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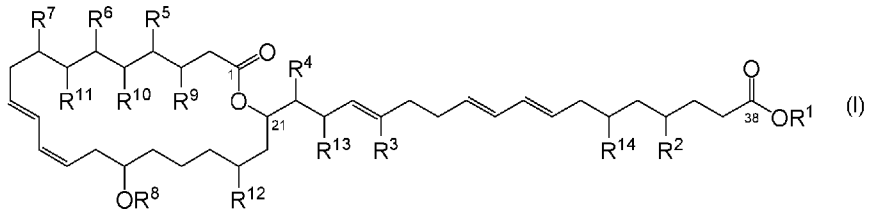
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(I)

(57) Abstract: The invention providesnovel macrolide compounds of formula (I) and their pharmaceutically acceptable salts, metabolites, isomers (e.g. stereoisomers), and prodrugs. In formula (I), R^1 is hydrogen or an ester-forming group; R^2 is methyl or oxo; R^3 is methyl or hydroxyl; R^4 to R^8 are each independently hydrogen or methyl; and R^9 to R^{14} are each independently hydroxyl or oxo. Methods for preparing such compounds, pharmaceutical compositions comprising them, and their use as antimicrobial agents also form part of the invention. The invention further provides a new strain of bacteria, Burkholderia gladioli strain LMG-P 26202, which is capable of producing the macrolide compounds.

ANTIMICROBIAL AGENTS

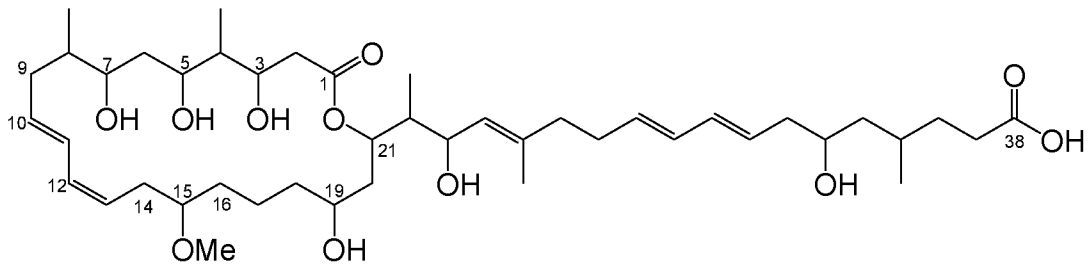
The present invention relates to novel macrolide compounds, their preparation, pharmaceutical compositions comprising them, and their use as antimicrobial agents. The invention further relates to a new strain of bacteria capable of producing such compounds.

Bacterial pathogens are prominent in many diseases and the treatment of bacterial infections has become increasingly difficult over recent years with the emergence of a number of antibiotic resistant bacterial strains. Examples include methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant Enterococci (VRE), and multidrug resistant Gram-negative bacteria such as *Stenotrophomonas maltophilia*. In addition to the emergence of antibiotic resistant strains, there are many bacterial infections that remain difficult to treat, for example, infections in immuno-compromised patients (e.g. those with AIDS).

There is therefore an ongoing need to identify new antimicrobial agents that can be used to treat microbial infections effectively, including those caused by drug resistant microbes, for example, infections caused by drug-resistant bacteria.

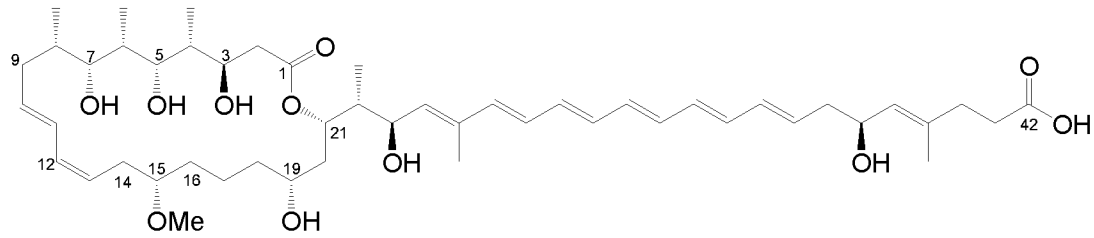
The present inventors have identified a new strain of *Burkholderia gladioli* which shows anti-microbial activity and, in particular, activity against bacteria that are resistant to known antimicrobial agents (e.g. MRSA and VRE). Bacteria of the genus *Burkholderia* are aerobic, catalase-positive, urease-positive, nonsporeformers. *Burkholderia gladioli* is a species that is indole-negative, nitrate-negative and lysine-decarboxylation negative. *Burkholderia gladioli* causes diseases in certain plants and may cause opportunistic infections in vulnerable humans.

A novel compound (herein referred to as "gladiolin") has been identified as responsible for the anti-microbial activity of the new *Burkholderia gladioli* strain. This compound has been isolated and its structure determined as shown below:



(This structure is intended primarily to illustrate the connectivity of the molecule and is not necessarily intended as an accurate representation of the cis/trans stereochemistry of the double bonds in the molecule).

Gladiolin has a macrolactone core that is similar in structure to that of etnangien, a macrolide lactone compound having antibiotic activity which is produced by a strain of *Sorangium cellulosum*. Etnangien has the following structural formula:



WO 2008/142046 describes etnangien and ester derivatives of etnangien having anti-bacterial and anti-viral activity. However, such compounds, especially etnangien itself, are acid-labile and light sensitive. As such, these are readily degraded by light. This chemical instability severely limits their clinical potential.

The present inventors have found that gladiolin and the derivatives of gladiolin which are herein described are light-stable. This improvement in stability represents a significant advance over the earlier known compounds. Furthermore, based on what is known about the structure-activity relationship for etnangien, it could not have been predicted that biological activity would be retained following modification of the C-21 side chain of the molecule. Indeed, it has been reported in the literature that truncation of the C-21 side chain of etnangien leads to significant loss of activity, suggesting that the polyene nature of the side chain is a significant part of the pharmacophore (Menche *et al.*, Bioorg. Med. Chem. Lett. (2010), 20:939-941).

The present invention also provides certain analogues of gladiolin. These may be produced using methods known in the art, for example techniques capable of modifying the enzymes responsible for gladiolin biosynthesis in order to produce recombinant microbes that synthesise the analogues. Such analogues are as herein described and may differ from gladiolin at key positions on the macrolactone core and the side-chain. Specific methods which may be used to produce

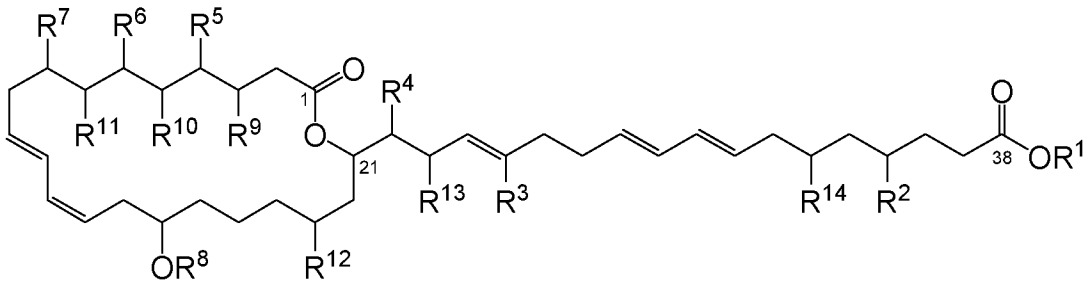
analogues of gladiolin may involve the use of recombinant gladiolin, or hybrid etnangien/gladiolin biosynthetic gene clusters.

Accordingly, the present invention provides novel macrolide compounds that are effective against a wide range of microbes, including bacteria, fungi, resistant bacteria and combinations thereof. The invention also provides a microorganism capable of producing such compounds.

The compounds of the invention, including but not limited to those specified in the examples, possess the ability to inhibit and/or prevent the growth of microbes. Such compounds may be useful in the treatment of a wide variety of microbial infections described herein. The present invention provides pharmaceutical compositions comprising one or more compounds according to the invention. In addition, compounds of the invention may be useful in the treatment of microbial infections described herein either when used alone or in combination with other therapeutic agents.

Further aspects of the present invention include: processes for the preparation of the compounds according to the invention; methods for the treatment of infections by microbes, including drug resistant strains thereof, comprising administering a compound according to the present invention; and uses of the compounds according to the present invention.

Viewed from a first aspect the invention provides a compound of formula (I) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(I)

wherein:

R^1 is hydrogen or an ester-forming group;

R^2 is methyl or oxo;

R^3 is methyl or hydroxyl;

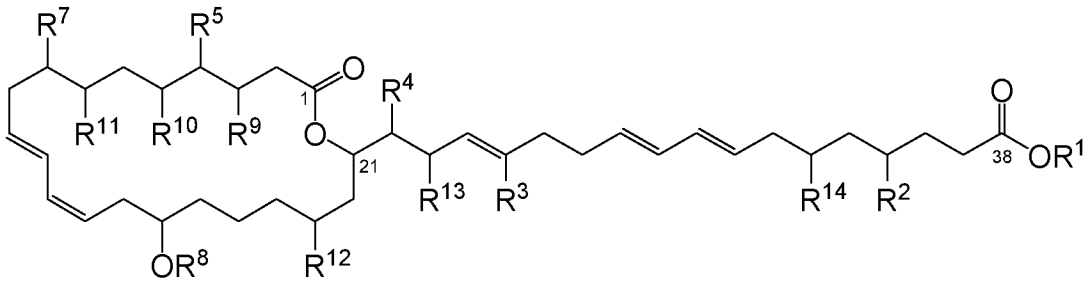
R^4 to R^8 are each independently hydrogen or methyl; and

R^9 to R^{14} are each independently hydroxyl or oxo.

Ester-forming groups for R^1 may include optionally substituted alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl and heteroaryl groups. Examples of such groups include optionally substituted C_{1-12}-alkyl, C_{1-12}-alkenyl, C_{3-10}-cycloalkyl, aryl and heteroaryl groups, wherein the aryl and heteroaryl groups may contain from 5 to 10 carbon atoms and the heteroaryl groups further contain one or more (e.g. 1, 2, 3 or 4) heteroatoms selected from N, O and S. Preferably, R^1 is an optionally substituted C_{1-6}-alkyl (especially methyl or ethyl), benzyl or phenyl group. Preferred optional substituents on the above-mentioned groups include halogens (e.g. -F, -Cl, -Br and -I), -CN, $-NO_2$, -OH and $-O-C_{1-4}$-alkyl.

In a preferred embodiment, R^2 is methyl. In a further preferred embodiment, R^3 is methyl. Especially preferably, R^2 and R^3 are both methyl.

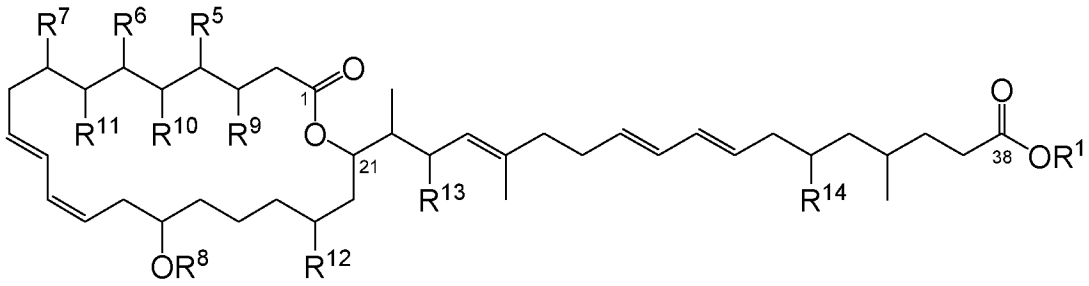
In one embodiment, R^6 is hydrogen. In this embodiment, the invention provides a compound of formula (Ia) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(Ia)

wherein R^1 to R^5 and R^7 to R^{14} are as herein defined.

In another embodiment, R^2, R^3 and R^4 are all methyl. In this embodiment, the invention provides a compound of formula (Ib) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(Ib)

wherein R^1 and R^5 to R^{14} are as herein defined.

In any of the compounds herein described, it is preferred that R^5 and R^7 are both methyl.

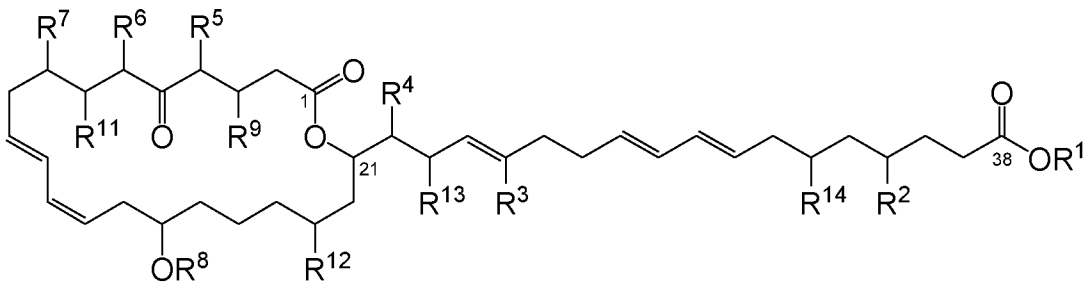
Other preferred compounds according to the invention are those of formula (I), (Ia) or (Ib) in which R^8 is methyl.

Any of the compounds herein described in which R^1 is hydrogen are particularly preferred.

In preferred embodiments of the invention, one or more of groups R^9 to R^{14} are hydroxyl groups, especially at least two, at least three or at least four. Especially preferably, at least five of groups R^9 to R^{14} are hydroxyl groups.

The inventors have observed that gladiolin can undergo a chemical rearrangement to an inactive isomer, for example upon prolonged standing in polar solvents such as DMSO and methanol. The hydroxyl group at C5 (i.e. position R^{10}) is believed to be involved in this rearrangement. Compounds of the invention in which R^{10} is an oxo group are less likely to undergo rearrangement to an inactive isomer and so display greater stability.

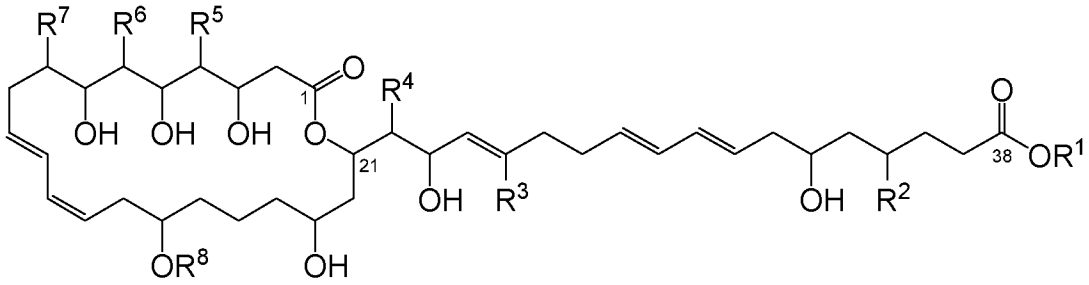
According to this embodiment, compounds of the invention are provided in which group R^{10} is oxo. Thus the invention provides a compound of formula (Ic) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(Ic)

wherein R^1 to R^9 and R^{11} to R^{14} are as herein defined.

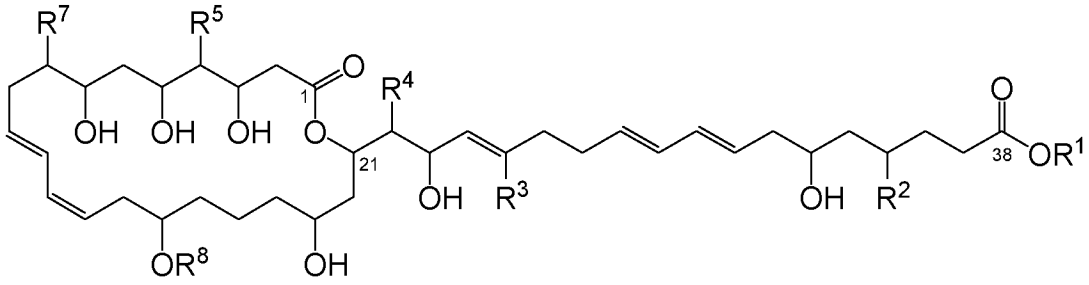
In further preferred embodiments, all of groups R^9 to R^{14} are hydroxyl groups. In this embodiment the invention provides a compound of formula (II) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(II)

wherein R^1 to R^8 are as herein defined.

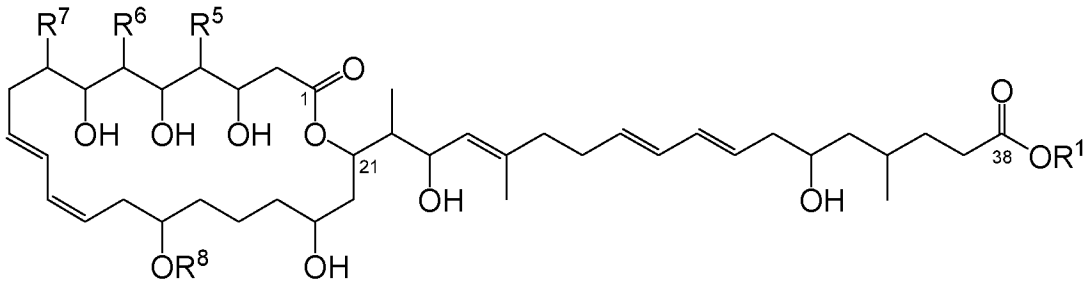
In one embodiment, R^6 is hydrogen. In this embodiment, the invention provides a compound of formula (IIa) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(IIa)

wherein R^1 to R^5, R^7 and R^8 are as herein defined.

In another embodiment, R^2, R^3 and R^4 are all methyl. In this embodiment, the invention provides a compound of formula (IIb) or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(IIb)

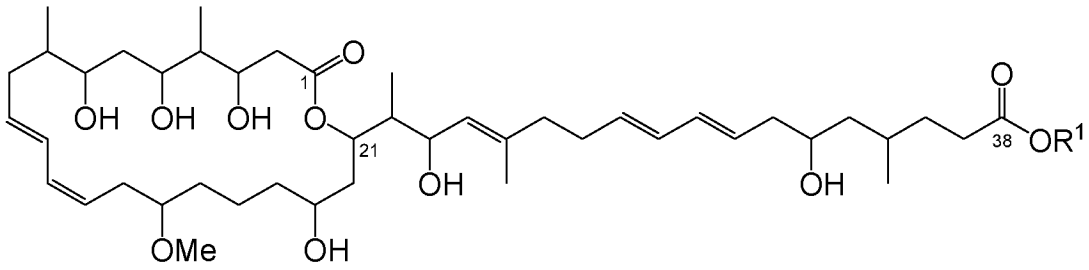
wherein R^1 and R^5 to R^8 are as herein defined.

In any of the compounds herein described, it is preferred that R^5 and R^7 are both methyl.

Other preferred compounds according to the invention are those of formula (I), (Ia), (Ib), (II), (IIa) or (IIb) in which R^8 is methyl.

Any of the compounds herein described in which R^1 is hydrogen are particularly preferred.

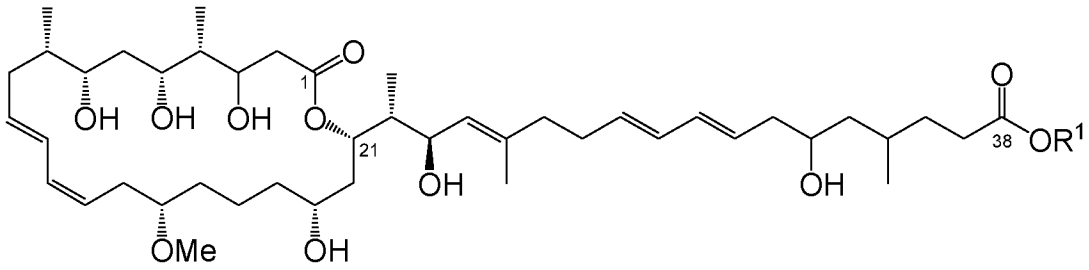
Particularly preferably, the invention provides a compound of formula (III), or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof:



(III)

wherein R^1 is as herein defined, preferably hydrogen or an ester-forming group selected from an optionally substituted C_{1-6}-alkyl, benzyl or phenyl group.

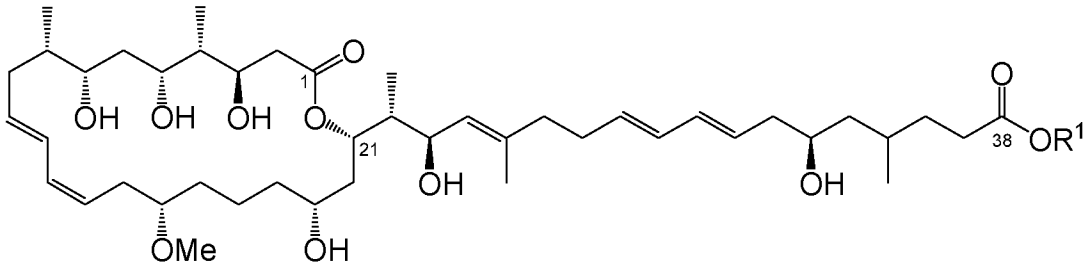
In one embodiment, the invention provides a compound of formula (IIIa), or a pharmaceutically acceptable salt, metabolite, stereoisomer or prodrug thereof:



(IIIa)

wherein R^1 is as herein defined, preferably hydrogen or an ester-forming group selected from an optionally substituted C_{1-6}-alkyl, benzyl or phenyl group.

In another embodiment, the invention provides a compound of formula (IIIb), or a pharmaceutically acceptable salt, metabolite, or prodrug thereof:



(IIIb)

wherein R^1 is as herein defined, preferably hydrogen or an ester-forming group selected from an optionally substituted C_{1-6}-alkyl, benzyl or phenyl group.

The C-10, C-11 double bond in the macrocyclic core of the compounds of the invention is preferably in the *trans* configuration, whereas the C-12, C-13 double bond is preferably in the *cis* configuration. It is also preferred that at least one (i.e. 1, 2 or 3, especially 2 or 3) of the double bonds in the tail of the compounds of the invention are in the *trans* configuration.

The compounds of the invention are suitable for pharmaceutical and medical uses, in particular they are useful as antimicrobial agents. More specifically, the compounds of the present invention provide new agents for application against bacteria, fungi, resistant bacteria and combinations thereof thus offering both separate and combination treatment potential. The compounds of the present invention have application for the treatment of various infections, for example including infections of the skin and skin structure, infections of the respiratory system, endocarditis, hospital acquired infections, infections of the

digestive system, urinary system, nervous system, blood infection, soft tissue infection, nasal canal infections and infection associated with cystic fibrosis. The compounds of the present invention also find application in relation to or for animal/veterinary illnesses.

Thus, in another aspect, the invention provides a pharmaceutical composition comprising a compound according to the invention or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer) or prodrug thereof along with one or more physiologically acceptable carriers, excipients or diluents.

Also provided are methods of treating infections (such as those listed above) comprising administration of one or more compounds of the invention, optionally in combination with one or more further active agents.

In a related aspect, the invention provides compounds as defined herein for use as a medicament or in therapy, e.g. for use in the treatment of infections such as those listed above. Preferably, the compounds of the invention are used to treat infections caused by a microbe which is resistant to known antimicrobial agents.

In another aspect, the invention provides a microorganism classified as *Burkholderia gladioli* BCC0238 having IDA accession number LMG-P 26202, or a variant and/or mutant thereof. This microorganism was recovered from a human patient in Minneapolis, Minnesota, USA.

In this aspect the term "variant" includes, but is not limited to, a bacterial strain that differs from the specified bacterial strain but which is able to produce the same antimicrobial agent according to the methods described herein. This term can also mean a bacterial strain that differs from the specified bacterial strain but which retains sufficient genotypic or phenotypic characteristics to maintain a taxonomic similarity.

In this aspect the term "mutant" includes, but is not limited to, a bacterial strain that has arisen as a result of a mutation of the specified bacterial strain but which is able to produce the same antimicrobial agent according to the methods described herein. This term can also mean a bacterial strain that differs from the specified bacterial strain as a result of a mutation, for example an altered gene, DNA sequence, enzyme, cell structure, etc.

Such mutants can be produced in a manner known in the art, for example by physical means such as irradiation (for example UV), by exposure to chemical mutagens or by genetic manipulation of DNA of the bacterium. Methods for

screening for mutants and isolating mutants will be known to a person skilled in the art.

Accordingly, the invention also provides an active agent, especially an antimicrobial agent, obtained or obtainable from *Burkholderia gladioli* sp. BCC0238. Preferably, the active agent is a macrolide agent, especially an agent having one or more of the characteristics identified below:

- a molecular formula of $C_{44}H_{74}O_{11}$;

- carbon (^{13}C) and hydrogen (^{1}H) NMR spectral signals substantially in accordance with Tables 1 and/or 2 and/or one or more of Figures 7 to 16;

- mass spectral signals substantially in accordance with one or more of Figures 3 to 6; and

- a macrolide structure having 21 carbon atoms and an oxygen atom in the ring members, wherein the structure is substituted with one or more groups independently selected from -OH, $-CH_3$ and $-OCH_3$, and further substituted with an unsaturated C_{17} side chain that includes a terminal carboxyl or ester group.

Where compounds of the invention are used in the treatment of an infection caused by a microbe, the microbe may be a bacterium. The bacterium may be a Gram-positive bacterium or a Gram-negative bacterium. Such infectious Gram-positive bacteria are preferably selected from *Mycobacterium* species, *Staphylococcus* species, *Enterococcus* species and *Bacillus* species. Such infectious Gram-negative bacteria are preferably selected from susceptible *Burkholderia* species, *Ralstonia* species and *Stenotrophomonas* species. The infectious microbe may also be a fungus, such as a *Candida* species, e.g. *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* or *Candida tropicalis*.

In preferred embodiments, the compounds according to the present invention are broad spectrum antibiotics that are able to inhibit or prevent the growth of Gram-positive and Gram-negative bacterial species and other microbes.

Preferably, the compounds according to the present invention are for use in the treatment of an infection caused by more than one type of microbe, for example, a bacterial species and a fungal species, or two or more different bacterial and/or fungal species.

Preferably, the compounds according to the present invention are for use in the treatment of an infection caused by a microbe that is resistant to at least one antimicrobial drug, for example an antimicrobial drug known in the art. The infection may be caused by one or more bacteria and/or fungi that show resistance

to common antimicrobial drugs. The bacterium or fungus may be multidrug-resistant. For example, the infection may be caused by the bacteria MRSA or VRE.

The antimicrobial drug against which the microbe has become resistant may be an antibacterial drug or an antifungal drug. The antibacterial drug may be selected from, but is not limited to: drugs of the penicillin family, drugs of the vancomycin family, drugs of the aminoglycoside family, drugs of the quinolone family, drugs of the daptomycin family, drugs of the cephalosporin family, drugs of the macrolide family and combinations thereof. Examples of such antibacterial drugs include penicillin, ampicillin, methicillin, vancomycin, gentamycin, ofloxacin, ciprofloxacin, daptomycin, Cefdimir, erythromycin, equivalents thereof, and combinations thereof. The antifungal drug may be selected from, but is not limited to: amphotericin B, nystatin, fluconazole, caspofungin, allylamines, equivalents thereof, and combinations thereof.

Preferably, the compounds according to the present invention are for use in the treatment of an infection in an animal, preferably a mammal, more preferably a human. Preferably, the compounds according to the present invention are for use in the treatment of an infection in a non-human mammal, such as a dog, cat, horse, etc. The compounds according to the present invention therefore have application in both human and veterinary medicine.

Preferably, the compounds according to the present invention are for use in the treatment of an infection of the respiratory system, digestive system, urinary system, nervous system, a blood infection, a soft tissue infection, a skin infection, a nasal canal infection, or combinations thereof.

Preferably, the compounds according to the present invention are for use in the treatment of a fungal infection of the skin (e.g., athletes foot), mouth, vagina (e.g. oral or vaginal candidosis), nails, intestine, lungs (e.g., respiratory aspergillosis), or combinations thereof.

Preferably, the compounds according to the present invention are for use in the treatment of a bacterial infection of the respiratory system or a portion thereof, for example, the upper respiratory system.

Preferably, the compounds according to the present invention are for use in the treatment of an infection associated with immuno-compromised individuals.

Preferably, the compounds and methods of the present invention may be used to treat, without limitation, otitis media, sinusitis, chronic bronchitis and

pneumonia, including pneumonia caused by drug-resistant *Streptococcus pneumoniae* or *Haemophilus influenza*.

Preferably, the compounds and methods of the present invention are for use in treating a variety of infections that comprise different types of Gram-positive or Gram-negative bacteria, including aerobic or anaerobic bacteria. These types of infections include intra-abdominal infections, pneumonia, bone and joint infections, and obstetrical/gynaecological infections and urinary tract infections.

The compounds and methods of the invention may also be used to treat an infection including, without limitation, endocarditis, nephritis, septic arthritis and osteomyelitis.

According to a further aspect of the present invention, there is provided a pharmaceutical composition comprising a compound according to the present invention, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, in combination with a pharmaceutically acceptable carrier. The pharmaceutical composition may further comprise one or more other therapeutic agents, for example selected from an anti-inflammatory agent, anti-cancer agent or immuno-modulatory agent, or different types of antibacterial and/or antifungal agents.

Preferably, a therapeutic agent, other than a compound of the present invention, may be administered concurrently with a compound of the present invention. In a preferred embodiment, an antibacterial and/or antifungal agent may be administered concurrently with a compound of the present invention. Co-administration of an antifungal agent and/or an antibacterial agent, other than the compounds of the present invention, may be useful for mixed infections such as those caused by different types of bacteria, or those caused by both bacteria and fungi. The different therapeutic agents may be administered sequentially, separately or simultaneously.

Antibacterial agents and classes thereof that may be co-administered with a compound of the present invention preferably include, without limitation, penicillins and related drugs, carbapenems, cephalosporins and related drugs, aminoglycosides, ceftriaxone, daptomycins and macrolides.

Antifungal agents that may be co administered with a compound according to the present invention preferably include, without limitation, caspofungen, polyenes, such as amphotericin, nystatin and pimaricin; azoles, such as fluconazole, itraconazole, ketoconazole, voriconazole and sertaconazole; and allylamines, such as naftifine and terbinafine.

Another aspect of the present invention relates to the use of a compound according to the present invention for inhibiting the growth or survival of a microbe. The microbe may be resistant to at least one antimicrobial agent. The microbe is preferably a bacterium, for example at least one bacterium selected from *Mycobacterium* species, *Burkholderia* species, *Pseudomonas* species, *Ralstonia* species, *Stenotrophomonas* species, *Staphylococcus* species, *Enterococcus* species and *Bacillus* species. Alternatively or additionally, the microbe may be a fungus, for example at least one yeast selected from *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*.

According to another aspect of the present invention, there is provided a process for the preparation of a compound according to the present invention. Preferably, the process comprises cultivating a microorganism capable of producing a compound as herein described, such as *Burkholderia gladioli* strain LMG-P 26202 or a mutant or variant thereof. Cultivation may be carried out in a culture or nutrient medium comprising a source of assimilable carbon, nitrogen, and inorganic salts, thereby producing a cultivation medium comprising the desired compound, especially a compound as herein defined wherein R^1 is hydrogen. Optionally, the desired compound may be recovered from the cultivation medium or fermentation broth. The process may further comprise converting any compound obtained wherein R^1 is hydrogen into an alternative compound according to the invention, for example, into a compound wherein R^1 is an ester-forming group as defined above. The process may also comprise converting the compound obtained into a pharmaceutically acceptable salt.

Conversion of the -COOH group at C38 to an ester derivative may be effected using methods which are known in the art (see, for example, March, J., Advanced Organic Chemistry, John Wiley & Sons, 4th edition, 1992). For example, gladiolin may be reacted with an optionally activated alkyl compound, such as a diazoalkane, to form the respective alkyl ester. Further methods for converting the -COOH group at C38 of the compounds according to the present invention to an ester group are described in WO 2008/142046, the content of which is incorporated herein by reference.

Gladiolin and its derivatives can be isolated and purified from the culture medium using known methods and taking account of the chemical, physical and biological properties of the natural substances. For the isolation, gladiolin may be extracted from both agar culture and liquid culture using an organic solvent, such as

methanol, and may be subjected to further purification. The further purification of gladiolin may be effected by chromatography on suitable materials, for example, on adsorber resins such as MCI or Amberlite XAD and subsequently on reverse phase HPLC resins.

Insofar as the gladiolins are present as stereoisomers, they can be separated using known methods, for example by means of separation using a chiral column.

Preferably, the producer microorganism is *Burkholderia gladioli* strain LMG-P 26202 or a variant or mutant thereof. A reference isolate of the gladiolin-producing strain of *Burkholderia gladioli* was deposited at the Belgium Coordinated Collection of Microorganisms (BCCM), which serves as International Depositary Authority (IDA) (see http://bccm.belspo.be/about/lmg.php), on 6 January 2011 under reference LMG-P 26202. Precise identification of the antibiotic producing *Burkholderia gladioli* strain described above can be achieved using, for example, the DNA sequence-based strain and species identification approach known as Multilocus Sequence Typing (MLST) (Spilker *et al.*, "Expanded multilocus sequence typing for *Burkholderia* species", J. Clin. Microbiol. (2009) 47:2607-2610).

Burkholderia gladioli strain LMG-P 26202 produces the novel compound gladiolin, which has the chemical structure of formula (III), (IIIa) and (IIIb) above, where R^1 is hydrogen. These structures were determined by the use of mass spectral and ^1H NMR and ^{13}C NMR spectral measurements.

Preferably, the nutrient medium in the process for the preparation of the compounds according to the present invention comprises glycerol as the sole carbon source. The glycerol may be present in an amount of between about 2 g/L and about 12 g/L, or between about 4 g/L and about 10g/L, such as about 5 g/L.

It is preferred that the nutrient or minimal media comprises yeast extract. In a preferred embodiment, the yeast extract is present in an amount of between about 0.01% w/v and about 0.1% w/v, such as between about 0.025% w/v and about 0.075% w/v, or about 0.05% w/v.

It is also preferred that the nutrient or minimal media comprises casamino acids. In a preferred embodiment, the casamino acids are present in an amount of between about 0.01% w/v and about 0.1% w/v, such as between about 0.025% w/v and about 0.075% w/v, or about 0.05% w/v.

Preferably, the bacterium is incubated at a temperature of between about 20ºC and about 37ºC, such as between about 28ºC and about 32ºC, or about 30ºC.

In some embodiments, the bacterium is incubated at a temperature of less than about 30ºC.

Preferably, the method comprises incubating the bacterium on nutrient or minimal media up to and including at least part of the stationary phase. In preferred embodiments, the method comprises incubating the bacterium on minimal media for between about 16 hours and about 120 hours, or for between about 48 hours and about 96 hours, or for between about 48 hours and about 72 hours. In further preferred embodiments, the method comprises incubating the bacterium on minimal media for at least about 16 hours, or at least about 48 hours, or about 48 hours.

Preferably, the nutrient or minimal medium comprises a basal salts medium (BSM). Preferably, the basal salts medium comprises the formulation originally described by Hareland *et al.* ("Metabolic function and properties of 4-hydroxyphenylacetic acid 1-hydroxylase from *Pseudomonas acidovorans*", J. Bacteriol. (1975) 121:272-285).

Preferably, the detection of *Burkholderia gladioli* antibiotics and the extraction thereof are carried out using a solid surface growth medium such as BSM agar. Growth in liquid media can also be used to isolate the *Burkholderia gladioli* antibiotics with cultures shaken or stirred to produce aeration during growth of the bacteria.

Preferably, the recovery of a compound according to the present invention from the fermentation broth comprises extraction of the compound with a solvent, preferably an organic solvent such as an alcohol, more preferably methanol. Preferably, the alcohol comprises between about 70% and about 90% methanol vol/vol., such as about 80% methanol vol/vol. In other embodiments, the alcohol comprises about 100% methanol vol/vol.

Preferably, the step of recovering the antimicrobial agent comprises drying the nutrient or minimal media after removal of bacteria, preferably freeze drying the nutrient or minimal media. In other embodiments, the step of recovering the antimicrobial agent comprises breaking up the nutrient or minimal media, preferably by grinding, prior to extraction of the antimicrobial agent using methanol.

Preferably, a neutral cross-linked polystyrene resin, such as Amberlite XAD-16 is used to isolate the antimicrobial agent. Extraction with the resin may be performed directly on the supernatant of liquid cultures where the bacteria have been removed by centrifugation. From agar surface cultures of the bacteria, an aqueous extraction of the antimicrobial agent is first performed to remove the

antimicrobial agent from the agar. The bacteria are grown on filters laid on the agar surface, these are then removed after growth, and the agar cut into blocks and mixed with water. The agar blocks are then removed by filtration and the resin, such as Amberlite XAD-16, added to the aqueous extract to bind the antimicrobial agents. The extracted antimicrobial agent can then be eluted from the resin using a solvent, preferably an organic solvent such as methanol.

As alluded to above, gladiolin and analogues thereof in accordance with the invention may also be prepared from recombinant (genetically modified) or hybrid microbial systems, conveniently, bacterial systems.

The gladiolin biosynthetic gene cluster has been identified from within the genome of *Burkholderia gladioli* LMG-P 26202 based on automated annotation, searching for high GC regions of DNA containing polyketide synthase (PKS) genes, and computer-aided analysis using antiSMASH 2.0 (Blin, K., M. H. Medema, D. Kazempour, M. A. Fischbach, R. Breitling, E. Takano, and T. Weber. 2013. antiSMASH 2.0 - a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res:DOI: 10.1093/nar/gkt1449), BLAST ((Altschul et al. (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402; and http://www.ncbi.nlm.nih.gov/BLAST)) and CD search (ref Marchler-Bauer A et al. (2011) "CDD: a Conserved Domain Database for the functional annotation of proteins.", Nucleic Acids Res.39(D)225-9). The gladiolin biosynthetic gene cluster (SEQ ID NO: 1) has been determined to have approximately 40 predicted genes, 6 of which encode PKSs: *gnbA1* to *gnbA6* (SEQ ID NOs 2, 4, 6, 8, 10 and 12, respectively) also referred to as *gln* genes. The functional domains within the PKSs are consistent with the production of gladiolin.

The entire cluster or the component genes thereof, including any of *gnbA1* to *gnbA6* may be used with recombinant techniques to prepare genetically modified ("recombinant") microorganisms capable of producing gladiolin and the gladiolin analogues according to the invention. Such microorganisms may be bacteria, in particular those which have, or are engineered to have, some or all components of another polyketide biosynthetic system (e.g. the etnangien biosynthetic system). Selective expression of the individual gene components of the gladiolin gene cluster, e.g. the *gnbA* genes, and/or the mutation (sequence modification) thereof, allows the design of tailored gladiolin compounds, e.g. the gladiolin analogues of the invention. Hybrid systems in which functionally complementary genes from

other polyketide biosynthetic systems are expressed together with some or all of the *gnbA* genes of the present invention, or some or all of the gladiolin biosynthetic genes cluster of the invention, provides for further control of the design of gladiolin analogues.

Thus, in a further aspect there is provided a nucleic acid molecule comprising:

(a) the nucleotide sequence set forth in SEQ ID NO: 1;

(b) a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence set forth in SEQ ID NO: 1 and which encodes a functional gladiolin biosynthetic gene cluster;

(c) a nucleotide sequence degenerate with the nucleotide sequence set forth in SEQ ID NO: 1;

(d) a nucleotide sequence encoding a functional component of the gladiolin biosynthetic gene cluster encoded by the nucleotide sequence set forth in SEQ ID NO: 1;

(e) a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence of (d) and which encodes a functional component of the gladiolin biosynthetic gene cluster encoded by the nucleotide sequence set forth in SEQ ID NO: 1; or

(f) the complement of any one of (a)-(e),

preferably operably associated with one or more regulatory elements.

The invention further provides a nucleic acid molecule comprising:

(a') the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12;

(b') a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12 and which encodes an enzymatically active gnbA protein;

(c') a nucleotide sequence which is a fragment of the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12 and which encodes an enzymatically active gnbA protein

(d') a nucleotide sequence degenerate with the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12; or

(e') the complement of (a'), (b'), (c') or (d'),

preferably operably associated with one or more regulatory elements.

In a further aspect the invention provides a polypeptide, wherein the amino acid sequence of the polypeptide:

(a) comprises the amino acid sequence set forth in any one of SEQ ID NOs: 3, 5, 7, 9, 11 or 13;

(b) comprises an amino acid sequence which has at least 70% sequence identity with any one of SEQ ID NOs: 3, 5, 7, 9, 11 or 13 and which is a enzymatically active gnbA protein;

(c) comprises an enzymatically active fragment of any one of SEQ ID NOs: 3, 5, 7, 9, 11 or 13;

(d) comprises the amino acid sequence encoded by the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12;

(e) comprises the amino acid sequence encoded by a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12 and which encodes a functional gnbA protein; or

(f) comprises the amino acid sequence of a functional component of the gladiolin biosynthetic gene cluster encoded by the nucleotide sequence set forth in SEQ ID NO: 1.

The invention also provides a nucleic acid vector comprising a nucleic acid molecule of the invention. Also provided is a host cell comprising a nucleic acid molecule of the invention or a nucleic acid vector of the invention.

As used herein, the term "nucleic acid molecule" refers to a DNA or RNA molecule, which might be single- or double-stranded. Preferably, the nucleic acid molecule is a DNA molecule, most preferably a double-stranded DNA molecule. In certain embodiments the nucleic acid molecule may be genomic DNA or cDNA. In other embodiments the nucleic is a single stranded RNA molecule carrying an above mentioned complementary sequence and said nucleic acid may be used in RNA interference methods or techniques.

The nucleic acid molecule of the invention is preferably isolated or purified. As used herein, the term "isolated nucleic acid" means that the nucleic acid molecule is not contiguous with other genes or nucleotide sequences with which it is normally associated in the natural source of the polypeptide-encoding nucleic acid. For example, an isolated nucleic acid of the invention comprising a gnbA gene will not be contiguous with a nucleic acid encoding other gnbA genes or other genes in the gladiolin biosynthetic gene cluster.

As used herein, the term "purified nucleic acid" means a nucleic acid molecule which is free or substantially free from other non-contiguous nucleic acids and/or is free or substantially free from one or more of the following: bacteria, agar, yeast extract, tryptone.

In some embodiments of the invention, the nucleic acid molecule is a recombinant nucleic acid or produced by other artificial means, i.e. not obtained from a natural source.

In preferred embodiments the nucleic acids of the invention defined in terms of percentage sequence identity to another nucleotide sequence have at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98%or 99% sequence identity to their reference sequences, e.g. with SEQ ID NOs: 1, 2, 4, 6, 8, 10 or 12, preferably as calculated by the BLASTN method of alignment.

Percentage sequence identity, for both nucleic acids and proteins, according to the invention can be also be calculated using any of the widely available algorithms, e.g. the BLAST methods of alignment (Altschul *et al.* (1997), "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402; and http://www.ncbi.nlm.nih.gov/BLAST) using default parameters, or the Clustal W2 multiple sequence alignment program (http://www.ebi.ac.uk/Tools/clustalW2) using default parameters (DNA Gap Open Penalty = 15.0; DNA Gap Extension Penalty = 6.66; DNA Matrix = Identity; Protein Gap Open Penalty = 10.0; Protein Gap Extension Penalty = 0.2; Protein matrix = Gonnet; Protein/DNA ENDGAP = -1; Protein/DNA GAPDIST = 4).

With regard to nucleotide sequence comparisons, MEGABLAST, discontiguous-megablast, and BLASTN may be also used to accomplish this goal. Preferably the standard or default alignment parameters are used. MEGABLAST is specifically designed to efficiently find long alignments between very similar sequences. Discontiguous MEGABLAST may be used to find nucleotide sequences which are similar, but not identical, to the nucleic acids of the invention. In some embodiments, the BLAST nucleotide searches can be performed with the BLASTN program, score=100, wordlength=12. The word size is adjustable in blastn and can be reduced from the default value to a minimum of 7 to increase search sensitivity. In other embodiments the discontiguous megablast page (www.ncbi.nlm.nih.gov/Web/Newsltr/FallWinter02/blastlab.html) is used. This page uses an algorithm which is similar to that reported by Ma *et al.* (Bioinformatics. 2002 Mar; 18(3): 440-5). Parameters unique for discontiguous megablast are: word size:

11 or 12; template: 16, 18, or 21; template type: coding (0), non-coding (1), or both (2).

The nucleic acid of the invention is preferably operably associated with one or more regulatory elements, e.g. a promoter and/or a terminator element. Preferably such elements will, in general, be functional in microbial, e.g. bacterial cells, but the skilled person would be able to select or design such elements to be compatible with the specific context in which the nucleic acids of the invention are being employed.

As used herein the term "operably associated" or "operably linked" with a promoter means that the polypeptide-encoding region is transcribable from that promoter. The polypeptide-encoding region may, for example, be immediately 3' to the promoter, in which case the promoter will direct the transcription of the coding sequence. Alternatively, the polypeptide-encoding region may be part of an operon or cluster in which case the associated or linked promoter will direct the transcription of all of the polypeptide-encoding regions within that operon/cluster.

The promoter or promoters are preferably ones which are operable in bacterial cells. More preferably, the promoters are bacterial promoters. Suitable promoters include inducible promoters, such as those that are inducible with specific sugars or sugar analogues, e.g. arabinose (e.g. lac, ara), those inducible with antibiotics (e.g. tetracycline, tet), those inducible with IPTG (e.g. trp, tac, Pspac), those inducible with heat (e.g. hsp70), those inducible with anaerobic induction (e.g. nisA, pfl, trc, IPL, IPR, T7), P11, ldh, sec (secDF), SV40 promoter, those inducible with xylose (e.g. Pxyl promoter), those inducible with osmotic shock, cell density (quorum sensing), anaerobicity, antibiotics, or growth phase. In some embodiments, the promoter is a constitutive promoter, e.g. the promoters for the thiolase gene (thl) or the permease operon (hfuC). In other embodiments, the promoter is one from Burkholderia, e.g. *Burkholderia gladioli*. In other embodiments, the promoter is one from a polyketide biosynthetic gene cluster, e.g. a Burkholderia biosynthetic gene cluster.

The nucleic acid molecule of the invention, with or without operable association with a regulatory element, will preferably be in the form of a nucleic acid vector, particularly an expression vector, or a plasmid. The vector or plasmid may comprise one or more selectable markers and/or other genetic elements. Preferably, the vector or plasmid is less than 100Kb, more preferably less than 90, 80, 70, 60, 50, 40, 30 or 20Kb. Preferably, the vector or plasmid additionally

comprises one or more antibiotic resistance genes. Examples of such genes include genes conferring resistance to ampicillin, erythromycin, neomycin/kanamycin, tetracycline, chloramphenicol, spectinomycin, bleomycin and puromycin. In some embodiments, the vector or plasmid also comprises one or more genes conferring tolerance to one or more heavy metals, e.g. mercury. Other selectable markers include auxotrophy genes, e.g. genes for essential amino acids.

The vector or plasmid may also comprise an origin of replication, for example a Gram positive and/or a Gram negative bacterial origin of replication. The vector or plasmid may also comprise one or more insertion sequences, e.g. Tn10, Tn5, Tn1545, Tn916 and/or ISCb.

The nucleic acid molecule of the invention, or the plasmid or vector, may be introduced into a host cell, e.g. a microorganism, preferably a yeast or bacterial cell. In certain embodiments the host cell will not be a human cell. The bacterial cell may, for example, be a Gram-positive or Gram-negative bacterium. In some embodiments, the bacterium will be a bacterium that has, or has been engineered to have, some or all components of another polyketide biosynthetic system (e.g. the etnangien biosynthetic system). In other embodiments the bacterium may be from the genus Burkholderia, Sorangium (e.g. *Sorangium cellulosum*) or Pseudomonas. In other embodiments standard experimental bacteria may be used as host, e.g. *E. coli* or Streptomyces. In some embodiments of the invention, the host cell is not from the genus Burkholderia, e.g. *Burkholderia gladioli*, in particular *Burkholderia gladioli* strain LMG-P 26202.

The invention further provides a process for making a recombinant host cell, e.g. bacterial host cell, comprising introducing a nucleic acid molecule of the invention, or a nucleic acid vector or plasmid of the invention, into a host cell. Methods of introducing nucleic acid molecules, plasmids and vectors into host cells are well known in the art. These include transformation, transfection and electroporation techniques.

The invention also provides a recombinant (genetically modified) host cell comprising a nucleic acid molecule of the invention, or a vector or plasmid of the invention. The nucleic acid molecule or vector or plasmid may be present in the cytoplasm of the host, or it may be integrated in the host genome. Preferably the host cell containing the nucleic acid molecule or vector of the invention produces gladiolin or a gladiolin analogue of the invention under conducive conditions.

The invention therefore provides a cell, preferably microorganism, e.g. a bacterium, comprising a nucleic acid molecule, a vector or plasmid of the invention, wherein the nucleic acid molecule, vector or plasmid is present in the cytoplasm of the cell.

The invention also provides a cell, preferably a microorganism, e.g. a bacterium, comprising a nucleic acid molecule of the invention or an operon or vector or plasmid of the invention, wherein the nucleic acid molecule, operon, vector or plasmid is present in (e.g. stably integrated into) the genome of the cell.

The polypeptides of the invention may be isolated and/or purified. In particular, the polypeptides of the invention may be in a form which is isolated from one or more of the following: bacteria, yeast extract, tryptone, agar, other enzymes or other polypeptides, in particular polypeptides that are not PKS polypeptides (e.g. encoded by the gladiolin gene cluster) e.g. gnbA polypeptides.

The polypeptides of the invention may be purified, i.e. the polypeptide may be substantially pure. In particular, the polypeptides may be at least 90%, preferably at least 95% and more preferably at least 99% pure. Purity may be assessed using SDS-PAGE or any other appropriate method.

The invention also provides variants or derivatives of any of the polypeptides of SEQ ID NOs: 3, 5, 7, 9, 11 or 13. The polypeptides of the invention may be altered in various ways including substitutions, deletions, truncations, and/or insertions of one or more (e.g. 2-5, 2-10) amino acids, preferably in a manner which does not substantially alter the biological activity of the polypeptides of the invention. Guidance as to appropriate amino acid changes that do not affect biological activity of the protein of interest may be found in the model of Dayhoff et al. (1978) Atlas of Protein Sequence and Structure (Nat'l. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may also be made. In particular, substitution of one hydrophobic amino acid such as isoleucine, valine, leucine or methionine for another may be made; or the substitution of one polar amino acid residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, may be made.

One or more (e.g. 1-5, 1-10) amino acids in the polypeptides of the invention may be substituted by their corresponding D-amino acids, preferably at the N- and/or C-terminus.

In particular, the invention provides variants of the any of the polypeptides of SEQ ID NOs: 3, 5, 7, 9, 11 or 13, wherein the amino acid sequence of the variants comprise or consist of an amino acid sequence having at least 70%, preferably at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% 95%, 96%, 97%, 98% or 99% sequence identity with the reference sequence, preferably using the blastp method of alignment.

Like other BLAST programs, blastp is designed to find local regions of similarity. When sequence similarity spans the whole sequence, blastp will also report a global alignment, which is the preferred result for protein identification purposes. Preferably the standard or default alignment parameters are used. In some instances, the "low complexity filter" may be taken off. BLAST protein searches may also be performed with the BLASTX program, score=50, wordlength=3. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) Nucleic Acids Res. 25: 3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules (see Altschul *et al.* (1997) *supra*). When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs may be used.

“Functional” in the context of the polypeptides of the invention refers to a role in the synthesis, transport or transfer of a macrolide antibiotic or polyketide moiety, preferably gladiolin or a gladiolin derivative or gladiolin-related molecule. Specifically in relation to gnbA proteins, it refers to a trans-acyl transferase activity.

The gnbA proteins of the invention are polyketide synthases belonging to the trans-acyl transferase class. An enzymatically active fragment or variant of a gnbA protein will therefore display the same catalytic effects (i.e. a trans-acyl transferase activity) and preferably the same or substantially the same levels of catalytic activity as the full length gnbA protein from which it derives, e.g. those as defined by SEQ ID NOs: 3, 5, 7, 9, 11 or 13. In these embodiments substantially the same may be expressed as at least 50% of the activity of the full length gnbA protein, e.g. at least 60%, 70%, 80%, 90, or 95% or the activity of the full length gnbA protein.

The polypeptides of the invention may be used in methods for the preparation of gladiolin or the gladiolin derivatives of the invention, e.g. in cell-free methods or in methods involving cell factories, or to modify gladiolin produced in cell factories.

In a further aspect of the invention there is provided a method for expressing a gladiolin biosynthetic gene cluster, e.g. that is encoded by the nucleic acids of the invention described above, or a functional component thereof in a host cell, said method comprising introducing a nucleic acid molecule, or a plasmid or vector comprising said nucleic acid molecule, into a host cell and subsequently culturing said host cell under conditions conducive to the expression of said cluster or component thereof, said nucleic acid comprising:

(a) the nucleotide sequence set forth in SEQ ID NO: 1;

(b) a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence set forth in SEQ ID NO:1 and which encodes a functional gladiolin biosynthetic gene cluster;

(c) a nucleotide sequence degenerate with the nucleotide sequence set forth in SEQ ID NO: 1;

(d) a nucleotide sequence encoding a functional component of the gladiolin biosynthetic gene cluster encoded by the nucleotide sequence set forth in SEQ ID NO: 1; or

(e) a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence of (d) and which encodes a functional component of the gladiolin biosynthetic gene cluster encoded by the nucleotide sequence set forth in SEQ ID NO: 1.

In a further aspect of the invention there is provided a method for expressing a gnb protein, e.g. a gnbA polypeptide of the invention described above, in a host cell, said method comprising introducing a nucleic acid molecule, or a plasmid or vector comprising said nucleic acid molecule, into a host cell and subsequently culturing said host cell under conditions conducive to the expression of said gnb protein, said nucleic acid comprising:

(a') the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12;

(b') a nucleotide sequence which has at least 70% sequence identity with the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12 and which encodes an enzymatically active gnbA protein;

(c') a nucleotide sequence which is a fragment of the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12 and which encodes an enzymatically active gnbA protein; or

(d') a nucleotide sequence degenerate with the nucleotide sequence set forth in any one of SEQ ID NOs: 2, 4, 6, 8, 10 or 12.

Culture conditions may be those as described above. The expression products or a portion thereof may be subsequently separated or isolated from said host cells and/or the media in which said cells have been cultured with any of the purification techniques for protein known in the art and widely described in the literature or any combination thereof. Such techniques may include, for example, precipitation, ultrafiltration, dialysis, various chromatographic techniques, e.g. gel filtration, ion-exchange chromatography, affinity chromatography, electrophoresis, centrifugation, etc. Likewise an extract of host cells may also be prepared using techniques well known in the art, e.g. homogenisation, freeze-thawing, etc. and from this extract the polypeptides of the invention can be purified. The host cells may be those described above.

It may be desirable to modify, e.g. mutate, for instance as described above, said nucleic acids in order to obtain modified versions of the gnb polypeptides or the gladiolin gene cluster.

In a further aspect of the invention there is provided a method for the preparation of gladiolin or a gladiolin analogue, e.g. those of the invention described above, said method comprising introducing at least one nucleic acid molecule of the invention, or a plasmid or vector comprising said at least one nucleic acid molecule, into a host cell and subsequently culturing said host cell under conditions conducive to the production of gladiolin or a gladiolin analogue of the invention. Alternatively the method may comprise culturing a recombinant (genetically modified) host cell of the invention as defined above under conditions conducive to the production of gladiolin or a gladiolin analogue of the invention. Culture conditions may be those as described above for *Burkholderia gladioli*. Recovery of the gladiolin or a gladiolin analogue or a portion thereof may conveniently be achieved as described above for *Burkholderia gladioli*. The host cells may be those described above. Gladiolin analogues obtained from such methods form a further aspect of the invention.

It may be desirable to modify, e.g. mutate, for instance as described above, said nucleic acids and/or said recombinant host cells prior to the culture step in order to obtain further gladiolin analogues. Mutation of host cells/nucleic acids can be achieved by routine means, e.g. exposure to radiation (e.g. UV) and/or chemical

mutagens. Gladiolin analogues obtained from such methods form a further aspect of the invention.

The compounds of the present invention may be used in therapy. As such, according to another aspect of the present invention, there is provided a method for the treatment of an infection, the method comprising administering to a subject in need thereof, a compound according to the present invention, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, wherein the infection is caused by a microbe, optionally wherein the microbe is resistant to an antimicrobial drug.

According to another aspect of the present invention, there is provided a method for the treatment of an infection, the method comprising administering to a subject in need thereof, a compound according to the present invention, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, wherein the infection is caused by at least one pathogenic bacterium and/or at least one pathogenic fungus that is susceptible to gladiolin, for example at least one bacterium selected from *Mycobacterium* species, *Burkholderia* species, *Ralstonia* species, *Stenotrophomonas* species, *Staphylococcus* species, *Enterococcus* species, and *Bacillus* species, and/or at least one fungus selected from those of the genus *Candida*, for example *Candida albicans*.

According to another aspect of the present invention, there is provided the use of a compound according to the present invention, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, preferably a therapeutically acceptable amount thereof, in the manufacture of a medicament for the treatment of a microbial infection.

Also provided is a method for inhibiting the growth of a microbe, the method comprising the microbe with a compound according to the present invention, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, or with a bacterium capable of producing the compound. The method may be performed *in vitro* or *in vivo*. In the case of contact with a bacterium capable of producing the compound, suitable conditions, such as those identified above, may be provided in order that the antimicrobial compound is produced.

It is preferred that a therapeutically effective amount a compound as herein described, in any stereochemical form, or a mixture of any stereochemical forms in any ratios, or a pharmaceutically acceptable salt, metabolite, or prodrug thereof, is present or is used in the above aspects of the invention.

The following definitions shall apply throughout the specification and the appended claims.

Within the context of the present application, the terms “comprises” and “comprising” are interpreted to mean “includes, among other things”. These terms are not intended to be construed as “consists of only”.

Unless otherwise stated or indicated, the term ”alkyl” means a linear or branched saturated aliphatic hydrocarbon chain of 1-20 carbon atoms, such as C_{1-10}, C_{1-8}, C_{1-6} or C_{1-4}, which may be unsubstituted or substituted. The group may be partially or completely substituted with substituents independently selected from one or more of halogen (F, Cl, Br or I), hydroxy, nitro and amino. Non-limiting examples of alkyl groups include methyl, ethyl, n-propyl, 1-methylethyl(isopropyl), n-butyl, n-pentyl, 1,1-dimethylethyl(t-butyl), and the like.

Unless otherwise stated or indicated, the term ”alkenyl” means a linear or branched unsaturated hydrocarbon chain of 2-20 carbon atoms, such as C_{2-10}, C_{2-8}, C_{2-6} or C_{2-4}, which may be unsubstituted or substituted, and containing at least one double bond. The group may be partially or completely substituted with substituents independently selected from one or more of halogen (F, Cl, Br or I), hydroxy, nitro and amino. Non-limiting examples of alkenyl groups include ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl–pentenyl, 3-pentenyl, 3-methyl-2-butenyl, 3-methylbut-2-enyl, 3-hexenyl, 1,1-dimethylbut-2-enyl, and the like.

Unless otherwise stated or indicated, the term "aryl" means phenyl, naphthyl or indene. The aryl group may be partially or completely substituted with substituents independently selected from one or more of halogen (F, Cl, Br or I), hydroxy, nitro and amino. Unless otherwise stated, the term "heteroaryl" means pyrrole, indole, thiazole, triazole or pyridine.

The term “antimicrobial” includes antibiotics and chemicals capable of inhibiting or preventing the growth of, or capable of killing, microbes, especially bacteria. An example of an antimicrobial chemical is a disinfectant.

The term “antibiotic” means an agent produced by a living organism, such as a bacterium, that is capable of inhibiting the growth of another living organism, for example another bacterium, or is capable of killing another living organism, for example another bacterium.

The term “therapeutically effective amount” means an amount of an agent or compound which provides a therapeutic benefit in the treatment of a microbial infection.

The term “treatment” includes prevention, reduction, amelioration or elimination or the disorder or condition.

The term “pharmaceutically acceptable” means being useful in preparing a pharmaceutical composition that is generally safe, non-toxic and neither biologically nor otherwise undesirable and includes being useful for veterinary use as well as human pharmaceutical use.

Suitable pharmaceutically acceptable salts may include acid addition salts which may, for example, be formed by mixing a solution of the antimicrobial agent with a solution of a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, fumaric acid, maleic acid, succinic acid, acetic acid, benzoic acid, citric acid, tartaric acid, carbonic acid or phosphoric acid. Furthermore, where the antimicrobial agents of the invention carry an acidic moiety, suitable pharmaceutically acceptable salts thereof may include alkali metal salts (e. g., sodium or potassium salts); alkaline earth metal salts (e. g., calcium or magnesium salts); and salts formed with suitable organic ligands (e. g., ammonium, quaternary ammonium and amine cations formed using counter-anions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, alkyl sulfonate and aryl sulfonate). Illustrative examples of pharmaceutically acceptable salts include but are not limited to acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, bromide, butyrate, calcium edetate, camphorate, camphorsulfonate, camsylate, carbonate, chloride, citrate, clavulanate, cyclopentanepropionate, digluconate, dihydrochloride, dodecylsulfate, edetate, edisylate, estolate, esylate, ethanesulfonate, formate, fumarate, gluceptate, glucoheptonate, gluconate, glutamate, glycerophosphate, glycolylarsanilate, hemisulfate, heptanoate, hexanoate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroiodide, 2- hydroxy-ethanesulfonate, hydroxynaphthoate, iodide, isothionate, lactate, lactobionate, laurate, lauryl sulfate, malate, maleate, malonate, mandelate, mesylate, methanesulfonate, methylsulfate, mucate, 2-naphthalenesulfonate, napsylate, nicotinate, nitrate, N-methylglucamine ammonium salt, oleate, oxalate, pamoate (embonate), pahnitate, pantothenate, pectinate, persulfate, 3-phenylpropionate, phosphate/diphosphate, picrate, pivalate, polygalacturonate, propionate, salicylate,

stearate, sulfate, subacetate, succinate, tannate, tartrate, teoclate, tosylate, triethiodide, undecanoate, valerate, and the like.

The term “metabolite” means any intermediate or product resulting from metabolism of a compound according to the present invention.

The term “prodrug” means a functional derivative of a compound according to the present invention, such as an ester or an amide, that is biotransformed in the body to form the active drug. Reference is made to Goodman and Gilman’s, The Pharmacological basis of Therapeutics, 8th ed., Mc-Graw-Hill, Int. Ed. 1992, “Biotransformation of Drugs”, p.13-15.

The term "isomer" used herein refers to all forms of structural and spatial isomers. In particular, the term "isomer" is intended to encompass both tautomers and stereoisomers, as well as tautomers of stereoisomers and *vice versa*. As will be readily appreciated, compounds of the invention may exist in tautomeric forms, i.e. in forms which readily convert by a chemical reaction, especially the migration of a hydrogen atom or proton, accompanied by a switch of a single bond and adjacent double bond. Compounds of the invention may, in particular, undergo keto-enol tautomerisation. This is especially expected in those compounds having 1,3-dione functionalities, e.g. in compounds of formula (I) wherein R^9 and R^{10}, or R^{10} and R^{11}, are both oxo groups. Dependent on the conditions, the compounds of the invention may exist predominantly in an enol form and the invention is not intended to be limited to the particular form shown in the structural formulae given herein. For example, in compounds of the invention denoted by any formula in which R^3 is hydroxyl, the term "isomer" and "tautomer" are expressly intended to cover compounds in which the enol may be replaced by the keto form, i.e. compounds having an oxo group at position C-25. All such tautomeric forms of the compounds of the invention are included within the present invention.

With regard to stereoisomers, the compounds of formulae (II), (IIa), (IIb), (III) and (IIIa) herein described may have one or more asymmetric carbon atoms and may occur as racemates, racemic mixtures and as individual enantiomers or diastereomers. All such isomeric forms are included within the present invention, including mixtures thereof. Cis (*E*) and trans (*Z*) isomerism may also occur. The present invention includes the individual stereoisomers of the compounds of the invention and where appropriate, the individual tautomeric forms thereof, together with mixtures thereof. Separation of diastereoisomers or *cis* and *trans* isomers may be achieved by conventional techniques, e.g. by fractional crystallisation,

chromatography or HPLC. A stereoisomeric mixture of the compounds may also be prepared from a corresponding optically pure intermediate or by resolution, such as by HPLC of the corresponding racemate using a suitable chiral support or by fractional crystallisation of the diastereoisomeric salts formed by reaction of the corresponding racemate with a suitable optically active acid or base, as appropriate.

The term “about” means plus or minus 20%, more preferably plus or minus 10%, even more preferably plus or minus 5%, most preferably plus or minus 2%.

The term “broad spectrum antibiotic” means an antimicrobial that is effective against a wide range of disease-causing bacteria, e.g. against both Gram-positive and Gram-negative bacteria.

The abbreviation “LMG” refers to Laboratorium voor Microbiologie, Universiteit Gent and is used in relation to accession numbers for microorganisms deposited at the Belgian Coordinated Collection of Microorganisms (BCCM) held at the LMG (http://bccm.belspo.be/about/lmg.php).

The term “potentially susceptible bacteria” or “potentially susceptible fungi” means bacteria/fungi which have the potential for their growth to be inhibited or destroyed by an antimicrobial agent produced by an antimicrobial producing bacterium. Examples include those listed herein whose growth may be inhibited by the antimicrobial agents of the present invention.

Antimicrobial agents of the invention can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise at least one antimicrobial of the invention and at least one pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral

(e.g., inhalation), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that syringability exists. It must be stable under the conditions of manufacture, transfer and storage. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminium mono stearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., an antimicrobial according to an embodiment of the invention) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the

preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavouring agent such as peppermint, methyl salicylate, or orange flavouring.

For administration by inhalation, the compounds can be delivered in the form of an aerosol spray of liquid, or powdered or formulated antibiotic (e.g. within liposomes as stated below) from pressured container or dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds can be formulated into ointments, salves, gels, or creams as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a

controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds which exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose

can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. According to this aspect, the invention provides a kit comprising at least one compound according to the invention or a pharmaceutical composition of the invention, optionally in addition to one or more further active agents as defined herein, preferably with instructions for the administration thereof in the therapeutic treatment of the human or animal body, e.g. the treatment of infection by one or more infectious organisms as hereinbefore defined.

When using the compounds according to the present invention, the dose can vary within wide limits and, as is customary and is known to the physician, is to be suited to the individual conditions in each individual case. It depends, for example, on the nature and severity of the disease to be treated, on the mode of administration, or on whether an acute or chronic condition is treated or whether prophylaxis is carried out. An appropriate dosage can be established using clinical approaches well known in the medical art. In general, the daily dosage for achieving the desired results in an adult weighing about 75 kg is from about 0.01 to about 100 mg/kg, preferably from about 0.1 to about 50 mg/kg, in particular from about 0.1 to about 10 mg/kg.

Within this specification embodiments have been described in a way which enables a clear and concise specification to be written, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the invention.

The invention will now be further illustrated by the following non-limiting examples and figures, in which:

Figure 1 shows the detection of antimicrobial activity in *Burkholderia gladioli* strain LMG-P 26202 (BCC0238). Antimicrobial overlay assays of *B. gladioli* LMG-P 26202 are shown as follows: (A) activity towards *Bacillus subtilis* strain 168 (genome sequence strain); (B) *Candida albicans* strain SC 5314 (genome sequence strain); (C) *Staphylococcus aureus* strain NCTC 12981 (antibiotic

susceptibility testing reference strain); and (D) *Stenotrophomonas maltophilia* strain LMG 958^T (Type strain for species). The overlay cultures shown in panels A and D contained triphenyl tetrazolium chloride (0.02%) as a metabolic dye to show the presence of growing cells and enhance the clarity of the zone of inhibition due to the secreted *Burkholderia* antimicrobials.

Figure 2 shows the antimicrobial activity of pure gladiolin from *Burkholderia gladioli* LMG-P 26202 (BCC0238). Antimicrobial susceptibility testing discs were loaded with 2, 10, 50 and 100 µg of pure gladiolin by addition of the antibiotic dissolved in methanol; the methanol was subsequently removed by air drying. An antimicrobial disc susceptibility testing assay was then performed as follows. *Staphylococcus aureus* strain NCTC 12981, *Candida albicans* strain SC 5314, *Enterococcus faecalis* strain ATCC 51299 (antimicrobial susceptibility testing reference strain), and *Burkholderia multivorans* ATCC 17616 were grown overnight in IsoSensitest broth and then diluted to an optical density of 0.5 units at 600 nm. The test bacteria were then spread onto an IsoSensitest agar using a sterile swab. After drying the gladiolin discs were placed onto the agar. The plates were incubated at 37°C overnight to detect antimicrobial activity of gladiolin at each concentration as shown. The zone of clearing indicates activity of gladiolin towards the test organism. Figure 2 shows the results of testing on: (A) *Staphylococcus aureus*; (B) *Candida albicans*; (C) *Enterococcus faecalis*; and (D) *Burkholderia multivorans*.

The results of NMR spectroscopy and mass spectrometry measurements are shown in Figures 3 to 15. Measurements were applied to gladiolin that had been extracted using Amberlite XAD-16 resin and then subjected to further purification by reverse phase HPLC as described below.

Figure 3 shows the positive ion mode ESI-TOF mass spectrum of gladiolin (top) and the simulated mass spectrum for the $C_{44}H_{75}O_{11}^+$ ion (bottom).

Figure 4 shows the positive ion mode ESI-TOF mass spectrum of gladiolin (top) and the simulated mass spectrum for the $C_{44}H_{74}O_{11}Na^+$ ion (bottom).

Figure 5 shows the negative ion mode ESI-TOF mass spectrum of gladiolin (top) and the simulated mass spectrum for the $C_{44}H_{73}O_{11}^-$ ion (bottom).

Figure 6 shows the negative ion mode ESI-TOF MS/MS spectrum of gladiolin.

Figure 7 shows the ^{1}H NMR spectrum of gladiolin (d_4-MeOH).

Figure 8 shows the COSY NMR spectrum of gladiolin (d_4-MeOH).

Figure 9 shows the HSQC NMR spectrum of gladiolin (d_4-MeOH).

Figure 10 shows the HMBC NMR spectrum of gladiolin (d_4-MeOH).

Figure 11 shows the TOCSY NMR spectrum of gladiolin (d_4-MeOH).

Figure 12 shows the ^{1}H NMR spectrum of gladiolin (d_6-DMSO).

Figure 13 shows the COSY NMR spectrum of gladiolin (d_6-DMSO).

Figure 14 shows the HSQC NMR spectrum of gladiolin (d_6-DMSO).

Figure 15 shows the HMBC NMR spectrum of gladiolin (d_6-DMSO).

Figure 16 shows the TOCSY NMR spectrum of gladiolin (d_6-DMSO).

and in which

SEQ ID No. 1 is the nucleotide sequence of the gladiolin biosynthesis gene cluster.

SEQ ID No. 2 is the nucleotide sequence of the *gnbA1* gene.

SEQ ID No. 3 is the amino acid sequence gnbA1.

SEQ ID No. 4 is the nucleotide sequence of the *gnbA2* gene.

SEQ ID No. 5 is the amino acid sequence gnbA2.

SEQ ID No. 6 is the nucleotide sequence of the *gnbA3* gene.

SEQ ID No. 7 is the amino acid sequence of gnbA3.

SEQ ID No. 8 is the nucleotide sequence of the *gnbA4* gene

SEQ ID No. 9 is the amino acid sequence of gnbA4.

SEQ ID No. 10 is the nucleotide sequence of the *gnbA5* gene.

SEQ ID No. 11 is the amino acid sequence of gnbA5.

SEQ ID No. 12 is the nucleotide sequence of the *gnbA6* gene.

SEQ ID No. 13 is the amino acid sequence gnbA6.

Sequence Listing Free Text

SEQ ID No. 1: <223> Gladiolin biosynthesis gene cluster

SEQ ID No. 2: <223> Polyketide synthase (PKS) gene: gnbA1

SEQ ID No. 4: <223> Polyketide synthase (PKS) gene: gnbA2

SEQ ID No. 6: <223> Polyketide synthase (PKS) gene: gnbA3

SEQ ID No. 8: <223> Polyketide synthase (PKS) gene: gnbA4

SEQ ID No. 10: <223> Polyketide synthase (PKS) gene: gnbA5

SEQ ID No. 12: <223> Polyketide synthase (PKS) gene: gnbA6

Examples

Example 1 - Production of gladiolin

Gladiolin is produced by the *Burkholderia gladioli* producer strain LMG-P 26202 (BCC0238) as described herein.

Preparation of BSM minimal media

BSM minimal media can be made using the following stock solutions: Phosphate Salts (20 x stock comprising di-potassium Hydrogen Orthophosphate Trihydrate [$K_2HPO_4.3H_2O$] 85 g/L and Sodium-di-Hydrogen Orthophosphate Monohydrate [$NaH_2PO_4.H_2O$] 20 g/L); Ammonium Chloride (20 x stock comprising NH_4Cl 40 g/l); Nitrilotriacetic Acid (100 x stock comprising $C_6H_9NO_6$ at 10 g/L); Metal Salts (100x stock comprising Magnesium Sulphate Heptahydrate [$MgSO_4.7H_2O$] 20 g/l, Ferrous Sulphate Heptahydrate [$FeSO_4.7H_2O$] 1.2 g/L, Manganese Sulphate monohydrate [$MnSO_4.H_2O$] 0.3 g/L, Zinc Sulphate Heptahydrate [$ZnSO_4.7H_2O$] 0.3 g/L, Cobalt Sulphate Heptahydrate [$CoSO_4.7H_2O$] 0.1 g/L). The stocks are combined as follows: 50 mL Phosphate stock, 50 mL Ammonium chloride stock, 10 mL Nitrilotriacetic acid stock, 10 mL Metal Salts, and made up to 900 mL with deionised water. Glycerol (4 g), yeast extract (0.5 g), casamino acids (0.5 g) and purified bacteriological agar (15 g) are then added to this mixture before the medium is made to a final volume of 1 litre and sterilised by autoclaving and poured into culture plates.

Preparation of Gladiolin - method 1

A starter culture of a gladiolin-positive *Burkholderia gladioli* producer strain was plated from a frozen stock onto Tryptic Soya Agar and incubated for 18 to 30 hours at 30°C. The area of confluent bacterial growth was resuspended into 5 ml of

minimal BSM medium (4 g/L glycerol; 0.05% casamino acids and 0.05% yeast extract) using a sterile swab. This suspension of starter culture was used to inoculate 1 L of the BSM medium contained in a 5 L sterile conical growth flask. The culture was then grown with either shaking or stirring (via a magnetic stirrer) for 8 to 120 hours to allow the production of gladiolin to occur.

After growth, the bacteria within the culture were removed by centrifugation and 50 g of Amberlite XAD-16 resin was added per L of the cleared supernatant. The resin and supernatant were mixed with stirring for 1 to 2 hours at room temperature to enable binding of the gladiolin. The resin was then harvested by filtration and washed with 2 L of deionised water. After washing, a concentrated suspension of the resin was made and poured into a chromatography column; after settling the resin was washed with 500 mL of water and then air blown through the column to remove as much residual water as possible. The bound gladiolin was then eluted from the resin by the addition of 150 mL methanol (3 times the volume of the resin) to the column followed by slow drainage of the eluent into a collecting vessel. The methanol eluent was then concentrated by rotary vacuum evaporation (with the temperature set to 30°C) and freeze dried down to a powder prior to further HPLC purification.

HPLC purification was effected by passage through a reverse phase (C18) HPLC column eluting with 30-100% methanol/0.1% formic acid in water/0.1% formic acid (see Example 3).

Preparation of Gladiolin - method 2

A starter culture of a gladiolin-positive *Burkholderia gladioli* producer strain was plated from a frozen stock onto Tryptic Soya Agar and incubated for 18 to 30 hours at 30°C. The area of confluent bacterial growth was resuspended into 5 ml of minimal BSM medium (4 g/L glycerol; 0.05% casamino acids and 0.05% yeast extract) using a sterile swab to created a starter culture. Using a sterile swab, 5 to 6 streaks of the *B. gladioli* starter culture were spread across each Petri dish (90 mm diameter; containing 20 ml BSM agar) with each strip approximately 1 cm apart; a total of 20 plates were processed as a single batch. The plates were sealed within a large plastic bag and incubated at 30°C for 72 hours. After growth, the bacterial growth was removed by scraping the plate surface with spatula. Residual bacteria were killed by incubation of the plates agar surface facing down in a chloroform vapour chamber for 2 minutes. After airing the plates for 10 minutes

to allow the chloroform vapour to disperse, the agar was cut into approximately 1 cm cubes and placed into a 5 L beaker; approximately 300 g of cubed agar resulted from each 20 plate batch. 1 litre of distilled and deionised water (used throughout the remaining preparation) was added to the agar cubes and the mixture stirred gently for 20 minutes using a large magnetic stirrer bar. The resulting aqueous extract containing gladiolin and other secreted metabolites was then poured through a fine sieve into another beaker. A second extraction of the agar cubes was then performed with another litre of water and the two extractions pooled. 100 g of Amberlite XAD-16 resin was added to the 2 litres of aqueous extract and mixed with stirring for 1 to 2 hours at room temperature to enable binding of the gladiolin.

The resin was then harvested by filtration and washed with 2 L of water. After washing, a concentrated suspension of the resin was made and poured into a large chromatography column (3 cm diameter; 70 cm length); after settling the resin was washed with 500 mL of water and then air blown through the column to remove as much residual water as possible. The bound gladiolin was then eluted from the resin by the addition of 200 mL ice cold methanol to the column followed by slow drainage of the eluent into a collecting vessel. The methanol eluent was then concentrated by rotary vacuum evaporation (with the temperature set to 30°C) and freeze dried down to a powder prior to further HPLC purification of the gladiolin.

HPLC purification was effected by passage through a reverse phase (C18) HPLC column eluting with 30-100% methanol/0.1% formic acid in water/0.1% formic acid.

Example 2 - Antimicrobial production by *B. gladioli*

The isolate *Burkholderia gladioli* LMG-P 26202 (BCC0238) was examined for antimicrobial production. This involved inoculating the isolate onto minimal media containing glycerol as the sole carbon source, leaving it to grow at 30°C until stationary phase (48 hours), and then overlaying it with soft-agar containing one of the four test susceptibility species: *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus* or *Stenotrophomonas maltophilia*. The results are shown in Figure 1. The diameter (mm) of the zone of clearing observed in the soft-agar overlay was 35, 44, 41 and 18 for *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus* and *Stenotrophomonas maltophilia*, respectively.

Figure 2 illustrates the activity of gladiolin purified from a 48 hour old liquid culture of *Burkholderia gladioli* strain LMG-P 26202 (BCC0238) using Amberlite XAD-16 resin. After cultivation, the BCC0238 culture was subjected to centrifugation to remove the bacteria. The cleared supernatant containing the gladiolin was filtered through a glass microfibre filter and 5 g of Amberlite XAD-16 anionic resin added per 100 mL of culture fluid. The gladiolin was allowed to bind the Amberlite particles for 2 hours with constant stirring used to keep the resin in suspension. After binding, the resin was separated from the culture supernatant by filtration onto a glass microfibre filter, and then washed with 2 litres of deionised water. The resin was resuspended in a small volume of water to form a slurry and poured into a chromatography column (25 mm diameter; 50 cm long) with a glass wool plug at the bottom. The resin was allowed to settle under gravity and then the water drawn off by opening the valve at the base of the column; the remaining water within the resin column was then blown out using air. The gladiolin was eluted from the Amerlite XAD-16 resin using methanol which was poured onto the column and allowed to drip slowly through the particles (2 x 50 ml methanol extractions). The methanol eluent was then concentrated using a rotary vacuum evaporator (45°C) and finally dried down into 14 ml samples tubes. The gladiolin was then purified using HPLC (see Example 3 below) and dried down prior to susceptibility testing. The dry weight was determined and the gladiolin dissolved in methanol to a concentration of 5 mg/ml. 2, 10, 50 and 100 µg of gladiolin extract was then spotted onto blank antibiotic susceptibility testing disks and the methanol was allowed to evaporate at 37°C. Isosensitest Agar plates were then spread with a standard inoculum (a 0.5 MacFarland standard) of fresh *S. aureus*, *B. multivorans*, *Candida albicans*, or *Enterococcus faecalis* and allowed to dry. Susceptibility testing disks containing 2, 10, 50 and 100 µg of gladiolin were then placed on each agar and the plates were incubated at 37°C. The zones of clearing around the disks show that gladiolin had activity on *Candida albicans* (minimal inhibitory concentration [MIC] below 2µg/ml), *S. aureus* (MIC below 2 µg/ml), *Enterococcus faecalis* (MIC between 2 and 10 µg/ml), and some activity towards the Gram negative species *B. multivorans* (MIC above 50 µg/ml).

Example 3 - Determination of the planar structure of gladiolin

The concentrated methanol eluate from the XAD resin was re-suspended in methanol and separated using semi-preparative HPLC on a reverse phase column (C18, 100 x 21 mm, fitted with a C18 pre-column 10 x 21 mm). The mobile phases used were A: water/0.1% formic acid and B: methanol/0.1% formic acid and the elution conditions were as follows: 0 minutes, 70% A / 30% B; 20 minutes, 100% B; 25 minutes 100% B. Absorbance was monitored at a wavelength of 240 nm. Fractions containing gladiolin were identified using ESI-MS and combined.

The combined fractions were evaporated under reduced pressure, and the residue was re-suspended in a small volume of 50% aqueous methanol and separated by semi-preparative HPLC on the same C18 column using the following elution conditions: 0 minutes, 40% A / 60% B; 15 minutes, 5% A / 95% B; 20 minutes, 100% B; 25 minutes, 100% B. Absorbance was monitored at 240 nm and fractions containing gladiolin were collected, combined and lyophilised. 5.6 mg of gladiolin was obtained from 85 mg of the concentrated eluate from the XAD resin.

High resolution ESI-TOF-MS analysis of purified gladiolin established its molecular formula as $C_{44}H_{74}O_{11}$ (calculated for $C_{44}H_{75}O_{11}^+$ ($[M+H]^+$): 779.5304, found: 779.5306; calculated for $C_{44}H_{74}O_{11}Na^+$ ($[M+Na]^+$): 801.5123, found: 801.5134; calculated for $C_{44}H_{73}O_{11}$ ($[M-H]^-$): 777.5158, found: 777.5154) (Figures 3-5). High resolution ESI-MS/MS of gladiolin in negative ion mode showed that the $[M-H]^-$ ion loses CO_2 (a neutral loss of 43.9910 was observed) indicating that it contains a carboxylic acid group (Figure 6).

^1H, COSY, TOCSY, HSQC and HMBC NMR spectra (measured in d_4-MeOH and d_6-DMSO, Figures 7-15) were used to establish the planar structure of gladiolin. Correlations in the COSY spectra established the structures of the C-2 to C-24 and C-26 to C-37 fragments. HMBC correlations between the protons attached to C-2, C-3 and C-21, respectively, and C-1 established the location of the lactone linkage. Several HMBC correlations linked the C-2/C-24 fragment to the C-26/C-37 fragment via an unsaturated C-25 bearing a methyl group, and defined the location of the C-15 methoxy group. The location of the carboxyl group at C-37 was also confirmed by HMBC correlations from the protons attached to C-36 and C-37 with C-38. The configurations of the double bonds between C-10/C-11, C-28/C-29 and C-30/C-31 were all established as E, and the configuration of the C-12/C-13 double bond was determined to be Z, on the basis of the coupling constants

observed in the ^{1}H NMR spectra. The configuration of the C-24/C-25 double bond is currently undefined. Also, the configurations of C-11/C-12 and C-29/C-30 bonds in the dienes were established as s-trans on the basis of coupling constants. Assignments for the signals observed in the ^{1}H and ^{13}C NMR spectra of gladiolin are given in Tables 1 and 2.

Table 1: Assignment of NMR signals observed for gladiolin in d_4-MeOH:

Position	^{1}H (ppm)†	^{13}C (ppm)*
1		173.4
2	2.43, 2.58 (dd, 4.1, 15.4; m)	41.2
3	4.10 (m)	71.6
4	1.62 (m)	44.6
4-Me	0.93 (d, 3.2)	10.2
5	3.97 (m)	71.4
6	1.66 (m)	39.8
7	3.71 (dd, 5.9, 11.3)	73.5
8	1.73 (m)	38.7
8-Me	0.92 (d, 3.1)	14.2
9	2.08, 2.28 (m; m)	37.2
10	5.75 (dt, 6.8, 14.8)	133.4
11	6.38 (dd, 11.3, 14.8)	128.0
12	6.08 (dd, 11.3, 11.0)	131.7
13	5.34 (dt, 7.7, 11.0)	125.8
14	2.36, 2.47 (m; m)	32.0
15	3.28 (dd, 5.5, 5.6)	82.4
15-OMe	3.35 (s)	56.7
16	1.47, 1.62 (m; m)	33.9
17	1.39, 1.48 (m; m)	22.2
18	1.47, 1.38 (m; m)	38.7
19	3.60 (m)	68.4
20	1.64, 1.70 (m; m)	38.0
21	5.39 (dd, 7.5, 10.5)	73.6
22	1.98 (m)	43.5
22-Me	0.81 (d, 7.1)	10.7
23	4.20 (dd, 9.1, 9.1)	69.9
24	5.16 (d, 9.4)	127.3
25		138.9
25-Me	1.68 (s)	16.7
26	2.12 (t, 6.5)	40.3
27	2.20 (m)	31.6
28	5.58 (dt, 7.2, 14.2)	132.8
29	6.04 (dd, 11.5, 14.2)	131.7
30	6.04 (dd, 11.5, 14.2)	133.7
31	5.59 (dt, 7.2, 14.2)	129.1
32	2.19 (m)	42.4
33	3.66 (m)	69.9
34	1.19, 1.43 (m; m)	44.8
35	1.70 (m)	29.9
35-Me	0.91 (d, 6.4)	18.5
36	1.46, 1.62 (dt, 2.8, 9.8; m)	34.2
37	2.28, 2.32 (m; m)	32.8
38		178.5

† multiplicities and coupling constants are given in brackets

* from HMBC and HSQC spectra

Table 2: Assignment of NMR signals observed for gladiolin in d_6-DMSO:

Position	^{1}H (ppm)†	^{13}C (ppm) *
1		170.9
2	2.22, 2.40 (m; m)	39.6
3	3.95 (m)	68.9
4	1.46 (m)	43.8
4-Me	0.79 (d, 6.4)	9.6
5	3.71 (m)	68.8
6	1.46 (m)	39.6
7	3.54 (m)	70.6
8	1.62 (m)	37.0
8-Me	0.78 (d, 6.2)	13.3
9	1.92, 2.18 (m; m)	35.8
10	5.71 (dt, 6.7, 14.9)	133.0
11	6.29 (dd, 10.9, 14.9)	126.1
12	6.02 (dd, 10.9, 11.2)	129.7
13	5.31 (dt, 6.8, 11.2)	125.0
14	2.26, 2.38 (m; m)	30.0
15	3.18 (dd, 5.5, 5.7)	79.6
15-OMe	3.23 (s)	55.5
16	1.32, 1.48 (m; m)	32.2
17	1.23, 1.36 (m; m)	21.1
18	1.32, 1.30 (m; m)	36.2
19	3.38 (m)	66.1
20	1.49, 1.61 (m; m)	36.3
21	5.30 (m)	71.8
22	1.80 (m)	42.1
22-Me	0.69 (d, 6.7)	10.4
23	3.98 (m)	67.8
24	5.06 (d, 8.5)	127.6
25		134.7
25-Me	1.59 (s)	16.2
26	2.02 (m)	38.9
27	2.12 (m)	30.1
28	5.54 (dt, 7.2, 14.8)	130.2
29	5.98 (dd, 10.9, 14.8)	131.1
30	5.96 (dd, 10.9, 14.8)	131.9
31	5.56 (dt, 7.0, 14.8)	129.2
32	2.08 (m)	41.3
33	3.49 (m)	67.1
34	1.04, 1.28 (m; m)	43.6
35	1.61 (m)	28.1
35-Me	0.80 (d, 6.7)	17.9
36	1.32, 1.47 (m; m)	32.3
37	2.14, 2.19 (m; m)	29.9
38		174.9

† multiplicities and coupling constants are given in brackets

* from HMBC and HSQC spectra

Example 4 - Antimicrobial activity against *Mycobacterium* species

The resazurin reduction assay was used to determine the activity of gladiolin against *Mycobacterium tuberculosis* (see Palomino *et al.*, Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in *Mycobacterium tuberculosis*, Antimicrob. Agents Chemother. 46**:** 2720-2722, 2002). From this assay an IC_{50} value of approximately 0.1 micrograms per mL was determined for pure gladiolin.

Example 5 - Genetic manipulation of producing strains and the production of gladiolin analogues

The gladiolin analogues can be prepared by manipulation of the gladiolin biosynthetic gene cluster of *Burkholderia gladioli* or other gladiolin-producing organisms. The gladiolin biosynthetic gene cluster can be identified and sequenced using methods well known in the art, for example standard cosmid cloning, sequencing and sequence compilation strategies.

Analogues lacking methyl groups at C-4, C-8 and/or C-22 can be produced by deletion or mutation of the methyltransferase domains within the appropriate modules of the polyketide synthase multienzymes responsible for assembly of gladiolin. Standard genetic engineering techniques analogous to those reported previously (e.g. Donadio *et al.*, *Science* (1991) 252:675-9; and Donadio *et al.*, *Proceedings of the National Academy of Sciences of the United States of America* (1993) 90:7119-23) can be used to achieve this. Similarly, the analogue with a keto group in place of the hydroxyl group at C-5 can be constructed by deletion or mutation of the ketoreductase domain within the appropriate module of the gladiolin polyketide synthase. Conversion of the C-25 and/or C-35 methyl groups to oxo groups may be achieved by deleting or mutating one or more genes responsible for introducing the methyl groups at C-25 and C-35, which genes encode enzymes that methylate using a HMG-CoA synthase mechanism operating *in trans* to the polyketide synthase.

Likewise, analogues comprising a methyl group at C-6 can be prepared by insertion of the appropriate methyltransferase domain from the corresponding

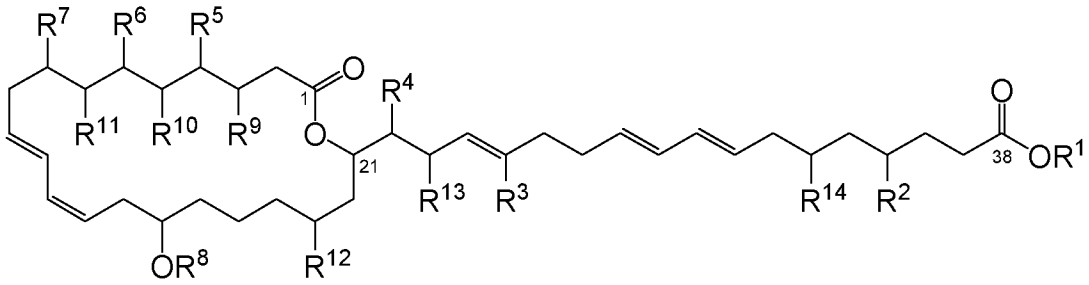
module of the etnangien polyketide synthase into the gladiolin synthase. A gladiolin analogue with a hydroxyl group in place of the O-methyl group at C-15 can be produced by deleting or mutationally inactivating the gene within the gladiolin biosynthetic gene cluster that encodes the O-methyl transferase responsible for post-polyketide synthase methylation of the C-15 hydroxyl group during gladiolin biosynthesis.

All references identified herein are incorporated herein by reference in their entirety.

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications are covered by the appended claims.

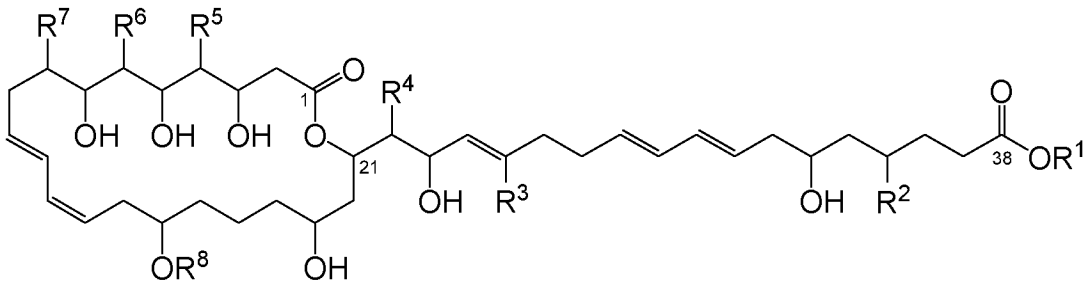
Claims:

1. A compound of formula (I):



or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer), or prodrug thereof, wherein R^1 is hydrogen or an ester-forming group; R^2 is methyl or oxo; R^3 is methyl or hydroxyl; R^4 to R^8 are each independently hydrogen or methyl; and R^9 to R^{14} are each independently hydroxyl or oxo.

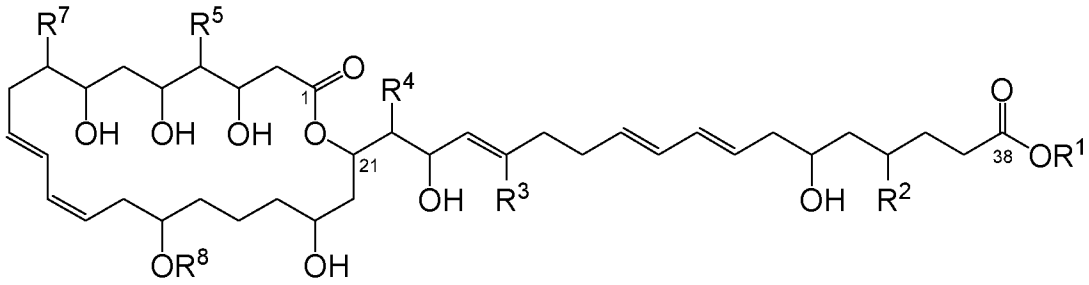
2. A compound as claimed in claim 1 of formula (II):



or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer), or prodrug thereof, wherein R^1 is hydrogen or an ester-forming group; R^2 is methyl or oxo; R^3 is methyl or hydroxyl; and R^4 to R^8 are each independently hydrogen or methyl.

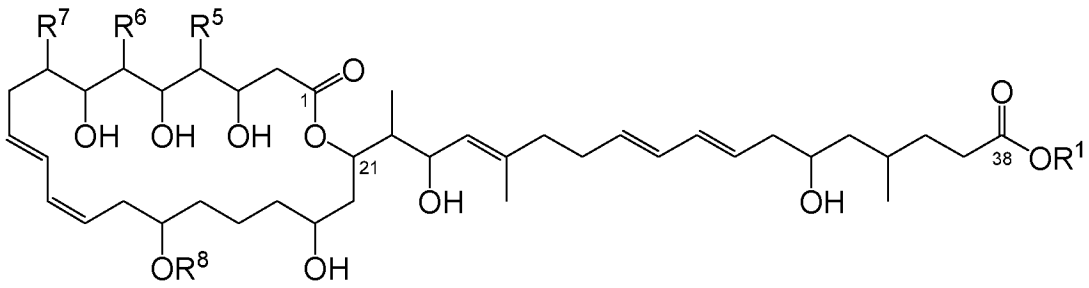
3. A compound as claimed in claim 1 or claim 2, wherein R^1 is a group selected from optionally substituted alkyl, alkenyl, cycloalkyl, cycloalkenyl, aryl and heteroaryl groups.

4. A compound as claimed in claim 2 of formula (IIa):



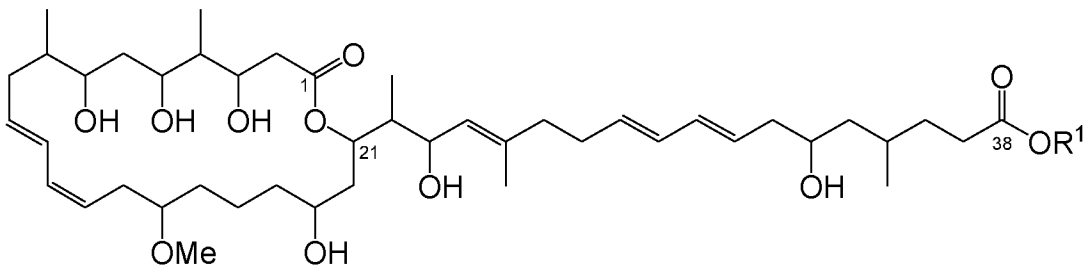
or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer), or prodrug thereof, wherein R^1 to R^5, R^7 and R^8 are as defined in claim 2 or claim 3.

5. A compound as claimed in claim 2 of formula (IIb):



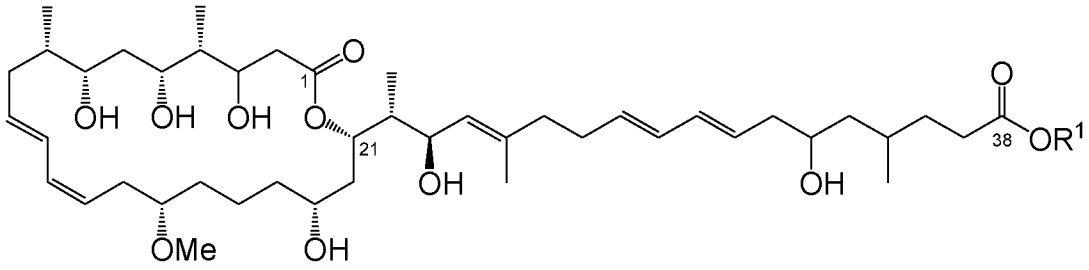
or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer), or prodrug thereof, wherein R^1 and R^5 to R^8 are as defined in claim 2 or claim 3.

6. A compound as claimed in claim 2 of formula (III):



or a pharmaceutically acceptable salt, metabolite, isomer (e.g. stereoisomer), or prodrug thereof, wherein R^1 is as defined in claim 2 or claim 3.

7. A compound as claimed in claim 6 of formula (IIIa):



or a pharmaceutically acceptable salt, metabolite stereoisomer or prodrug thereof, wherein R^1 is as defined in claim 2 or claim 3.

8. A compound as claimed in any one of claims 1 to 7, wherein R^1 is a group selected from an optionally substituted C_{1-6}-alkyl, a benzyl or a phenyl group.

9. A compound as claimed in any one of claims 1 to 7, wherein R^1 is hydrogen.

10. A compound as claimed in any one of claims 1 to 9 for use as a medicament.

11. A compound as claimed in any one of claims 1 to 9 for use as an antimicrobial and/or antiviral agent.

12. A compound as claimed in any one of claims 1 to 9 for use in the treatment of an infection caused by at least one microbe, preferably wherein the microbe is a bacterium, or a fungus, or a combination of a bacterium and a fungus.

13. The compound of claim 12 for use in the treatment of an infection, wherein the bacterium is a Gram-positive bacterium, e.g. selected from *Mycobacterium* species, *Staphylococcus* species, *Enterococcus* species and *Bacillus* species.

14. The compound of claim 12 for use in the treatment of an infection, wherein the bacterium is a Gram-negative bacterium, e.g. selected from *Burkholderia* species, *Ralstonia* species and *Stenotrophomonas* species.

15. The compound of any one of claims 12 to 14 for use in the treatment of an infection, wherein the fungus is selected from *Candida albicans*, *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*.

16. The compound of any one of claims 12 to 15 for use in the treatment of an infection caused by at least one microbe which is resistant to at least one antimicrobial drug.

17. The compound of claim 16 for use in the treatment of an infection, wherein the antimicrobial drug is selected from drugs of the penicillin family, drugs of the vancomycin family, drugs of the aminoglycoside family, drugs of the quinolone family, drugs of the daptomycin family, drugs of the cephalosporin family, drugs of the macrolide family, and combinations thereof.

18. The compound of claim 17 for use in the treatment of an infection, wherein the antimicrobial drug is selected from penicillin, ampicillin, methicillin, vancomycin, gentamycin, ofloxacin, ciprofloxacin, daptomycin, Cefdimir, erythromycin, equivalents thereof, and combinations thereof.

19. The compound of claim 16 for use in the treatment of an infection, wherein the antimicrobial drug is an antifungal drug selected from amphotericin B, nystatin, fluconazole, caspofungin, allylamines, equivalents thereof, and combinations thereof.

20. Use of a compound as claimed in any one of claims 1 to 9 in the manufacture of a medicament for use in treating an infection caused by at least one microbe as defined in any one of claims 12 to 19.

21. A compound as claimed in any one of claims 12 to 19 for use in the treatment of infection, or a use as claimed in claim 20, wherein the infection is an infection of the respiratory system, digestive system, urinary system, nervous system, a blood infection, a soft tissue infection, a skin infection, a nasal canal infection, or combinations thereof.

22. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

23. A pharmaceutical composition as claimed in claim 22, further comprising at least one other therapeutically active agent.

24. A pharmaceutical composition as claimed in claim 23, wherein the compound according to any one of claims 1 to 9 and the other therapeutically active compound are for sequential, separate or simultaneous administration.

25. Use of a compound as claimed in any one of claims 1 to 9, for inhibiting the growth or survival of a microbe, e.g. a microbe as defined in any one of claims 12 to 15, optionally wherein the microbe is resistant to at least one antimicrobial agent, e.g. as defined in any one of claims 17 to 19.

26. The microorganism *Burkholderia gladioli* sp. BCC0238 deposited under IDA accession number LMG-P 26202, or a variant or mutant thereof.

27. An active agent, especially an antimicrobial agent, obtained or obtainable from a microorganism as defined in claim 26.

28. The active agent of claim 27 having mass spectral and/or NMR spectroscopic properties substantially according to one or more of Figures 3 to 16 and/or Tables 1 and 2.

29. A process for the preparation of a compound as claimed in any one of claims 1 to 9 wherein R^1 is hydrogen, comprising cultivating a microorganism capable of producing said compound, in a culture medium comprising a source of assimilable carbon, nitrogen, and inorganic salts and, optionally, recovering said compound from the culture medium and, optionally, further converting the compound into a pharmaceutically acceptable salt thereof.

30. A process as claimed in claim 29, wherein the microorganism is a strain of *Burkholderia gladioli*, e.g. as defined in claim 26.

31. A process as claimed in claim 29 or claim 30, further comprising converting the compound wherein R^1 is hydrogen, into a compound wherein R^1 is an ester-forming group as defined in claim 1 or claim 2 and, optionally, further converting the resultant compound into a pharmaceutically acceptable salt thereof.

32. A method for the treatment of an infection, the method comprising administering to a subject in need thereof a compound as claimed in any one of claims 1 to 9, wherein the infection is caused by at least one microbe, optionally wherein the microbe is resistant to an antimicrobial drug.

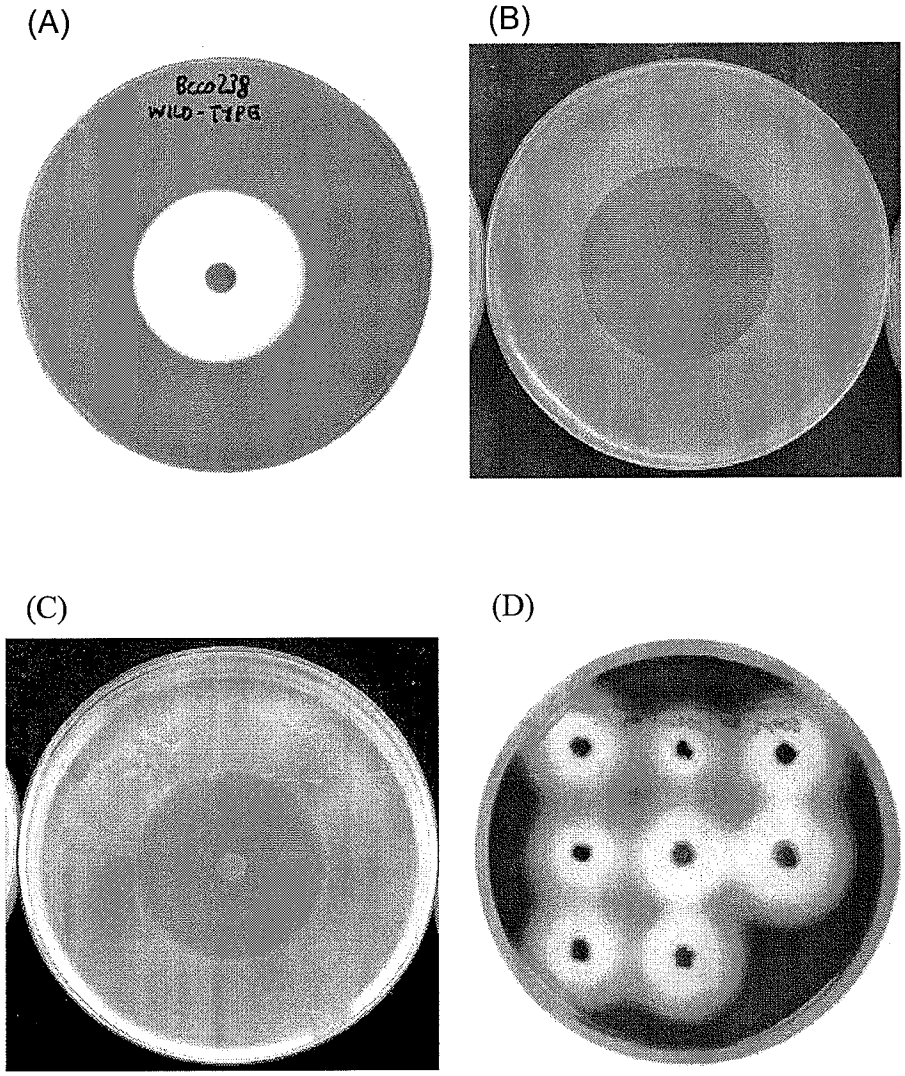


FIG. 1

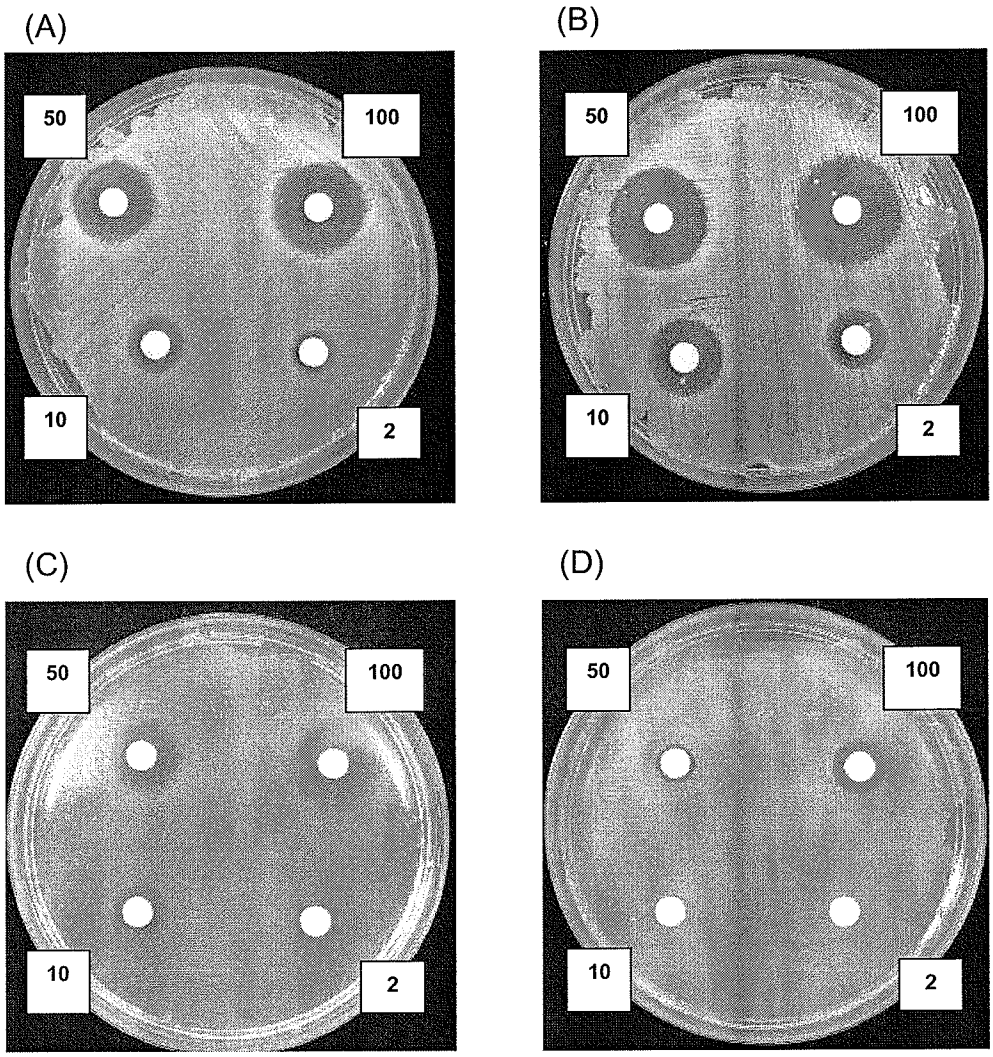


FIG. 2

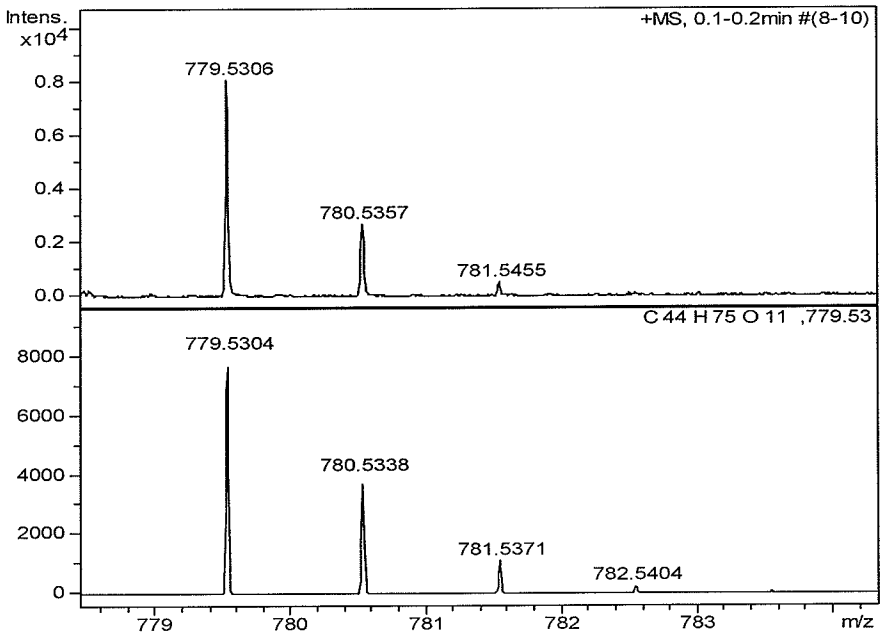

Intens.
x10^4
+MS, 0.1-0.2min #(8-10)
779.5306
780.5357
781.5455
C 44 H 75 O 11 ,779.53
779.5304
780.5338
781.5371
782.5404
m/z

FIG. 3

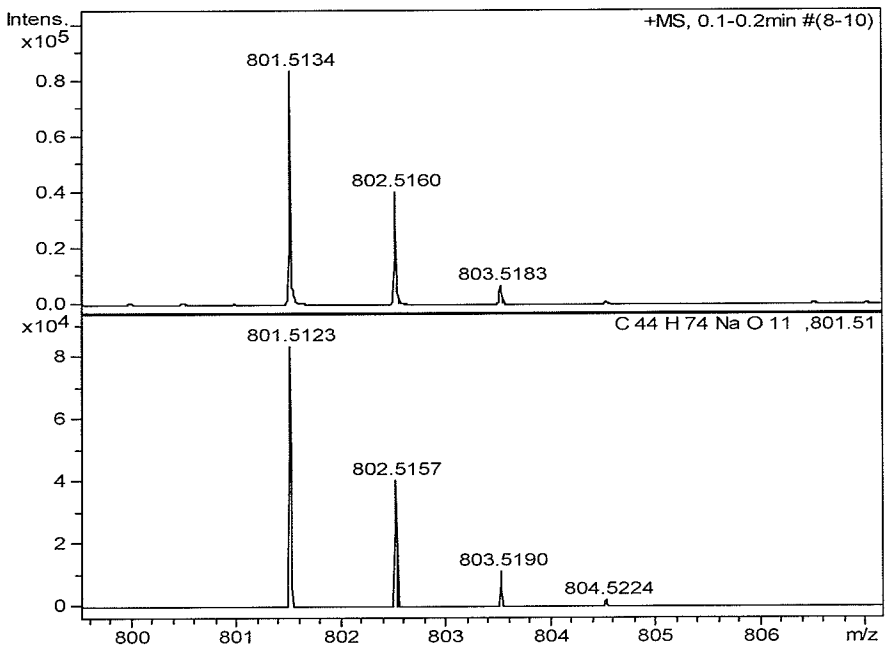

Intens.
x10^5
+MS, 0.1-0.2min #(8-10)
801.5134
802.5160
803.5183
x10^4
C 44 H 74 Na O 11 ,801.51
801.5123
802.5157
803.5190
804.5224
m/z

FIG. 4

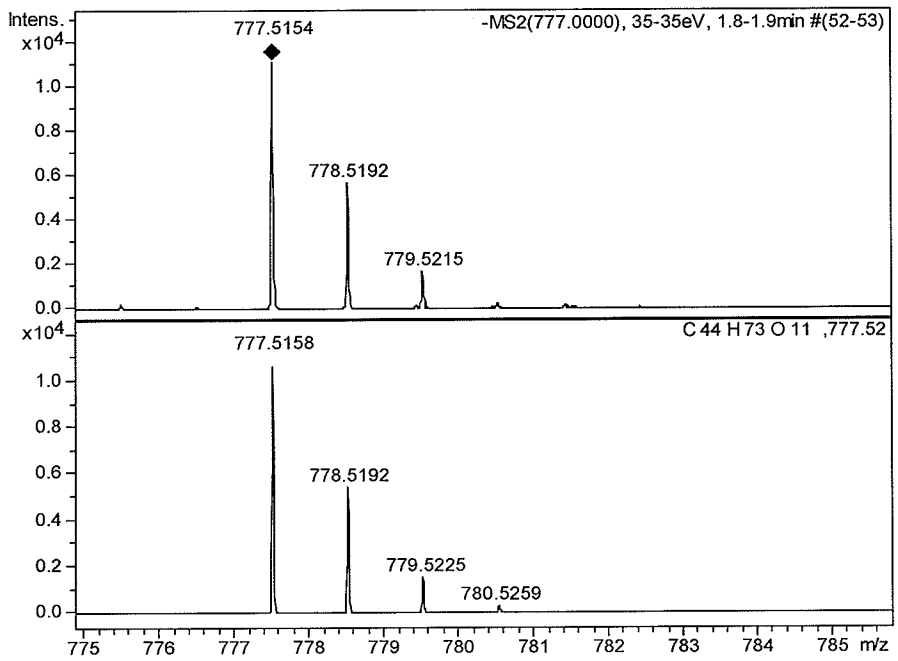

Intens.
x10^4
-MS2(777.0000), 35-35eV, 1.8-1.9min #(52-53)
777.5154
778.5192
779.5215
C 44 H 73 O 11 ,777.52
777.5158
778.5192
779.5225
780.5259
m/z

FIG. 5

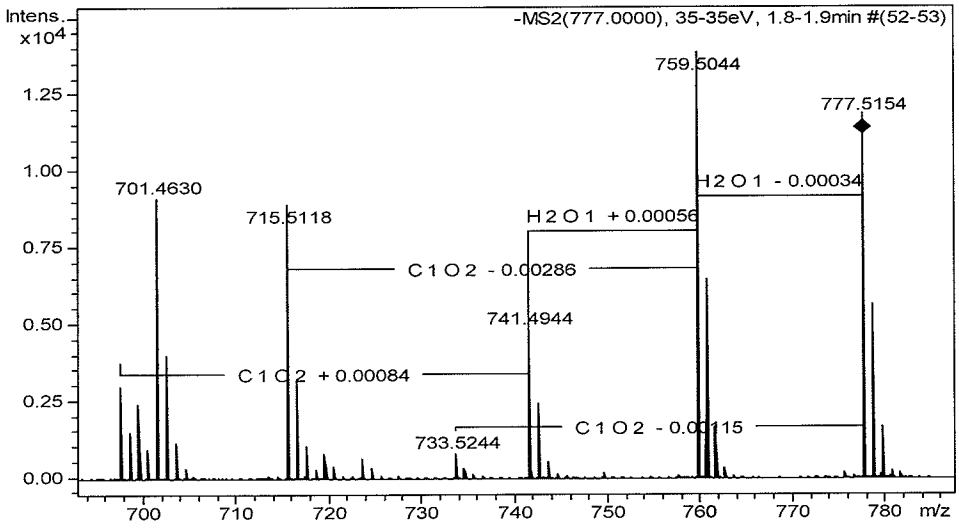

Intens.
x10^4
-MS2(777.0000), 35-35eV, 1.8-1.9min #(52-53)
759.5044
777.5154
701.4630
715.5118
H 2 O 1 - 0.00034
H 2 O 1 + 0.00056
C 1 O 2 - 0.00286
741.4944
C 1 O 2 + 0.00084
C 1 O 2 - 0.00115
733.5244
m/z

FIG. 6

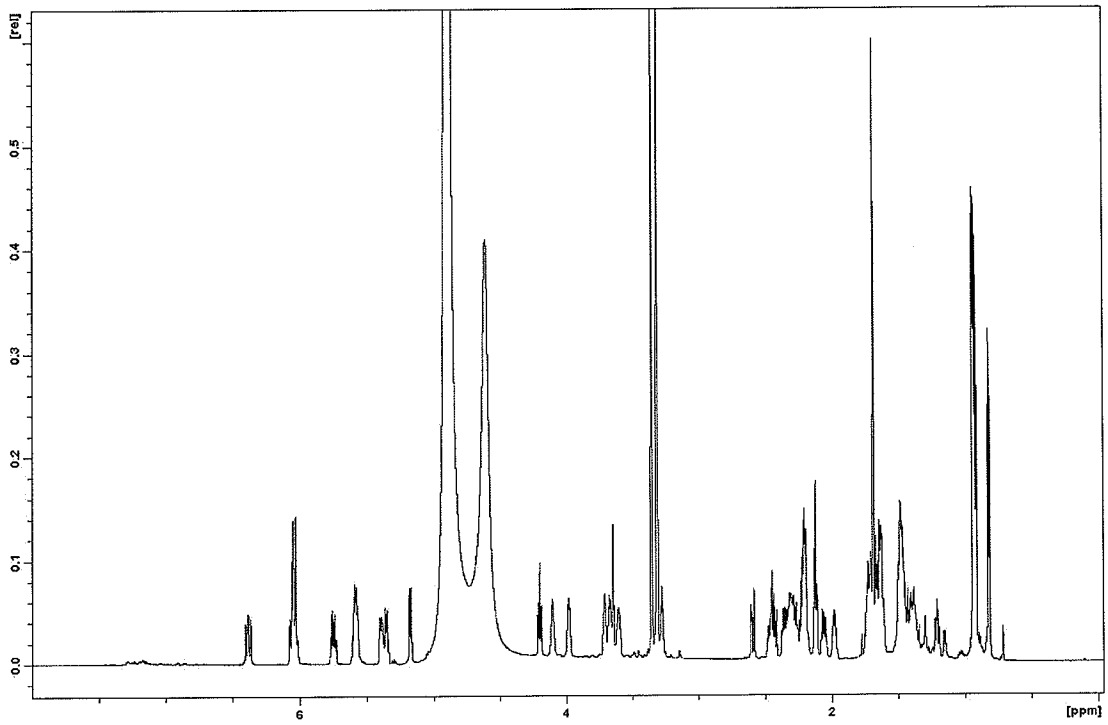


FIG. 7

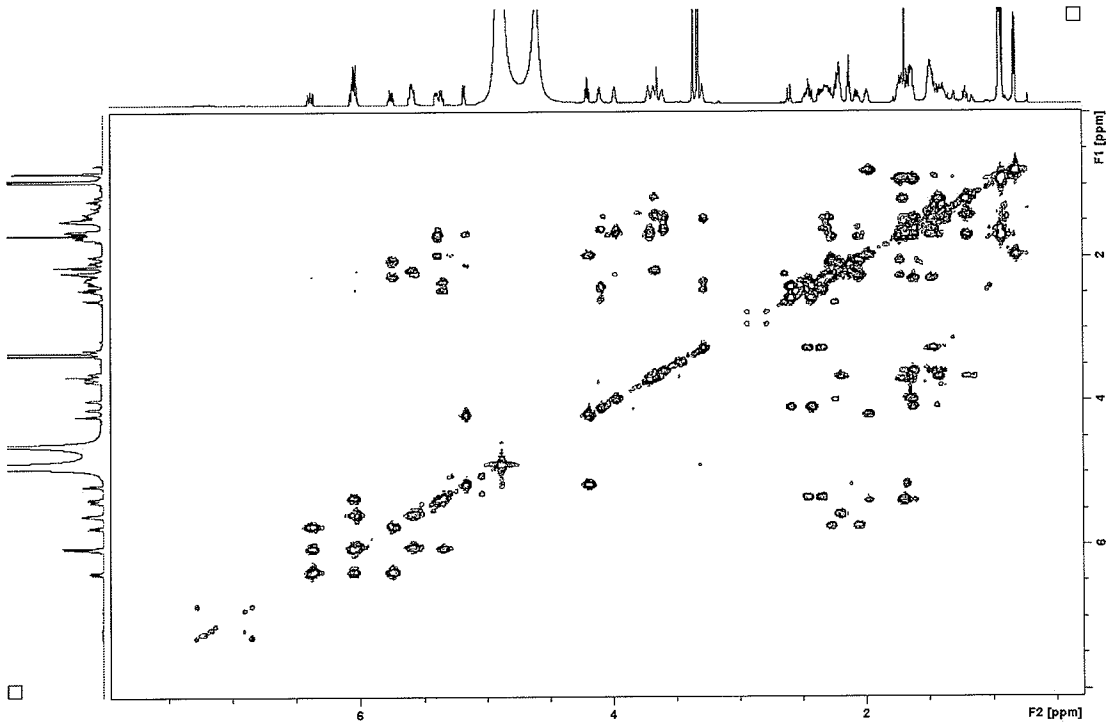


FIG. 8

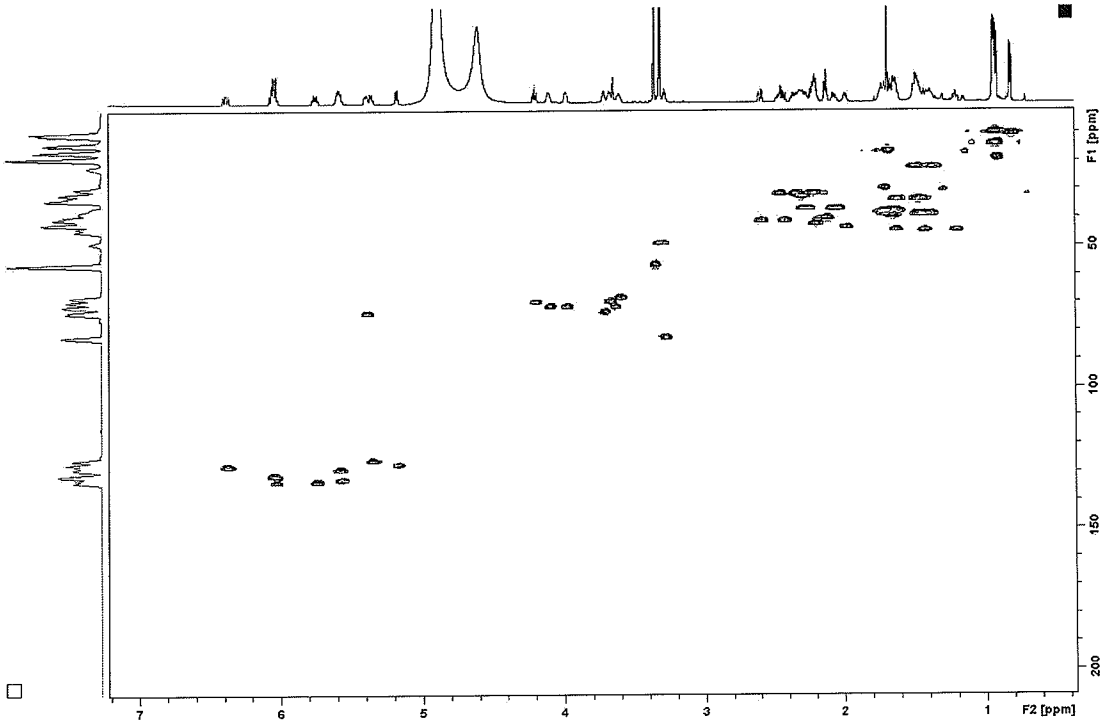

F1 [ppm]
50
100
150
200
7
6
5
4
3
2
1
F2 [ppm]

FIG. 9

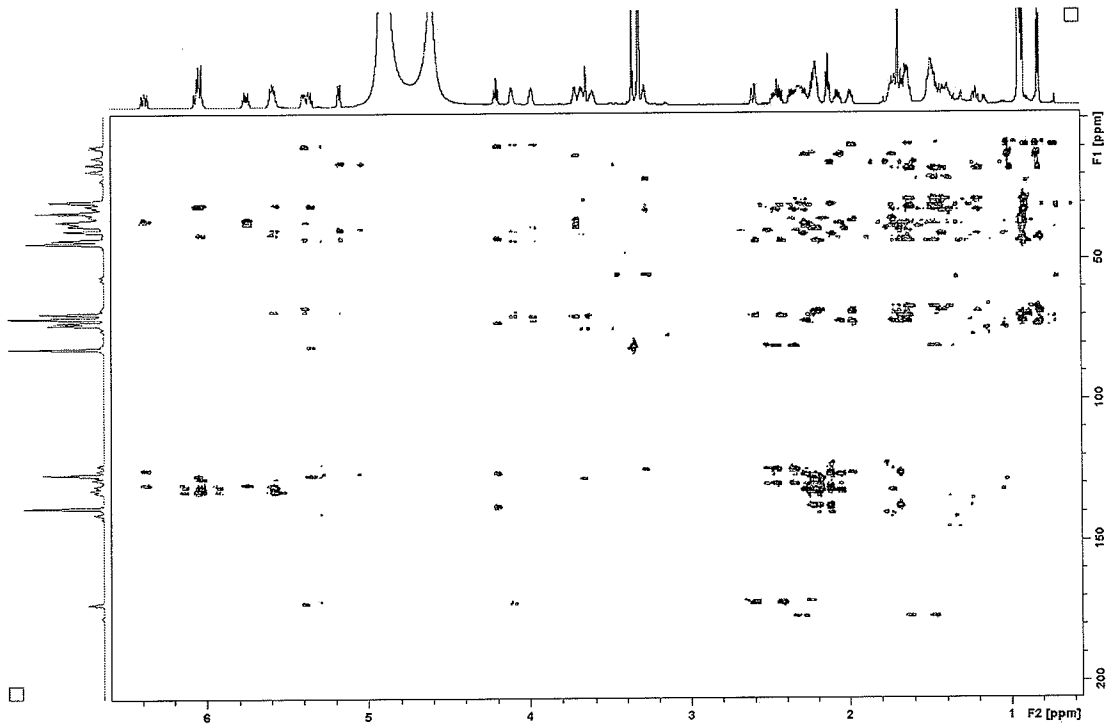

F1 [ppm]
50
100
150
200
6
5
4
3
2
1
F2 [ppm]

FIG. 10

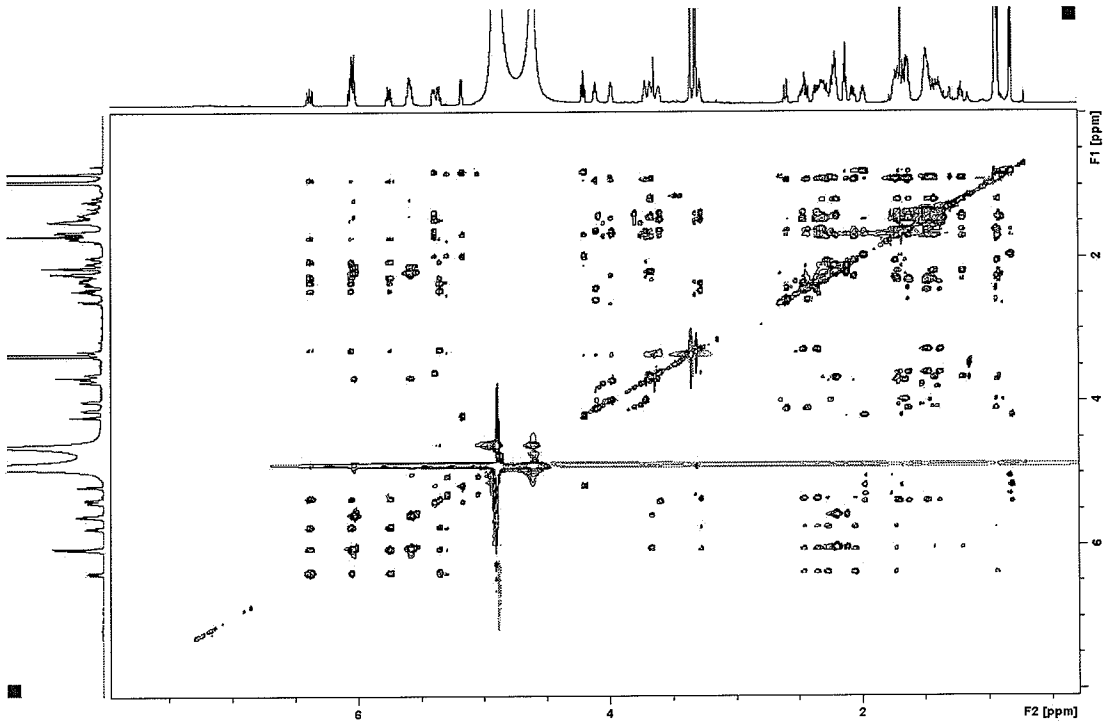


FIG. 11

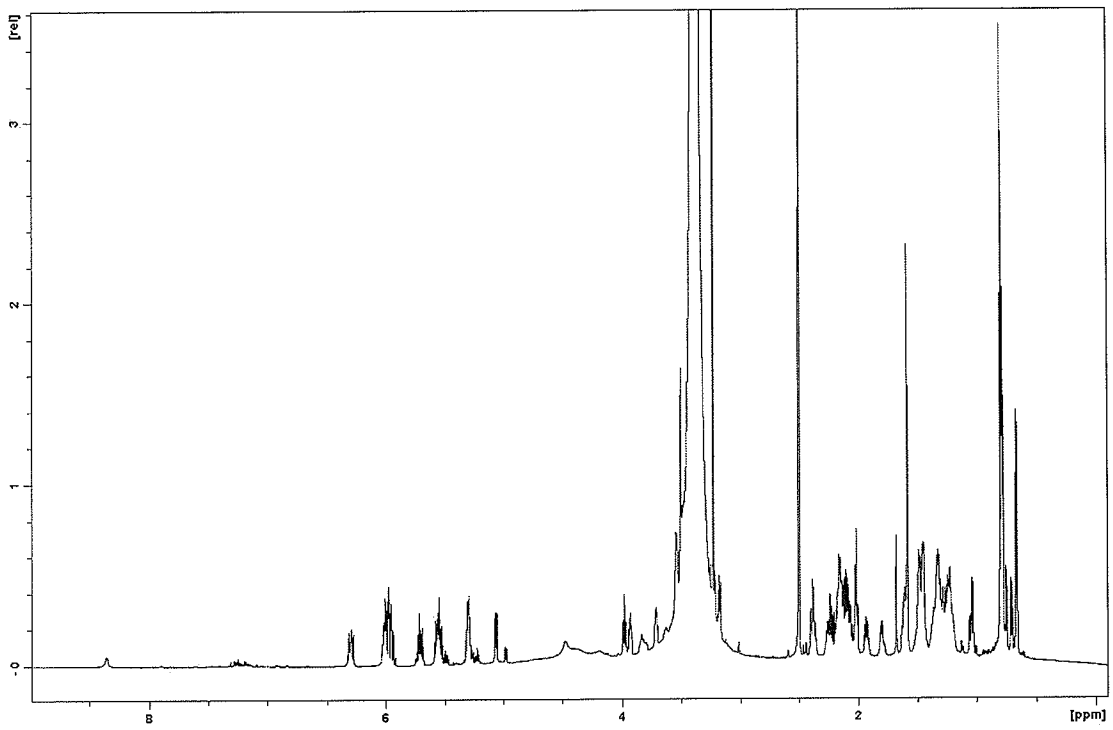


FIG. 12

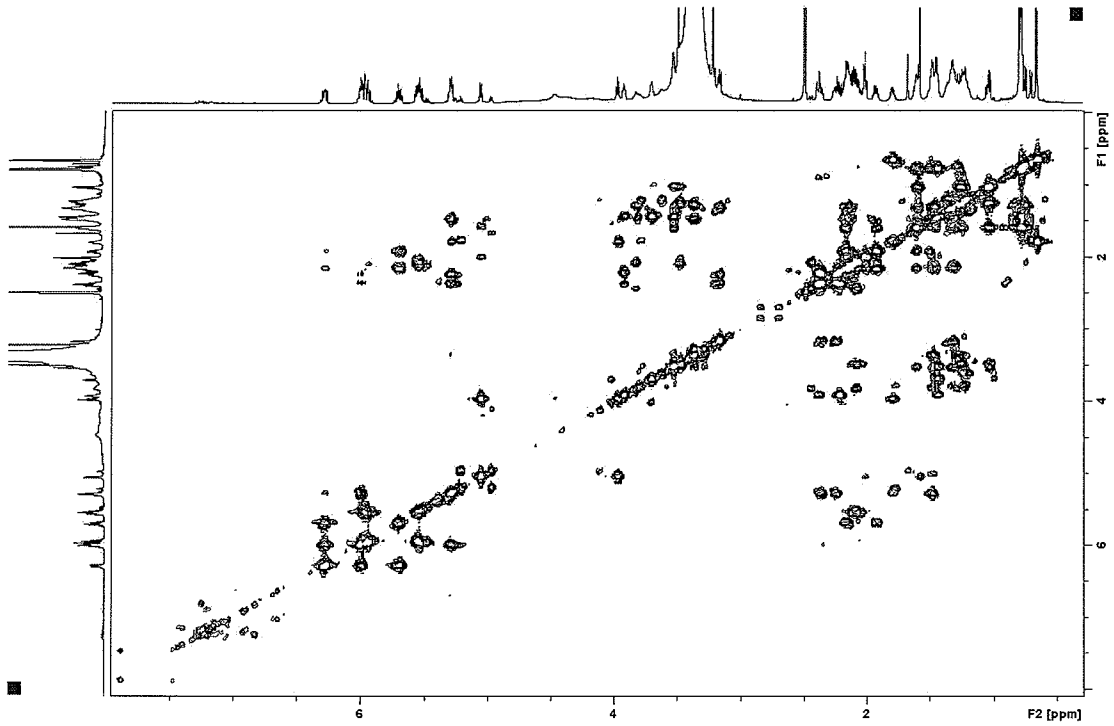


FIG. 13

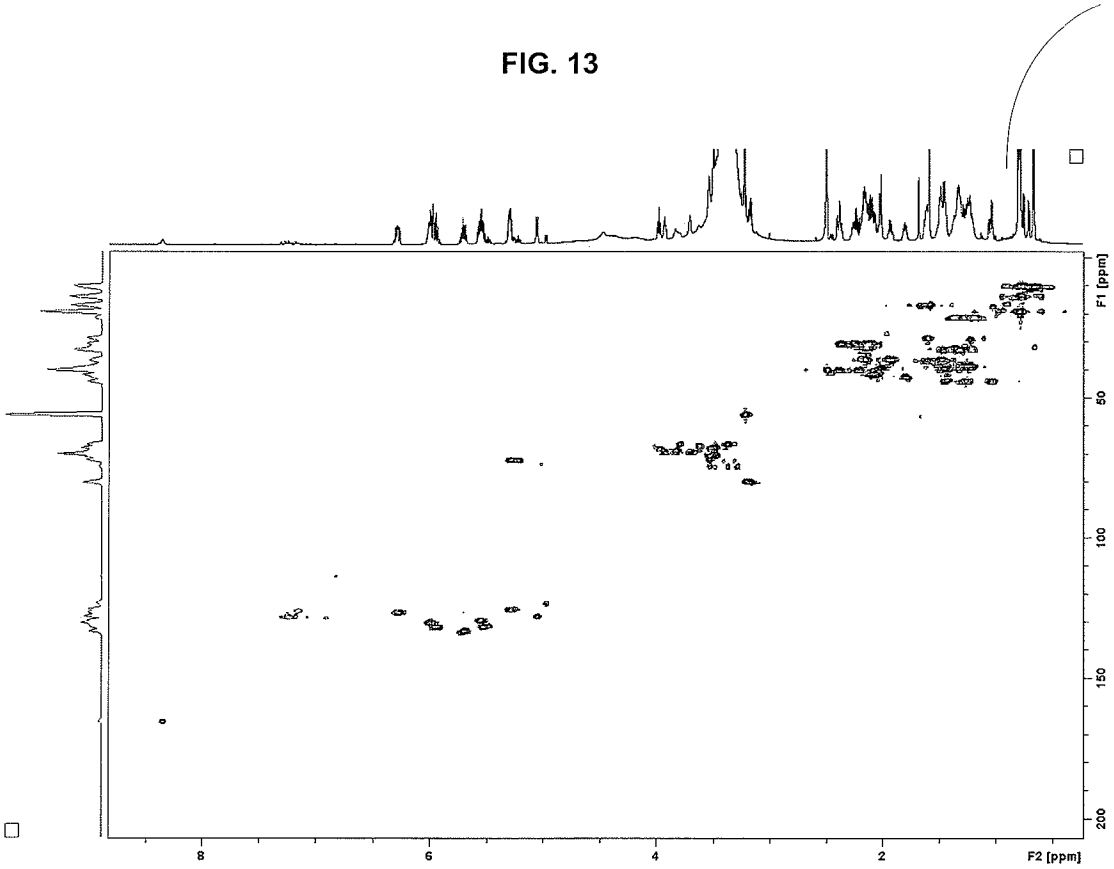


FIG. 14

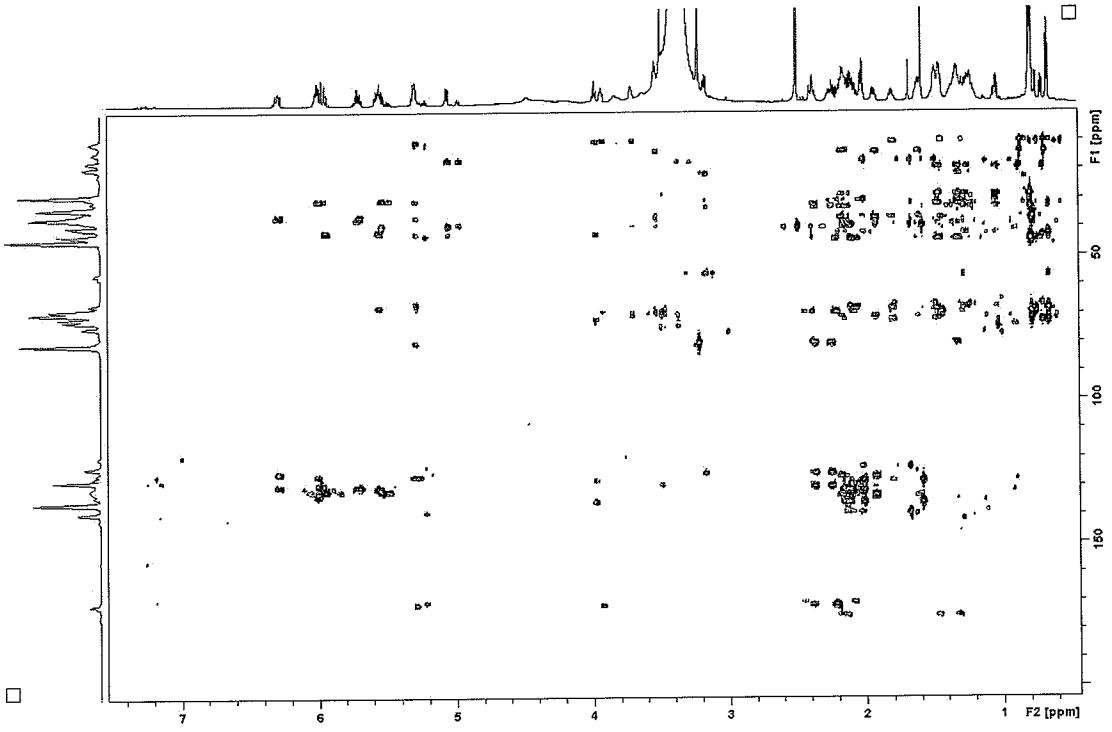


FIG. 15

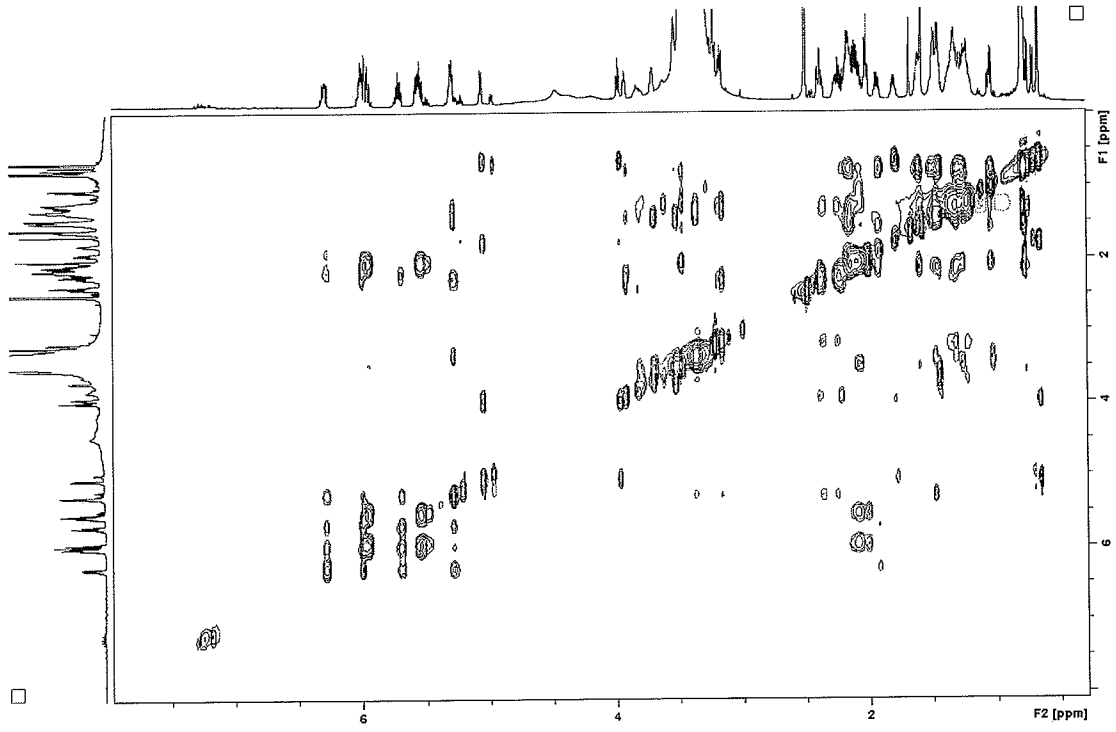


FIG. 16

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/050896

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D313/00 A61K31/365 A61P31/04 C12N1/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D C12N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, 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	WO 2008/142046 A1 (HELMHOLTZ INFEKTIONSFORSCHUNG [DE]; MENCHE DIRK [DE]; HASSFELD JORMA []) 27 November 2008 (2008-11-27) cited in the application claim 1 ----- -/--	1-32

[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

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2015	16/06/2015

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	Bérillon, Laurent

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2015/050896

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MENCHE D ET AL: "Design, synthesis and biological evaluation of simplified analogues of the RNA polymerase inhibitor etnangien", BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, PERGAMON, AMSTERDAM, NL, vol. 20, no. 3, 1 February 2010 (2010-02-01), pages 939-941, XP026861838, ISSN: 0960-894X [retrieved on 2010-01-19] cited in the application the whole document ----	1-32
X	PAYNE GEORGE W. ET AL.: "Development of a recA Gene-Based Identification Approach for the Entire Burkholderia Genus", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 71, no. 7, 1 July 2005 (2005-07-01), pages 3917-3927, XP002740217, table 3 ----	26

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2015/050896

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008142046 A1	27-11-2008	AT 440830 T	15-09-2009
		EP 2014655 A2	14-01-2009
		WO 2008142046 A1	27-11-2008