NOVEL COMPOUNDS EXHIBITING ANTIMICROBIAL ACTIVITY

Technical Field

The present invention generally relates to novel compounds which exhibit antimicrobial activity and which are useful as antimicrobial agents.

Background

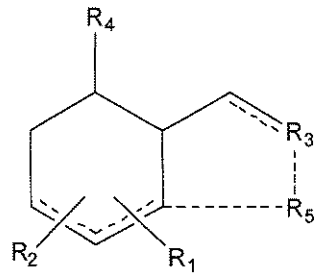
Antimicrobial agents are chemical compositions that inhibit or destroy the growth of microorganisms, such as bacteria, protozoa or fungi including mold, mildew, yeasts, and algae. Inhibition of microbial growth is desirable as microbial growth leads to problems such as unpleasant odors, unsightly stains, discoloration, deterioration of surfaces and materials and various disorders in humans.

Antimicrobial agents prevent microbiological contamination by inhibiting, killing and/or removing or neutralizing the various negative effects mentioned above. The first widely used antimicrobial agent used was isolated from the fungi in the genus *Penicillium* and the bacteria of the genus *Streptomyces*. Currently, antimicrobial agents are used in various areas of applications like cosmetics, disinfectants, sanitizers, wood preservation, food, animal feed, cooling water, metalworking fluids, hospital/medical uses, plastics, resins, petroleum, pulp and paper, textiles, latex, adhesives, leather and hides, and paint slurries.

With the increasing use of antimicrobial agents, an increase of antimicrobial resistance has in recent times also been observed. Therefore, there is a need to provide a novel antimicrobial agent.

Summary

According to a first aspect there is provided a compound represented by the following formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein

--- the single dotted line indicates the optional presence of a single bond;

--- the dotted line adjacent the single line indicate the optional presence of a double bond;

R_1, R_2 and R_3 are independently selected from the group consisting of hydrogen, oxygen, sulfur, hydroxyls, optionally substituted ketones, cyclic ketones, aldehydes, cyclic aldehydes, optionally substituted carbonyls, cyclic carbonyls, optionally substituted esters, cyclic esters, carbonate esters, cyclic carbonate esters, and optionally substituted ethers;

R_4 is selected from the group consisting of optionally substituted, straight chain or branched, alkyls alkenyls and alkynyls;

R_5 is selected from the group consisting of oxygen, sulfur, hydroxyls, optionally substituted ketones, aldehydes, carbonyls, esters, carbonate esters, and ethers,

with the proviso that when one of R_1 and R_2 is hydrogen, the other is not hydrogen.

Definitions

The following are some definitions that may be helpful in understanding the description of the present invention. These are intended as general definitions and should in no way limit the scope of the present invention to those terms alone, but are put forth for a better understanding of the following description.

Unless the context requires otherwise or specifically stated to the contrary, integers, steps, or elements of the invention recited herein as singular integers, steps or elements clearly encompass both singular and plural forms of the recited integers, steps or elements.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated step or element or integer or group of steps or elements or integers, but not the exclusion of any other step or element or integer or group of elements or integers. Thus, in the context of this specification, the term "comprising" means "including principally, but not necessarily solely".

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any

and all combinations or any two or more of said steps or features.

The following words and terms used herein shall have the meaning indicated:

The term "ketones" as used herein refers to organic compounds having the general formula: -R(CO)R', having from 3 to 12 carbon atoms wherein R and R' are the same or different and aliphatic compounds.

The term "cyclic ketone" as used herein refers to any optionally substituted cyclic ketone having from 3 to 12 carbon atoms in the ring. The optional substituents may be lower alkyls. Exemplary cycloketones include cyclopropanone, cyclobutanone, cyclopentanone and cyclohexanone.

The term "aldehydes" as used herein refers to organic compounds having a terminal carbonyl group with the general formula O=CH-R, having from 3 to 12 carbon wherein R may or may not be aliphatic compounds.

The term "cyclic aldehylde" as used herein refers to any optionally substituted cyclic aldehyde having from 3 to 12 carbon atoms in the ring, wherein one carbon has a double bond with an oxygen atom.

The term "hydroxyl" as used herein refers to the radical -OH.

The term "carbonyl" as used herein refers to the compounds with carbon atom double bonded to an oxygen atom, wherein the carbon atom has two free valences, thus having the general formula of R-(C=O)-R', having 3 to 12 carbon atoms wherein R and R' are the same or different and aliphatic compounds.

The term "cyclic carbonyl" as used herein refers to optionally substituted cyclic carbonyl having at least 3 to 12 carbon atoms forming an enclosed ring structure,

wherein at least one of said carbons has a double bond with an oxygen atom.

The term "ester" refers to a functional group that may comprise an inorganic or organic acid in which at least one hydroxyl group is replaced by an alkoxy group. Exemplary esters include acetate, propionate, butyrate, and phenylcarbamate.

The term "cyclic esters" as used herein refers to the optionally substituted cyclic esters having at least 3 to 12 carbon atoms forming an enclosed ring structure.

The term "carbonate ester" as used herein refers to the organic compound wherein a carbonyl group is flanked by two alkoxy groups, thus having a general formula of R-O(C=O)O-R' and they are related to esters R-O(C=O)-R' and ethers R-O-R' and also to the inorganic carbonates.

The term "cyclic carbonate ester" as used herein refers to the optionally substituted cyclic carbonate ester having at least 3 to 12 carbon atoms forming an enclosed ring structure.

The term "ether" as used herein refers to the compounds wherein an oxygen atom is connected to two (substituted) alkyl or aryl groups, thus having a general formula R-O-R'.

As used herein, the term "alkyl group" includes within its meaning monovalent ("alkyl") and divalent ("alkylene") straight chain or branched chain saturated aliphatic groups having from 1 to 10 carbon atoms, eg, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. For example, the term alkyl includes, but is not limited to, methyl, ethyl, 1-propyl, isopropyl, 1-butyl, 2-butyl, isobutyl, tert-butyl, amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, pentyl, isopentyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 3,3-

dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, 2-ethylpentyl, 3-ethylpentyl, heptyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, 5-methylheptyl, 1-methylheptyl, octyl, nonyl, decyl, and the like.

The term "alkenyl group" includes within its meaning monovalent ("alkenyl") and divalent ("alkenylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms, eg, 2, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms and having at least one double bond, of either E, Z, cis or trans stereochemistry where applicable, anywhere in the alkyl chain. Examples of alkenyl groups include but are not limited to ethenyl, vinyl, allyl, 1-methylvinyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 2-methyl-1-propenyl, 1-butenyl, 2-butenyl, 3-butentyl, 1,3-butadienyl, 1-pentenyl, 2-pententyl, 3-pentenyl, 4-pentenyl, 1,3-pentadienyl, 2,4-pentadienyl, 1,4-pentadienyl, 3-methyl-2-butenyl, 1-hexenyl, 2-hexenyl, 3-hexenyl, 1,3-hexadienyl, 1,4-hexadienyl, 2-methylpentenyl, 1-heptenyl, 2-heptentyl, 3-heptenyl, 1-octenyl, 1-nonenyl, 1-decenyl, and the like.

The term "alkynyl group" as used herein includes within its meaning monovalent ("alkynyl") and divalent ("alkynylene") straight or branched chain unsaturated aliphatic hydrocarbon groups having from 2 to 10 carbon atoms and having at least one triple bond anywhere in the carbon chain. Examples of alkynyl groups include but are not limited to ethynyl, 1-propynyl, 1-butynyl, 2-butynyl, 1-methyl-2-butynyl, 3-methyl-1-butynyl, 1-pentynyl, 1-

hexynyl, methylpentynyl, 1-heptynyl, 2-heptynyl, 1-octynyl, 2-octynyl, 1-nonyl, 1-decynyl, and the like.

The term "cycloalkyl" as used herein refers to cyclic saturated aliphatic groups and includes within its meaning monovalent ("cycloalkyl"), and divalent ("cycloalkylene"), saturated, monocyclic, bicyclic, polycyclic or fused polycyclic hydrocarbon radicals having from 3 to 10 carbon atoms, eg, 3, 4, 5, 6, 7, 8, 9, or 10 carbon atoms. Examples of cycloalkyl groups include but are not limited to cyclopropyl, 2-methylcyclopropyl, cyclobutyl, cyclopentyl, 2-methylcyclopentyl, 3-methylcyclopentyl, cyclohexyl, and the like.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the

possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Schema

The compounds disclosed herein may be synthesised utilizing known methodologies disclosed in texts well known to those skilled in the art such as Advanced Organic Chemistry, 3rd Ed.; John Wiley: New York (1985) and J. March, Advanced Organic Chemistry - Reactions, Mechanisms and Structure, John Wiley & Sons, New York, 1992.

Formulations

In accordance with the present invention, when used for the treatment or prevention of microbial infection, compound(s) of the invention may be administered alone. Alternatively, the compounds may be administered as a pharmaceutical, veterinarial, agricultural, or industrial formulation which comprises at least one compound according to the invention. The compound(s) may also be present as suitable salts, including pharmaceutically acceptable salts.

In accordance with the present invention, the compounds of the invention may be used in combination with other known treatments or antimicrobial agents, including antifungal treatments, antibiotics, disinfectants, etc. Suitable agents are listed, for

example, in the Merck Index, An Encyclopoedia of Chemicals, Drugs and Biologicals, 12th Ed.,1996, the entire contents of which are incorporated herein by reference.

Combinations of active agents, including compounds of the invention, may be synergistic.

By pharmaceutically acceptable salt it is meant those salts which, within the scope of sound medical judgment, are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

For instance, suitable pharmaceutically acceptable salts of compounds according to the present invention may be prepared by mixing a pharmaceutically acceptable acid such as hydrochloric acid, sulfuric acid, methanesulfonic acid, succinic acid, fumaric acid, maleic acid, benzoic acid, phosphoric acid, acetic acid, oxalic acid, carbonic acid, tartaric acid, or citric acid with the compounds of the invention. Suitable pharmaceutically acceptable salts of the compounds of the present invention therefore include acid addition salts.

S. M. Berge et al. describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66:1-19. The salts can be prepared in situ during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Representative acid addition salts include acetate, adipate, alginate, ascorbate, asparate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate,

camphorsulfonate, citrate, digluconate, cyclopentanepropionate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, triethanolamine and the like.

Convenient modes of administration include injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, topical creams or gels or powders, or rectal administration. Depending on the route of administration, the formulation and/or compound may be coated with a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the therapeutic activity of the compound. The compound may also be administered parenterally or intraperitoneally.

Dispersions of the compounds according to the invention may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use,

pharmaceutical preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injection include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Ideally, the composition is stable under the conditions of manufacture and storage and may include a preservative to stabilise the composition against the contaminating action of microorganisms such as bacteria and fungi.

In one embodiment of the invention, the compound(s) of the invention may be administered orally, for example, with an inert diluent or an assimilable edible carrier. The compound(s) and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into an individual's diet. For oral therapeutic administration, the compound(s) may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Suitably, such compositions and preparations may contain at least 1% by weight of active compound. The percentage of the compound(s) of formula (I) and/or (II) in pharmaceutical compositions and preparations may, of course, be varied and, for example, may conveniently range from about 2% to about 90%, about 5% to about 80%, about 10% to about 75%, about 15% to about 65%; about 20% to about 60%, about 25% to about 50%, about 30% to about 45%, or about 35% to about 45%, of the weight of the dosage unit. The amount of compound in therapeutically useful compositions is such that a suitable dosage will be obtained.

The language "pharmaceutically acceptable carrier" is intended to include solvents, dispersion media, coatings, anti-bacterial and anti-fungal agents, isotonic and absorption delaying agents, and the like. 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 compound, use thereof in the therapeutic compositions and methods of treatment and prophylaxis is contemplated. Supplementary active compounds may also be incorporated into the compositions according to the present invention. It is especially advantageous to formulate 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 individual to be treated; each unit containing a predetermined quantity of compound(s) is calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The compound(s) may be formulated for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in an acceptable dosage unit. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

In one embodiment, the carrier may be an orally administrable carrier.

Another form of a pharmaceutical composition is a dosage form formulated as enterically coated granules, tablets or capsules suitable for oral administration.

Also included in the scope of this invention are delayed release formulations.

Compounds of the invention may also be administered in the form of a "prodrug". A prodrug is an inactive form of a compound which is transformed in vivo to the active form. Suitable prodrugs include esters, phosphonate esters etc, of the active form of the compound.

In one embodiment, the compound may be administered by injection. In the case of injectable solutions, 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), suitable mixtures thereof, and vegetable oils. 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. Prevention of the action of microorganisms can be achieved by including various anti-bacterial and/or anti-fungal agents. Suitable agents are well known to those skilled in the art and include, for example, parabens, chlorobutanol, phenol, benzyl alcohol, ascorbic acid, thimerosal, and the like. In many cases, it may be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and 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 monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the analogue 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 analogue into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above.

Tablets, troches, pills, capsules and the like can also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier. Various other materials can be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules can be coated with shellac, sugar or both. A syrup or elixir can contain the analogue, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the analogue can be incorporated into sustained-release preparations and formulations.

Preferably, the pharmaceutical composition may further include a suitable buffer to minimise acid hydrolysis. Suitable buffer agent agents are well known to those skilled in the art and include, but are not limited to, phosphates, citrates, carbonates and mixtures thereof.

Single or multiple administrations of the pharmaceutical compositions according to the invention

may be carried out. One skilled in the art would be able, by routine experimentation, to determine effective, non-toxic dosage levels of the compound and/or composition of the invention and an administration pattern which would be suitable for treating the diseases and/or infections to which the compounds and compositions are applicable.

Further, it will be apparent to one of ordinary skill in the art that the optimal course of treatment, such as the number of doses of the compound or composition of the invention given per day for a defined number of days, can be ascertained using convention course of treatment determination tests.

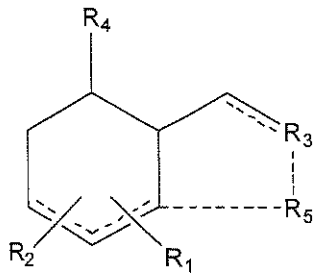
Generally, an effective dosage per 24 hours may be in the range of about 0.0001 mg to about 1000 mg per kg body weight; suitably, about 0.001 mg to about 750 mg per kg body weight; about 0.01 mg to about 500 mg per kg body weight; about 0.1 mg to about 500 mg per kg body weight; about 0.1 mg to about 250 mg per kg body weight; or about 1.0 mg to about 250 mg per kg body weight. More suitably, an effective dosage per 24 hours may be in the range of about 1.0 mg to about 200 mg per kg body weight; about 1.0 mg to about 100 mg per kg body weight; about 1.0 mg to about 50 mg per kg body weight; about 1.0 mg to about 25 mg per kg body weight; about 5.0 mg to about 50 mg per kg body weight; about 5.0 mg to about 20 mg per kg body weight; or about 5.0 mg to about 15 mg per kg body weight.

Alternatively, an effective dosage may be up to about 500mg/m^2. For example, generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, about 25 to about 350mg/m^2, about 25 to about 300mg/m^2, about 25 to about 250mg/m^2, about 50 to about 250mg/m^2, and about 75 to about 150mg/m^2.

Disclosure of Embodiments

Exemplary, non-limiting embodiments of the chemical compounds, will now be disclosed.

In the first embodiment, a compound is represented by the following formula (I):



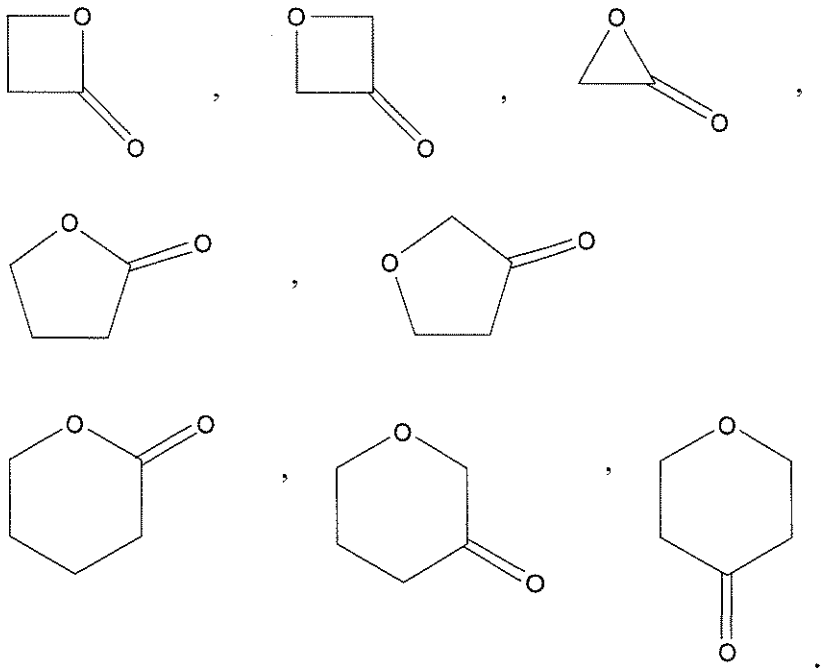
(I)

R_2 may be hydrogen and R_1 may be selected from the group consisting of -OH, C_2-C_6 ethers and C_2-C_6 cyclic esters. R_1 may be selected from the group consisting of -OH and $-CH_2-O-CH_3$, $-CH_2CH_2-O-CH_3$, $-CH_2-O-CH_2CH_3$, $-CH_2CH_2CH_2-O-CH_3$, $-CH_2CH_2-O-CH_2CH_2CH_3$, and $-CH_2CH_2CH_2-O-CH_2CH_2CH_2CH_3$. R_1 may be selected from the group consisting of $-CH_2OH$, $-CH_2CH_2OH$ and $CH_2CH_2CH_2OH$. R_1 may be a ring selected from the group consisting of oxiranone, oxetanone, furanone and pyranone.

R_1 may be furan-2(5*H*)-one or furan-3(2*H*)-one.

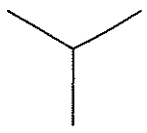
R_3 may be selected from the group consisting of substituted ketone, esters and cyclic esters. R_3 may be selected from the group consisting of a C_1-C_6 carboxyl, C_2-C_6 cyclic ethers and C_3-C_6 cyclic esters. R_3 may be a C_3-C_4 cyclic esters.

The cyclic ester may have one of the following general structures:



R_4 may be optionally substituted, straight chain or branched, C_1-C_8 alkyls, C_2-C_8 alkenyls and C_2-C_8 alkynyls. R_4 may be a branched C_3-C_8 alkyls, C_3-C_8 alkenyls and C_3-C_8 alkynyls.

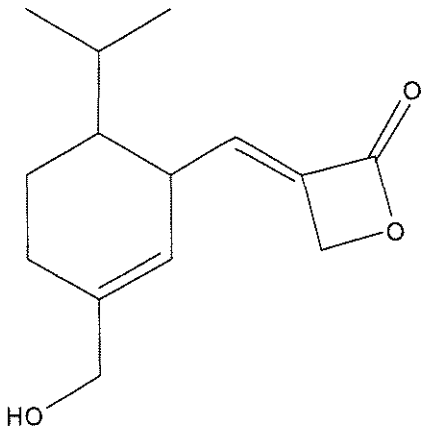
R_4 may have the general structure



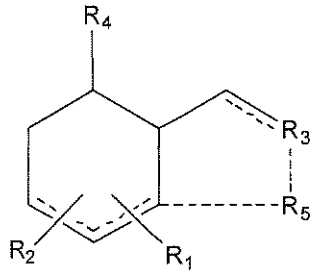
R_4 may have the stereochemical structure:



In this particular embodiment, the compound may have the general formula:



In a second embodiment, a compound is represented by the following formula (I):



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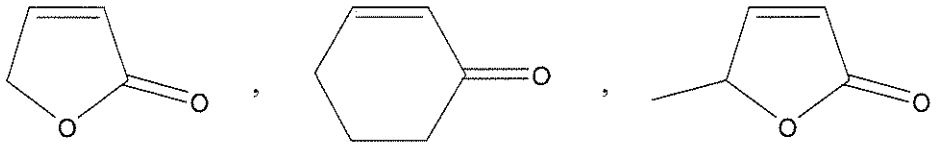
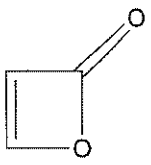
In this second embodiment, R_2 may be a hydrogen and R_1 may be selected from the group consisting of substituted ketone groups, cyclic ketones, ether, esters and cyclic esters. R_1 may be selected from the group consisting of a C_1-C_6 cyclic ether, C_2-C_6 cyclic carbonyl, and C_3-C_6 cyclic esters.

R_1 may be a C_3-C_4 cyclic esters. The cyclic ester may have one of the following general structures:



or



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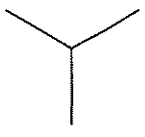
R_3 may be selected from the group consisting of C_2-C_6 carbonyls, C_2-C_6 esters and C_2-C_6 carboxyls. R_3 may be C_3-C_6 hydroxy carboxyl. R_3 may be $-CH_2-C(COH)-C=O(OH)$ or $=C(COH)-C=O(OH)$.

R_4 may be optionally substituted, straight chain or branched, C_1-C_8 alkyls, C_2-C_8 alkenyls and C_2-C_8 alkynyls. R_4 may be a branched C_3-C_8 alkyls, C_3-C_8 alkenyls and C_3-C_8 alkynyls.

R_4 may have the general structure



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R_4 may have the stereochemical structure:



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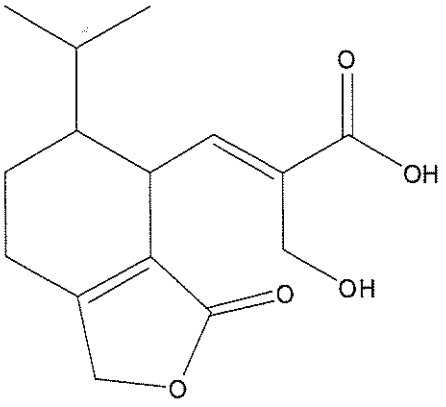
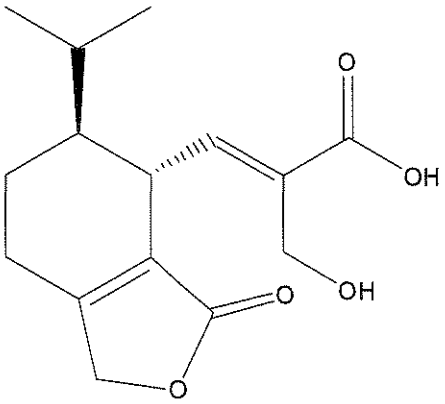
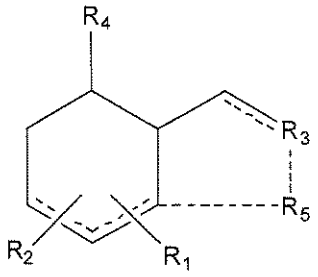
In this second embodiment, the compound may have the general formula:



In this second embodiment, the compound may have the stereochemical structure:



In a third embodiment, a compound is represented by the following formula (I):

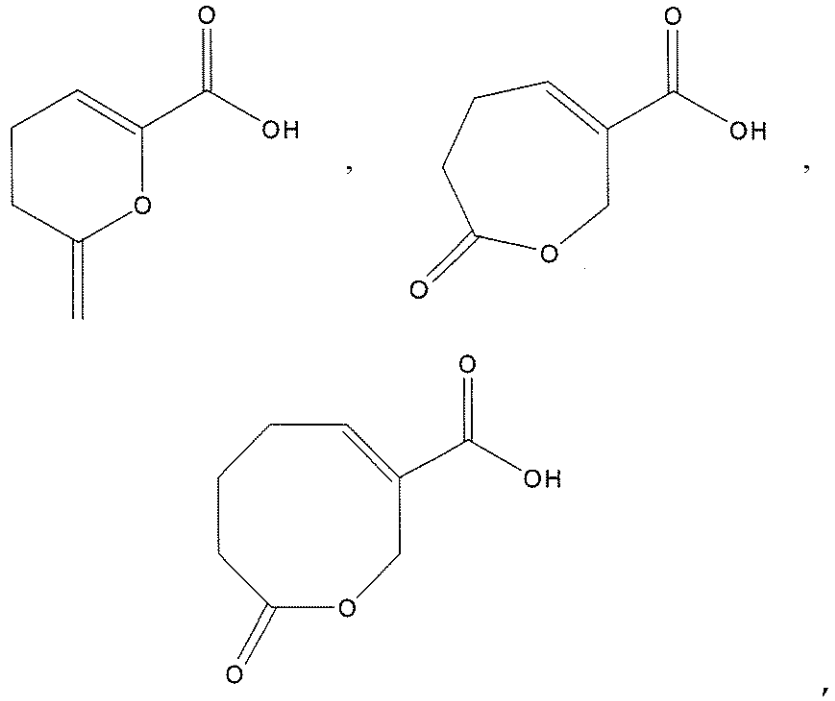


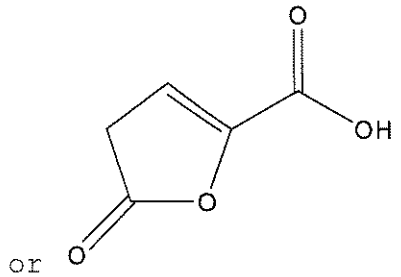
(I)

R_2 and R_1 may be selected from the group consisting of -OH and C_1-C_6 ethers. R_2 may be a hydroxyl group. R_1 may be selected from the group consisting of -OH and -CH_2-O-CH_3, -CH_2CH_2-O-CH_3, -CH_2-O-CH_2CH_3, -$CH_2CH_2CH_2$-O-CH_3, -CH_2CH_2-O-$CH_2CH_2CH_3$, and -$CH_2CH_2CH_2$-O-$CH_2CH_2CH_2CH_3$. R_1 may be selected from the group consisting of CH_2-O-CH_3, -CH_2CH_2-O-CH_3, -CH_2-O-CH_2CH_3. R_1 may be CH_2-O-CH_3 or -CH_2CH_2-O-CH_3.

R_3 may be selected from the group consisting of substituted ketone groups, carboxyl groups, and cyclic carboxyl esters groups. R_3 may be selected from the group consisting of a C_1-C_8 carboxyl, C_2-C_7 cyclic ester and C_4-C_7 cyclic carboxyl esters.

The cyclic carboxyl ester may have one of the following general structures:

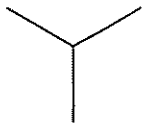




or .

R_4 may be optionally substituted, straight chain or branched, C_1-C_8 alkyls, C_2-C_8 alkenyls and C_2-C_8 alkynyls. R_4 may be a branched C_3-C_8 alkyls, C_3-C_8 alkenyls and C_3-C_8 alkynyls.

R_4 may have the general structure



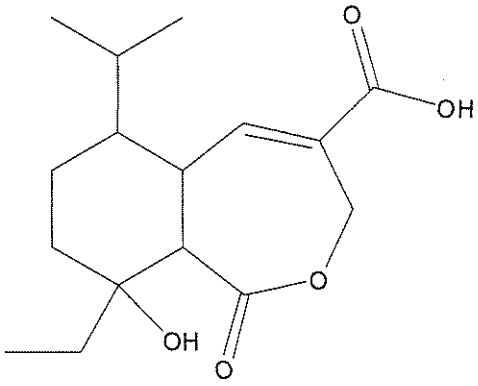
.

R_4 may have the stereochemical structure:



.

In this third embodiment, the compound may have the general formula:



.

In any of the above embodiments, the compound may be part of a pharmaceutical composition. In other

embodiments, the compound may be part of an anti-bacterial or antimicrobial composition. The compound may be used in the manufacture of a medicament for treating a condition associated with bacteria or other microorganisms.

Examples

Non-limiting examples of the invention, including the best mode, and a comparative example will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Example 1

Isolation of novel compounds from Trichoderma sp.

Freshly grown mycelia are inoculated in a solid phase media consists of trytone, dextrose, yeast extracts and wheat. Methanol is added into the solid phase fermented *Trichoderma sp* to extract the secondary metabolites after it has fully grown and turned into slight green in colour. After 24 hours, methanol extracts are collected, pooled and dried via rotary evaporation technique.

The compounds are purified from methanol extract by three-steps preparative HPLC. First step, acetonitrile (ACN) and water are used as mobile phase and C-18 as stationary phase, ACN gradient from 10% to 100% in 50 mins; second step, methanol and water are used as mobile phase and C-18 as stationary phase, methanol gradient from 30% to 90% in 30 mins; third step, ACN and water are used as mobile phase and Phenyl/Hexyl as stationary

phase. ACN gradient from 10% to 75% in 30 mins. The structures of the compounds are solved by 2D-NMR and MS.

Compounds isolated includes:-

A. Compound A

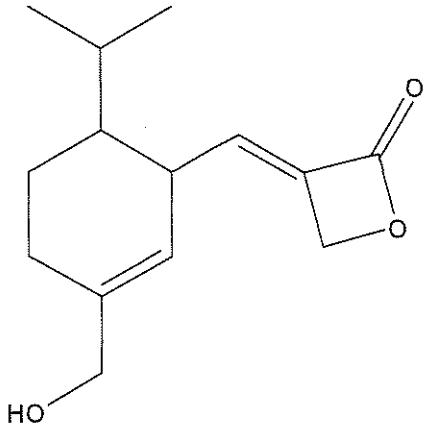


Table 1. ^{1}H and ^{13}C NMR assignment data for Compound A. d_6-DMSO; 500 Hz NMR

	^{1}H	^{13}C
1	1.69 (m, 1H)	21.3
	1.23 (m, 1H)	
2	1.97 (m, 2H)	25.7
3		140.2
4	5.22 (s, 1H)	122.0
5	3.17 (m, 1H)	38.5
6	1.27 (m, 1H)	45.3
7	6.37 (d, J=10.3, 1H)	147.8
8		133.3
9		169.0
11	4.17 (d, J=10.8; 1H)	55.8
	4.13 (d, J=10.8; 1H)	
13	1.60 (m, 1H)	28.7
14	0.77 (d, J=9.1, 3H)	17.2
15	0.90 (d, J=9.1, 3H)	21.3

16	3.78 (br s, 2H)	65.1

ESI-MS $[2M+Na]^+$ 495.1

APCI-MS $[M+H]^+$ 237.3

B. Compound B

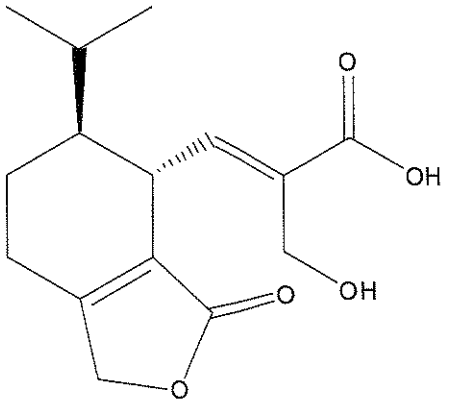


Table 2. ^1H and ^{13}C NMR assignment data for Compound B. d_6-Acetone; 500 Hz NMR

	^1H	^{13}C
1	1.96 (m, 1H)	20.2
	1.58 (m, 1H)	
2	2.5 (m, 2H)	22.7
3		165.2
4		124.6
5	3.58 (br t, 1H)	34.3
6	1.56 (m, 1H)	45.4
7	6.48 (d, J=10.8, 1H)	143.7
8		134.6
9		168.4
11	4.43 (s, 2H)	56.3
13	1.76 (m, 1H)	27.7
14	0.91 (d, J=6.85, 3H)	17.0
15	1.02 (d, J=6.85, 3H)	21.1
16	4.88 (d, J=17.5, 1H)	71.7

	4.83 (dd, J=17.5, 2.5; 1H)	
18		173.8
ESI-MS $[M+Na]^+$ 303.3		

C. Compound C

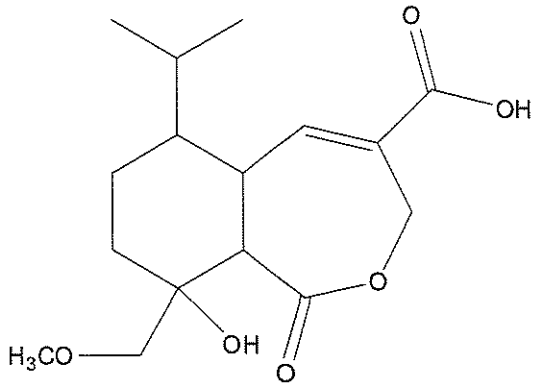


Table 3. ^1H and ^{13}C NMR assignment data for Compound C. d_6-Acetone; 500 Hz NMR

	^1H	^{13}C
1	1.73 (m, 1H)	20.8
	1.38 (m, 1H)	
2	2.26 (m, 2H)	35.5
3		73.0
4	3.44 (d, J=12.7, 1H)	53.0
5	2.79 (m, 1H)	40.3
6	1.62 (m, 1H)	47.9
7	7.30 (d, J=4.0, 1H)	147.0
8		130.0
9		166.2
11	5.01 (d, J=14.5, 1H)	61.1
	5.28 (d, J=14.5, 1H)	
13	2.16 (m, 1H)	27.6
14	0.85 (d, J=6.85, 3H)	14.7
15	1.02 (d, J=6.85, 3H)	20.8
16	3.71 (d, J=12.8, 1H)	74.2

	4.18 (d, J=12.8, 1H)	
17	3.38 (s, 3H)	58.5
18		172.0

ESI-MS $[M+Na]^+$ 335.1

Example 2

Antimicrobial activity of isolated compounds

Bacteria strain *Staphylococcus aureus* and *Escherichia coli* are innoculated in LB grow medium for 12 hours at 37°C. The bacteria are plated at 100µl into a 96-well microtiter plates at 10 mg/ml of BSA bacterial concentration per well Compound A in combination with Compound B, or Compound C singly are added into each bacterial culture well at 50mg/ml and incubated for 24 hours at 37°C. All compounds are suspended in ethanol fraction and tested in triplicate. At the end of 24 hours, 15µl of 10 mg/ml rezurine fluorescent stock solution (Philips Harris, UK) is added and gently mixed for 1 hour in the dark at room temperature (RT). Fluorescence is recorded using Tecan plate reader. High fluorescence corresponds to higher bacterial growth and antimicrobial EC50 values are determined using GraphPrism 4 (Graphprism software)

Table 4. Antimicrobial activity of Compound A, B and C

Compound	EC50 (*S.aureus*)	EC50 (*E.coli*)
A B	> 20mg/ml	>20mg/ml
C	> 15.6mg/ml	>12.5mg/ml
A B	> 20mg/ml	
B	> 20mg/ml	

Example 3

Cytotoxicity of isolated compounds

Hela, HepG2, MDCK, MCF-7, HFN cells are cultured at 37°C in 5% CO^2 in DMEM (Sigma-Aldrich, United States of America) containing 10% fetal bovine serum (PAA laboratory, UK), 2mM L-glutamine. Cell lines ($1x10^6$ cells/well) are incubated overnight at 37°C in 96-well plates. Medium alone or compound serially diluted in 100% DMSO is added to the cell lines in triplicate and incubated for 24 hours at 37°C. At the end of 24 hours incubation, 15µl of rezurine fluorescent reagent (Philips chemicals) is added and gently mixed for 1 hour in the dark at RT. Fluroscence is recorded using Tucan plate reader and CC_{50} values are determined using GraphPrism 4 (Graphprism software).

Table 5. Compounds A, B and C exhibit weak cytotoxicity towards cells tested

Compound	Cytotoxicity (Hela)	Cytotoxicity (HepG2)	Cytotoxicity (MDCK)	Cytotoxicity (MCF-7)	Cytotoxicity (HFN)
A	> 5mM	> 5mM	> 5mM	> 5mM	> 5mM
B	> 5mM	> 5mM	> 5mM	> 5mM	> 5mM
C	> 5mM	> 5mM	> 5mM	> 5mM	> 5mM

Applications

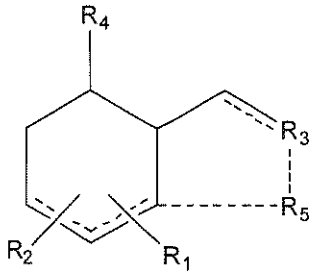
The disclosed chemical compounds are newly discovered and useful antimicrobial agent. More advantageously, the compounds are shown to be non-

cytotoxic against cell lines derived from human, canine and mouse.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Claims

1. A compound represented by the following formula (I):



(I)

or a pharmaceutically acceptable salt thereof, wherein

--- the single dotted line indicates the optional presence of a single bond;

<u>---</u> the dotted line adjacent the single line indicate the optional presence of a double bond;

R_1, R_2 and R_3 are independently selected from the group consisting of hydrogen, oxygen, sulfur, hydroxyls, optionally substituted ketones, cyclic ketones, aldehydes, cyclic aldehydes, optionally substituted carbonyls, cyclic carbonyls, optionally substituted esters, cyclic esters, carbonate esters, cyclic carbonate esters, and optionally substituted ethers;

R_4 is selected from the group consisting of optionally substituted, straight chain or branched, alkyls alkenyls and alkynyls;

R_5 is selected from the group consisting of oxygen, sulfur, hydroxyls, optionally substituted ketones, aldehydes, carbonyls, esters, carbonate esters, and ethers,

with the proviso that when one of R_1 and R_2 is hydrogen, the other is not hydrogen.

2. A compound as claimed in claim 1, wherein R_2 is hydrogen and R_1 is selected from the group consisting of -OH, C_2-C_6 ethers and C_2-C_6 cyclic esters.

3. A compound as claimed in claim 1, wherein R_1 is selected from the group consisting of -OH and $-CH_2-O-CH_3$, $-CH_2CH_2-O-CH_3$, $-CH_2-O-CH_2CH_3$, $-CH_2CH_2CH_2-O-CH_3$, $-CH_2CH_2-O-CH_2CH_2CH_3$, and $-CH_2CH_2CH_2-O-CH_2CH_2CH_2CH_3$.

4. A compound as claimed in claim 1, wherein R_1 is selected from the group consisting of $-CH_2OH$, $-CH_2CH_2OH$ and $CH_2CH_2CH_2OH$.

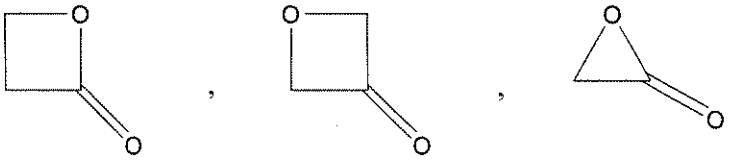
5. A compound as claimed in claim 1, wherein R_1 is a ring selected from the group consisting of oxiranone, oxetanone, furanone and pyranone.

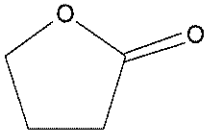
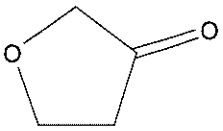
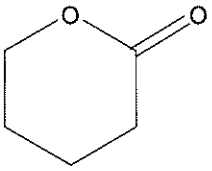
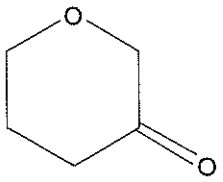
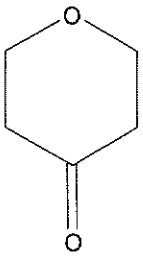
6. A compound as claimed in claim 5, wherein R_1 is furan-2(5*H*)-one or furan-3(2*H*)-one.

7. A compound as claimed in claim 1, wherein R_3 is selected from a C_1-C_6 carboxyl, C_2-C_6 cyclic ethers and C_3-C_6 cyclic esters.

8. A compound as claimed in claim 7, wherein R_3 is a C_3-C_4 cyclic esters.

9. A compound as claimed in claim 8, wherein the cyclic ester has one of the following general structures:

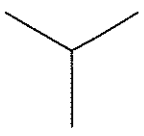


10. A compound as claimed in claim 1, wherein R_4 is optionally substituted, straight chain or branched, C_1-C_8 alkyls, C_2-C_8 alkenyls and C_2-C_8 alkynyls.

11. A compound as claimed in claim 1, wherein R_4 is a branched C_3-C_8 alkyls, C_3-C_8 alkenyls and C_3-C_8 alkynyls.

12. A compound as claimed in claim 1, wherein R_4 has the general structure

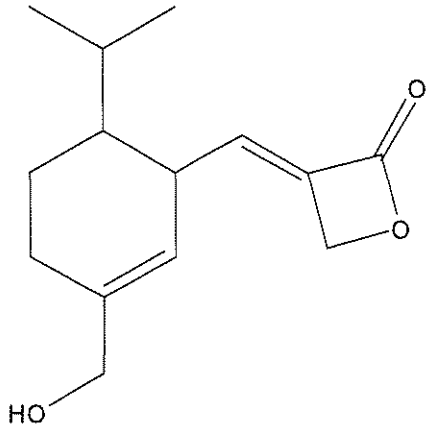


13. A compound as claimed in claim 1, wherein R_4 has the stereochemical structure:



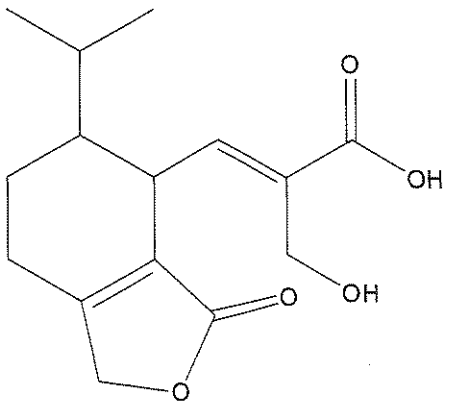
.

14. A compound as claimed in claim 1, having the general formula:



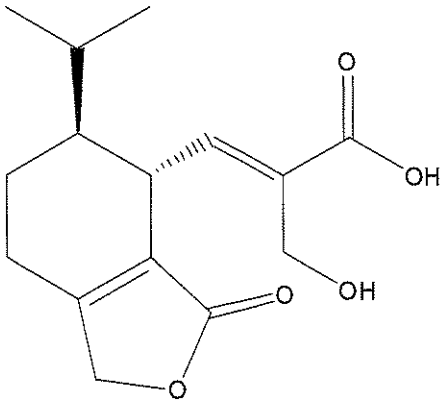
.

15. A compound as claimed in claim 1, having the general formula:

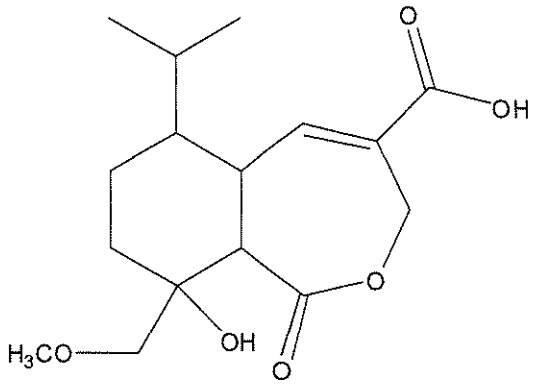


.

16. A compound as claimed in claim 15, having the stereochemical structure:



17. A compound as claimed in claim 1, having the general formula:



18. A pharmaceutical composition comprising a compound as defined in any one of the above claims.

19. An anti-bacterial composition comprising a compound as defined in any one of the above claims.

20. Use of a compound as defined in any one of the above claims, in the manufacture of a medicament for treating a condition associated with bacteria.