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(54)	Title	New antimicrobial compound, a method of producing it, pharmaceutical compositions and uses in therapy
(56)	References Cited:	DE 102006002427 A1, WINTER, DANA K. et al. "Polycyclic xanthone natural products: structure, biological activity and chemical synthesis", Nat. Prod. Rep., 2013, 30, page 382-391, DOI: 10.1039/C3NP20122Hy, WO 2007/095696 A1, TERUI, Y et al "Xantholipin, a novel inhibitor of HSP47 gene expression produced by Streptomyces sp", Tetrahedron Letters, 2003, vol 44(29), page 5427-5430, WEIKE ZHANG et al "Unveiling the Post-PKS Redox Tailoring Steps in Biosynthesis of the Type II Polyketide Antitumor Antibiotic Xantholipin", CHEMISTRY AND BIOLOGY, 2012, vol. 19 (3), page 422-432, ENGELHARDT K. et al "Production of a New Thiopeptide Antibiotic, TP-1161, by a marine Nocardiopsis Species", Applied and Environmental Microbiology, 2010, vol. 76 (15), p. 4969-4976
(57)	Abstract	

The invention provides a new compound, a method to produce it and use of it as an antimicrobial agent, as well as pharmaceutical compositions comprising it. It is also disclosed use of actinomycetes bacteria to produce the compound.

INTRODUCTION

The invention provides a new compound, a method to produce it and uses as an antimicrobial agent.

BACKGROUND

5 Natural products remain the most prolific source of new antimicrobials, and the chemical diversity of natural compounds is still unmatched by combinatorial chemistry approaches (Newman and Cragg, 2012). While the latter has been successfully applied for lead optimization, it basically failed to deliver genuinely new pharmacophores, especially in the field of antimicrobials. This is mainly due to
10 limitations in the structural variety of compounds represented in combinatorial libraries. Most of the antibiotics in clinical use today have been developed from compounds isolated from bacteria and fungi, with members of the actinobacteria being the dominant source (Peláez F, 2006). Actinobacteria-derived antibiotics that are important in medicine include aminoglycosides, anthracyclines,
15 chloramphenicol, macrolides, tetracyclines, etc. Traditionally, most of these antimicrobials have been isolated from soil-derived actinomycetes of the genus *Streptomyces*.

However, isolation strategies in recent years have been directed to unexploited
20 environments like marine sources. Bioprospecting efforts focusing on the isolation and screening of actinobacteria from ocean habitats have added new biodiversity to the order Actinomycetales and revealed a range of novel natural products of potential pharmacological value (Mincer 2001). The existence of marine actinobacterial species that are physiologically and phylogenetically distinct from
25 their terrestrial relatives is now widely accepted, and new taxonomic groups of marine actinomycetes have been described for at least six different families within the order Actinomycetales (Fenical et al 2006).

Apart from being phylogenetically distinct from their terrestrial relatives, marine
30 isolates have been shown to possess specific physiological adaptations (e.g., to high salinity/osmolarity and pressure) to their maritime surroundings. The immense diversity of this habitat along with its underexploitation is the fundamental reason for attracting researchers toward it for discovering novel metabolite producers. There is an occurrence of distinct rare genera in the marine ecosystem, and many
35 were found to produce novel and chemically diverse secondary metabolites (Riedlinger 2004), (Zotchev, 2012), (Manivasagan et al., 2014).

Most streptomycetes and other filamentous actinomycetes possess numerous gene clusters for the biosynthesis of secondary metabolites (Bentley et al 2002), and genome sequence studies have revealed that large portions of their genomes are devoted to secondary metabolite biosynthesis. Several gene clusters coding for known or predicted secondary metabolites has been identified in the genome of different *Streptomyces* strains (Brautaset et al 2003), and the marine actinomycete *Salinispora* (Bode et al, 2002). Many medicinally important natural products, including antibacterials and antifungals, are synthesized by these multimodular assembly lines, and genome mining for secondary metabolite gene clusters has become a common tool to assess the genetic capability of bacteria to produce novel bioactive compounds (Fischbach and Walsh, 2006).

However, even for well-studied model antibiotic producers like *Streptomyces coelicolor* A3(2), discrepancies between the number of known metabolites on the one hand and the number of pathways identified from genomic data on the other hand are tremendous (Bentley et al 2002). These discrepancies can only be explained by the facts that most gene clusters for secondary metabolites are silenced under standard laboratory cultivation conditions and that an expression or upregulation of these pathways is only triggered in response to certain environmental signals. It has been shown that by cultivating bacteria under a range of conditions, it is possible to obtain products of many of these “orphan” biosynthetic pathways (Bode, 2002).

In Engelhardt et al (2010), twenty-seven marine sediment- and sponge-derived actinomycetes were classified at the genus level using molecular taxonomy. As described, PCR-screenings for genes involved in polyketide and non-ribosomal peptide antibiotic synthesis was used for analyzing the actinomycetes potential to produce bioactive secondary metabolites.

DE 102006002427 A1 describes lysolipin derivatives with a general formula (I) which have been produced from *Streptomyces*, and exhibit improved pharmacological properties.

WINTER, DANA K. et al. “Polycyclic xanthone natural products: structure, biological activity and chemical synthesis” *Nat. Prod. Rep.*, 2013, 30, 382-391 DOI:10.1039/C3NP20122Hy describes polycyclic xanthone natural products such as lysolipin I, which inhibits cellwall biosynthesis and therefore are active against both Gram-positive and Gram-negative bacteria. It has been shown that polycyclic xanthenes possess wide spectrum of biological activities from potent antifungal to heat shock protein inhibition.

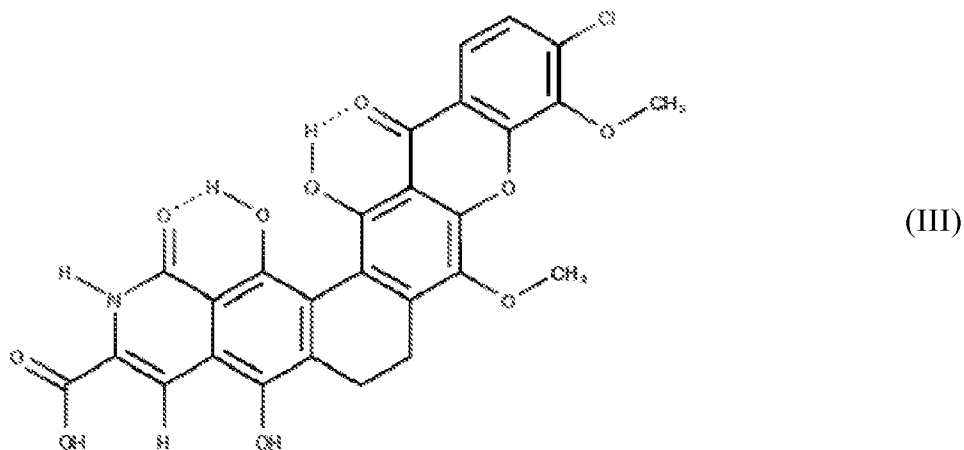
WO 2007/095696 A1 relates to polyketide xanthenes of the kibelone class of general formula (I) and their use as anticancer or antibacterial agents.

Most of the antibiotics in clinical use today were discovered more than 5 decades ago. Over the last 10 years, only two new antibacterial agents with new mechanisms of action (the synthetic oxazolidinone linezolid and the natural-product-based lipopeptide daptomycin) have been approved. Loss of efficacy of existing drugs due to emerging multidrug resistant pathogens threatens to outpace the development of new antimicrobials. The majority of all anti-infective drugs are either derived from or inspired by natural products. Accordingly, new antibiotics are most likely to come from natural product-based research since neither genomics-derived target based research nor combinatorial chemistry has so far provided drugs that have actually entered the market.

Thus, mining microbial diversity represents the most promising source for obtaining new and diverse antimicrobial leads to meet the challenges with emerging multidrug-resistance.

SUMMARY OF INVENTION

The invention provides a new compound, having the structure according to formula III.



and derivatives, salts and solvates thereof.

A method for producing the compound is also provided comprising the following steps:

a) cultivating a bacterium selected from the group consisting of:

i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 32287; and

ii) a bacterium which is closely related to the bacterial isolate in i) such as a strain with similar genotypic and/or phenotypic characteristics to the isolated bacterium;

5 in a suitable culture medium comprising sea water;

b) extracting the compound of claim 1 from the culture.

10 In one embodiment of the method for producing the compound the bacterium is a bacterium which comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical to the sequence depicted in SEQ ID NO 1.

15 Further it is specified that step b) of the method may comprise centrifugation of the cultivated bacteria to obtain a cell pellet, from which the compound is extracted, and the step of extracting the compound from the cell pellet using dimethyl sulfoxide (DMSO).

20 In one embodiment the culture medium is PM6, optionally with artificial sea water.

It is also provided use of an isolated bacterium to produce the compound according to claim 1, wherein the bacterium is selected from the group consisting of:

25 i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 32287; and

30 b) a bacterium which is closely related to the bacterial isolate of a) such as a bacterium with similar genotypic and/or phenotypic characteristics to the isolated bacterium.

35 It is further provided use of isolated bacteria wherein it is specified that the closely related bacterium comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical to the sequence depicted in SEQ ID NO 1.

40 Another aspect of the invention is a pharmaceutical composition comprising the compound of the invention, and pharmaceutically acceptable one or more carriers and/or excipients, and the compound for use in therapy, such as an antimicrobial agent, more specifically an antibacterial agent.

It is also described a method for treating a bacterial infection in a subject comprising administering the compound or the pharmaceutical composition to said

subject.

According to another embodiment, the bacterial infection is caused by a multidrug resistant bacterium, such as a Gram-positive and/or Gram-negative bacterium
5 multiresistant bacterium.

A method for killing or inhibiting the growth of a bacterium comprising the step of bringing the compound or the pharmaceutical composition in contact with the bacteria to be killed or inhibited is also described.

10 The invention also includes non-medical use of the compound as antibacterial agent.

BRIEF DESCRIPTION OF FIGURES

Figure 1 shows LC-DAD-isoplot (upper spectre) and MS spectra at ESI+ (middle)
15 and MS spectra of ESI- (bottom) of the active fraction of the MP127-ig17 extract fractionated on HPLC.

Figure 2 shows the structur of MBL-AB01 with key NMR and MS data supporting the structure.

DETAILED DESCRIPTION

The present invention provides a new antimicrobial agent. The inventors have analyzed marine sediment-derived actinomycete isolates, thereby identifying new bacteria able to produce antimicrobial secondary metabolites.

25 By using microwell, shake flask and fermentor cultures, the inventors were able to identify culturing conditions for the production of antibacterial and antifungal compounds. The approach led to the identification of a new antimicrobial compound, MBL-AB01.

Accordingly, the invention provides a new antimicrobial compound, such as MBL-
30 AB01.

Compounds like MBL-AB01 belong to a group of compounds produced by microorganisms often referred to as secondary metabolites.

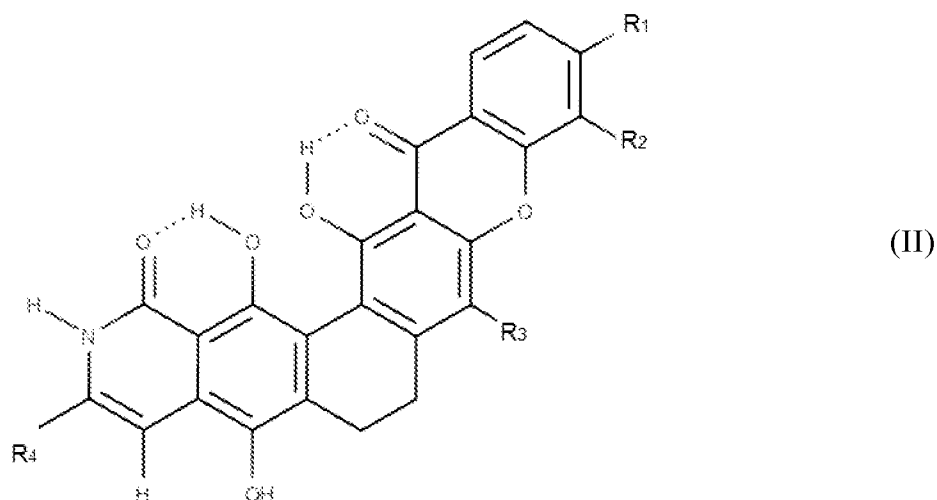
By “secondary metabolites” we mean compounds that the microorganisms can synthesize. They are not essential for basic metabolic processes, such as growth and
35 reproduction. Secondary metabolites may have other useful characteristics, such as

anti-cancer and/or antimicrobial activity, such as anti-fungi and anti-bacterial activity (Behal, 2000; Bennett and Bentley, 1989)

Structure elucidation of the compound MBL-AB01 has revealed that the compound is a new compound belonging to the Xanthon class of compounds. The molecular formula is as shown in formula I:

Molecular formulae: $C_{27}H_{18}ClNO_{10}$ (I)

A general molecular structure of the present compound is shown in formula II:

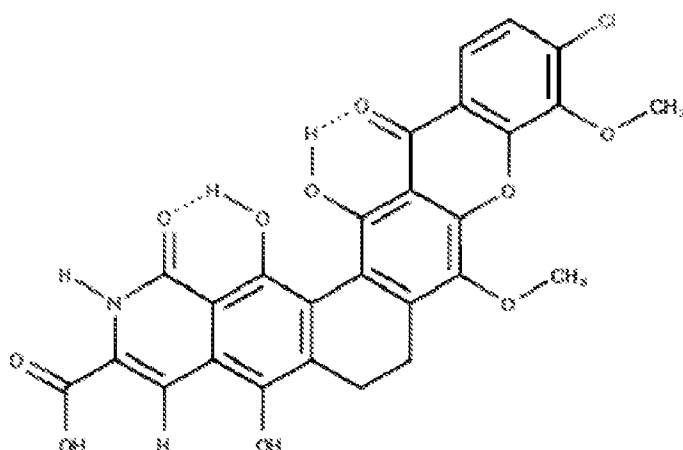


R_1 may be a halogen atom, selected from chlorine, bromine and iodine, R_2 and R_3 may be $-O-CH_3$. R_4 may be $-COOH$, $-C(O)OR_1$ and $-C(O)NR_1R_2$, and R_1 and R_2 are independently a hydrogen, a C_1 - C_4 -alkyl group, a C_2 - C_4 -alkenyl group, a C_2 - C_4 alkynyl group and a phenyl group.

The compound according to the invention is a xanthone compound with the general structure formula II.

In a particular embodiment, the invention has the molecular formula I and the structure as shown in formula III, and derivatives, solvates and/or hydrates thereof.

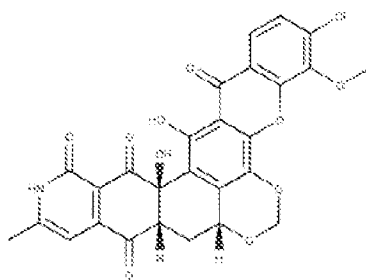
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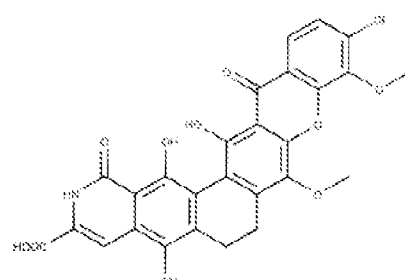
(III)

A former known compound, Xantholipin, has the same molecular formula as depicted in formula I, however a comparison of the structure revealed significant differences between the molecules Xantholipin and the invention MBL-AB01. The differences are summarized below:

5



Xantholipin



MBL-AB01

No. of labile hydrogens	3	5
Predicted logP	2.6	4.8
Chiral centers	3	0
Lowest predicted pKA	9.0	2.9

The invention is a compound having a structure according to the formula III, and derivatives, solvates and/or hydrates thereof. As described herein, derivatives may be compounds with a structure according to formula II, where R₁ is a halogen atom, selected from chlorine, bromine and iodine, R₂ and R₃ is -O-CH₃. R₄ is -COOH, -C(O)OR₁ and -C(O)NR₁R₂, and R₁ and R₂ are independently a hydrogen, a C₁-C₄-alkyl group, a C₂-C₄-alkenyl group, a C₂-C₄ alkynyl group and a phenyl group.

10

The term "solvate" refers to a solid compound that has one or more solvent molecules associated with its solid structure. Solvates can form when a solid compound is crystallized from a solvent, wherein one or more solvent molecules become an integral part of the solid crystalline matrix. The compounds of the formulas described herein can be solvates. Another type of a solvate is a hydrate. A "hydrate" likewise refers to a solid compound that has one or more water molecules intimately associated with its solid or crystalline structure at the molecular level. A hydrate is a specific type of a solvate. Hydrates can form when a compound is solidified or crystallized in water, wherein one or more water molecules become an integral part of the solid crystalline matrix. The compounds of the formulas described herein can be hydrates.

The new antibacterial compound according to the invention is produced by actinomycete bacteria, such as a strain in the genera *Actinalloteichus*. In one particular embodiment the antibacterial compound of the invention is produced by culturing of the marine sediment-derived bacterial isolate MP127-ig17 or closely related strains.

By "closely related strains" we mean any strain which shares similar genotypic and/or phenotypic characteristics to the isolated strain. In particular this phrase encompasses slightly modified forms of the strain which retain substantially the same functional activities. Thus, for example some amino acid or nucleotide additions, deletions or alterations have very little effect; if any, on the functional ability to produce a compound according to the invention. A definition of the term "closely related strains" is provided in Peak et al, which may be used herein.

Further, the invention provides a method for producing an antimicrobial agent, such as MBL-AB01, comprising the step of cultivating actinomycetes bacteria, such as a strain in the genera *Actinalloteichus*. In one particular embodiment the compound is produced by a bacterium selected from the group consisting of i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date 7th April 2016 under deposit number DSM 32287; and ii) a bacterium which is closely related to the bacterial isolate of i) such as a bacterium with similar genotypic and/or phenotypic characteristics to the isolated bacterium.

The compound-producing bacterium, as described herein, may be a bacterium which comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical, such as at least 82%, 83%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 98.7%, 98.8% or 98.9% or 99% identical, to the sequence set forth in SEQ ID NO: 1.

The skilled person will expect that considerable alterations may be introduced into a sequence defined in SEQ ID NOs: 1 and subsequences thereof without significantly altering its overall structure, function and properties.

5 By "phenotypic characteristics" we mean the ability to produce the secondary metabolite according to the invention, i.e the compound with a molecular structure according to any one of formula II and/or III.

10 By "genotypic characteristics" we mean characteristic features of genetic molecules such as nucleic acids and amino acids, such as the 16S rRNA molecule, for example known as sequence identity. As referred to herein "strains with similar genotypic characteristics" include bacteria comprising a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical, such as at least 82%, 83%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 98.7%, 98.8% or 98.9% or 99% identical, to the sequence set forth in SEQ ID NO: 1.

15 The expression "bacterial isolate" is often used to define a culture of one bacterial strain. The isolate may be purified and isolated by different means known to the skilled person in the art. A "bacterial isolate" or "bacterial strain", as used herein, refers to a genotypic and phenotypic unique microorganism, traceable back to a colony, typical derivable from blotting a purified sample into a suitable medium.
20 The expressions isolate, strain and bacterium are used interchangeably.

According to the method of the invention, the bacteria are cultivated in a microbiological culture with a suitable culture medium, known to the skilled person. In one embodiment the culture medium comprises sea water.

25 A "microbiological culture", or microbial culturing, is a method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions. The term "culture" is more generally used informally to refer to "selectively growing" a specific microorganism as a bacterium in the lab.

30 The compound according to the invention is obtainable from a bacterial culture as described herein.

Accordingly, the invention provides a method for producing the compound of formula III, and derivatives, solvates and/or hydrates thereby, by cultivating bacteria as described herein in a culture with a suitable culture medium.

35 In one embodiment the culture medium is a commercially available growth medium commonly used for culturing bacteria, such as Trypton Soya broth (Oxoid). In another embodiment the culture medium is a standard culture medium such as Luria-Bertani (LB) medium. Yet another embodiment is a a complex medium

designed for production of secondary metabolite by Actinomycetes such as PM6 described in Engelhardt et al., 2010. In yet another embodiment the culture medium is supplemented with artificial sea water. In one embodiment, an inoculum culture with the compound-producing bacterial strain is produced in flasks filled with
5 trypton soya broth medium with seawater.

The culture comprising the cultured bacteria may optionally be a production culture. The production cultures may be inoculated from seed cultures. The production cultures may be produced in flasks filled with a suitable culture medium, such as PM6 medium with artificial sea water.

10 By "suitable culture medium" we mean any medium known to the skilled person suitable to grow the bacterium in question. As used herein, the expressions "culture medium" or "fermentation medium" or "cell culturing" refer to a nutrient solution used for growing and shall refer to all kinds of media which are used in the context of culturing the isolates. Typically, a culture medium comprises a carbon source
15 such as sugars, starch, flour or yeast extract, a nitrogen source such as flour containing proteins and amino acids or ammonium sulphate and minerals such as inorganic salts.

The culture media may be chemically defined such as MR6 (Illing et al., 1989), complex media such as PM4, PM5 and PM6 described in Engelhardt et al., 2010 or
20 standard culture media such as ISP2. Other typical examples of culture media for the production of antibiotic compounds are R2YE (Thompson et al., 1980), R5 (Illing et al., 1989) and AMP (Wendt-Pienkowski et al., 2005).

The method of the invention further comprises the step of isolating the antibacterial compound from the culture. The isolation of the compound from the cultured
25 bacteria may be done by means well known to the skilled person.

One method of obtaining the compound is by extracting it from the production culture and/or from the cell pellet which is collected by centrifugation of the production culture. This may be done by harvesting the dry matter which is collected by centrifugation of the culture. Optionally the dry matter can be washed
30 with methanol to extract compounds that are not related to the active compound.

The compound may be extracted by a suitable solvent known to the skilled person.

In one particular embodiment, the dry matter from the production culture may be collected by centrifugation and optionally fractionated or lysed by means familiar to the skilled person, for example by freeze-drying the cell pellet.

35 Further, the compound may be extracted by a suitable solvent, such as by DMSO or DMSO added trifluoroacetic acid (TFA) to 0.1 %. The compound may also be extracted by other organic solvents such as alcohols and alkanes. Undissolved

matter is optionally removed by filtration. In one embodiment, the compound is further separated by chromatography, such as HPLC. One embodiment the separation is done by HPLC at basic conditions.

5 To optionally avoid degradation of the compound, pH of the fractions can be adjusted, for example by adding a buffer, such as an ammonium acetate buffer with pH=4, to each of the fraction collector vials prior to fractionation. The active compound in the fractions is further bound to a solid phase column, conditioned with an alcohol, such as methanol, optionally acidified with ammonium acetate buffer at pH=4. After the compound is bound to the column, impurities are washed
10 out of the column with acidified alcohol, such as methanol.

The compound is further eluted from the column with an alcohol, such as methanol, optionally also added ammonium acetate buffer pH adjusted to pH=8.0. Further, the method of isolating the compound may comprises the step of removing the alcohol or other solvents by vacuum centrifuge, before the compound is added water and
15 freeze dried.

A method for identifying the compound of the invention is by use of high-performance liquid chromatography (HPLC), such as HPLC-MS or HPLC-UV and high resolution mass spectroscopy (MS).

20 In Engelhardt et al (2010), an isolation process of marine actinomycete bacteria is described. The study provided the molecular taxonomy and phylogenetic analyses of 27 different actinomycete bacteria. In table 1, one isolate referred to as TSI127-17 derived from sponge is described. Sequence analyses of the 16S rRNA gen revealed that TSI127-ig17 had a 98.97 % gene similarity to the *Actinoalloteichus hymeniacidonis* HPA177 with GenAccession no. DQ144222. In Engelhart et al.
25 (2010), PCR screening for PKS/NRPS genes was used to investigate the potential of these actinomycete isolates to synthesize polyketide- and nonribosomal peptide-derived secondary metabolites, thus indicating the potential of these actinomycetes isolates to synthesize secondary metabolites.

30 Herein, the deposited bacterium (DSM 32287) is provided comprising in its genome a 16S rRNA molecule having the sequence depicted in SEQ ID NO 1.

An aspect of the invention is the use of new bacterial isolates of actinomycete bacteria to produce an antimicrobial compound as MBL-AB01.

35 Thus, the invention is use of a bacterium in the genera *Actinalloteichus* to produce secondary metabolites, such as the antimicrobial compound MBL-AB01. The bacteria to be used may be the antimicrobial compound-producing strain *Actinalloteichus hymeniacidonis*.

In a particular embodiment, the bacteria according to this aspect of the invention is the bacterial isolate (DSM 32287) designated MP127-ig17, or closely related strains, as defined herein.

5 In another particular embodiment, the invention is the use of a bacterium selected from the group consisting of i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date 7th April 2016 under deposit number DSM 32287; and ii) a bacterium which is closely related to the bacterial isolate of i) such as a bacterium with similar genotypic and/or phenotypic characteristics to the isolated bacterium to produce secondary metabolites, such as compounds having a structure
10 according to any one of the formula I and/or II and/or III, and derivatives, solvates and/or hydrates thereof with the same functional properties as MBL-AB01.

The compound-producing bacterium, as described herein, may be a bacterium which comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical, such as at least 82%,
15 83%, 85%, 86%, 87%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 98.5%, 98.7%, 98.8% or 98.9% or 99% identical, to the sequence set forth in SEQ ID NO: 1.

The structural and biological features of the new antimicrobial compound, MBL-AB01, have been characterized. It has been shown that the compound of the
20 invention is a powerful antibacterial agent shown to inhibit growth of a variety of bacterial strains, including multidrug resistant bacteria.

The antibacterial activity has been determined by *in vitro* studies, such as described in Example 5.

It has also been demonstrated *in vitro* that MBL-AB01 is less cytotoxic than
25 comparable compounds such as Xantholipin, as described in Example 6. Thus, MBL-AB01 is a very attractive candidate as an antimicrobial agent, useful in different pharmaceutical compositions.

Accordingly, the invention also provides the use of the compound of the invention in medical applications, such as in therapy. The invention includes a compound of
30 the formula I and/or II and/or III, or a pharmaceutically acceptable salt or solvates thereof, for use in therapy, in particular for the treatment of bacterial infections.

The terms "treating", "treat" and "treatment" include (i) preventing a disease, pathologic or medical condition from occurring (e.g., prophylaxis); (ii) inhibiting the disease, pathologic or medical condition or arresting its development; (iii)
35 relieving the disease, pathologic or medical condition; and/or (iv) diminishing symptoms associated with the disease, pathologic or medical condition. Thus, the terms "treat", "treatment", and "treating" can extend to prophylaxis and can include

prevent, prevention, preventing, lowering, stopping or reversing the progression or severity of the condition or symptoms being treated. As such, the term "treatment" can include medical, therapeutic, and/or prophylactic administration, as appropriate.

- 5 The terms "inhibit", "inhibiting", and "inhibition" refer to the slowing, halting, or reversing the growth or progression of a disease, infection, condition, or group of cells. The inhibition can be greater than about 20%, 40%, 60%, 80%, 90%, 95%, or 99%, for example, compared to the growth or progression that occurs in the absence of the treatment or contacting.
- 10 The compounds of the invention and pharmaceutically acceptable salts or solvates thereof may be used on their own but will generally be administered in the form of a pharmaceutical composition in which the compound/salt/solvate (active ingredient) is in association with pharmaceutically acceptable excipients, diluents or carriers. Such pharmaceutical compositions are provided by the invention.
- 15 The compounds described herein can be used to prepare therapeutic pharmaceutical compositions, for example, by combining the compounds with a pharmaceutically acceptable diluent, excipient, or carrier. The compounds may be added to a carrier in the form of a salt or solvate. For example, in cases where compounds are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration
- 20 of the compounds as salts may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids that form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartrate, succinate, benzoate, ascorbate, α -ketoglutarate, and 0 - glycerophosphate. Suitable inorganic salts may also be formed, including
- 25 hydrochloride, halide, sulfate, nitrate, bicarbonate, and carbonate salts (Berge et al 1997).

The invention still further provides a process for the preparation of a pharmaceutical composition of the invention, which comprises mixing a compound of either one of the formula I, II, and /or III or a pharmaceutically acceptable salt or

30 solvate thereof with a pharmaceutically acceptable diluent, excipient or carrier. The skilled person will be able to identify suitable pharmaceutical excipients dependent upon the administration route.

The compound for use as a medicament, such as an antimicrobial agent, more specifically an antibacterial agent, is provided.

- 35 The in vitro antibacterial activity of the invention was determined against a panel of bacterial strains. As shown in the examples, a compound according to the invention is active against multiresistant Gram-positive bacterial, including vancomycin-resistant *Enterococcus faecium*.

The use of a bacterium, such as the deposited bacterium isolate or a closely related strain, for producing the compound is also provided.

5 The invention provides therapeutic methods of treating infections in a mammal, which involve administering to a mammal having an infection an effective amount of a compound or composition described herein. A mammal includes a primate, human, rodent, canine, feline, bovine, ovine, equine, swine, caprine, bovine and the like. The infection can be a bacterial infection, for example, one caused by a bacterium described herein. A method for killing or inhibiting the growth of a bacterium is also provided, comprising the step of bringing the compound according to the invention into contact with the bacterium to be killed. This method may additionally comprise the step of contacting the bacterium with a pharmaceutical composition as described herein.

15 The ability of a compound of the invention to treat a bacterial infection may be determined by using assays well known to the art. For example, the design of treatment protocols, toxicity evaluation, data analysis, quantification of cell kill, and the biological significance of the use of antibacterial screens are known. In addition, ability of a compound to treat a bacterial infection or kill or inhibit bacteria may be determined using the assays as described herein.

20 References in the specification to "one embodiment", "an embodiment", etc., indicate that the embodiment described may include a particular aspect, feature, structure, moiety, or characteristic, but not every embodiment necessarily includes that aspect, feature, structure, moiety, or characteristic. Moreover, such phrases may, but do not necessarily, refer to the same embodiment referred to in other portions of the specification. Further, when a particular aspect, feature, structure, moiety, or characteristic is described in connection with an embodiment, it is within the knowledge of one skilled in the art to affect or connect such aspect, feature, structure, moiety, or characteristic with other embodiments, whether or not explicitly described.

30 The singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a compound" includes a plurality of such compounds, so that a compound X includes a plurality of compounds X. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for the use of exclusive terminology, such as "solely," "only," and the like, in connection with the recitation of claim elements or use of a "negative" limitation.

40 The term "and/or" means any one of the items, any combination of the items, or all of the items with which this term is associated.

The term "about" can refer to a variation of $\pm 5\%$, $\pm 10\%$, $\pm 20\%$, or $\pm 25\%$ of the value specified. For example, "about 50" percent can in some embodiments carry a variation from 45 to 55 percent.

5 As will be understood by the skilled person, all numbers, including those expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, are approximations and are understood as being optionally modified in all instances by the term "about." These values can vary depending upon the desired properties sought to be obtained by those skilled in the art utilizing the teachings of the descriptions herein. It is also understood that such values inherently contain
10 variability necessarily resulting from the standard deviations found in their respective testing measurements.

EXAMPLES

Example 1: Isolation of MP127-ig17

5 Isolation and taxonomy of MP127-ig17

The isolation of a bacterial isolate belonging to *Actinoalloteichus hymeniacidonis* (TSI127-17) has been described previously (Engelhardt et al., 2010).

10 Shortly, sponge samples were collected from the Tautra ridge (63°36'53"N, 10°31'22"E, Trondheim Fjord, Norway) at 60 m depth. Homogenized material was plated on different agar medium, and the isolate, denoted MP127-ig17, was isolated from agar medium IM18: 3 g/l crab flour, 2 g/l sea weed flour, 20 g/l agar, pH 8.0, prepared with 0.5x natural sea water and 1 ml/l vitamin B solution (5 mg/l each of
15 thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, p-aminobenzoic acid, 2.5 mg/l biotin). MP127-ig17 did not grow on this medium in the absence of sea water-

Sequencing of the 16S rDNA showed 98.97 % gene similarity to *Actinoalloteichus hymeniacidonis* HPA177.
20

MP127-ig17 has been deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 32287.

25 Screening for bioactivity

The isolates from the sponge sample were cultivated in different production media at 25°C, and extracts were screened using agar diffusion assay as described previously (Engelhardt et al., 2010). The isolate designated MP127-ig17 was
30 cultivated in PM6 medium (soluble starch 10 g/l; yeast extract 2 g/l; glucose 10 g/l; glycerol 10 g/l; cornsteep powder 2.5 g/l; peptone 2.0 g/l; CaCO₃ 3.0 g/l) with 25 % 2 x artificial sea water. 2 x artificial sea water was prepared as follows: 1.34 g/l KCl, 2.72 g/l CaCl₂ x 2H₂O, 12.58 g/l MgSO₄ x 7H₂O, 9.32 g/l MgCl₂ x 6H₂O, 0.36 g/l sodium bicarbonate, pH=7.8. The solution was sterilized by filtration.

35 It was shown that the extracts of MP127-ig17 were able to inhibit growth of *Micrococcus luteus* ATCC9341, *Enterococcus faecium* CCUG37832, *Candida albicans* ATCC10231 and *Candida albicans* CCUG39434.

40

Example 2: Identification of bioactive compound, MBL-AB01

Inoculum of MP127-ig17 was produced in 500 ml shake flasks filled with 100 ml trypton soya broth medium (Oxoid) with 0.5 x artificial sea water. The culture was incubated at 30°C for 5 days. Production cultures were inoculated (3%, vol/vol) from seed cultures. The production was performed in 500 ml shake flasks filled with 125 ml PM6 medium with 0.5 x artificial sea water. The culture was incubated at 25°C for 12 days. The culture was freeze dried and extracted with DMSO.

The DMSO extract was fractionated on an Agilent 1100 series high-performance liquid chromatography (HPLC) system with a Zorbax Bonus-RP column (2.1 x 50 mm, 3.5 µm) connected to a diode array detector (DAD) and a fraction collector system. Methanol and 10 mM ammonium acetate (pH 4) were used as the mobile phase, and the methanol gradient was linearly increased from 10 to 90% for 24 min. Fractions were sampled every minute for the whole run. The fractions were dried in a vacuum centrifuge and re-dissolved in DMSO.

The fractions were tested for activity in a robotic liquid based bioassay with *Micrococcus luteus* ATCC9341 and *Enterococcus faecium* CCUG37832 as indicator organisms. Active fractions were analyzed on an Agilent HPLC system with a Zorbax Bonus-RP column (2.1 by 50 mm, 3.5 µm) connected to a DAD and a time-of-flight (TOF) apparatus to determine the accurate mass of the bioactive compound. 10 mM ammonium acetate (pH 7) and acetonitrile were used as the mobile phases, and electrospray ionization was performed in the negative mode.

An UV absorption peak at 395 nm in the active fraction correlated with peaks in LCMS Q-ToF chromatograms with positive and negative electrospray ionization consistent with the molecular mass of MBL-AB01. After obtaining the correct molecular structure the molecular mass has been calculated to 551.061927.. The spectra are shown in figure 1.

Example 3: Characterization of active compound, MBL-AB01.

Isotope labelling and determination of molecular formula

The molecular formula of MBL-AB01 was determined by production in media with ¹³C, ¹⁵N or ¹³C and ¹⁵N labeled compounds. Seeds were produced in a two stage cultivation. First, MP127-ig17 was inoculated in TSB broth supplemented with 50 % sea water and incubated for 4 days, then the seed were re-inoculated in E. coli-OD2 medium with wither ¹³C, ¹⁵N or ¹³C+¹⁵N labeling (Silantes) supplemented with 50 % sea water and incubated for 6 days. Seeds were transferred to production media with the following composition: E. coli-OD2 medium with wither ¹³C, ¹⁵N or

$^{13}\text{C}+^{15}\text{N}$ labeling (Silantes); 537 ml/l, unlabeled or ^{15}N labeled $(\text{NH}_4)_2\text{SO}_4$; 0.34 g/l, $\text{MgSO}_4 \times 7\text{H}_2\text{O}$; 0.17 g/l, CaCO_3 ; 2.1 g/l, KH_2PO_4 ; 0.086 g/l, unlabeled or ^{13}C labeled glucose; 10 g/l, TMS1 (Olga Sekurova Håvard Sletta 1999); 1.3 ml/l and incubated for 11 days. The culture was freeze dried, extracted with DMSO and analyzed as described above. The mass in negative mode (M-H) of unlabeled, ^{13}C labeled, ^{15}N labeled and ^{13}C and ^{15}N labeled MBL-AB01 was 550.05, 577.05, 551.05 and 578.14 respectively. Thus, the increased atomic mass due to ^{13}C and ^{15}N labeling demonstrates that MBL-AB01 has 27 carbons and 1 nitrogen.

10 Determination of molecular formula and structure elucidation with FT-ICR

Characterization of MBL-AB01 was performed by direct infusion into a Bruker Solarix 12T FT ICR MS equipped with an ESI source. MS spectra were recorded in positive and negative ESI mode. The most abundant ions in the spectra were isolated and fragmented by CID. Mass calibration was performed externally using an NaTFA standard. The sample was diluted in methanol/water.

The Bruker Compass Data analysis software was used to predict possible molecular formula compositions for the ions detected. The prediction was performed allowing for the presence of C, H, N, O for all ions. In addition other elements; S, Br, Cl, P, etc. were included in searches for which the isotope pattern suggested the presence of other atoms than C, H, N, and O. The prediction was initially performed allowing for 2 ppm mass error. Theoretical isotope patterns of the suggested molecular formulas were compared to the signals in the MS spectra. HDX analyses were performed by diluting a sample of MBL-AB01 in d4-methanol, and CID spectra were recorded after 60, 120 min and 240 hours.

The isotope pattern shows the presence of Cl. The molecular formula was identified as $\text{C}_{27}\text{H}_{18}\text{ClNO}_{10}$ and this formula is consistent with the results from the fermentation labeling experiment and the isotopic distribution observed with both LC-QTOF and FT-ICR. Fragmentation experiments show the loss of CO_2 followed by water from the suggested molecular ion. The HDX analyses show the presence of a maximum of 5 exchangeable protons.

35 Structural elucidation with NMR

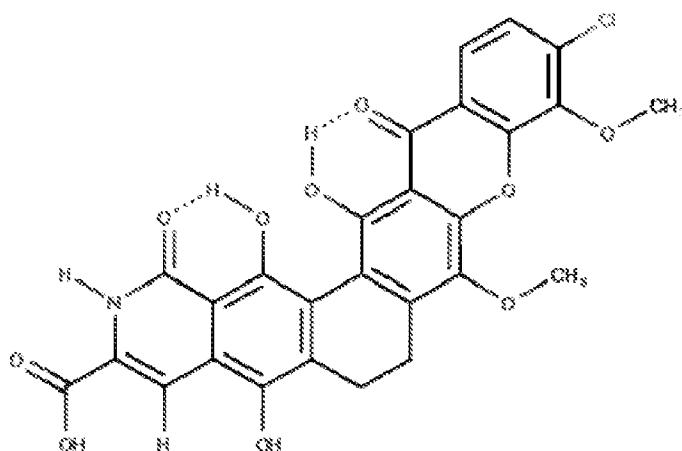
The aim was to identify the chemical structure of the molecule identified as the active compound in Example 2, with a mass of 551.061927 Da, and the chemical formula of $\text{C}_{27}\text{H}_{18}\text{ClNO}_{10}$. Xantholipin is the only molecule disclosed in the public domain with this chemical formula, but data clearly show that MBL-AB01 is not identical to Xantholipin.

A vial of 0.7 milligram of purified MP127-ig17 was obtained. The solid material was stored at -18°C until the NMR experiments were.

The sample was dissolved in 120 µl DMSO-d₆ in the vial. The solution was transferred to a 3 mm NMR sample, PN027-20-02. An additional 60 µl of DMSO-d₆ was used to rinse the vial and this washing solution was also transferred to the NMR tube. The tube was flushed with nitrogen gas before the cap was put on. NMR experiments were performed on the 800 MHz spectrometer with a TCO cryoprobe (¹³C inner coil, i.e. optimized for carbon detection).

The 1D ¹H spectrum revealed a very pure sample of MBL-AB01. The sample was further analyzed by a number of additional NMR spectra (see figure 2).

The proposed structure of MBL-AB01 (see formula III) is supported by and consistent with all NMR and MS data available. The structure is also considered reasonable with respect to data and publications on biosynthetic pathways of related compounds (e.g. Xantholipin) as well as fully realistic from a general organic chemistry perspective.



(III)

Example 4: Isolation of the active compound from bacterial culture with 0.5 x artificial sea water

Cultivation of MP127-ig17 for the production of MBL-AB01 was performed in 3-litre Applicon fermentors with 1.65 litre PM6_MOD3 media in batch fermentation (The composition of the PM6_MOD3 media used: soluble starch 30 g/l; yeast extract 2 g/l; cornsteep liquor 2.5 g/l; peptone 2.0 g/l; CaCO₃ 3.0 g/l). Fermentations were run for 8 days at 25 °C with 0.25 vvm aeration (gas volume

flow per unit of liquid volume per minute) from start, then reduced to 15 vvm for the rest of the cultivation and agitation. Dissolved oxygen was above 30 %. Seed cultures for the fermentations were prepared in 500-ml baffled shake flasks with 100 ml tryptone soy broth medium (Oxoid).

5 The dry matter from PM6_MOD3 fermentation broth was collected by centrifugation and then freeze dried. The resulting powder was then washed with 50 ml methanol/g powder and the extracted twice with 5 and 10 ml DMSO/ g powder respectively. The two DMSO extracts were mixed and then freeze dried. The dried extract was resuspended in a small amount of DMSO, and undissolved matter was removed by filtration.

The extract was separated on an Agilent HPLC system with a Zorbax eclipse XBD-C18, 5 μ m, 9.4x250 mm column connected to a diode array detector and a fraction collector. 20 mM ammonium acetate added 0.4 ml 25 % NH_3/l [A] and methanol were used as mobile phases. The HPLC was run isocratic at 76 % [B] for the first 15 7.5 min. From 7.6 to 9.0 min 100 % [B] was applied. The active compound eluted at approximately 5.5 min. To avoid degradation of the compound, 0.01 x the fraction volume of 50 g/l ammonium acetate pH=4 was added to each of the fraction collector vials prior to fractionation. The active compound in the fractions was bound to a solid phase column (60 mg Oasis HLB) that was conditioned with 100 % 20 methanol, then 76 % methanol added 0.1 % 50 g/l ammonium acetate pH4. After the compound was bound to the column, the column was washed with 1.5 ml 85 % methanol pH=4, then with 5 ml 76 % methanol pH=4. The compound was eluted from the column with methanol added 0.1 % of 50 g/l ammonium acetate pH=8. Methanol was removed in a vacuum centrifuge; the compound was added water and 25 freeze dried.

Example 5: *In vitro* antibacterial activity (MIC determination) of MBL-AB01, a comparison with other known antimicrobial compounds.

30 MBL-AB01 was tested against a panel of Gram-negative and Gram-positive pathogens. MICs for all Gram-positive and Gram-negative bacterial strains were determined by standardized microdilution tests using Mueller-Hinton broth (Acumedia). Bacterial inoculums containing 5×10^5 CFU/ml were incubated for 19 35 h at 35°C in the presence of different antibiotic concentrations according to Clinical and Laboratory Standards Institute protocols. The bacterial strains were obtained from the culture collections ATCC (American Type Culture Collection), NCTC (National Collection of Type Cultures) and CCUG (culture collection university of Göteborg Sweden).

The MICs of MBL-AB01, ranging from below 0.032 to 0.5 µg/ml for most Gram-positive strains, were comparable to or lower than those of the reference antibiotic vancomycin, gentamicin, streptomycin and daptomycin (Table 1). MBL-AB01 also inhibited the growth of vancomycin-resistant bacterial strains represented by *Enterococcus faecalis* CCUG 37832 and *E. faecium* CTC 492, with a MIC of 0.25 and 0.5 µg/ml, respectively.

	MIC µg/ml				
	MBL-AB (+)01	Vancomycin	Gentamicin	Streptomycin	Daptomycin
<i>E. faecium</i> CCUG 37832*	0.25	>16	16	>16	>16
<i>E. faecium</i> CTC 492	0.5	1	16	>16	>16
<i>M. luteus</i> , ATCC 9341	0.063	1	4	8	0,5
<i>S. aureus</i> ATCC 29213	<0.032	2	4	16	4
<i>S. aureus</i> ATCC 43300 (MRSA)	0.032	2	16	16	4
<i>S. aureus</i> NCTC 6571	0.032	2	4	16	4

*) *Enterococcus faecium* CCUG37832: multiresistant, vanA-positive clinical isolate. Mic (µg/ml): Ampicillin(20), Chlortetracycline (>10), erythromycin (>20), Lincomycin (>10), Vancomycin (>20), Apramycin (>20), Bacitracin (>8), Cycloserine (>8), Spectinomycin (>8), Sensitive: Gramicidin (0.01)

Table 1. *In vitro* antibacterial activity of compound MBL-AB01 against different bacterial strains.

Example 6: Determination of *In vitro* cytotoxicity of MBL-AB01 and Xantolipin for IMR90 fibroblast cells

The cytotoxicity of MBL-AB01 and Xantolipin was evaluated in *in vitro* assays with IMR90 Human Lung Fibroblast (ATCC CCL-186) cells.

MBL-AB01 was isolated as described in example 5 above. Xantolipin was obtained from Shanghai Jiao Tong University, China. A stock solution of Xantolipin was established in methanol, and the concentration of the Xantolipin stock solution was correlated to MBL-AB01 on basis of UV/vis absorption by assuming that the compounds have similar extinction coefficients at 395 nm.

IMR90 cells were grown in DMEM – low glucose (Sigma) supplemented with 2 mM L-Glutamine, 1% MEM NEAA (Sigma), 1mM Sodium Pyruvate, 10mM HEPES and 100U/mL Pen-Strep. The cells were subcultured twice or thrice a week at ratios between 1:2 and 1:8, depending on confluency. The day before exposure of the cells to the compounds, 30 µl of cell suspension with 1.2×10^5 IMR90 cells per ml was seeded into 384-well plates (Corning Assay Plate, 3712) a using Tecan EVO robotic workstation with MCA384 pipetting unit using disposable tips (Tecan MCA

125 µl, 300 51 808). The microplates with cell suspension were shaken at 1600rpm with 2.5 mm amplitude (Bioshake) for 20 seconds after seeding. The cell suspension was transferred to the microplates from a stirred reservoir (Reservoir flat base 300mL 10723363) with sterile magnetic stirring bars (15x4.5mm VWR 442-4522) at stirring at 350 rpm positioned on the on Tecan EVO. The microplates with the IMR90 cells were incubated at 37°C with 5 % CO₂ atmosphere.

At the day of the exposure of the cells serial dilutions of MBL-AB01 and Xantholipin were made in DMSO. The serial dilutions with the compounds were further diluted in cell culture medium and transferred to the assay wells, giving a total DMSO concentration in the assay wells of 0.6 %.

After exposure, the assay plates with the IMR90 cells were further incubated at 37°C with 5 % CO₂ atmosphere for 24 hours. The viability of the cells after incubation was measured using the Promega CellTiter-GLO 2.0 viability assay. EC₅₀ values for MBL-AB01 and Xantolipin were estimated based on the viability of exposed cell relative to the viability of control wells added growth medium with similar DMSO concentration. The EC₅₀ value of MBL-AB01 was estimated to 20 µg/ml and the EC₅₀ value of Xantholipin was estimated to 1 µg/ml for IMR90 cells in this assay.

DEPOSIT AND EXPERT SOLUTIONS

The applicant request that a sample of the deposited micro-organisms deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 32287 may only be made available to an expert, until the date on which the

5 patent is granted.

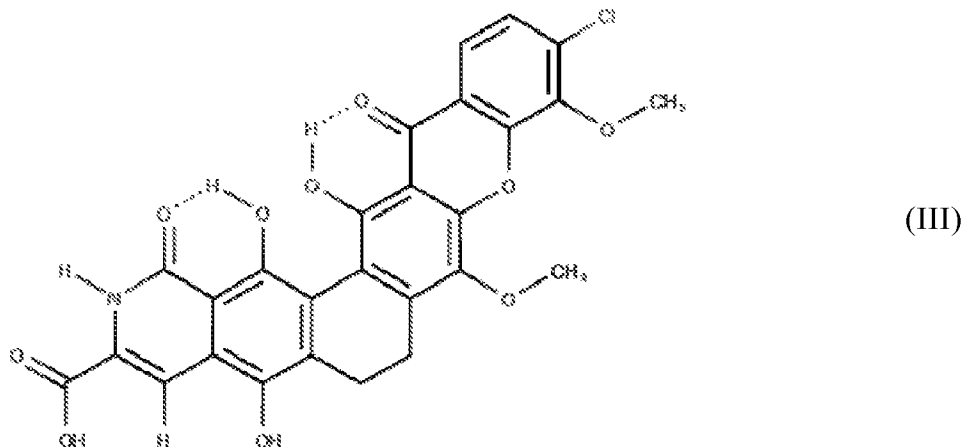
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CLAIMS

1. A compound, having the structure according to formula III.



and derivatives, salts and solvates thereof.

2. A method for producing the compound according to claim 1, comprising the following steps:

a) cultivating a bacterium selected from the group consisting of:

i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 32287; and

ii) a bacterium which comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical to the sequence depicted in SEQ ID NO;;

in a suitable culture medium comprising sea water;

b) extracting the compound from the culture.

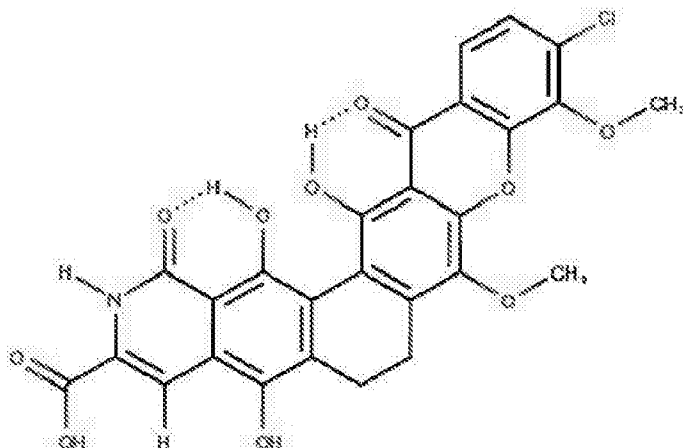
3. The method according to claim 2 , wherein step b) comprises centrifugation of the cultivated bacteria obtaining a cell pellet, from which the compound is extracted.

4. The method according to any one of the claim 2-3, wherein the compound is extracted from the cell pellet using dimethyl sulfoxide (DMSO).

5. The method according to any one of the claim 2-4, wherein the culture medium is PM6, optionally with artificial sea water.
- 5 6. Use of an isolated bacterium to produce the compound according to claim 1, wherein the bacterium is selected from the group consisting of:
- 10 i) a bacterial isolate deposited under the Budapest Treaty with DSMZ on date April 7th, 2016 under deposit number DSM 3228); and
- 10 b) a bacterium which is closely related to the bacterial isolate of a) such as a bacterium with similar genotypic and/or phenotypic characteristics to the isolated bacterium.
- 15 7. Use according to claim 6, wherein the closely related bacterium comprises in its genome a 16S rRNA which by reverse transcription and 2nd strand synthesis provides a sequence which is at least 80 % identical to the sequence depicted in SEQ ID NO 1.
- 20 8. A pharmaceutical composition comprising the compound of claim 1, and one or more pharmaceutically acceptable carriers and/or excipients.
9. The compound according to claim 1 for use in therapy.
- 25 10. The compound according to claim 1 for use as an antimicrobial agent.
11. The compound for use according to claim 11 wherein the antimicrobial agent is an antibacterial agent.
- 30 12. The compound according to claim 1 or the pharmaceutical composition according to claim 8 for treating a bacterial infection in a subject.
13. The use according to claim 12, wherein the bacterial infection is caused by a multidrug resistant bacterium.
- 35 14. The use according to claim 12, wherein the bacterial infection is caused by a Gram-positive or Gram-negative bacterium.
15. The compound of claim 1 or the composition according to claim 9 for killing or
- 40 inhibiting the growth of a bacterium, wherein the compound or the composition is brought in contact with the bacteria to be killed or inhibited.

NORSKE KRAV

1. En forbindelse med en struktur tilsvarende formell III.



(III)

og derivativer, salter og solvater derav.

5

2. En metode for å produsere en forbindelse ifølge krav 1, omfattende de følgende trinnene:

a) kultivering av en bakterie selektert fra gruppen bestående av:

10

i) et bakterielt isolat som deponert under Budapest-konvensjonen hos DSMZ den 7. april, 2016 med deponeringsnummer DSM 32287; og

15

ii) en bakterie som omfatter i sitt genom et 16S rRNA som ved revers transkriptase og andre streng syntese tilveiebringer en sekvens som minster 80 % identisk til sekvensen som beskrevet i SEKV ID NO 1;

i et egnet dyrkingsmedium inneholdende sjøvann;

b) ekstrahere forbindelsen fra kulturen.

20

3. Metoden ifølge krav 2, hvori trinn b) omfatter sentrifugering av den kultiverte bakterien for å oppnå en celle pellet, som forbindelsen ekstraheres fra.

25

4. Metoden ifølge ethvert av kravene 2-3, hvori forbindelsen blir ekstrahert fra celle pelleten ved å bruke dimetyl sulfokside (DMSO).

5. Metoden ifølge ethvert av kravene 2-4, hvori dyrkingsmediet er PM6, valgfritt tilsatt kunstig sjøvann.

6. Anvendelse av en isolert bakterie for å produsere forbindelsen ifølge krav 1, hvori bakterien er selektert fra gruppen bestående av:

i) et bakterielt isolat som deponert under Budapest-konvensjonen hos
5 DSMZ den 7. april, 2016, med deponeringsnummer DSM 32287; og

ii) en bakterie som er nært beslektet med det bakterielle isolatet i i), så som en bakterie med lignende genotypiske og/eller fenotypiske karakteristikk til den isolerte bakterien,

10 7. Anvendelse ifølge krav 6, hvori den nært beslektede bakterien omfatter i sitt genom et 16S rRNA som ved revers transkriptase og andre streng syntese tilveiebringer en sekvens som minster 80 % identisk til sekvensen som beskrevet i SEKV ID NO.

15 8. En farmasøytisk sammensetning omfattende forbindelsen ifølge krav 1, og en eller flere farmasøytisk akseptable bærere og/eller eksipienter.

9. Forbindelsen ifølge krav 1 for anvendelse i terapi. 20

10. Forbindelsen ifølge krav 1 for anvendelse som en antimikrobiell agent.

11. Forbindelsen for anvendelse ifølge krav 11, hvori den antimikrobielle agenten er en antibakteriell agent.

25 12. Forbindelsen ifølge krav 1 eller farmasøytisk sammensetning ifølge krav 8 for behandling av en bakteriell infeksjon i et subjekt.

30 13. Forbindelse eller sammensetning for anvendelse ifølge krav 12, hvori den bakterielle infeksjonen er forårsaket av en multiresistent bakterie.

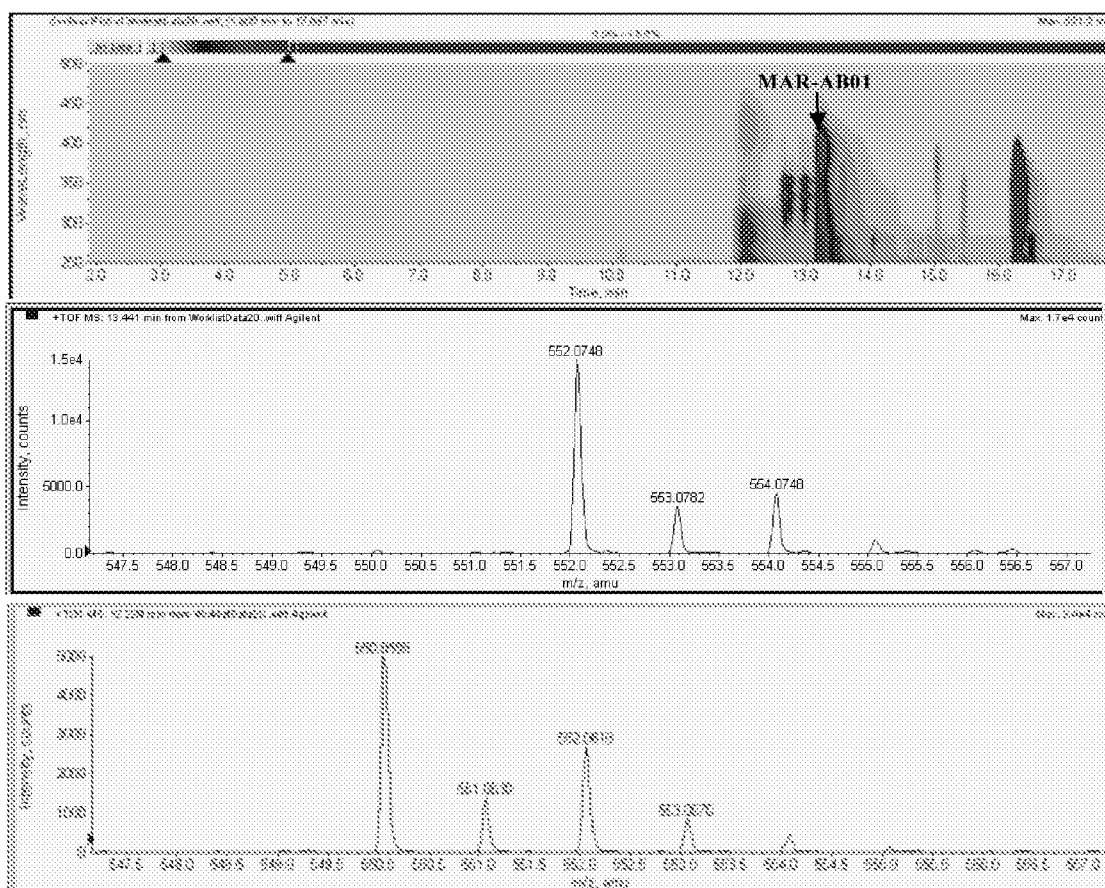
14. Anvendelse ifølge krav 12, hvori den bakterielle infeksjonen er forårsaket av en Gram-positiv eller Gram-negative bakterie.

35 15. Forbindelse ifølge krav 1 eller sammensetning ifølge krav 8 for dreping eller inhibering av vekst av en bakterie, hvori forbindelsen eller sammensetningen blir brakt i kontakt med bakterien som skal drepes eller inhiberes.

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 gtaatacgta ggggtgcgagc gttgtccgga attattgggc gtaaagagct cgtaggcggt 540
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 acttgagttc ggcaggggag actggaattc ctggtgtagc ggtgaaatgc gcagatatca 660
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 tgtgggggat ttccacgtcc tccgtgccgt agctaacgca ttaagcgccc cgctgggga 840
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	gtgaatacgt tcccgggcct tgtacacacc gcccgtcacg tcacgaaagt cggtaacacc	1380
	cgaagcccat ggccaaccc gtagggggg gagtggtcga aggtgggact ggcgattggg	1440
10	acgaagtcgt aacaaggt	1458

1/2



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Figure 1: LC-DAD-isoplot (upper) and MS spectra at ESI+ (middle) and MS spectra of ESI- (bottom) of the active fraction of the MP127-ig17 extract fractionated on HPLC.

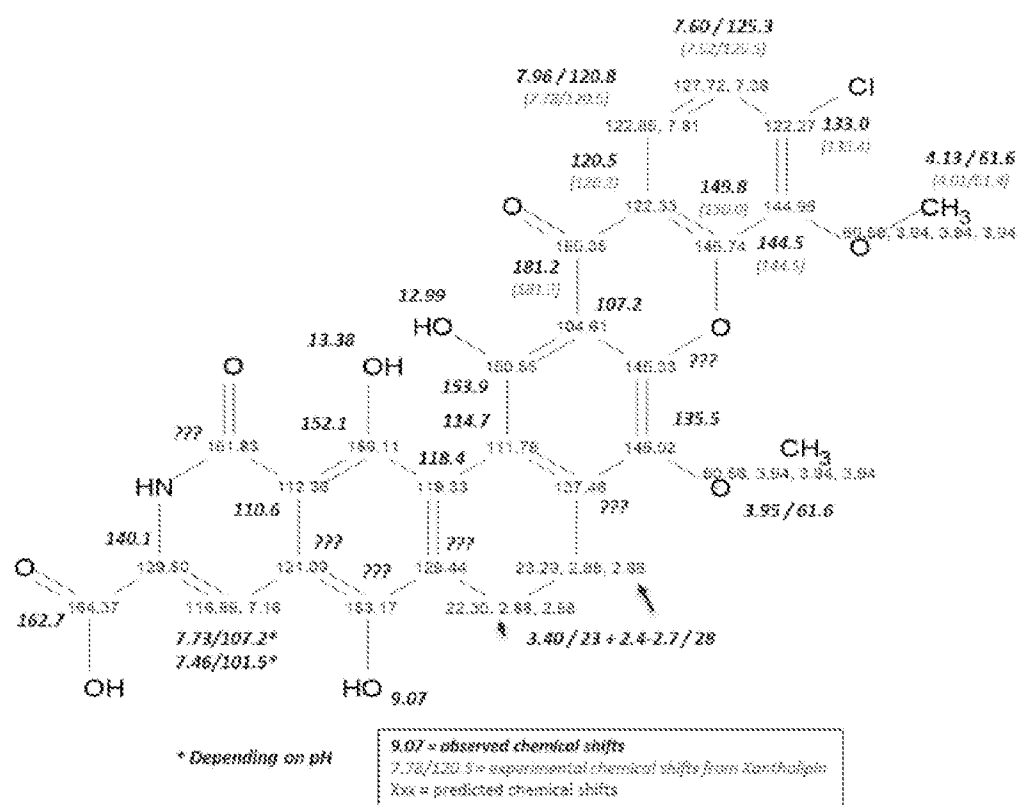


Figure 2: Key NMR and MS data supporting the structure of MBL-AB01.