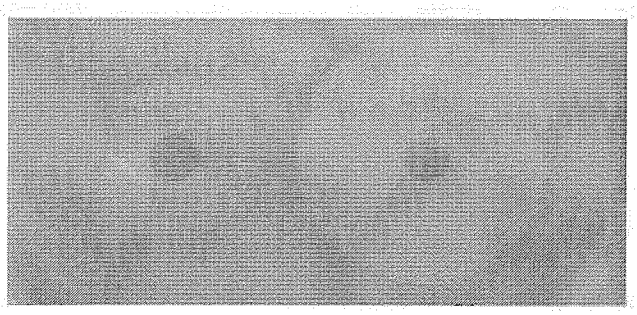

3e+6
3e+6
2e+6
2e+6
1e+6
5e+5
0
Initial velocity
0
200
400
600
800
1000
1200
1400
1600
I5 (µM)

Figure 6

Ex.2 (32 µg) Nisin (8 µg)



Ex.2 (16 µg) Ex.1 (32 µg)



Moenomycin (8µg) Ampicillin (10µg)

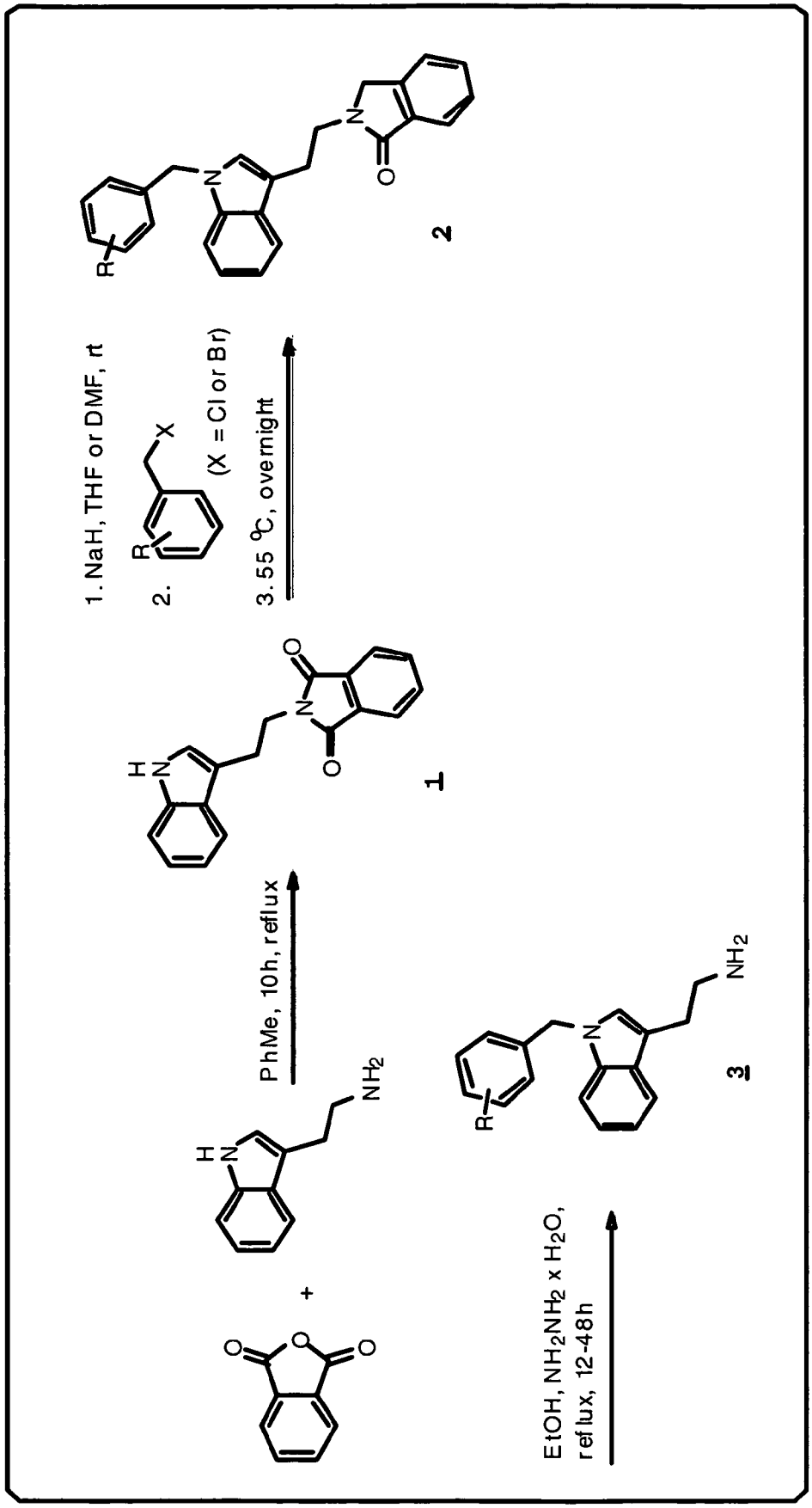

1. NaH, THF or DMF, rt
2.
(X = Cl or Br)
3. 55 °C, overnight
2
1
PhMe, 10h, reflux
NH2
+
EtOH, NH2NH2 x H2O,
reflux, 12-48h
3
NH2

Figure 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/063405

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K31/4045 C07D209/16 A61P31/04
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)
C07D A61K

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 practical, search terms used)
EPO-Internal, BEILSTEIN Data, BIOSIS, CHEM ABS Data, EMBASE, SCISEARCH, 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	US 3 074 960 A (SYDNEY ARCHER) 22 January 1963 (1963-01-22) examples 13, 16	9,10, 12-17
X	GASSNER NADINE C ET AL: "Accelerating the discovery of biologically active small molecules using a high-throughput yeast halo assay." JOURNAL OF NATURAL PRODUCTS MAR 2007 LNKD-PUBMED:17291044, vol. 70, no. 3, March 2007 (2007-03), pages 383-390, XP002606572 ISSN: 0163-3864 figure 3 table 1 page 389, left-hand column, paragraph 2-3	15-17

-/--

[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 document 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
25 October 2010	04/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Herdemann, Matthias

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2010/063405

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CINGOLANI G ET AL: "Indolizine derivatives with biological activity VI 1-(2-aminoethyl)-3-benzyl-7-methoxy-2-methylindolizine, benanserin structural analogue" EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR LNKD- DOI:10.1016/0223-5234(90)90138-S, vol. 25, no. 8, 1 October 1990 (1990-10-01), pages 709-712, XP023870905 ISSN: 0223-5234 [retrieved on 1990-10-01] scheme 1; page 709, left-hand column, paragraph 1	15-17
X	GB 1 021 422 A (BRITISH SCHERING LTD) 2 March 1966 (1966-03-02) page 3, lines 70-99 claim 19	15-17
X	WO 2007/062399 A2 (UNIV TEXAS [US]; FANG BINGLIANG [US]; LIU JINSONG [US]; GUO WEI [US];) 31 May 2007 (2007-05-31)	15-17
Y	table 1; compounds K005, K045 example 1	1-29
Y	WO 2008/110690 A2 (UNIV JOSEPH FOURIER [FR]; CENTRE NAT RECH SCIENT [FR]; DENIS JEAN-NOEL) 18 September 2008 (2008-09-18) cited in the application page 106, line 1 - page 109, last line claim 1	1-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2010/063405

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3074960 A	22-01-1963	NONE	
GB 1021422 A	02-03-1966	NONE	
WO 2007062399 A2	31-05-2007	AU 2006318212 A1	31-05-2007
		CA 2631082 A1	31-05-2007
		CN 101360422 A	04-02-2009
		EP 1956910 A2	20-08-2008
		JP 2009517401 T	30-04-2009
		US 2009286847 A1	19-11-2009
WO 2008110690 A2	18-09-2008	AU 2008224776 A1	18-09-2008
		CA 2677146 A1	18-09-2008
		CN 101652346 A	17-02-2010
		EP 2121601 A2	25-11-2009
		FR 2912133 A1	08-08-2008
		JP 2010517983 T	27-05-2010
		US 2010144726 A1	10-06-2010