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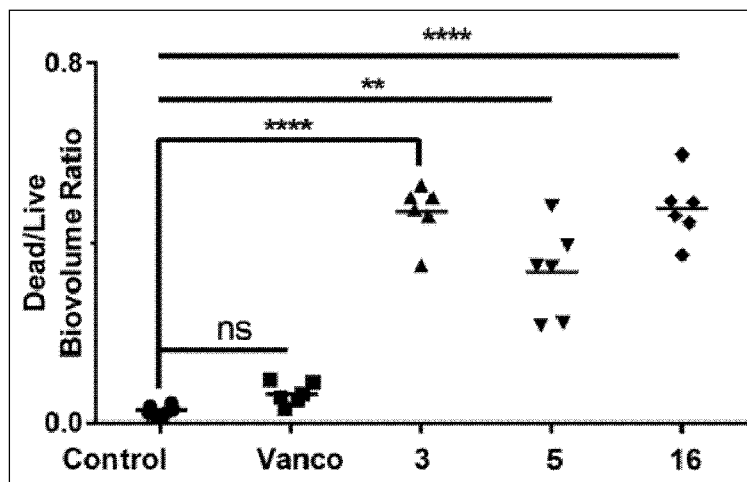
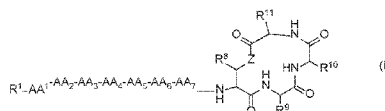


Figure 1c



(57) Abstract: The invention provides a compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, for use in killing, inhibiting, or preventing the growth of a microbial biofilm, formula (I) wherein R¹, AA¹, AA², AA³, AA⁴, AA⁵, AA⁶, AA⁷, R⁸, R⁹, R¹⁰, R¹¹ and Z have meanings given in the description. Preferably, the biofilms are produced by *Staphylococcus aureus* or *Staphylococcus epidermidis*.

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Field of the Invention

5 The present invention relates to new uses of teixobactin analogues and, in particular, the use of such compounds in the killing, inhibition or prevention of microbial biofilms. Biofilm treatment or prevention is primarily useful in a clinical setting, particularly with patients that are suffering from biofilm infections, though the invention includes the treatment or prevention of biofilms in non-living materials, such as in medical devices.

Background

The formation of biofilms is a universal bacterial survival strategy. Biofilms occur on both inert and living supports, in natural environments and in industrial installations.

15 A biofilm is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface. Biofilms are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances.

20 Single-celled organisms generally exhibit two distinct modes of behavior. The first is the familiar free floating, or planktonic, form in which single cells float or swim independently in some liquid medium. The second is an attached state in which cells are closely packed and firmly attached to each other and usually form a solid surface. A change in behavior is triggered by many factors, including quorum sensing, as well as other mechanisms that vary between species. When a cell switches modes, it undergoes a phenotypic shift in behavior in which large suites of genes are up- and down-regulated.

Formation

30 Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion structures such as pili. The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able

to communicate via quorum sensing. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development and is the stage in which the biofilm is established and may only change in shape and size. This development of biofilm allows for the cells to become more antibiotic resistant. Bacterial biofilms are thought to be refractive to antibiotic action for at least two reasons; the biofilm forms a physical barrier preventing antibiotic penetration to the bacteria, and secondly the bacteria within biofilms tend to grow more slowly, hence providing a lower metabolic profile for antibiotics to target.

Properties

Biofilms are usually found on solid substrates submerged in or exposed to some aqueous solution, although they can form as floating mats on liquid surfaces and also on the surface of leaves, particularly in high humidity climates. Given sufficient resources for growth, a biofilm will quickly grow to be macroscopic. Biofilms can contain many different types of microorganism, e.g. bacteria, archaea, protozoa, fungi and algae; each group performing specialized metabolic functions. However, some organisms will form monospecies films under certain conditions.

Biofilms appear able to defend themselves against disinfectants and antibiotics, phagocytes and the human immune system.

Extracellular matrix

The biofilm is held together and protected by a matrix of excreted polymeric compounds called EPS. EPS is an abbreviation for either extracellular polymeric substance or exopolysaccharide. This matrix protects the cells within it and facilitates communication among them through biochemical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signaling molecules. This matrix is strong enough that under certain conditions, biofilms can become fossilized.

Bacteria living in a biofilm usually have significantly different properties from free-floating bacteria of the same species, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. In some cases antibiotic resistance can be increased 1000 fold (see Stewart P, Costerton J, 2001, *Lancet* **358** (9276):135–8).

The tolerance of biofilms for antibiotics can be attributed to limited drug diffusion through the extra-cellular polymeric substances (EPS) and cell conversion from the planktonic to dormant state, with altered metabolic activities resulting in phenotypic antimicrobial tolerance. The release of EPS and formation of the biofilm matrix is a fundamental step in the process of biofilm development, as it protects the cells from threats in the surrounding environment (e.g. immune cells and antibiotics) and provides mechanical and structural support. The EPS mostly constitutes of proteins, polysaccharides, extracellular DNA and lipids, which have a wide range of functions including adhesion, water retention, structural integrity and enzymatic activity. Subsequently, in addition to being a physical barrier for antibiotics penetration, the biofilm matrix plays a role in antibiotic decomposition by enzymatic action, low pH and high concentration of metals. Bacterial cells inside the biofilm have been shown to be 1000 times more tolerant to antibiotics than planktonic cells in some cases.

Biofilms and infectious diseases

Biofilms have been found to be involved in a wide variety of microbial infections in the body, by one estimate 80% of all infections (see "Research on microbial biofilms (PA-03-047)", NIH, National Heart, Lung, and Blood Institute, 2002-12-20). Infectious processes in which biofilms have been implicated include common problems such as urinary tract infections, catheter infections, middle-ear infections, formation of dental plaque, gingivitis, coating contact lenses, and less common but more lethal processes such as endocarditis, infections in cystic fibrosis, and infections of permanent indwelling devices such as joint prostheses and heart valves.

It has been shown that biofilms are present on the removed tissue of 80% of patients undergoing surgery for chronic sinusitis. The patients with biofilms were shown to have been denuded of cilia and goblet cells, unlike the controls without biofilms who had normal cilia and goblet cell morphology. Biofilms were also found on samples from two of 10 healthy controls mentioned. The species of bacteria from interoperative cultures did not correspond to the bacteria species in the biofilm on the respective patient's tissue. In other words, the cultures were negative though the bacteria were present.

Mortality relating to antimicrobial resistance (AMR) in 2019 was estimated as 1.27 million deaths based on data gathered from 204 countries. Most of the mortalities were attributed to methicillin-resistant *Staphylococcus aureus* (MRSA) with nearly half a million deaths.

Numbers are estimated to reach 10 million annually by 2050 if no firm actions are taken to resolve the problem.

Furthermore, biofilm cultures are typically highly refractory to eradication with chemotherapy, without developing genotypic resistance. Consequently, the number of therapeutic options is limited and the development of novel antimicrobial agents with antibiofilm activity is increasingly important.

Hence, there is a need for new methods of killing, inhibiting or preventing the growth of a microbial biofilms.

Teixobactin is a recently-discovered depsipeptide antibiotic that acts through a novel mechanism of action (Ling L.L *et al.*, *Nature*, 2015, 517, 455-459). Teixobactin inhibits bacterial cell wall synthesis by binding to precursors of essential cell wall components. As such, it is likely to induce resistance at a considerably slower rate than antibacterials that act at intracellular protein targets.

Teixobactin's unusual structure comprises D-amino acid residues, and an L-*allo*-enduracididine residue. The manufacture of Teixobactin in a commercial scale is difficult and expensive, in part due to the presence of the L-*allo*-enduracididine residue.

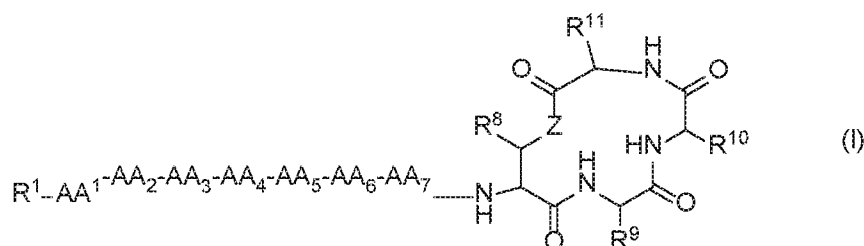
Various analogues of Teixobactin have been described in the literature, including in Wu C., *et al.*, *RSC Adv.*, 2017, **7**, 1923-1926; Yang H., *et al.*, *ACS Chem. Biol.* 2016, 11, 1823-1826; Abdel Monaim S. A. H., *ACS Omega* 2016, **1**, 1262-1265; Parmar A., *et al.*, *Chem. Commun.* 2017, **53**, 2016-2019; Parmar A., *et al.*, *Chem. Commun.* 2016, **52**, 6060-6063; Jad Y. E., *et al.*, *Org. Lett.*, 2015, **17** (24), 6182-6185; Yang H. *Chem. Commun.*, 2017, **53**, 2772-2775; and international patent publication no. WO 2018/162922.

Jarkhi A. *et al.* *Microorganisms* **2022**, 10, 1099 discusses antimicrobial effects of L-Chg₁₀-Teixobactin against *Enterococcus faecalis* *in vitro*.

The listing or discussion of an apparently prior published document in this specification should not necessarily be taken as an acknowledgement that the disclosure of the document is part of the state of the art or is common general knowledge.

Summary of the Invention

According to a first aspect of the invention, there is provided a compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, for use in killing, inhibiting or preventing the growth of a microbial biofilm,



wherein:

R¹ represents H, C₁₋₆ alkyl, C₁₋₆ acyl, benzyl or benzoyl;

AA¹ represents any hydrophobic proteinogenic or hydrophobic non-proteinogenic amino acid;

AA², AA⁵ and AA⁶ each independently represents an isoleucine or an *allo*-isoleucine residue;

AA³ and AA⁴ each independently represents a proteinogenic or non-proteinogenic amino acid;

AA⁷ represents a serine residue;

R⁸ represents hydrogen or C₁₋₄ alkyl;

R⁹ represents a proteinogenic or non-proteinogenic amino acid side chain;

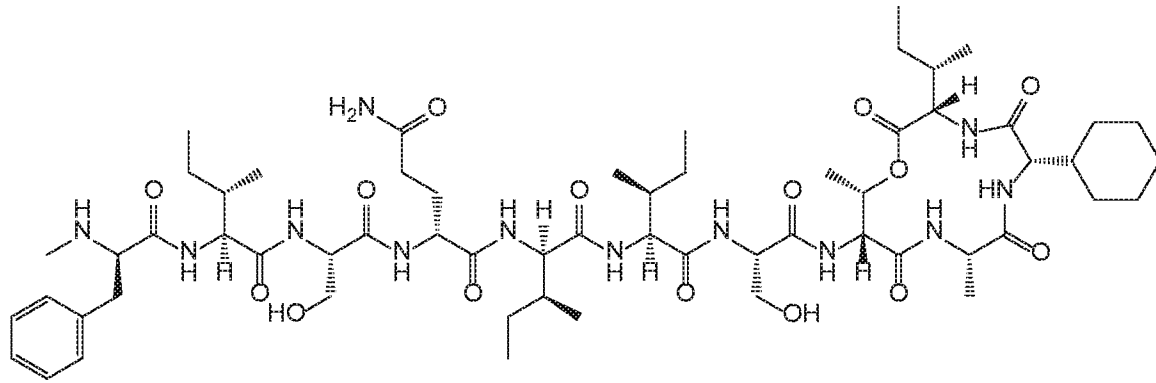
R¹⁰ represents, a hydrophobic proteinogenic or hydrophobic non-proteinogenic amino acid side chain;

R¹¹ represents a proteinogenic or non-proteinogenic amino acid side chain; and

Z is -O- or -NH-;

Provided that the compound of formula (I), or the pharmaceutically-acceptable salt, solvate or clathrate thereof, is not L-Chg₁₀-teixobactin.

For the avoidance of doubt, the proviso serves to exclude the compound referred to as "L-Chg₁₀-teixobactin" in Jarkhi A. et al. *Microorganisms* **2022**, 10, 1099. L-Chg₁₀-teixobactin may be described as having the following structure:



In an alternative, the invention relates to a compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, as defined above, provided that the compound of formula (I), or the pharmaceutically-acceptable salt, solvate or clathrate thereof, is not:

(R)-N1-((2R,3S)-1-(((2S,3S)-1-(((S)-1-(((3S,6S,9S,12R,13S)-3-((S)-sec-butyl)-6-cyclohexyl-9,13-dimethyl-2,5,8,11-tetraoxo-1-oxa-4,7,10-triazacyclotridecan-12-yl)amino)-3-hydroxy-1-oxopropan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)-2-((S)-3-hydroxy-2-((2S,3S)-3-methyl-2-((R)-2-(methylamino)-3-phenylpropanamido)pentanamido)propanamido)pentanediamide.

Compounds, salts, solvates, and clathrates of formula (I) are referred to hereinafter as the "compounds of the invention".

By "pharmaceutically-acceptable salt" we mean an acid addition or base addition salt suitable for use in pharmaceuticals. Such salts may be formed by conventional means, for example by reaction of a free acid or a free base form of a compound of the invention with one or more equivalents of an appropriate acid or base, optionally in a solvent, or in a medium in which the salt is insoluble, followed by removal of said solvent, or said medium, using standard techniques (e.g. in vacuo, by freeze-drying or by filtration). Salts may also be prepared by exchanging a counter-ion of a compound of the invention in the form of a salt with another counter-ion, for example using a suitable ion exchange resin.

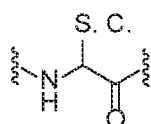
Examples of pharmaceutically acceptable addition salts include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulphuric acids; from organic acids, such as tartaric, acetic, trifluoroacetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic and arylsulphonic acids; and from metals such as sodium, magnesium, or preferably, potassium and calcium. Particularly preferred salts include those derived from acetic, trifluoroacetic, hydrochloric and tartaric acids.

By "solvate" we mean a solid form wherein the relevant compound (e.g. a compound of formula (I)) is associated with one or more solvent molecules. The term solvate includes hydrates and other solvates of pharmaceutically acceptable solvents. A preferred solvent for solvate formation is DMSO.

By "clathrate" we mean a solid form wherein the relevant compound (e.g. a compound of formula (I)) forms a lattice that contains a guest molecule (e.g. a pharmaceutically-acceptable solvent) within the lattice structure.

By "biofilm" we include microbial (e.g. bacterial, fungal, algal) communities, typically enveloped by an extracellular matrix produced by the microbial cells, which can adhere to the interface of a liquid and a surface (for example, on a mucosal membrane within the body, any host tissue or organ, or on the surface of a permanent or semi-permanent implanted medical device (e.g. venous catheter)).

By "amino acid" and "residue" (for example D-phenylalanine "residue") we mean the dehydrated portion of an amino acid present in polypeptide chains and represented by the following formula



wherein S. C. represents an amino acid side chain. For the avoidance of doubt, the term "amino acid" includes non-proteinogenic amino acids unless otherwise specified.

By "amino acid side chain" or "side chain of an amino acid" we mean the group attached to the position α to the carboxyl and amino groups in α -amino acids, including non-proteinogenic α -amino acids and particularly proteinogenic amino acids. The skilled person will understand that the most common natural amino acids are known by their trivial names and will be aware of the side chain groups present in these amino acids.

"Proteinogenic" amino acids are the 22 amino acids that may be naturally encoded or naturally found in the genetic code of organisms. "Non-proteinogenic" amino acids are those not naturally encoded or found in the genetic code of any organism. The set of non-proteinogenic amino acids is generally considered to include all organic compounds with an amine (-NH₂) and a carboxylic acid (-COOH) functional group linked via a single additional carbon atom, as well as a side chain and a hydrogen bound to that single additional carbon atom, but excluding selenocysteine, pyrrolysine and the 20 standard amino acids that are incorporated into proteins during translation. Non-proteinogenic amino acids include those amino acids that are intermediates in biosynthesis, those that are post-translationally formed in proteins, and those that possess a physiological role (e.g. components of bacterial cell walls, neurotransmitters, and toxins). References to hydrophobic non-proteinogenic amino acid side chains are references to hydrophobic side chains (particularly those formed primarily of alkyl and/or aryl groups in the absence of polar groups) which are capable of being bound to an amino acid backbone. References to polar non-proteinogenic amino acid side chains are references to polar side chains (particularly those comprising a hydroxyl group or an amide functional group (e.g. wherein one or more of said groups is bound to the amino acid via a linear, branched, cyclic or part cyclic C₁₋₈ alkylene group)) which are capable of being bound to an amino acid backbone.

The amino acid residues may be provided in their naturally occurring stereochemical configuration (e.g. the L-configuration), or the alternative stereochemical configuration. It is preferred that the amino acid residues are provided in their naturally occurring stereochemical configuration.

Various structural features, such as L² represent linking groups which form a bridge between two separate portions of the molecule. In each case, such linking groups include -OC(O)-. For L², the left-hand hyphen in such linking groups represents the point of attachment to L¹, and the right-hand hyphen in such linking groups represents the point of attachment to L³.

Unless otherwise specified, alkyl groups as defined herein may be straight-chain or, when there is a sufficient number (i.e. a minimum of three) of carbon atoms, be branched-chain and/or cyclic. Further, when there is a sufficient number (i.e. a minimum of four) of carbon atoms, such alkyl groups may also be part cyclic/acyclic. Such alkyl groups may also be saturated or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be unsaturated. Unless otherwise specified, alkyl groups may also be substituted with one or more halo, and especially fluoro, atoms.

Unless otherwise specified, alkylene groups as defined herein may be straight-chain or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be branched-chain. Such alkylene chains may also be saturated or, when there is a sufficient number (i.e. a minimum of two) of carbon atoms, be unsaturated. Unless otherwise specified, alkylene groups may also be substituted with one or more halo atoms.

The term "heterocyclyl group" as used herein refers to a 3- to 6- membered aromatic, saturated or part saturated heterocyclic ring containing one or two heteroatoms selected from O and N.

The term "acyl" as used herein refers to alkyl groups having a carbonyl group attached to the carbon which forms the point of attachment to the rest of the molecule.

When the stereochemistry of a chiral centre is not explicitly defined herein (i.e. by the use of wedged/hashed bonds) it should be understood that the stereocentre may be present in the R- or S-configuration, or a mixture of both configurations.

The skilled person will realise that all references herein to particular aspects of the invention include references to all embodiments and combinations of one or more embodiments that make up that aspect of the invention. Thus, all embodiments of particular aspects of the inventions may be combined with one or more other embodiments of that aspect of the invention to form further embodiments without departing from the teaching of the invention.

In one embodiment of the invention, R^1 represents H, C_{1-6} alkyl, C_{1-6} acyl, benzyl or benzoyl. For example, R^1 may represent H, C_{1-4} alkyl, C_{1-4} acyl, benzyl or benzoyl. In a particular embodiment, R^1 represents H, methyl, or acetyl; preferably methyl.

In another embodiment of the invention, AA^1 represents any hydrophobic proteinogenic amino acid, such as alanine, valine, leucine, isoleucine, methionine, tyrosine, tryptophan or, particularly, phenylalanine. In a particular embodiment, AA^1 represents an L- or D-phenylalanine residue; preferably a D-phenylalanine residue.

In one embodiment of the invention, AA^2 , AA^5 and AA^6 each individually represents an L-, L-*allo*-, D- or D-*allo*-isoleucine residue. In particular examples, AA^2 and AA^6 each independently represents an L- or D-isoleucine residue, preferably an L-isoleucine residue. In another embodiment of the invention, AA^5 represents a D-*allo*-isoleucine or a D-isoleucine residue.

In yet another embodiment of the invention, AA⁷ represents an L- or D-serine residue, preferably an L-serine residue.

- 5 Adjacent AA groups may be linked together via an amide bond between the C1 (carbon number one) of one amino acid with the nitrogen attached to the alpha carbon in the adjacent amino acid, as in a so-called eupeptide bond. Alternatively, any adjacent AA groups may be linked via an isopeptide bond; that is, the side chain of at least one of these amino acids may form part of the backbone of the polypeptide chain. For example,
10 a serine residue (e.g. at the AA⁷ position) may link to the carboxyl group of an adjacent amino acid (e.g. at the AA⁶ position) via the oxygen atom in the HO-CH₂- side chain of serine, thereby forming an O-acyl linkage between AA⁶ and AA⁷.

- The incorporation of isopeptide bonds may be advantageous for one or more properties of
15 the compound of the invention as, for example, it may improve the solubility of the compound. Isopeptide bonds are also capable of being converted into eupeptide bonds under suitable conditions.

- In one embodiment of the invention, the AA⁶ and AA⁷ groups are linked via a eupeptide or
20 O-acyl isopeptide bond, preferably via a eupeptide bond.

In another embodiment of the invention, the AA² and AA³ groups are linked via a eupeptide or O-acyl isopeptide bond, preferably via a eupeptide bond.

- 25 In a particular embodiment of the invention, the AA⁶ and AA⁷ groups are linked via an O-acyl isopeptide bond, and/or the AA² and AA³ groups are linked via an O-acyl isopeptide bond.

- In certain embodiments of the invention, O-acyl isopeptide bonds are formed via the
30 oxygen atom in the HO-CH₂- side chain of a serine residue in the AA⁷ or AA³ position.

- It has been surprisingly found that structural variation is tolerated to a much greater extent at positions represented by AA¹, AA³ and AA⁴. Thus, in a preferred embodiment, AA² represents an L-isoleucine residue, AA⁵ represents a D-*allo*-isoleucine or D-isoleucine
35 residue, AA⁶ represents an L-isoleucine residue, and AA⁷ represents an L-serine residue, whereas AA¹, AA³ and AA⁴ may be varied as described herein.

As structural variation is better tolerated at AA³ and AA⁴, the serine and glutamine residues normally present at these positions in native teixobactin may be more readily substituted. The solubility of the compound can be improved by using a polar amino acid at one or both of these positions (and also at R⁹). Uncharged polar amino acids such as serine, threonine, asparagine and glutamine may therefore be used. Particular amino acids that may be mentioned in this respect are positively charged amino acids, such as arginine, histidine, lysine, ornithine, 2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid. Other similar structures that are described herein may also be used at these positions.

In one embodiment of the invention, the side chain of AA³ represents a moiety selected from the group consisting of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a fragment of formula -L¹-L²-L³-X¹; wherein

L¹ represents a linear or branched C₁₋₁₂ alkylene linker;

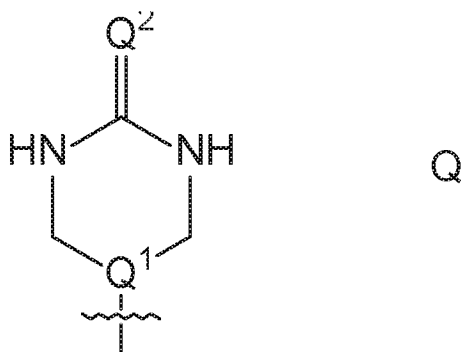
L² is selected from the group consisting of -O-, -N(X^a)-, -[N(X^a)₂]⁺-, -C(O)-, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)N(X^a)-, -OC(O)N(X^a)-, -NHC(O)O-, or -NHC(O)N(X^a)-;

X^a represents hydrogen or -L³-X¹;

L³ represents a direct bond or a linear or branched C₁₋₁₂ alkylene linker;

each X¹ is independently selected from the group consisting of -C(O)-C₁₋₁₂ alkyl, -C(O)-NH₂, -C(S)-NH₂, a fragment of formula Q, and a C₁₋₁₂ alkyl group optionally substituted by one or more X² substituents;

wherein the fragment of formula Q is:



in which Q¹ represents either CH or N, and Q² represents O, S or NH;

each X² independently represents -NH₂, -OH, -NHC(O)NH₂, -NHC(S)NH₂, -NHC(=NH)NH₂, or a 3- to 12- membered heterocycl group;

or -L²-L³-X¹ together represent a fragment of formula Q.

In a further embodiment of the invention, the side chain of AA³ represents a side chain of
5 an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine,
2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid; preferably arginine, even more
preferably L-arginine.

In yet another embodiment of the invention, AA³ represents an L- or D-serine residue,
10 preferably an L-serine residue.

Similarly, the side chain of AA⁴ may represent a moiety selected from the group consisting
of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a
fragment of formula -L¹-L²-L³-X¹ as defined hereinabove. In a further embodiment, the
15 side chain of AA⁴ represents a side chain of an amino acid selected from the group
consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropanoic acid, and 2,4-
diaminobutyric acid; preferably arginine, even more preferably D-arginine.

In yet another embodiment, AA⁴ represents an L- or D-glutamine residue, preferably a D-
20 glutamine residue.

While it may be advantageous for at least one of the side chains of AA³ and AA⁴ to be a
moiety selected from the group consisting of positively charged amino acid side chains, -
CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a fragment of formula -L¹-L²-L³-X¹ as defined
25 hereinabove, in one embodiment the side chains of both AA³ and AA⁴ are (independently)
moieties selected from this group. Compounds of the invention have hydrophobic groups
elsewhere on the molecule, e.g. at R¹⁰, and the presence of the above-mentioned moieties
at AA³ and/or AA⁴ (and optionally also at R⁹) has been found to enhance the efficacy of
the compounds. Without wishing to be bound by theory, it is believed that the balance of
30 polarities in the molecule helps improve its solubility, which in turn benefits its efficacy.

The compounds of the invention are proposed as analogues of Teixobactin. Consequently,
it is preferred that the sequence of amino acids represented by AA¹ to AA⁷ is structurally
similar to the corresponding amino acid sequence in Teixobactin. Thus, in embodiments
35 of the invention:

AA¹ may represent an L- or D-phenylalanine residue;

AA² may represent an L- or D-isoleucine residue;

AA⁵ may represent an L-, L-*allo*-, D- or D-*allo*-isoleucine residue;

AA⁶ may represent an L- or D-isoleucine residue; and/or
 AA⁷ may represent an L- or D-serine residue.

In an embodiment of the invention, R⁸ represents hydrogen or C₁₋₄ alkyl, preferably
 5 hydrogen or a methyl group, even more preferably a methyl group. Irrespective of the
 group that R⁸ represents, it is preferred that the carbon to which this group is attached is
 in the D-configuration, i.e. so that R⁸ forms part of a D-amino acid residue, as is found in
 teixobactin.

10 In compounds of formula (I), R⁹ represents a proteinogenic or non-proteinogenic amino
 acid side chain. In particular embodiments, R⁹ represents -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-
 NH₂, a hydroxy group, an amide functional group (which latter two groups are bound to
 the remainder of the molecule via a linear, branched, cyclic or part cyclic C₁₋₈ alkylene
 15 group), the side chain of an amino acid selected from the group consisting of histidine,
 lysine, arginine, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine,
 tryptophan, norvaline, cyclohexylglycine, cyclohexylalanine, phenylglycine,
 biphenylglycine, biphenylalanine, naphthylglycine naphthylalanine, serine, threonine,
 asparagine and glutamine, or a fragment of formula -L¹-L²-L³-X¹ as defined above.

20 Structural variation at R⁹ has been found to be well tolerated, and the presence of a polar
 group at this position has been found to improve biological efficacy, similar to that
 observed when polar groups are attached to the side chains of AA³ and AA⁴.

In a further embodiment of the invention, R⁹ represents a moiety selected from the group
 25 consisting of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-
 NH₂, and a fragment of formula -L¹-L²-L³-X¹ as defined hereinabove. In a more particular
 embodiment, R⁹ represents a side chain of an amino acid selected from the group
 consisting of arginine, lysine, and histidine; preferably arginine. Alternatively, R⁹ may
 represent methyl, in line with the structure of teixobactin.

30 In compounds of formula (I), R¹⁰ represents a hydrophobic proteinogenic or hydrophobic
 non-proteinogenic amino acid side chain. Preferably R¹⁰ is a hydrophobic side chain bound
 to the rest of the molecule in the L-configuration. Particular hydrophobic side chains that
 may be mentioned include C₁₋₆ alkyl groups (e.g. methyl, ethyl, propyl, butyl, pentyl,
 35 hexyl, cyclopentyl and cyclohexyl), phenyl, hydroxyphenyl, benzyl, indolylmethyl and
 CH₃SCH₂CH₂-. Further hydrophobic side chains that may be mentioned include -C₁₋₆
 alkylene-NH(R^{10a}) and -C₁₋₆ alkylene-NHCH₂(R^{10a}), wherein R^{10a} represents a C₁₋₆ alkyl

group (e.g. methyl, ethyl, propyl, butyl, pentyl, hexyl, cyclopentyl or cyclohexyl) or phenyl.

In yet another embodiment of the invention, R^{10} represents a linear or branched C_{1-6} alkyl, optionally substituted by $-NH(R^{10a})$ or $-NHCH_2(R^{10a})$. In yet another embodiment of the invention, R^{10} represents a linear or branched C_{1-6} alkyl. It has surprisingly been found that compounds having a non-cyclic alkyl group at the R^{10} position have better anti-biofilm properties in certain circumstances (e.g. against *S. epidermidis* biofilms) compared to compounds with a cyclic group at this position. Therefore, particularly preferred R^{10} groups include branched and linear propyl and butyl groups (e.g. *n*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl). Other particularly preferred R^{10} groups include branched and linear propyl and butyl groups substituted by $-NH(R^{10a})$ or $-NHCH_2(R^{10a})$. Most preferably, R^{10} groups include linear propyl and butyl groups. In one embodiment, R^{10} is butyl.

In a particular embodiment of the invention, R^{10} represents a cyclic C_{1-6} alkyl, preferably cyclohexyl. It has surprisingly been found that compounds with a cyclic alkyl group at this position have better anti-biofilm properties in certain circumstances (e.g. against *S. aureus* biofilms) compared to compounds with a non-cyclic alkyl group at this position.

In one embodiment, R^{11} represents a hydrophobic proteinogenic or hydrophobic non-proteinogenic amino acid side chain (such as that of alanine (i.e. a methyl group), valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, norvaline, cyclohexylglycine, cyclohexylalanine, phenylglycine, biphenylglycine, biphenylalanine, naphthylglycine or naphthylalanine). The group at R^{11} may be bound in either the D- or L-configuration, though it is preferred that it is present in the L-configuration. Thus, for example, when R^{11} represents a butyl group (e.g. the side chain of isoleucine), the chiral carbon to which R^{11} is bound is preferably in the S-configuration (thus corresponding to the L-isoleucine that is present at this position for Teixobactin).

In a further embodiment, R^{11} represents an L- or D-isoleucine side chain, preferably an L-isoleucine side chain.

Irrespective of the group that R^9 , R^{10} and R^{11} represent, it is preferred that the carbon to which each group is attached is in the L-configuration, i.e. the configuration most commonly seen in naturally occurring amino acids.

In one embodiment Z is $-O-$ or $-NH-$, preferably $-O-$.

In one embodiment:

AA¹ represents a D-phenylalanine residue;

AA² represents an L-isoleucine residue;

AA⁵ represents a D-*allo*-isoleucine or D-isoleucine residue;

5 AA⁶ represents an L-isoleucine residue;

AA⁷ represents an L-serine residue;

R⁸ represents a methyl group;

R¹¹ represents an L-isoleucine side chain; and

Z is -O-.

10

As is mentioned elsewhere herein, the presence of a polar group at the AA³ and/or AA⁴ side chains and/or at the R⁹ position has been found to improve biological efficacy. As is shown in the examples, *S. epidermidis* biofilms exhibited increased sensitivity to teixobactin analogues with increasing overall net charge of the peptides.

15

In one embodiment, one or more of R⁹, the side chain of AA³ and the side chain of AA⁴ represents a moiety selected from the group consisting of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a fragment of formula -L¹-L²-L³-X¹ as defined hereinabove. Preferably, one or more of R⁹, the side chain of AA³ and the side chain of AA⁴, represents a side chain of an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropionic acid, and 2,4-diaminobutyric acid. More preferably, one or more of R⁹, the side chain of AA³ and the side chain of AA⁴, represents an arginine side chain. In yet another embodiment, AA⁴ represents an arginine residue. All of these compounds are particularly suited to combating *S. epidermidis* biofilms.

25

Further increasing the number of positively charged groups at these positions (i.e. providing two or three such groups on a teixobactin analogue) has also been found to further improve efficacy. Therefore, particular compounds of the invention that may be mentioned are those in which two or more of R⁹, the side chain of AA³ and the side chain of AA⁴ (or preferably all three) represent a moiety selected from the group consisting of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a fragment of formula -L¹-L²-L³-X¹ as defined hereinabove. These compounds are particularly suited to combating *S. epidermidis* biofilms.

35

In a preferred embodiment, two or more of R⁹, the side chain of AA³ and the side chain of AA⁴, represent a side chain of an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid. More

preferably, two or more of R^9 , the side chain of AA^3 and the side chain of AA^4 , represent an arginine side chain. In yet another embodiment, AA^4 and AA^3 represent an arginine residue.

- 5 In a more preferred embodiment, R^9 , the side chain of AA^3 and the side chain of AA^4 each independently represent a moiety selected from the group consisting of a positively charged amino acid side chain, $-CH_2-NH_2$, $-(CH_2)_2-NH_2$, $-(CH_2)_3-NH_2$, and a fragment of formula $-L^1-L^2-L^3-X^1$ as defined hereinabove (e.g. an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid). It is most preferred that R^9 , the side chain of AA^3 and the side chain of AA^4 , are all arginine side chains. These compounds are particularly suited to combating *S. epidermidis* biofilms.

- 15 The combination of positively charged groups at one or more of R^9 , AA^3 and AA^4 together with a hydrophobic group at R^{10} is found to be particularly effective, and it is believed that this arises from an improved overall solubility for the compound due to the balance of charges. Therefore, particular compounds that may be mentioned include those in which one or more of R^9 , the side chain of AA^3 and the side chain of AA^4 represents a side chain of an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid; and R^{10} represents a linear or branched C_{1-6} alkyl optionally substituted by $-NH(R^{10a})$ or $-NHCH_2(R^{10a})$, preferably a linear or branched propyl or butyl group, even more preferably a linear propyl or butyl group.

- 25 In another embodiment, at least two of R^9 , the side chain of AA^3 and the side chain of AA^4 represents a side chain of an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropanoic acid, and 2,4-diaminobutyric acid; and R^{10} represents a linear or branched C_{1-6} alkyl or a cyclic C_{3-6} alkyl, preferably a linear or branched propyl or butyl group, even more preferably a linear propyl or butyl group.

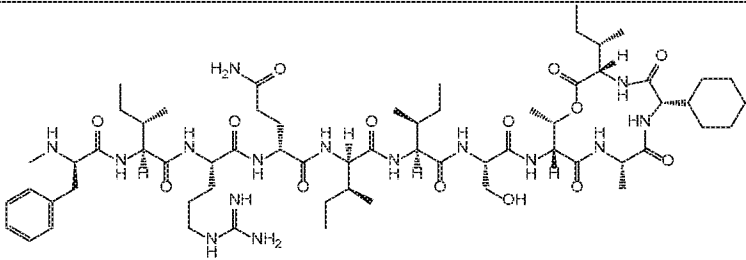
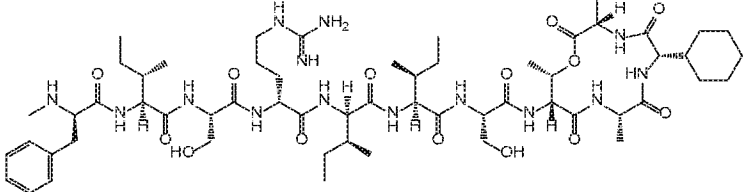
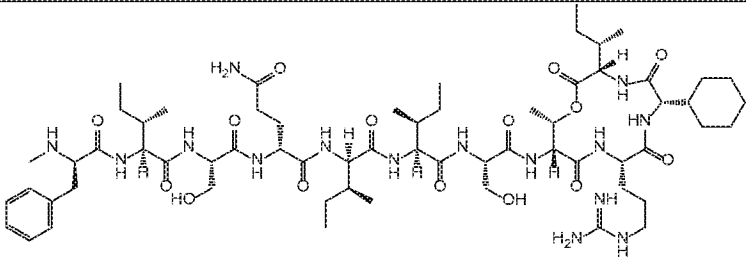
- 30 In a further embodiment, one or more of R^9 , the side chain of AA^3 and the side chain of AA^4 represents an arginine side chain; and R^{10} represents a linear or branched C_{1-6} alkyl, preferably a linear or branched propyl or butyl group, even more preferably a linear propyl or butyl group.

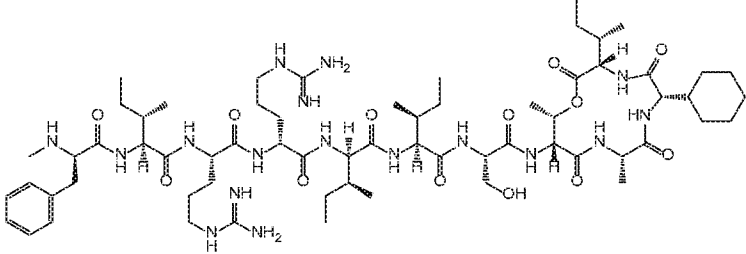
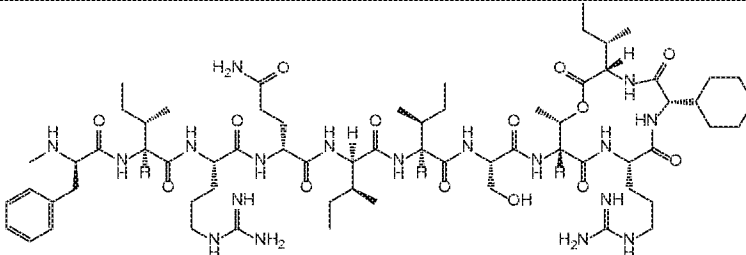
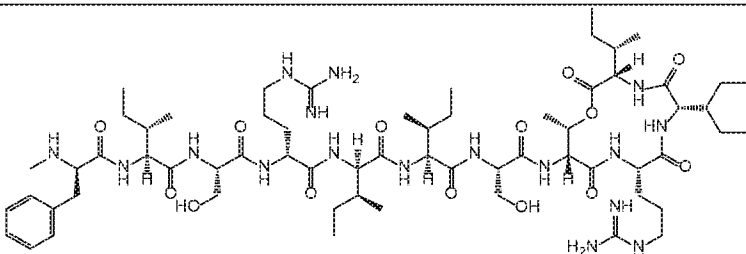
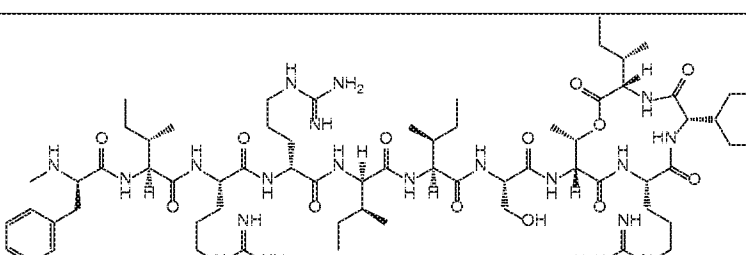
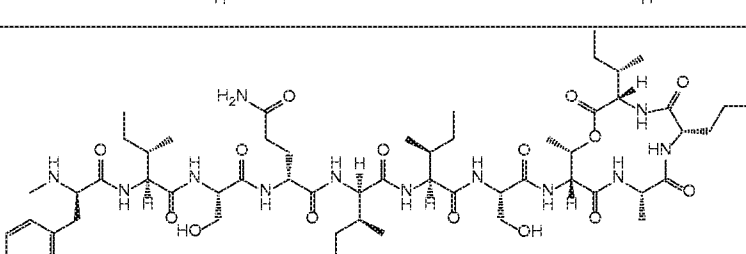
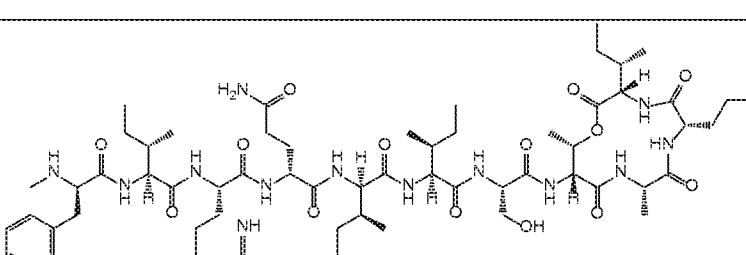
- 35 In yet another embodiment, R^9 represents an alanine side chain, AA^3 represents a serine residue, AA^4 represents a glutamine residue, and R^{10} represents a linear or branched C_{1-6} alkyl, preferably a linear or branched propyl or butyl group, even more preferably a linear

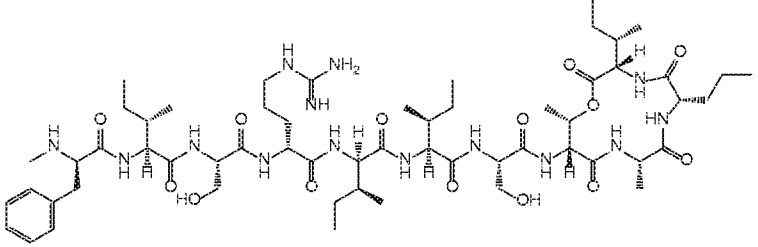
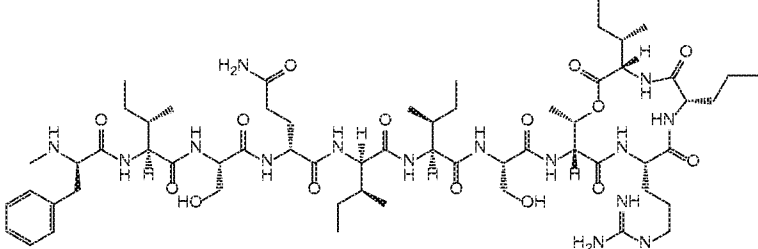
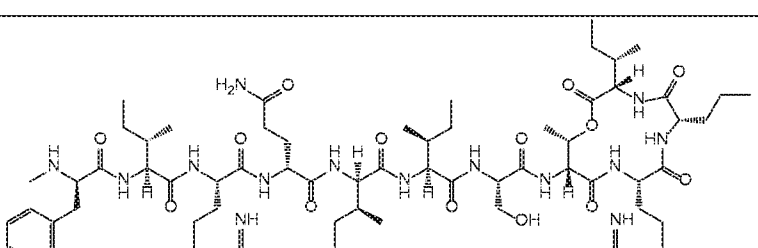
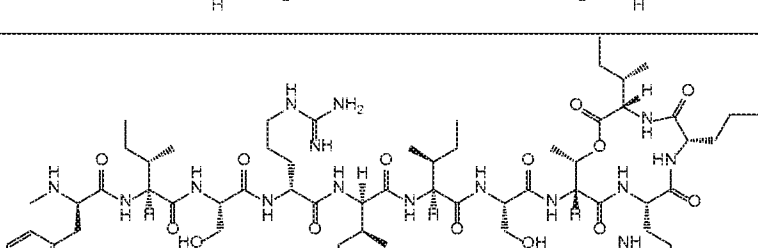
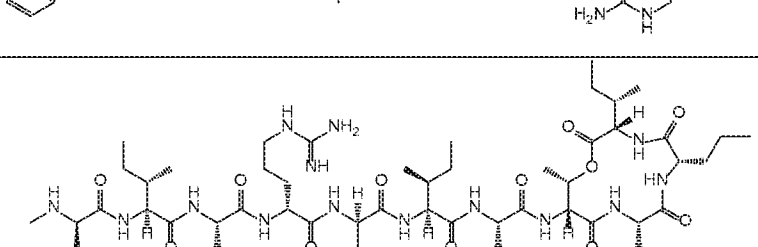
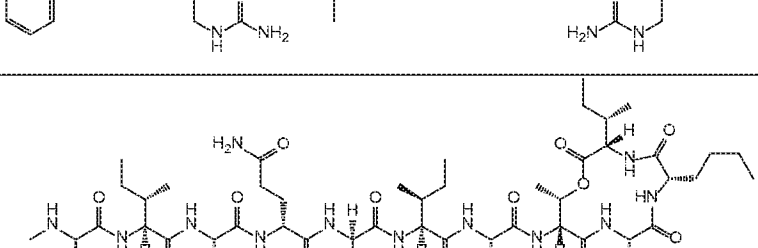
propyl or butyl group. These compounds have been found to particularly suited to combating *S. epidermidis* biofilms.

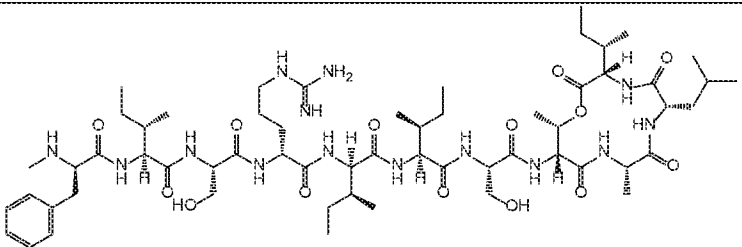
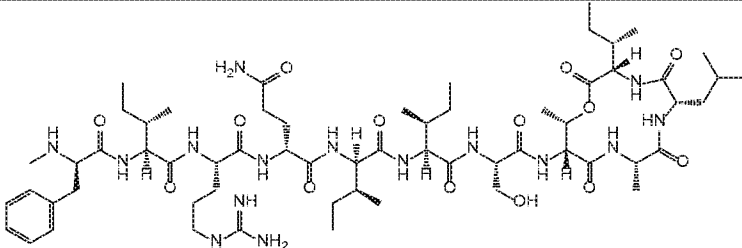
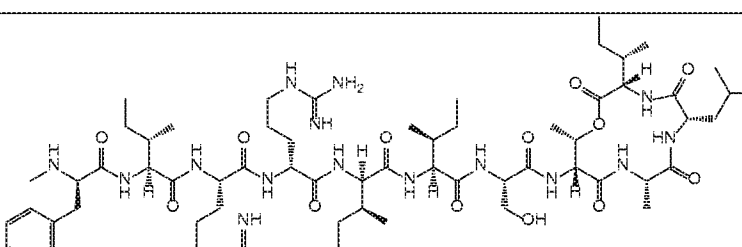
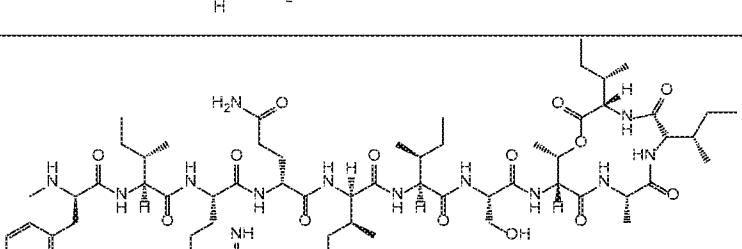
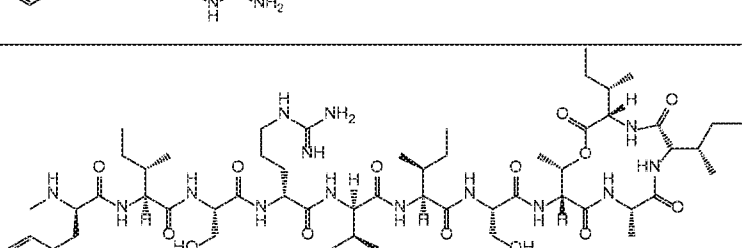
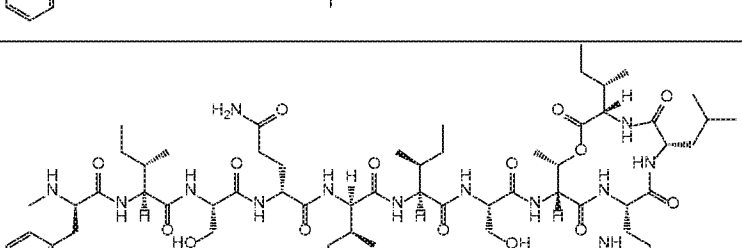
5 In a further embodiment, R⁹ represents an alanine side chain, AA³ represents a serine residue, AA⁴ represents a glutamine residue, and R¹⁰ represents a cyclic C₁₋₆ alkyl, preferably a cyclohexyl group. These compounds have been found to particularly suited to combating *S. aureus* biofilms. In this respect, the present invention also relates to the use of Chg₁₀-Teixobactin (or a pharmaceutically-acceptable salt, solvate or clathrate thereof) in killing, inhibiting, or preventing the growth of a microbial biofilm caused by *S.*
10 *aureus*.

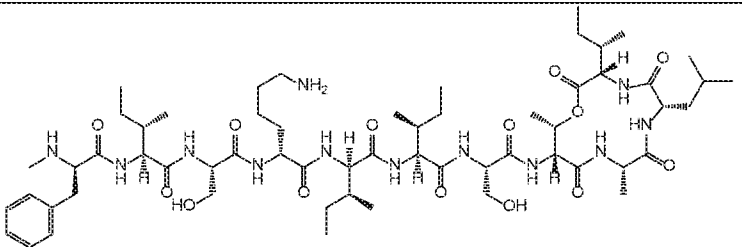
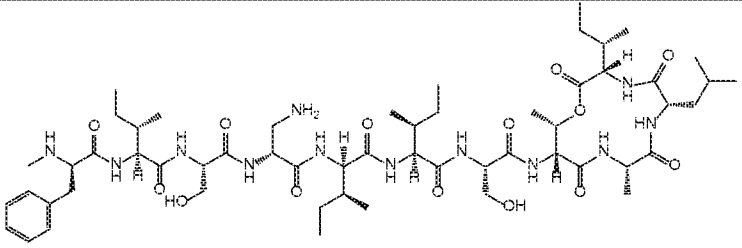
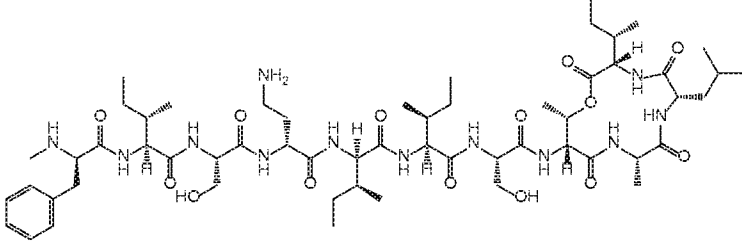
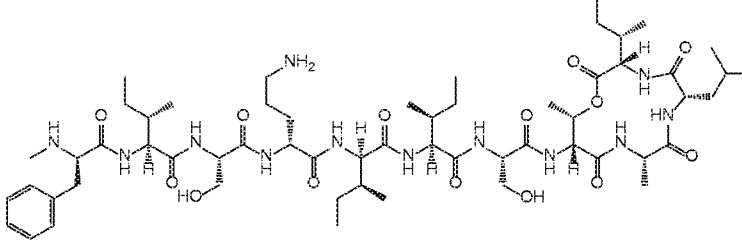
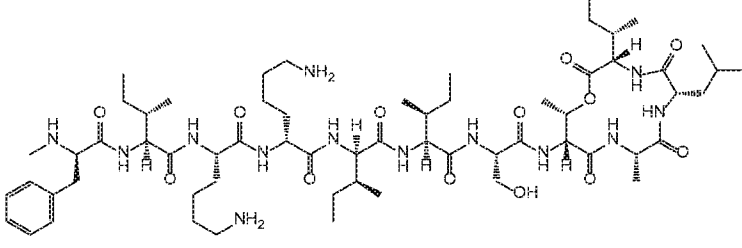
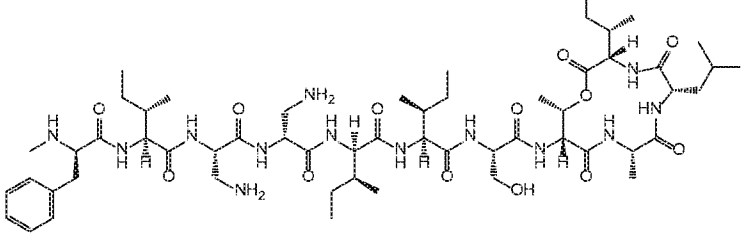
In one embodiment of the invention, the compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, is selected from the group consisting of the compounds in the following table:

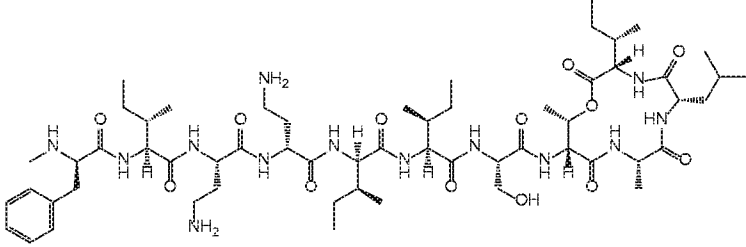
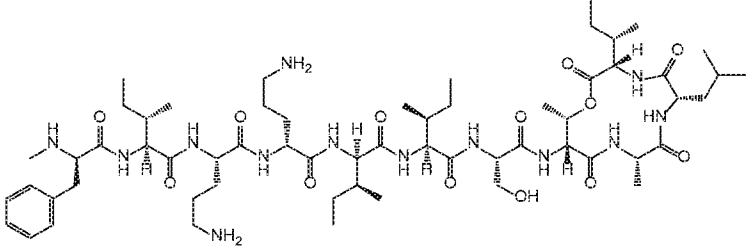
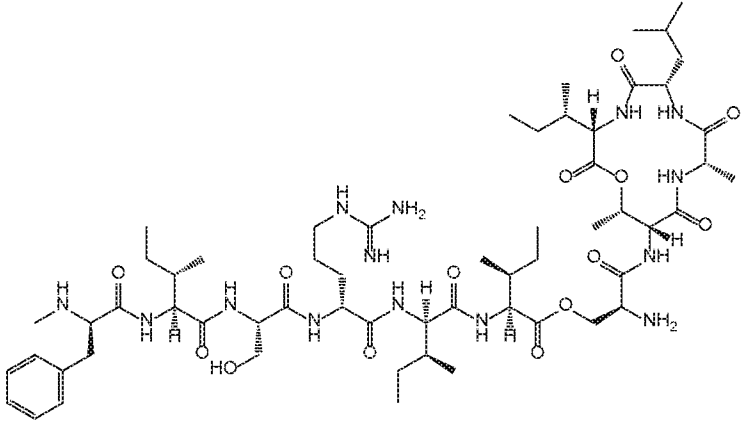
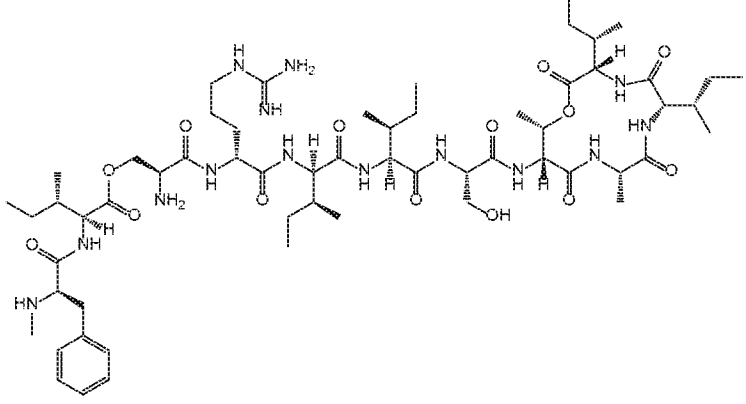
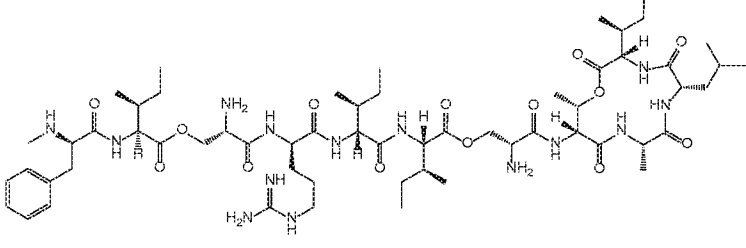
Compound Number	Structure	Name
2		Arg ₃ -Chg ₁₀ -teixobactin
3		D-Arg ₄ -Chg ₁₀ -teixobactin
4		Arg ₉ -Chg ₁₀ -teixobactin

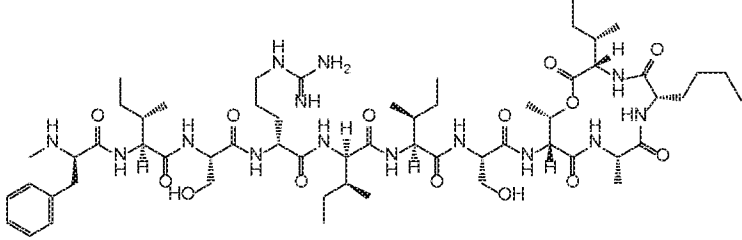
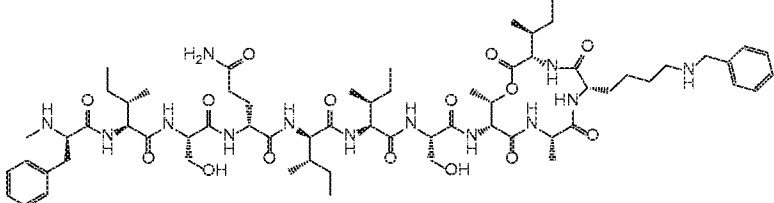
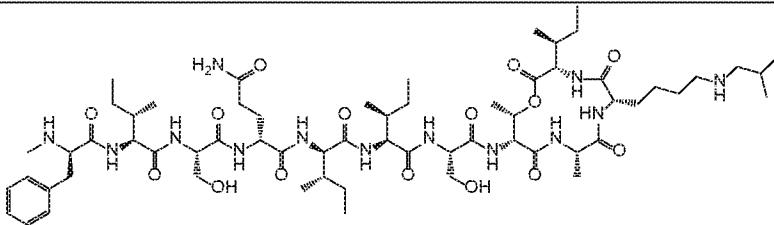
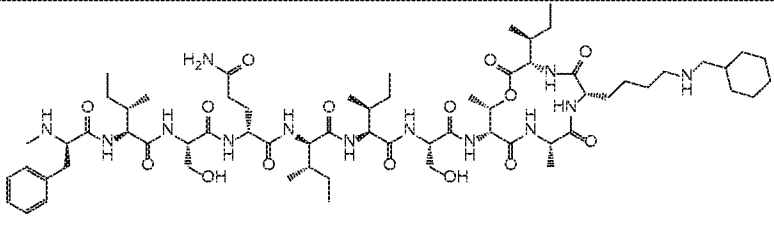
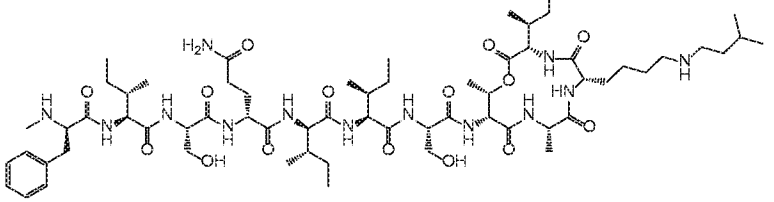
5		Arg ₃ -D-Arg ₄ - Chg ₁₀ - teixobactin
6		Arg ₃ -Arg ₉ - Chg ₁₀ - teixobactin
7		D-Arg ₄ -Arg ₉ - Chg ₁₀ - teixobactin
8		Arg ₃ -D-Arg ₄ - Arg ₉ -Chg ₁₀ - teixobactin
9		Nva ₁₀ - teixobactin
10		Arg ₃ -Nva ₁₀ - teixobactin

11		D-Arg ₄ - Nva ₁₀ - teixobactin
12		Arg ₉ -Nva ₁₀ - teixobactin
13		Arg ₃ -Arg ₉ - Nva ₁₀ - teixobactin
14		D-Arg ₄ -Arg ₉ - Nva ₁₀ - teixobactin
15		Arg ₃ -D-Arg ₄ - Arg ₉ -Nva ₁₀ - teixobactin
16		Nle ₁₀ - teixobactin

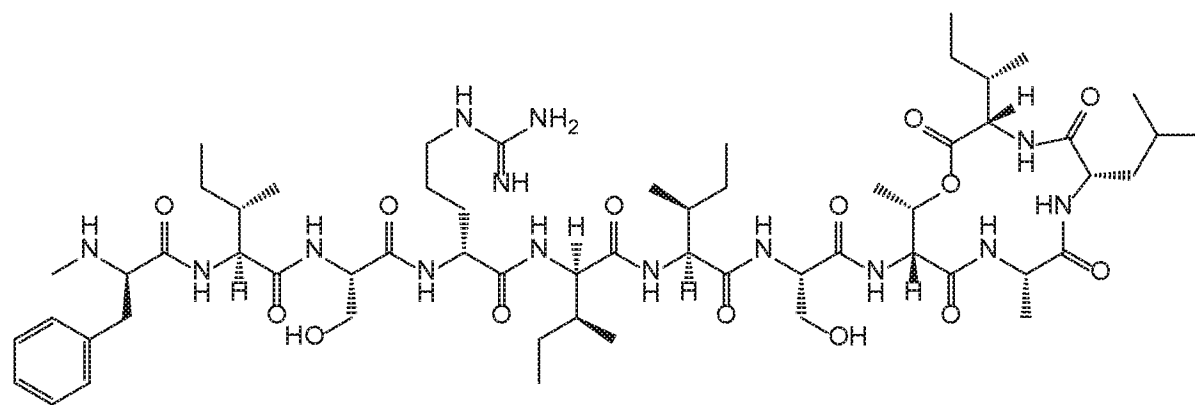
17		D-Arg ₄ -Leu ₁₀ - teixobactin
18		Arg ₃ -Leu ₁₀ - teixobactin
19		Arg ₃ -D-Arg ₄ - Leu ₁₀ - teixobactin
20		Arg ₃ -Ile ₁₀ - teixobactin
21		D-Arg ₄ -Ile ₁₀ - teixobactin
22		Arg ₉ -Leu ₁₀ - teixobactin

23		D-Lys ₄ -Leu ₁₀ - teixobactin
24		D-Dap ₄ - Leu ₁₀ - teixobactin
25		D-Dab ₄ - Leu ₁₀ - teixobactin
26		D-Orn ₄ - Leu ₁₀ - teixobactin
27		Lys ₃ -D-Lys ₄ - Leu ₁₀ - teixobactin
28		Dap ₃ -D- Dap ₄ -Leu ₁₀ - teixobactin

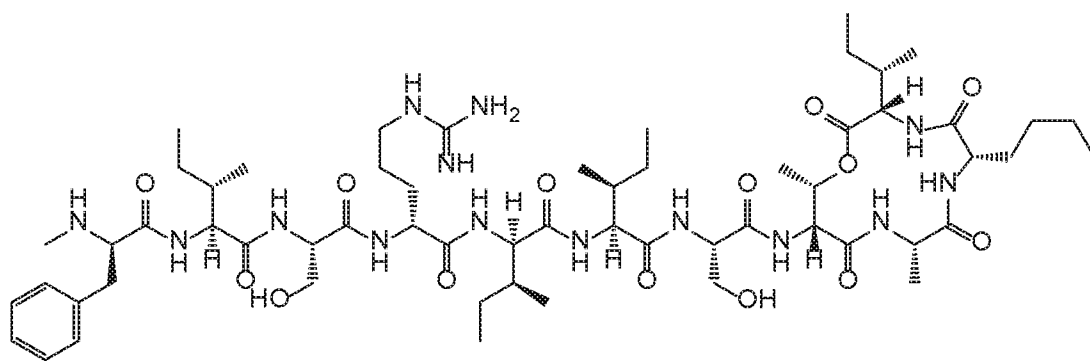
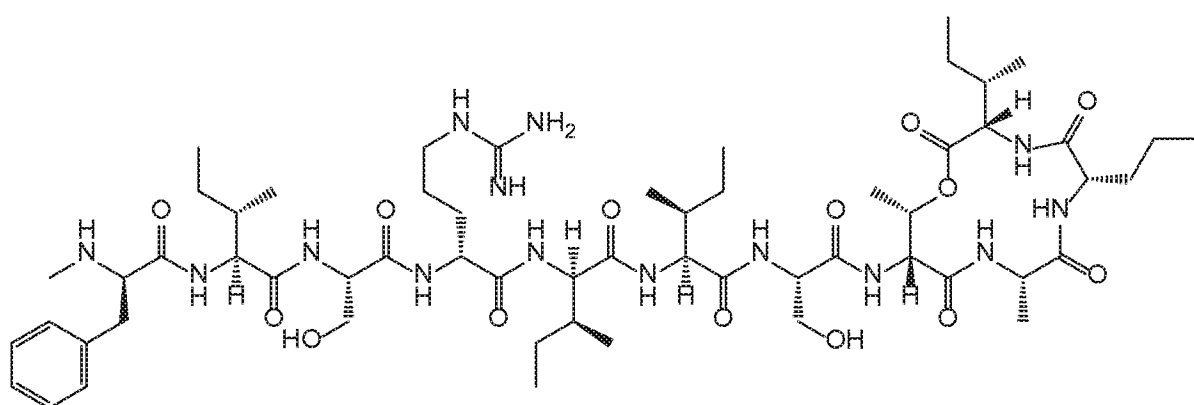
29		Dab ₃ -D-Dab ₄ -Leu ₁₀ -teixobactin
30		Orn ₃ -D-Orn ₄ -Leu ₁₀ -teixobactin
31		D-Arg ₄ -O-acylSer ₇ -Leu ₁₀ -teixobactin
32		D-Arg ₄ -O-acylSer ₃ -Ile ₁₀ -teixobactin
33		D-Arg ₄ -O-acylSer ₃ -O-acylSer ₇ -Leu ₁₀ -teixobactin

34		D-Arg ₄ -Nle ₁₀ -teixobactin
35		Lys ₁₀ (benzyl)-teixobactin
36		Lys ₁₀ (isobutyl)-teixobactin
37		Lys ₁₀ (cyclohexylmethyl)-teixobactin
38		Lys ₁₀ (isopentyl)-teixobactin

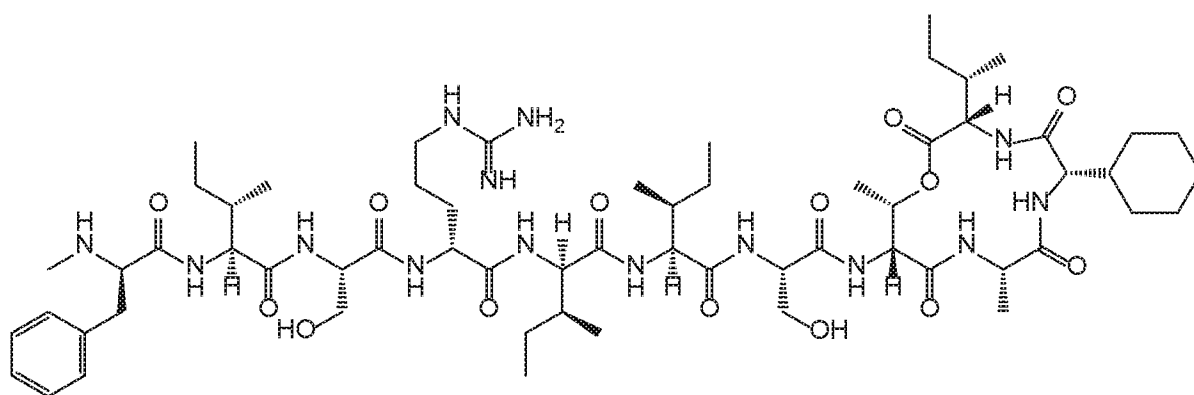
In another embodiment of the invention, the compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, is selected from the group consisting of:



(D-Arg₄-Leu₁₀-teixobactin);

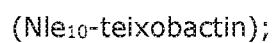
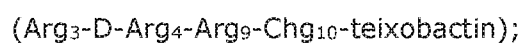
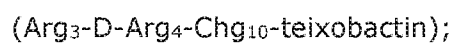
(D-Arg₄-Nle₁₀-teixobactin);

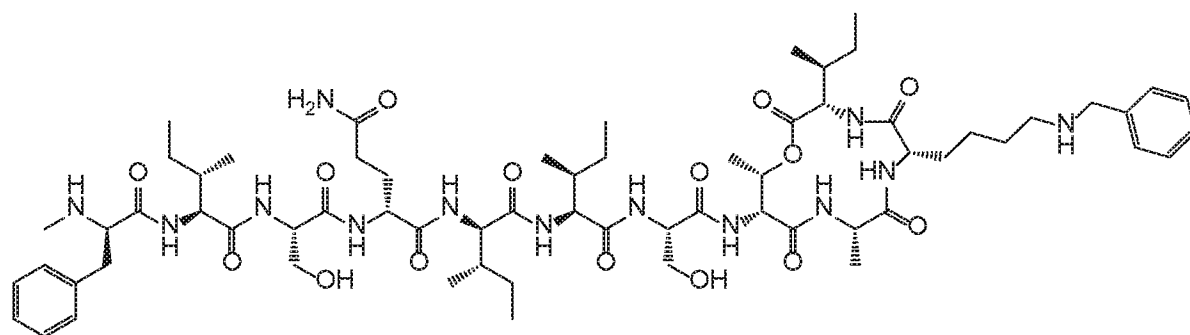
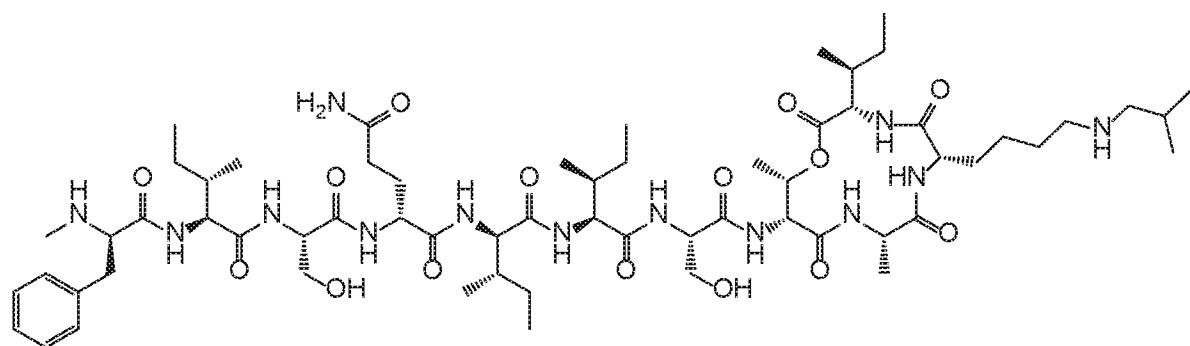
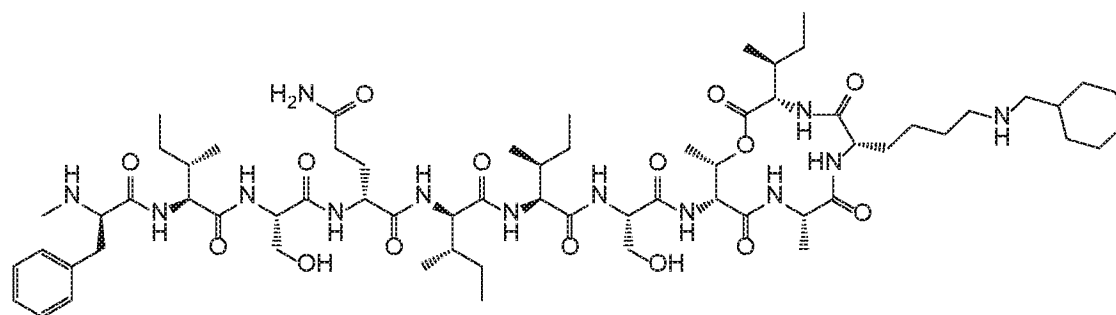
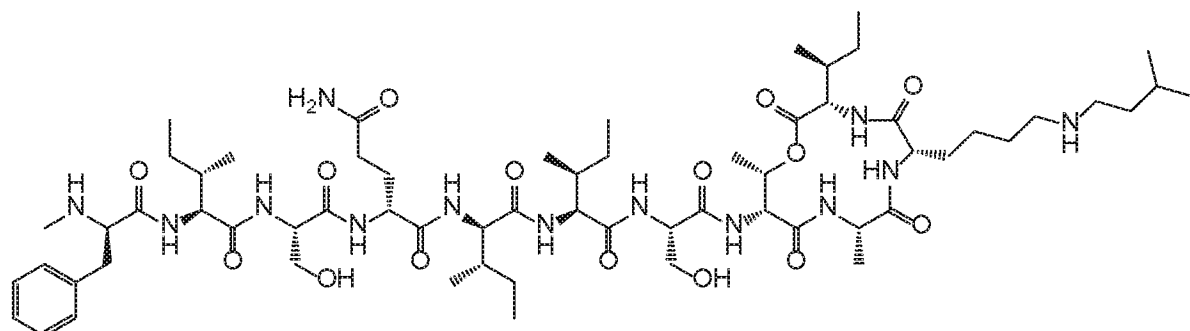
5

(D-Arg₄-Nva₁₀-teixobactin);

10

(D-Arg₄-Chg₁₀-teixobactin);



(Lys₁₀(benzyl))-teixobactin);5 (Lys₁₀(isobutyl))-teixobactin);(Lys₁₀(cyclohexylmethyl))-teixobactin); and

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(Lys₁₀(isopentyl))-teixobactin).

In the case of a discrepancy between the names and structures of any of the compounds disclosed herein, the structures provided should prevail.

Preparation

The compounds of the invention may be prepared in accordance with techniques known to those skilled in the art, for example as described in WO 2018/162922.

The novel compounds of the invention, including for example Arg₄-Nle₁₀-teixobactin and Nle₁₀-teixobactin, can be synthesised using this method. In order to synthesise Nle₁₀-teixobactin (compound 16), the amino acid at position 10 is replaced with norleucine during the synthesis of the compound (e.g. during step (a) in Example 2 of WO 2018/162922). Similarly, in order to synthesise Arg₄-Nle₁₀-teixobactin, the amino acid at position 10 may be replaced with norleucine and the amino acid at position 4 is replaced with arginine.

For compounds of the invention that contain one or more isopeptide bonds (e.g. Compounds 31-33), amino acid dimers in which the relevant isopeptide bonding mode is already present may be obtained from commercial sources and used as a reagent in a peptide coupling reaction in order to incorporate the isopeptide bonding mode into the molecule.

Uses and Pharmaceutical Preparations

According to the first aspect of the invention hereinbefore described, a compound of the invention, i.e. a compound of formula (I) or a pharmaceutically-acceptable salt, solvate or clathrate thereof as defined above, is used for killing, inhibiting or preventing the growth of a microbial biofilm.

The compounds of the invention are useful because they possess pharmacological activity. They are therefore indicated as pharmaceuticals. There is provided a pharmaceutical composition comprising a compound of the invention in combination with a pharmaceutically-acceptable adjuvant diluent or carrier. Such formulations are referred to hereinafter as the "formulations of the invention".

The use of compounds or formulations of the invention in medicine includes their use as pharmaceuticals (both for human and veterinary use). The compositions of the present

invention may also be useful in other fields of industry. For example, the compositions may be useful in hygiene and sterilisation procedures (e.g. in scientific laboratories).

The compounds of the invention may be formulated at various concentrations, depending on the efficacy/toxicity of the compound being used and the purpose for which it is being used.

It will be appreciated by persons skilled in the art that, when used in medicine, the compound will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice (for example, see *Remington: The Science and Practice of Pharmacy*, 19th edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA). Suitable routes of administration are discussed below, and include topical, intravenous, oral, pulmonary, nasal, aural, ocular, bladder and CNS delivery.

It will be appreciated that the compounds for use according to the invention may be employed for killing a number of types of biofilm-forming microorganisms, including bacteria, archaea, protozoa, fungi and algae. Such microorganisms may be resistant to one or more conventional antibiotics, such as methicillin (e.g. MRSA). In one embodiment, the microorganisms are bacteria.

When used herein, the terms "*bacteria*" (and derivatives thereof, such as "*bacterial infection*") includes references to organisms (or infections due to organisms) of the following classes and specific types:

Gram-positive cocci, such as

Staphylococci (e.g. *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *Staph. auricularis*, *Staph. capitis capitis*, *Staph. c. ureolyticus*, *Staph. caprae*, *Staph. cohnii cohnii*, *Staph. c. urealyticus*, *Staph. equorum*, *Staph. gallinarum*, *Staph. haemolyticus*, *Staph. hominis hominis*, *Staph. h. novobiosepticus*, *Staph. hyicus*, *Staph. intermedius*, *Staph. lugdunensis*, *Staph. pasteurii*, *Staph. saccharolyticus*, *Staph. schleiferi schleiferi*, *Staph. s. coagulans*, *Staph. sciuri*, *Staph. simulans*, *Staph. warneri* and *Staph. xylosus*) and

Streptococci (e.g.

beta-haemolytic, pyogenic streptococci (such as *Strept. agalactiae*, *Strept. canis*, *Strept. dysgalactiae dysgalactiae*, *Strept. dysgalactiae equisimilis*, *Strept. equi equi*, *Strept. equi zooepidemicus*, *Strept. iniae*, *Strept. porcinus* and *Strept. pyogenes*),

microaerophilic, pyogenic streptococci (*Streptococcus* "milleri", such as *Strept. anginosus*, *Strept. constellatus constellatus*, *Strept. constellatus pharyngidis* and *Strept. intermedius*),

oral streptococci of the "mitis" (alpha-haemolytic - *Streptococcus* "viridans", such as *Strept. mitis*, *Strept. oralis*, *Strept. sanguinis*, *Strept. cristatus*, *Strept. gordonii* and *Strept. parasanguinis*), "salivarius" (non-haemolytic, such as *Strept. salivarius* and *Strept. vestibularis*) and "mutans" (tooth-surface streptococci, such as *Strept. criceti*, *Strept. mutans*, *Strept. ratti* and *Strept. sobrinus*) groups,

Strept. acidominimus, *Strept. bovis*, *Strept. faecalis*, *Strept. equinus*, *Strept. pneumoniae* and *Strept. suis*,

or Streptococci alternatively classified as Group A, B, C, D, E, G, L, P, U or V *Streptococcus*);

Gram-negative cocci, such as *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Neisseria cinerea*, *Neisseria elongata*, *Neisseria flavescens*, *Neisseria lactamica*, *Neisseria mucosa*, *Neisseria sicca*, *Neisseria subflava* and *Neisseria weaveri*;

Bacillaceae, such as *Bacillus anthracis*, *Bacillus subtilis*, *Bacillus thuringiensis*, *Bacillus stearothermophilus* and *Bacillus cereus*;

Enterobacteriaceae, such as

Escherichia coli,

Enterobacter (e.g. *Enterobacter aerogenes*, *Enterobacter agglomerans* and *Enterobacter cloacae*)

Citrobacter (such as *Citrob. freundii* and *Citrob. divernis*),

Hafnia (e.g. *Hafnia alvei*),

Erwinia (e.g. *Erwinia persicinus*),

Morganella morganii,

Salmonella (*Salmonella enterica* and *Salmonella typhi*),

Shigella (e.g. *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*),

Klebsiella (e.g. *Klebs. pneumoniae*, *Klebs. oxytoca*, *Klebs. ornitholytica*, *Klebs. planticola*, *Klebs. ozaenae*, *Klebs. terrigena*, *Klebs. granulomatis* (*Calymmatobacterium granulomatis*) and *Klebs. rhinoscleromatis*),

Proteus (e.g. *Pr. mirabilis*, *Pr. rettgeri* and *Pr. vulgaris*),

Providencia (e.g. *Providencia alcalifaciens*, *Providencia rettgeri* and *Providencia stuartii*),

Serratia (e.g. *Serratia marcescens* and *Serratia liquifaciens*), and

Yersinia (e.g. *Yersinia enterocolitica*, *Yersinia pestis* and *Yersinia pseudotuberculosis*);

Enterococci (e.g. *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus cecorum*, *Enterococcus dispar*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus flavescens*, *Enterococcus gallinarum*, *Enterococcus hirae*, *Enterococcus malodoratus*, *Enterococcus mundtii*, *Enterococcus pseudoavium*,
5 *Enterococcus raffinosus* and *Enterococcus solitarius*);

Helicobacter (e.g. *Helicobacter pylori*, *Helicobacter cinaedi* and *Helicobacter fennelliae*);

Acinetobacter (e.g. *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. Iwoffii* and *A. radioresistens*);

10 *Pseudomonas* (e.g. *Ps. aeruginosa*, *Ps. maltophilia* (*Stenotrophomonas maltophilia*), *Ps. alcaligenes*, *Ps. chlororaphis*, *Ps. fluorescens*, *Ps. luteola*, *Ps. mendocina*, *Ps. montellii*, *Ps. oryzihabitans*, *Ps. pertucinogena*, *Ps. pseudocaligenes*, *Ps. putida* and *Ps. stutzeri*);

Bacteriodes fragilis;

15 *Peptococcus* (e.g. *Peptococcus niger*);

Peptostreptococcus;

Clostridium (e.g. *C. perfringens*, *C. difficile*, *C. botulinum*, *C. tetani*, *C. absonum*, *C. argentinense*, *C. baratii*, *C. bifermentans*, *C. beijerinckii*, *C. butyricum*, *C. cadaveris*, *C. carnis*, *C. celatum*, *C. clostridioforme*, *C. cochlearium*, *C. coccleatum*, *C. fallax*, *C. ghonii*,
20 *C. glycolicum*, *C. haemolyticum*, *C. hastiforme*, *C. histolyticum*, *C. indolis*, *C. innocuum*, *C. irregulare*, *C. leptum*, *C. limosum*, *C. malenominatum*, *C. novyi*, *C. oroticum*, *C. paraputrificum*, *C. piliforme*, *C. putrefasciens*, *C. ramosum*, *C. septicum*, *C. sordellii*, *C. sphenoides*, *C. sporogenes*, *C. subterminale*, *C. symbiosum* and *C. tertium*);

Mycoplasma (e.g. *M. pneumoniae*, *M. hominis*, *M. genitalium* and *M. urealyticum*);

25 *Mycobacteria* (e.g. *Mycobacterium tuberculosis*, *Mycobacterium avium*, *Mycobacterium fortuitum*, *Mycobacterium marinum*, *Mycobacterium kansasii*, *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Mycobacterium leprae*, *Mycobacterium smegmitis*, *Mycobacterium africanum*, *Mycobacterium alvei*, *Mycobacterium asiaticum*, *Mycobacterium aurum*, *Mycobacterium bohemicum*,
30 *Mycobacterium bovis*, *Mycobacterium branderi*, *Mycobacterium brumae*, *Mycobacterium celatum*, *Mycobacterium chubense*, *Mycobacterium confluentis*, *Mycobacterium conspicuum*, *Mycobacterium cookii*, *Mycobacterium flavescens*, *Mycobacterium gadium*, *Mycobacterium gastri*, *Mycobacterium genavense*, *Mycobacterium gordonae*, *Mycobacterium goodii*, *Mycobacterium haemophilum*, *Mycobacterium hassicum*,
35 *Mycobacterium intracellulare*, *Mycobacterium interjectum*, *Mycobacterium heidelbergense*, *Mycobacterium lentiflavum*, *Mycobacterium malmoense*, *Mycobacterium microgenicum*, *Mycobacterium microti*, *Mycobacterium mucogenicum*, *Mycobacterium neoaurum*, *Mycobacterium nonchromogenicum*, *Mycobacterium peregrinum*, *Mycobacterium phlei*,

Mycobacterium scrofulaceum, *Mycobacterium shimoidei*, *Mycobacterium simiae*, *Mycobacterium szulgai*, *Mycobacterium terrae*, *Mycobacterium thermoresistabile*, *Mycobacterium triplex*, *Mycobacterium triviale*, *Mycobacterium tusciae*, *Mycobacterium ulcerans*, *Mycobacterium vaccae*, *Mycobacterium wolinskyi* and *Mycobacterium xenopi*);

5 *Haemophilus* (e.g. *Haemophilus influenzae*, *Haemophilus ducreyi*, *Haemophilus aegyptius*, *Haemophilus parainfluenzae*, *Haemophilus haemolyticus* and *Haemophilus parahaemolyticus*);

Actinobacillus (e.g. *Actinobacillus actinomycetemcomitans*, *Actinobacillus equuli*, *Actinobacillus hominis*, *Actinobacillus lignieresii*, *Actinobacillus suis* and *Actinobacillus*
10 *ureae*);

Actinomyces (e.g. *Actinomyces israelii*);

Brucella (e.g. *Brucella abortus*, *Brucella canis*, *Brucella melintensis* and *Brucella suis*);

Campylobacter (e.g. *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*
15 and *Campylobacter fetus*);

Listeria monocytogenes;

Vibrio (e.g. *Vibrio cholerae* and *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus* and *Vibrio vulnificus*);

20 *Erysipelothrix rhusopathiae*;

Corynebacteriaceae (e.g. *Corynebacterium diphtheriae*, *Corynebacterium jeikeum* and *Corynebacterium urealyticum*);

Spirochaetaceae, such as *Borrelia* (e.g. *Borrelia recurrentis*, *Borrelia burgdorferi*, *Borrelia afzelii*, *Borrelia andersonii*, *Borrelia bissettii*, *Borrelia garinii*, *Borrelia japonica*,
25 *Borrelia lusitaniae*, *Borrelia tanukii*, *Borrelia turdi*, *Borrelia valaisiana*, *Borrelia caucasica*, *Borrelia crocidurae*, *Borrelia duttoni*, *Borrelia graingeri*, *Borrelia hermsii*, *Borrelia hispanica*, *Borrelia latyschewii*, *Borrelia mazzottii*, *Borrelia parkeri*, *Borrelia persica*, *Borrelia turicatae* and *Borrelia venezuelensis*) and *Treponema* (*Treponema pallidum* ssp. *pallidum*, *Treponema pallidum* ssp. *endemicum*, *Treponema pallidum* ssp. *pertenue* and
30 *Treponema carateum*);

Pasteurella (e.g. *Pasteurella aerogenes*, *Pasteurella bettyae*, *Pasteurella canis*, *Pasteurella dagmatis*, *Pasteurella gallinarum*, *Pasteurella haemolytica*, *Pasteurella multocida multocida*, *Pasteurella multocida gallicida*, *Pasteurella multocida septica*, *Pasteurella pneumotropica* and *Pasteurella stomatis*);

35 *Bordetella* (e.g. *Bordetella bronchiseptica*, *Bordetella hinzii*, *Bordetella holmseii*, *Bordetella parapertussis*, *Bordetella pertussis* and *Bordetella trematum*);

Nocardiaceae, such as *Nocardia* (e.g. *Nocardia asteroides* and *Nocardia brasiliensis*);

Rickettsia (e.g. *Rickettsia* or *Coxiella burnetii*);

Legionella (e.g. *Legionella anisa*, *Legionella birminghamensis*, *Legionella bozemanii*, *Legionella cincinnatiensis*, *Legionella dumoffii*, *Legionella feeleyi*, *Legionella gormanii*, *Legionella hackeliae*, *Legionella israelensis*, *Legionella jordanis*, *Legionella lansingensis*, *Legionella longbeachae*, *Legionella maceachernii*, *Legionella micdadei*, *Legionella oakridgensis*, *Legionella pneumophila*, *Legionella sainthelensi*, *Legionella tucsonensis* and *Legionella wadsworthii*);

Moraxella catarrhalis;

Stenotrophomonas maltophilia;

Burkholderia cepacia;

Francisella tularensis;

Gardnerella (e.g. *Gardnerella vaginalis* and *Gardnerella mobiluncus*);

Streptobacillus moniliformis;

Flavobacteriaceae, such as *Capnocytophaga* (e.g. *Capnocytophaga canimorsus*, *Capnocytophaga cynodegmi*, *Capnocytophaga gingivalis*, *Capnocytophaga granulosa*, *Capnocytophaga haemolytica*, *Capnocytophaga ochracea* and *Capnocytophaga sputigena*);

Bartonella (*Bartonella bacilliformis*, *Bartonella clarridgeiae*, *Bartonella elizabethae*, *Bartonella henselae*, *Bartonella quintana* and *Bartonella vinsonii arupensis*);

Leptospira (e.g. *Leptospira biflexa*, *Leptospira borgpetersenii*, *Leptospira inadai*, *Leptospira interrogans*, *Leptospira kirschneri*, *Leptospira noguchii*, *Leptospira santarosai* and *Leptospira weilli*);

Spirillum (e.g. *Spirillum minus*);

Bacteroides (e.g. *Bacteroides caccae*, *Bacteroides capillosus*, *Bacteroides coagulans*, *Bacteroides distasonis*, *Bacteroides eggerthii*, *Bacteroides forsythus*, *Bacteroides fragilis*, *Bacteroides merdae*, *Bacteroides ovatus*, *Bacteroides putredinis*, *Bacteroides pyogenes*, *Bacteroides splanchnicus*, *Bacteroides stercoris*, *Bacteroides tectus*, *Bacteroides thetaiotaomicron*, *Bacteroides uniformis*, *Bacteroides ureolyticus* and *Bacteroides vulgatus*);

Prevotella (e.g. *Prevotella bivia*, *Prevotella buccae*, *Prevotella corporis*, *Prevotella dentalis* (*Mitsuokella dentalis*), *Prevotella denticola*, *Prevotella disiens*, *Prevotella enoeca*, *Prevotella heparinolytica*, *Prevotella intermedia*, *Prevotella loeschii*, *Prevotella melaninogenica*, *Prevotella nigrescens*, *Prevotella oralis*, *Prevotella oris*, *Prevotella oulora*, *Prevotella tannerae*, *Prevotella venoralis* and *Prevotella zoogloformans*);

Porphyromonas (e.g. *Porphyromonas asaccharolytica*, *Porphyromonas cangingivalis*, *Porphyromonas canoris*, *Porphyromonas cansulci*, *Porphyromonas catoniae*, *Porphyromonas circumdentaria*, *Porphyromonas crevioricanis*, *Porphyromonas*

endodontalis, *Porphyromonas gingivalis*, *Porphyromonas gingivicanis*, *Porphyromonas levii* and *Porphyromonas macacae*);

Fusobacterium (e.g. *F. gonadiaformans*, *F. mortiferum*, *F. naviforme*, *F. necrogenes*, *F. necrophorum necrophorum*, *F. necrophorum fundiliforme*, *F. nucleatum nucleatum*, *F. nucleatum fusiforme*, *F. nucleatum polymorphum*, *F. nucleatum vincentii*, *F. periodonticum*, *F. russii*, *F. ulcerans* and *F. varium*);

Chlamydia (e.g. *Chlamydia trachomatis*);

Chlamydophila (e.g. *Chlamydophila abortus* (*Chlamydia psittaci*), *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*) and *Chlamydophila psittaci* (*Chlamydia psittaci*));

Leuconostoc (e.g. *Leuconostoc citreum*, *Leuconostoc cremoris*, *Leuconostoc dextranicum*, *Leuconostoc lactis*, *Leuconostoc mesenteroides* and *Leuconostoc pseudomesenteroides*);

Gemella (e.g. *Gemella bergeri*, *Gemella haemolysans*, *Gemella morbillorum* and *Gemella sanguinis*); and

Ureaplasma (e.g. *Ureaplasma parvum* and *Ureaplasma urealyticum*).

Thus, compounds of the invention may be used to kill any of the above-mentioned bacterial organisms.

It will be further appreciated by skilled persons that the compounds may be used to prevent and/or treat infection with such microorganisms, *i.e.* the compounds are suitable for prophylactic and/or therapeutic treatment. For example, the compounds may be used to prevent or reduce the spread or transfer of a pathogen to other subjects, *e.g.* patients, healthcare workers, *etc.*

In another embodiment, the bacteria are Gram-positive bacteria, such as those selected from the group consisting of *Staphylococci* and *Enterococci*.

For example, the bacteria may be *Staphylococci*, such as *Staphylococcus aureus*, (e.g. methicillin-resistant *Staphylococcus aureus*, MRSA).

Alternatively, the bacteria are *Staphylococcus epidermidis*.

Other *Staphylococci* include *Staph. aureus*, *Staph. epidermidis*, *Staph. saprophyticus*, *Staph. auricularis*, *Staph. capitis capitis*, *Staph. c. ureolyticus*, *Staph. caprae*, *Staph. cohnii cohnii*, *Staph. c. urealyticus*, *Staph. equorum*, *Staph. gallinarum*, *Staph. haemolyticus*, *Staph. hominis hominis*, *Staph. h. novobiosepticus*, *Staph. hyicus*, *Staph. intermedius*, *Staph. lugdunensis*, *Staph. pasteurii*, *Staph. saccharolyticus*, *Staph. schleiferi schleiferi*, *Staph. s. coagulans*, *Staph. sciuri*, *Staph. simulans*, *Staph. warneri* and *Staph. Xylosus*.

Enterococci include *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus cecorum*, *Enterococcus dispar*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus flavescens*, *Enterococcus gallinarum*, *Enterococcus hirae*,
5 *Enterococcus malodoratus*, *Enterococcus mundtii*, *Enterococcus pseudoavium*, *Enterococcus raffinosus* and *Enterococcus solitarius*.

In another embodiment, the bacteria are Gram-negative bacteria, such as those selected from the group consisting of *Acinetobacter* (e.g. *A. baumannii*, *A. calcoaceticus*, *A. haemolyticus*, *A. johnsonii*, *A. junii*, *A. Iwoffii* and *A. radioresistens*); Enterobacteriaceae
10 such as *Escherichia coli*, *Klebsiella* (e.g. *Klebs. pneumoniae* and *Klebs. oxytoca*) and *Proteus* (e.g. *Pr. mirabilis*, *Pr. rettgeri* and *Pr. vulgaris*); and *Pseudomonas* (e.g. *Ps. aeruginosa*, *Ps. maltophilia* (*Stenotrophomonas maltophilia*), *Ps. alcaligenes*, *Ps. chlororaphis*, *Ps. fluorescens*, *Ps. luteola*, *Ps. mendocina*, *Ps. monteilii*, *Ps. oryzae*, *Ps. pertucinogena*, *Ps. pseudocaligenes*, *Ps. putida* and *Ps. stutzeri*).

Dosages of the compound for use according to the invention will depend on several factors; including the particular compound used, the formulation, route of administration and the indication for which the compound is used. Typically, however, dosages will range from 0.01 to 20 mg of compound per kilogram of body weight, preferably from 0.1 to 15 mg/kg,
20 for example from 1 to 10 mg/kg of body weight.

It will be appreciated by persons skilled in the art that the compounds described herein may be used to kill, inhibit or prevent the growth of a microbial biofilm in any environment in which such biofilms may be found. Thus, biofilm may be associated with either an inert
25 support or a living support.

In one embodiment, the biofilm is associated with a living support. For example, the biofilm may grow or be susceptible to growth on a surface within the human or animal body.
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Thus, the invention provides a compound as defined above for use in the treatment or prevention of a condition or disorder associated with the presence or growth of a biofilm on or in the body of a living mammal.

A further aspect of the invention provides a method for treating or preventing a condition or disorder associated with the presence or growth of a biofilm on or in the body of a living mammal, said method comprising administering a compound as described herein to a
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patient suffering from or susceptible to a disease or condition associated with or caused by a biofilm.

A yet further aspect of the invention provides the use of a compound of formula (I) as described herein in the manufacture of a medicament for treating or preventing a condition or disorder associated with the presence or growth of a biofilm on or in the body of a living mammal.

In another embodiment, the invention provides a compound for use in killing, inhibiting, or preventing the growth of a microbial biofilm as hereinbefore defined, wherein the biofilm is on or in the body of a living mammal.

In a further embodiment, the mammal is human.

For example, the compounds described herein may be used to treat or prevent a disorder or condition associated with the growth of a biofilm (e.g. a microbial biofilm) at one of the following sites within the body:

(a) The oral cavity, including the surfaces of the teeth and gums (for example, dental plaque, gingivitis, endodontic infections, oral candidiasis, oral aspergillosis, periodontitis).

(b) The urinary tract (for example, cystitis).

(c) The sinuses (for example, chronic sinusitis).

(d) The ear (for example, middle ear infections).

(e) The heart (for example, endocarditis).

(f) The prostate (for example, chronic bacterial prostatitis).

(g) The bone (for example, osteomyelitis)

(h) The lungs (for example, infections in cystic fibrosis such as pneumonia)

(i) The kidneys (for example, infectious kidney stones and in peritoneal dialysis).

(j) The skin.

In a further embodiment, the biofilm is associated with an inert support, e.g. an inert support within the body. Thus, the biofilm may grow or be susceptible to growth on the surface of a device implanted or otherwise inserted within the human or animal body.

For example, the compounds described herein may be used to treat or prevent an infection associated with the growth of a microbial biofilm on one of the following inert surfaces within the body:

(a) A catheter (for example, for intravascular or urinary tract use).

(b) A stent (for example, a coronary stent).

(c) A shunt (for example, a cerebrospinal shunt).

(d) An intubating or tracheotomy tube.

(e) An ophthalmic device (for example, contact lenses, scleral buckles and intraocular lenses).

(f) A joint prosthesis (*i.e.* arthroplasty and implantation of other orthopaedic devices).

(g) An artificial heart valve.

(h) A breast implant.

In one embodiment of the invention, an implantable medical device is provided which is impregnated, coated or otherwise treated with a compound as described herein.

For example, the implantable medical device may be selected from the group consisting of intravascular devices, catheters, shunts, intubating and tracheotomy tubes, ophthalmic devices, joint prostheses, artificial heart valves and breast implants. By "implantable device" we include devices attached to surface of body, e.g. contact lenses.

Preferably, the implantable medical device is packaged in a sealed and sterile container prior to use.

It will be appreciated by persons skilled in the art that, when used in medicine, the compound will generally be administered in admixture with a suitable pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice (for example, see Remington: The Science and Practice of Pharmacy, 19th edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA). Suitable routes of administration are discussed below, and include topical, intravenous, oral, pulmonary, nasal, aural, ocular, bladder and CNS delivery.

For example, for application topically, e.g. to the skin or a wound site, the compounds can be administered in the form of a lotion, solution, cream, gel, ointment or dusting powder (for example, see Remington, *supra*, pages 1586 to 1597). Thus, the compounds can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, e-lauryl sulphate, an alcohol (e.g. ethanol, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol) and water.

Formulations suitable for topical administration in the mouth further include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

The medicament for use may also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray, or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler

or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Aerosol or dry powder formulations are preferably arranged so that each metered dose or "puff" contains at least 1 mg of a compound for delivery to the patient. It will be appreciated that the overall dose with an aerosol will vary from patient to patient and from indication to indication, and may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, other conventional administration routes known in the art may also be employed; for example the medicament for use according to the invention may be delivered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-, delayed- or controlled-release applications. The medicament may also be administered intra-ocularly (see below), intra-aurally or via intracavernosal injection.

The medicament may also be administered parenterally, for example, intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously (including via an array of fine needles or using needle-free Powderject® technology), or they may be administered by infusion techniques. They are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

The medicament may also be administered by the ocular route, particularly for treating diseases of the eye. For ophthalmic use, the compounds can be formulated as micronised suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

For veterinary use, a compound is administered as a suitably acceptable formulation in accordance with normal veterinary practice and the veterinary surgeon will determine the dosing regimen and route of administration which will be most appropriate for a particular animal.

The medicaments may be stored in any suitable container or vessel known in the art. It will be appreciated by persons skilled in the art that the container or vessel should preferably be airtight and/or sterilised. Advantageously, the container or vessel is made of a plastics material, such as polyethylene.

The compounds for use according to the invention may also be employed to kill, inhibit or prevent the growth of microbial biofilms *in vitro*. For example, the compounds may also be used in the form of a sterilising solution or wash to prevent the growth of microbial biofilms on a surface or substrate, such as in a domestic environment (e.g. kitchen work surfaces, showers, pipes, floors, etc.) or a commercial or industrial environment (e.g. within cooling systems, pipes, floor surfaces, etc.) environment.

Preferably, such a medicament comprises the antimicrobial compound in solution at a concentration of 1 to 100 µg/ml.

Preferably, the solution further comprises a surface-active agent or surfactant. Suitable surfactants include anionic surfactants (e.g. an aliphatic sulphonate), amphoteric and/or zwitterionic surfactants (e.g. derivatives of aliphatic quaternary ammonium, phosphonium and sulfonium compounds) and nonionic surfactants (e.g. aliphatic alcohols, acids, amides or alkyl phenols with alkylene oxides)

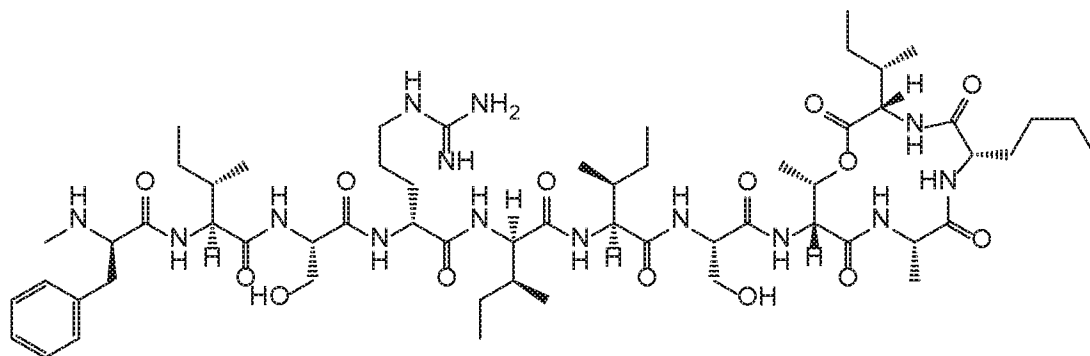
Conveniently, the surface-active agent is present at a concentration of 0.5 to 5 weight percent.

In both *in vitro* and *in vivo* uses, the compounds for use in the first and second aspects of the invention are preferably exposed to the target surface for at least five minutes. For

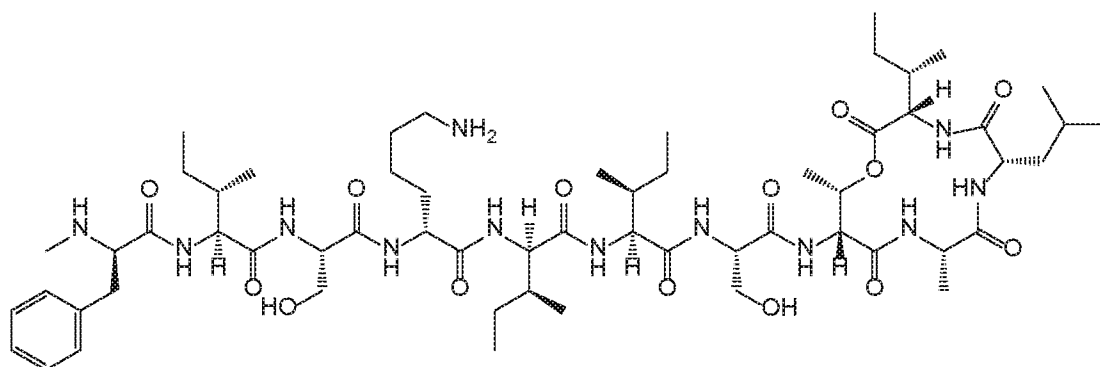
example, the exposure time may be at least 10 minutes, 20 minutes, 30 minutes, 40 minutes, 50 minutes, 1 hour, 2 hours, 3, hours, 5 hours, 12 hours and 24 hours.

According to a second aspect of the invention, there is provided a compound selected from

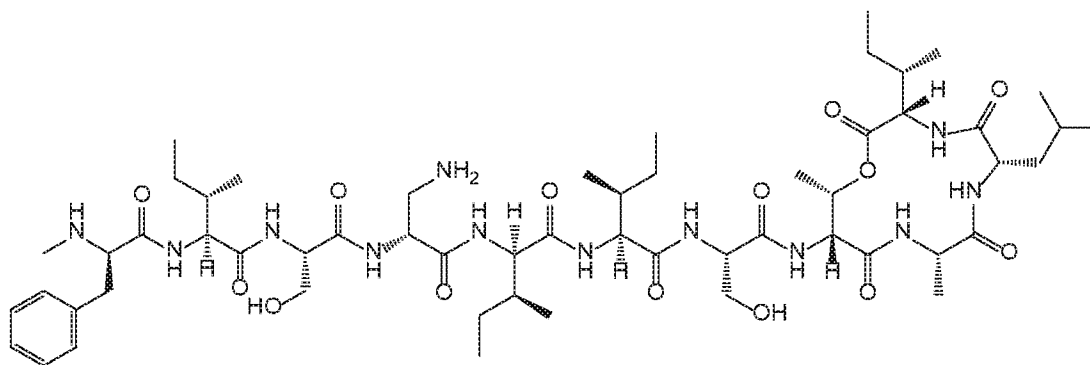
5 the group consisting of



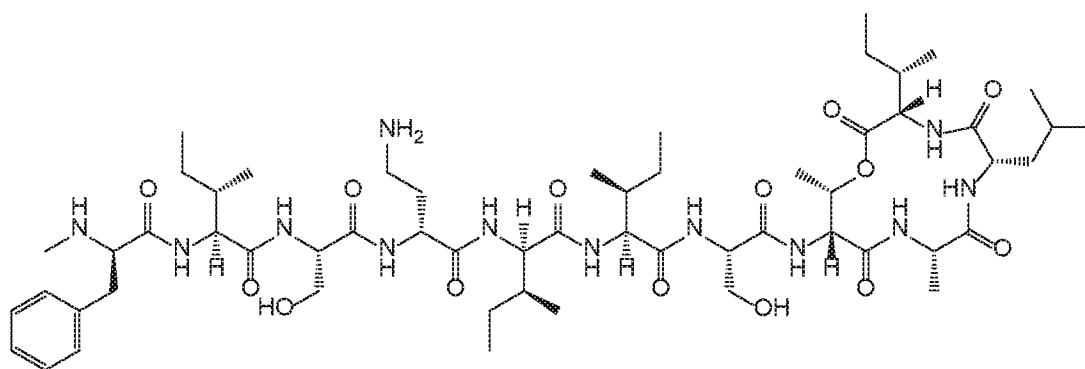
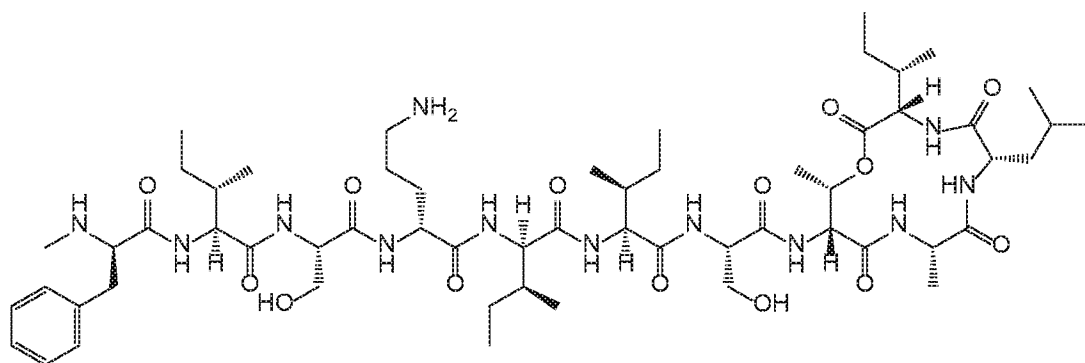
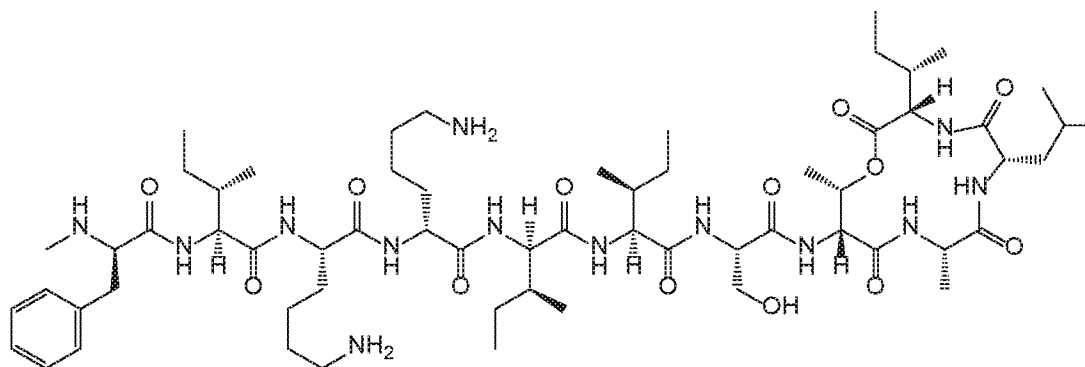
(D-Arg₄-Nle₁₀-teixobactin),



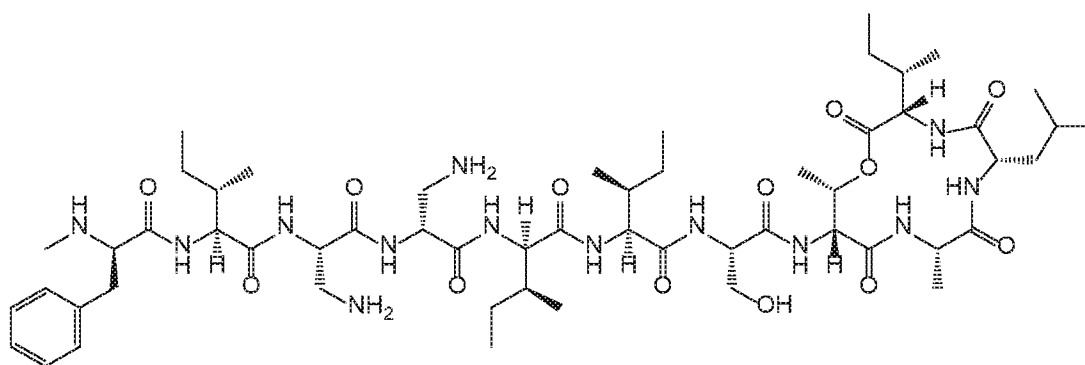
10 (D-Lys₄-Leu₁₀-teixobactin),

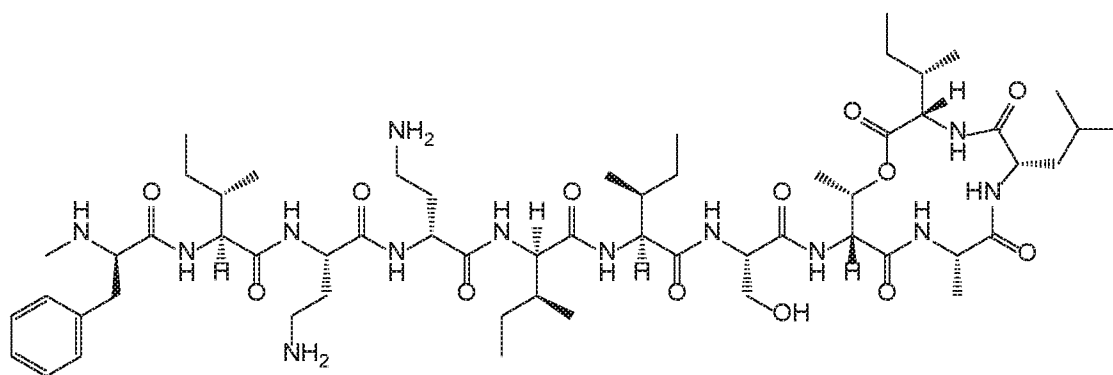
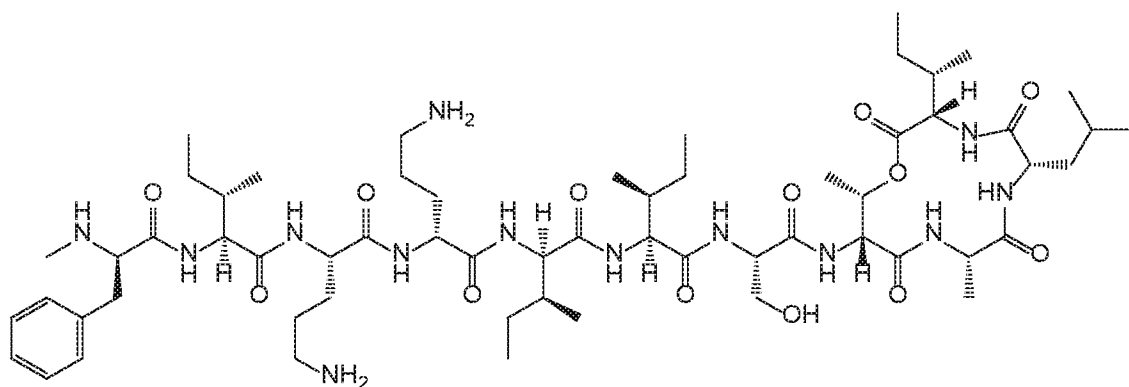
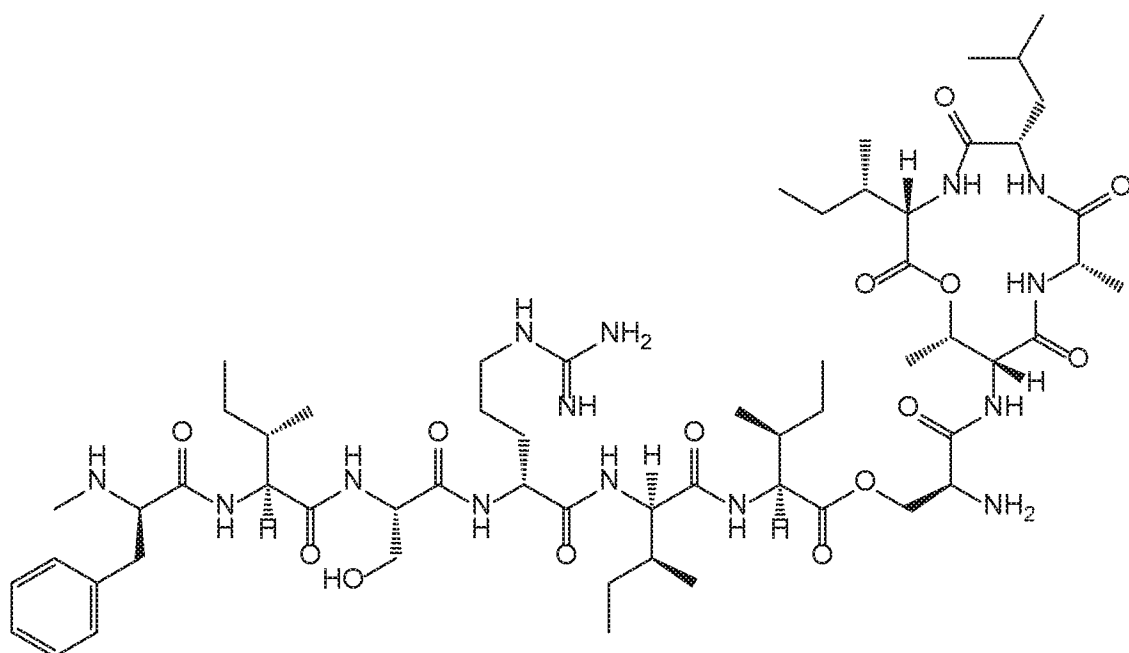


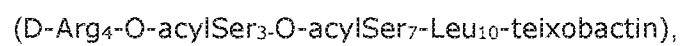
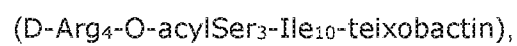
(D-Dap₄-Leu₁₀-teixobactin),

(D-Dab₄-Leu₁₀-teixobactin),(D-Orn₄-Leu₁₀-teixobactin),

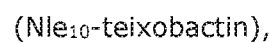
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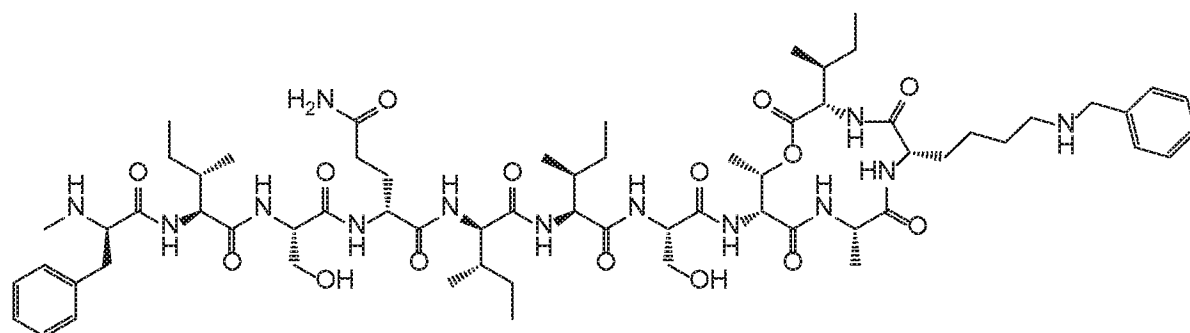
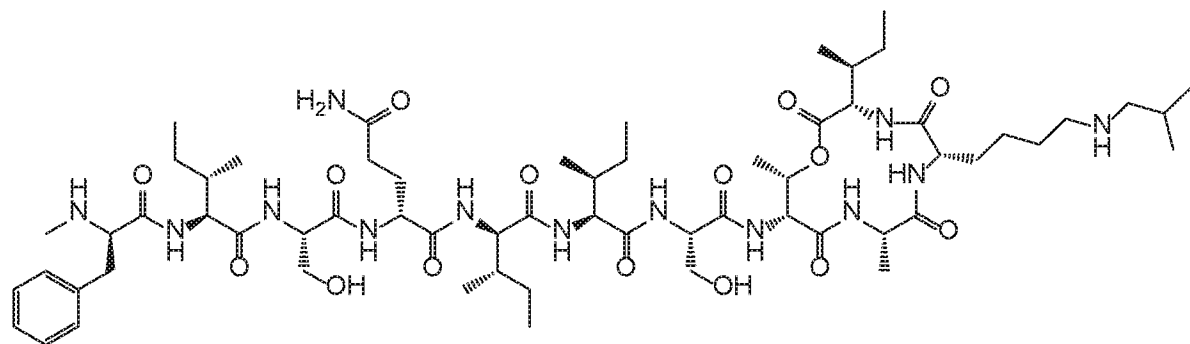
(Lys₃-D-Lys₄-Leu₁₀-teixobactin),(Dap₃-D-Dap₄-Leu₁₀-teixobactin),

(Dab₃-D-Dab₄-Leu₁₀-teixobactin),(Orn₃-D-Orn₄-Leu₁₀-teixobactin),(D-Arg₄-O-acylSer₇-Leu₁₀-teixobactin),

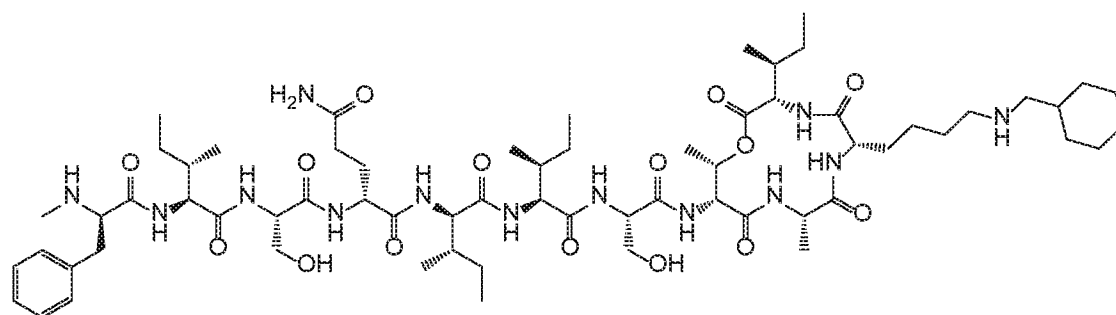
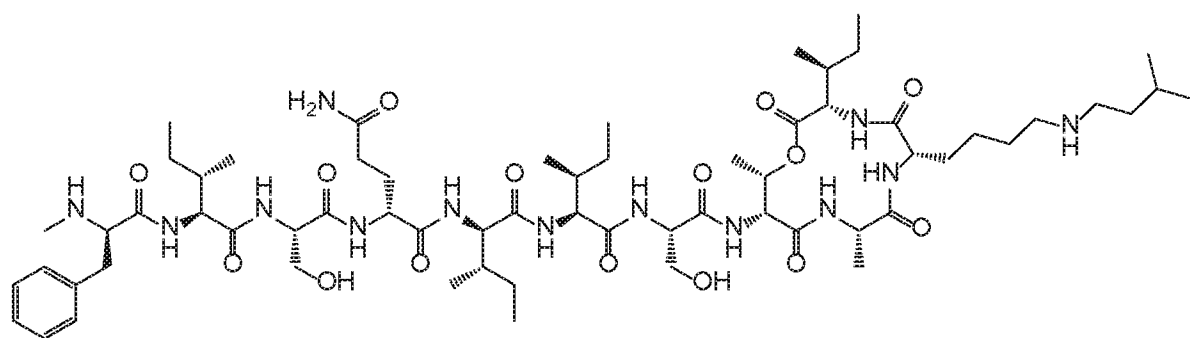


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(Lys₁₀(benzyl)-teixobactin),(Lys₁₀(isobutyl)-teixobactin),

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(Lys₁₀(cyclohexylmethyl)-teixobactin), and10 (Lys₁₀(isopentyl)-teixobactin),

or a pharmaceutically-acceptable salt, solvate or clathrate thereof.

The invention is illustrated by the following examples, with reference to the accompanying drawings in which:

Figure 1 shows representative confocal images of a) *S. epidermidis* 1457 and b) *S. aureus* 15981 biofilms stained with live/dead fluorescent probes; and scatter plots displaying dead/live biovolume ratio quantitatively estimated from 6 representative images for c) *S. epidermidis* 1457 biofilms and d) *S. aureus* 15981 biofilms. The biofilms were treated with either the teixobactin analogues (10 µg/ml) or vancomycin (10 µg/ml) for 5 h. The results were analyzed by two-way ANOVA, Turkey's multiple comparison test. ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$ and ****, $p \leq 0.0001$.

Figure 2 shows: a) Structure-activity correlation in teixobactin peptides showing the effect of side chain alkyl group on geometric mean minimum inhibitory concentrations (GM-MIC) values, which are determined from the MIC values against 16 different Gram-positive strains. Note a decrease in the values with increasing number of carbons in the side chain. b) Effect of two arginine substitution on GM-MIC values for Chg₁₀- and Nva₁₀-teixobactins. c) Effect of overall cationic charge on antimicrobial properties of teixobactins. Note that for Chg₁₀-teixobactin a linear increase in GM-MIC was observed whereas Nva₁₀- and Leu₁₀-teixobactins a non-linear relationship was observed. d) Concentration-dependent haemolytic activity of teixobactins for rabbit erythrocytes. The prolific pore-forming peptide, melittin was used as negative control.

Figure 3 shows the *in vivo* efficacy of teixobactin analogue in a murine non-neutropenic subcutaneous foreign body implant model (Teflon catheter) of *Staphylococcus aureus* ATCC 43300 infection.

The invention will now be described in more detail by reference to the following non-limiting Examples.

Examples

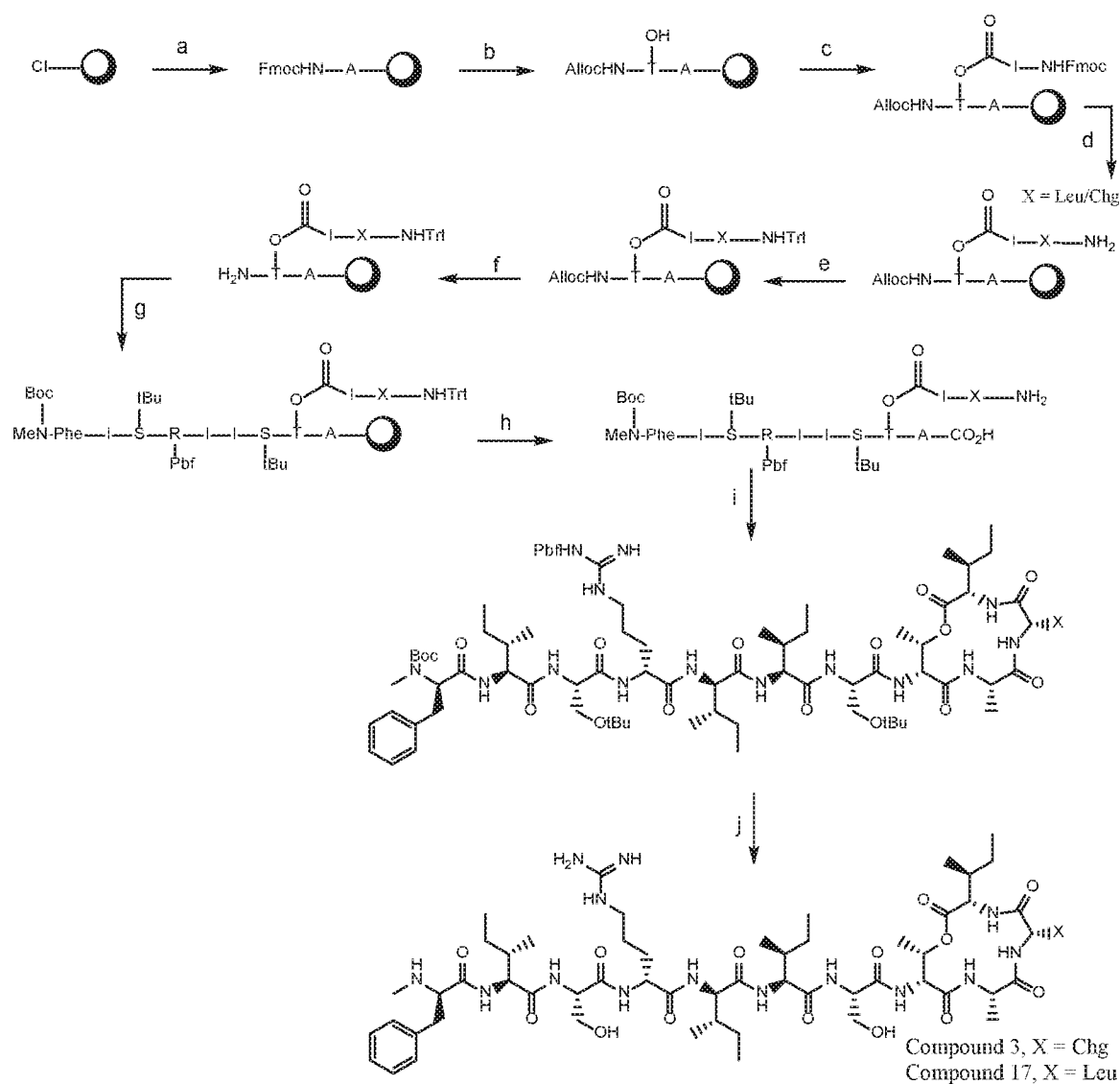
Abbreviations

AA	amino acid
ACN	acetonitrile
Alloc	allyloxycarbonyl
Boc	butyloxycarbonyl
CFU	colony forming unit
Chg	cyclohexylglycine
CLSM	confocal laser scanning microscopy

	DCM	dichlorometane	
	DIC	N,N'-diisopropylcarbodiimide	
	DIPEA	N,N-diisopropylethylamine	
	DMAP	4-dimethylaminopyridine	
5	DMF	dimethyl formamide	
	DMSO	dimethyl sulphoxide	
	Eq	equivalents	
	Et3N	triethylamine	
	Fmoc	fluorenylmethyloxycarbonyl	
10	HATU	1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate	
	MHB	Meuller-Hinton broth	
	MIC	minimum inhibitory concentration	
	Nle	Norleucine	
15	Nva	Norvaline	
	Pbf	2,2,4,6,7-pentamethyldihydrobenzofurane	
	PhSiH ₃	phenylsilane	
	RP-HPLC	reverse phase high performance liquid chromatography	
	TFA	trifluoroacetic acid	
20	TIS	triisopropylsilane	
	Trt	trityl	

Example 1 - Syntheses of Compounds 3 and 17

Teixobactin analogues (3 and 17) were synthesised by using the procedure given below.



Scheme 1: Synthesis of D-Arg₄-Chg₁₀-teixobactin (3) and D-Arg₄-Leu₁₀-teixobactin (17)

(step a) Commercially available 2-Chlorotriptyl chloride resin (manufacturer's loading = 1.2 mmol/g, 160 mg resin) was swelled in DCM in a reactor. To this resin was added 4 eq. Fmoc-Ala-OH/8 eq. DIPEA in DCM and the reactor was shaken for 3h. The loading determined by UV absorption of the piperidine-dibenzofulvene adduct was calculated to be 0.6 mmol/g, (160mg resin, 0.096 mmol). Any unreacted resin was capped with MeOH:DIPEA:DCM = 1:2:7 by shaking for 1h. (step b) The Fmoc protecting group was deprotected using 20% piperidine in DMF by shaking for 3 min, followed by draining and shaking again with 20% piperidine in DMF for 10 min. AllocHN-D-Thr-OH was then coupled to the resin by adding 3 eq. of the AA, 3 eq. HATU and 6 eq. DIPEA in DMF and shaking for 1.5h at room temperature. (step c) Esterification was performed using 10 eq. of Fmoc-Ile-OH, 10 eq. DIC and 5 mol% DMAP in DCM and shaking the reaction for 2h. This was followed by capping the unreacted alcohol using 10% Ac₂O/DIPEA in DMF shaking for 30 min and Fmoc was removed using protocol described earlier in step (b). (step d) Fmoc-

Chg-OH was coupled using 4 eq. of AA, 4 eq. HATU and 8 eq. DIPEA in DMF and shaking for 1h followed by Fmoc deprotection using 20% piperidine in DMF as described earlier. (step e) The N terminus of Chg was protected using 10 eq. Trt-Cl and 15% Et₃N in DCM and shaking for 1h. The protection was verified by the Ninhydrin colour test. (step f) The Alloc protecting group of D-Thr was removed using 0.2 eq. [Pd(PPh₃)]⁰ and 24 eq. PhSiH₃ in dry DCM under argon for 20 min. This procedure was repeated again increasing the time to 45 min and the resin was washed thoroughly with DCM and DMF to remove any Pd stuck to the resin. (step g) All amino acids were coupled using 4 eq. Amino Acid, 4 eq. DIC/Oxyma using a microwave peptide synthesizer. Coupling time was 10 min. Deprotection cycles were performed as described earlier. (step h) The peptide was cleaved from the resin without cleaving off the protecting groups of the amino acid side chains using TFA:TIS:DCM = 2:5:93 and shaking for 1h. (step i) The solvent was evaporated and the peptide was redissolved in DMF to which 1 eq. HATU and 10 eq. DIPEA were added and the reaction was stirred for 30 min to perform the cyclization. (step j) The side-chain protecting groups were then cleaved off using TFA:TIS:H₂O = 95:2.5:2.5 by stirring for 1h. The peptide was precipitated using cold Et₂O (-20°C) and centrifuging at 7000 rpm to obtain a white solid. This solid was further purified using RP-HPLC using the protocols described below.

Example 2 – Synthesis of Further Teixobactin Analogues (Compounds 1, 2, 4-16, 18-30)

Teixobactin analogues 1, 2, and 4-16 and 18-30 were synthesised according to the above procedure.

Compound Number	Name	Chemical formula	Exact mass	Mass found [M +H ⁺]
1*	Chg ₁₀ -teixobactin	C ₆₀ H ₉₈ N ₁₂ O ₁₅	1226.7275	1227.7357
2	Arg ₃ -Chg ₁₀ -teixobactin	C ₆₃ H ₁₀₅ N ₁₅ O ₁₄	1295.7965	1296.8052
3	D-Arg ₄ -Chg ₁₀ -teixobactin	C ₆₁ H ₁₀₂ N ₁₄ O ₁₄	1254.7700	1255.7777
4	Arg ₉ -Chg ₁₀ -teixobactin	C ₆₃ H ₁₀₅ N ₁₅ O ₁₅	1311.7915	1312.8022
5	Arg ₃ -D-Arg ₄ -Chg ₁₀ -teixobactin	C ₆₄ H ₁₀₉ N ₁₇ O ₁₃	1323.8391	1324.8438
6	Arg ₃ -Arg ₉ -Chg ₁₀ -teixobactin	C ₆₆ H ₁₁₂ N ₁₈ O ₁₄	1380.8605	1381.8714
7	D-Arg ₄ -Arg ₉ -Chg ₁₀ -teixobactin	C ₆₄ H ₁₀₉ N ₁₇ O ₁₄	1339.8340	1340.8285
8	Arg ₃ -D-Arg ₄ -Arg ₉ -Chg ₁₀ -teixobactin	C ₆₇ H ₁₁₆ N ₂₀ O ₁₃	1408.9031	1409.9061
9	Nva ₁₀ -teixobactin	C ₅₇ H ₉₄ N ₁₂ O ₁₅	1186.6962	1187.7044
10	Arg ₃ -Nva ₁₀ -teixobactin	C ₆₀ H ₁₀₁ N ₁₅ O ₁₄	1255.7652	1256.7739
11	D-Arg ₄ -Nva ₁₀ -teixobactin	C ₅₈ H ₉₈ N ₁₄ O ₁₄	1214.7378	1215.7493
12	Arg ₉ -Nva ₁₀ -teixobactin	C ₆₀ H ₁₀₁ N ₁₅ O ₁₅	1271.7602	1272.7729

Compound Number	Name	Chemical formula	Exact mass	Mass found [M + H ⁺]
13	Arg ₃ -Arg ₉ -Nva ₁₀ -teixobactin	C ₆₃ H ₁₀₈ N ₁₈ O ₁₄	1340.8292	1341.8318
14	D-Arg ₄ -Arg ₉ -Nva ₁₀ -teixobactin	C ₆₁ H ₁₀₅ N ₁₇ O ₁₄	1299.8027	1300.8132
15	Arg ₃ -D-Arg ₄ -Arg ₉ -Nva ₁₀ -teixobactin	C ₆₄ H ₁₁₂ N ₂₀ O ₁₃	1368.8718	1387.8879
16	Nle ₁₀ -teixobactin	C ₅₈ H ₉₆ N ₁₂ O ₁₅	1200.7118	1201.7234
17	D-Arg ₄ -Leu ₁₀ -teixobactin	C ₅₉ H ₁₀₀ N ₁₄ O ₁₄	1228.7543	1229.30
18	Arg ₃ -Leu ₁₀ -teixobactin	C ₆₁ H ₁₀₃ N ₁₅ O ₁₄	1269.7809	1270.7913
19	Arg ₃ -D-Arg ₄ -Leu ₁₀ -teixobactin	C ₆₂ H ₁₀₈ N ₁₇ O ₁₃	1297.8234	1298.8325
20	Arg ₃ -Ileu ₁₀ -teixobactin	C ₆₁ H ₁₀₄ N ₁₅ O ₁₄	1269.7809	1270.7896
21	D-Arg ₄ -Ileu ₁₀ -teixobactin	C ₅₉ H ₁₀₁ N ₁₄ O ₁₄	1228.7543	1229.7607
22	Arg ₉ -Leu ₁₀ -teixobactin	C ₆₁ H ₁₀₄ N ₁₅ O ₁₅	1285.7758	1286.7843
23	D-Lys ₄ -Leu ₁₀ -teixobactin	C ₅₉ H ₁₀₀ N ₁₂ O ₁₄	1200.7482	1202.00
24	D-Dap ₄ -Leu ₁₀ -teixobactin	C ₅₆ H ₉₄ N ₁₂ O ₁₄	1158.7012	1159.96
25	D-Dab ₄ -Leu ₁₀ -teixobactin	C ₅₇ H ₉₆ N ₁₂ O ₁₄	1172.7169	1174.00
26	D-Orn ₄ -Leu ₁₀ -teixobactin	C ₅₈ H ₉₈ N ₁₂ O ₁₄	1186.7325	1188.04
27	Lys ₃ -D-Lys ₄ -Leu ₁₀ -teixobactin	C ₆₂ H ₁₀₇ N ₁₃ O ₁₃	1241.8111	1243.04
28	Dap ₃ -D-Dap ₄ -Leu ₁₀ -teixobactin	C ₅₆ H ₉₅ N ₁₃ O ₁₃	1157.7172	1158.95
29	Dab ₃ -D-Dab ₄ -Leu ₁₀ -teixobactin	C ₅₈ H ₉₉ N ₁₃ O ₁₃	1185.7485	1187.17
30	Orn ₃ -D-Orn ₄ -Leu ₁₀ -teixobactin	C ₆₀ H ₁₀₃ N ₁₃ O ₁₃	1213.7798	1215.11

*reference compound

Table 1: Compound number, name, chemical formula, exact mass and mass found for compounds 1-30.

Materials

All L amino acids, Fmoc-D-Arg(pbf)-OH, Fmoc-D-Gln(Trt)-OH, Boc-N-methyl-D-phenylalanine, 1 [Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium-3-oxidhexafluorophosphate (HATU), Phenylsilane (PhSiH₃), Tetrakis(triphenylphosphine)palladium(0) [Pd(PPh₃)], Diisopropylcarbodiimide (DIC) and Triisopropylsilane (TIS) were purchased from Fluorochem, UK. Fmoc-D-*allo*-Ile-OH and oxyma pure were purchased from Merck Millipore. The side chain protecting groups for the amino acids are tBu for Ser, Pbf for Arg and Trt for Gln and Thr unless specified otherwise. Diisopropylethylamine (DIPEA), supplied as extra dry, redistilled, 99.5 % pure, Acetic anhydride, allyl chloroformate, CDCl₃ and polysorbate 80 and were purchased from Sigma Aldrich. Tritylchloride and 4-(Dimethylamino)pyridine were purchased from Alfa Aesar.

Dimethylformamide (DMF) peptide synthesis grade was purchased from Rathburn chemicals. Triethylamine, Diethyl ether (Et₂O), Dimethylsulfoxide (DMSO), Dichloromethane (DCM), Tetrahydrofuran (extra dry with molecular sieves), Formic acid 98-100% purity and Acetonitrile (HPLC grade) were purchased from Fisher Scientific.

5 Water with the Milli-Q grade standard was obtained in-house from an ELGA Purelab Flex system. 2-Chlorotriylchloride resin (manufacturer's loading: 1.20 mmol/g) was purchased from Fluorochem. All chemicals were used without further purification.

Equipment used for the analysis and purification of compounds

10 All peptides were analysed on a Thermo Scientific Dionex Ultimate 3000 RP-HPLC equipped with a Phenomenex Gemini NX C18 110 Å (150 x 4.6 mm) column using the following buffer systems: A: 0.1% HCOOH in milliQ water. B: ACN using a flow rate of 1 ml/min. The column was flushed with 95% A for 5 min prior to an injection and was flushed for 5 min with 95% B and 5% A after the run was finished.

15 Peptides were dissolved in (1:1) 0.1% HCOOH buffer in water and acetonitrile (ACN) and analysed using the following gradient: 95% A for 2 min. 5-95% B in 25 min. 95% B for 5 min. 5% A for 4 min. Peptides were dissolved in 0.1% HCOOH buffer in water and in ACN (10-30% ACN) and purified using the same gradient as mentioned above, on a Thermo

20 Scientific Dionex Ultimate 3000 RP-HPLC with a flow rate of 5 mL/min using a Phenomenex Gemini NX C18 110 Å (150 x 10 mm) semi-prep column.

Example 3 – MIC Testing

25 Bacterial cultures were grown overnight in Mueller-Hinton Agar (MHA) plates and adjusted to a final concentration of 10⁵ – 10⁶ CFU/ml. 100 µl of inoculum in Mueller-Hinton broth (MHB) was mixed with equal volume of peptides (dissolved in MHB) at 2x their concentration in a 96 well plate. In parallel experiments, MIC values were determined in the media containing polysorbate 80 (0.002%, v/v) to prevent non-specific adsorption of

30 the peptides to plastic surfaces. The final peptides concentrations ranged from 0.0625 – 32 µg/ml (for lower range 0.031 – 16 µg/ml was used). Positive and negative controls contained 200 µl of inoculum without any peptide dissolved in broth, respectively. The 96 well plates were then incubated at 37 °C for 24 h. All the experiments were performed in two independent duplicates and the MIC was determined as the lowest concentration in

35 which no visible growth was observed.

The antimicrobial properties of the synthetic peptides were investigated against a panel of 16 Gram-positive strains (Tables 1A and 1B). To gain better insight into the structure-activity relationship, we determined the geometric mean MIC (GM-MIC) values for all of the peptides and compared the results. A clear correlation between the number of carbons in the sidechain at position 10 and GM-MIC was observed (**Fig. 2a**). The best overall results were obtained for compounds containing a Chg₁₀ substitution (compounds 1-8), which showed highly potent antibacterial activity against all bacteria tested with MIC values in the range of 0.0625-0.125 µg/mL and a GM-MIC of 0.07 µg/mL. Thus, natural or unnatural residues carrying bulky side chains of ≥4 carbons (Chg or Ile) at position 10 resulted in the lowest GM-MIC values (**Fig. 2a**). This was further supported by the fact that substitution of residues containing linear alkyl groups (Nle) (16) resulted in 2-fold higher GM-MIC values than Ile₁₀-teixobactin (18). Among the amino acids containing isopropyl or n-propyl (Val or Nva) side chains at position 10; Val₁₀-teixobactin had a two-fold higher GM-MIC than Nva₁₀-teixobactin (9). Next, we investigated the importance of the overall net charge of the peptides on GM-MIC values. In particular, we focused our attention on the substitution of L-Arg residues at positions 3 and 9 and D-Arg at position 4 in Chg₁₀-teixobactin and Nva₁₀-teixobactin. The results indicated that substitution of cationic residues at all three positions did not affect the GM-MIC values for Chg₁₀ and Nva₁₀ teixobactin analogues. These results are consistent with the results obtained for Leu₁₀-teixobactin (**Fig. 2b**). However, substitution of L-Arg at position 3 or 9 increased the GM-MIC values for Ile₁₀-teixobactin, suggesting that Chg₁₀/Nva₁₀-teixobactin analogues have broader scopes for the substitution of cationic residues.

Tables 2 to 4 - MICs (in µg/ml) of compounds **1-16** tested against a panel of Gram-positive bacteria. Table 2 compares compounds **1-8** which contain a Chg₁₀ substitution. Table 3 compares compounds **9-15** which have a Nva₁₀ substitution, while compound **16** has an Nle₁₀ substitution. Both tables compare the synthesized teixobactin analogues to daptomycin as the control in the experiment. Table 4 shows MICs (in µg/ml) of compounds **17-30** tested against MRSA.

Table 2

	1	2	3	4	5	6	7	8	Dapt
saprophyticus ATCC BAA 750	0.0625	0.0625	0.0625	0.0625	<0.0625	<0.0625	0.125	<0.0625	0.12
Staphylococcus saprophyticus ATCC 15305	<0.0625	<0.0625	<0.0625	<0.0625	<0.0625	<0.0625	<0.0625	<0.0625	0.12
Staphylococcus saprophyticus ATCC 49453	<0.0625	<0.0625	<0.0625	0.125	0.125	0.0625	0.0625	0.0625	0.12

Staphylococcus saprophyticus ATCC 49907	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.12
VRE 1014	0.25	0.0625	0.0625	0.25	1	1	1	2	2
VRE ATCC 700802	0.0625	0.0625	0.0625	0.5	2	1	1	4	1
VRE ATCC 29212	0.125	0.125	0.0625	1	2	1	1	2	1
MRSA ATCC 700699	0.125	0.0625	0.0625	0.5	0.25	0.5	0.5	1	2
MRSA 42412	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.125	1	1
MRSA 21455	0.0625	0.125	0.25	0.5	0.25	0.5	0.25	2	0.5
MRSA 1003	0.0625	0.0625	0.0625	0.125	0.5	0.5	0.5	1	2
Staphylococcus aureus 29213	0.0625	0.0625	0.0625	0.0625	0.0625	0.25	0.5	0.5	0.25
Staphylococcus aureus 4299	0.0625	0.0625	0.0625	0.0625	0.125	0.0625	0.5	1	0.25
Staphylococcus epidermidis 12228	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.12
Bacillus Cereus ATCC 11788	0.0625	0.0625	0.0625	0.25	2	1	1	0.5	0.25
Bacillus Subtilis ATCC 6633	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.12

Dapt = Daptomycin

Table 3

	9	10	11	12	13	14	15	16	Dapt
Staphylococcus saprophyticus ATCC BAA 750	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.5	0.0625	0.12
Staphylococcus saprophyticus ATCC 15305	0.0625	0.0625	0.0625	0.0625	0.125	0.0625	0.25	0.0625	0.12
Staphylococcus saprophyticus ATCC 49453	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.25	0.0625	0.12
Staphylococcus saprophyticus ATCC 49907	0.0625	0.0625	0.0625	0.0625	0.0625	0.0625	0.5	0.0625	0.12
VRE 1014	1	1	1	2	2	2	8	1	2
VRE ATCC 700802	1	1	0.5	1	2	1	32	0.5	1
VRE ATCC 29212	1	2	1	2	2	2	32	1	1
MRSA ATCC 700699	0.5	0.25	0.0625	0.5	0.25	0.5	32	0.5	2
MRSA 42412	0.0625	0.0625	0.0625	0.0625	0.125	0.125	32	0.125	1
MRSA 21455	0.125	0.0625	0.125	0.25	0.5	0.5	16	0.5	0.5
MRSA 1003	0.25	0.125	0.125	1	1	1	>32	0.5	2

Staphylococcus aureus 29213	0.125	0.125	0.125	0.5	0.5	0.25	16	0.0625	0.25
Staphylococcus aureus 4299	0.0625	0.0625	0.0625	0.25	0.25	0.0625	16	0.25	0.25
Staphylococcus epidermidis 12228	0.125	0.0625	0.0625	0.0625	0.0625	0.0625	2	0.0625	0.12
Bacillus Cereus ATCC 11788	0.0625	0.0625	0.5	0.5	1	1	2	0.0625	0.25
Bacillus Subtilis ATCC 6633	0.0625	0.0625	0.0625	0.0625	0.0625	0.5	16	0.0625	0.12

Dapt = Daptomycin

Table 4 - MICs (in µg/ml) of compounds **17-30** tested against MRSA.

Compound	MIC (ug/mL) MRSA ATCC 33591
17	0.125
18	0.125
19	0.25
20	0.25
21	0.125
22	0.25
23	0.125
24	0.5
25	0.125
26	0.125
27	0.25
28	0.25
29	0.25
30	0.5

5 Results are shown in **Fig. 2**.

Increasing the net cationicity by substituting two and three arginine residues still maintained high antibacterial potency (MIC lower than 1 µg/ml, MIC of 4 µg/ml or lower is clinically relevant) of modified teixobactin analogues. (**Fig. 2c**). Arginine substitutions in case of Chg₁₀-teixobactin had the lowest effect on the GM-MIC values compared to Leu₁₀-
 10 and Nva₁₀-teixobactin analogues, making it easier to balance the amphipathic nature of Chg₁₀-teixobactin.

Most of the analogues showed potent antibacterial activity (MIC) against MRSA/*S. aureus* strains (0.125-2 µg/mL). To determine the effect of cationicity on bactericidal properties,
 15 we determined the minimum bactericidal concentration (MBC) of teixobactin analogues

against tested MRSA/ *S. aureus* strains. In Chg₁₀-teixobactin analogues, a clear decrease in the MBC was observed as the number of charged residues increased, suggesting possible membrane perturbation.

To determine if substitution of charged residues altered the cytotoxicity, we determined the hemolytic activity levels of peptides **1**, **3**, **5**, **8** and **16** for rabbit erythrocytes. The results suggested that peptides **1**, **3** and **16** did not show appreciable hemolytic activity ($\leq 2\%$), even at 512 $\mu\text{g/mL}$, suggesting their excellent microbial cell selectivity (**Fig. 2d**). Peptides **5** and **8** displayed good microbial cell selectivity (3.7%, 5.4% hemolytic activity) at 64 $\mu\text{g/mL}$ (128 times higher than the MIC). However, peptides **5** and **8** displayed 18% and 25% hemolytic activity at 256 $\mu\text{g/mL}$, respectively. These results suggest that increasing the cationicity of Chg₁₀-teixobactin above +2 resulted in heightened hemolytic activity. Nevertheless, the peptides still required $>200\times$ GM-MIC to achieve a cytotoxic effect for rabbit erythrocytes. It is likely that the increase of cationicity may induce variable interactions with the zwitterionic phospholipids present in the cytoplasmic membranes of mammalian cells, thus causing membrane perturbation and eventual hemolytic activity. The stability of the compounds was also assessed in serum and plasma and the compounds were showed excellent stability.

Example 4 – *in vitro* Biofilm Studies

Study

Commensal biofilm former pathogens such as *S. aureus* and *S. epidermidis* are known to colonize implants at the insertion sites and they are challenging to treat due to their inherent resistance to antimicrobial therapies and the host immune responses. To test the potency of teixobactin peptides, we determined their anti-biofilm properties against prolific biofilm forming strains *S. aureus* 15981 and *S. epidermidis* 1457 using a static biofilm model (Mol Microbiol. 2003 May;48(4):1075-87 and J Bacteriol. 2005 Aug;187(15):5318-29). The preformed biofilms were treated with teixobactin analogues (**1**, **3**, **5**, **8** and **16**) for 5 h as detailed below.

Method

The bacterial strains were cultured overnight in 2 ml of MH11 media supplemented with 0.002% Tween (MHII-PS80) at 37°C shaking incubator (200 rpm). The overnight cultures were diluted 1: 100x with fresh media. Two hundred microlitres of the diluted culture was added into each well of the μ -Slide 8 Well (catalogue number: 80826, ibidi®, Germany) and was placed in 37°C incubator for 18 h for biofilm development. The supernatant, which consists of the unbound cells, was removed and fresh medium containing either

vancomycin (10 µg/ml) or teixobactin analogue test substance (10 µg/ml) was added to the biofilms. After 5 h of treatment, the medium was removed and fresh medium containing 3.34 µM SYTO9 and 10 µM propidium iodide (LIVE/DEAD™ BacLight™ Bacterial Viability Kit) was added to the well to stain the live and dead cells respectively for 15 min.

5 The confocal images of the biofilms were captured and acquired using Zeiss LSM780 confocal laser scanning microscopy (CLSM; Carl Zeiss, Germany) with x100 objective lens. Green and red fluorescence were observed using 488 nm laser and 561 nm laser respectively. The acquired images were processed using IMARIS version 9 software (Bitplane AG, Zurich, Switzerland) to obtain the biovolumes of the live and dead cell
10 populations and the dead-to-live biovolume ratio graphs were plotted using GraphPad Prism 7 software (United States).

Results

The teixobactin analogues displayed potent antibiofilm properties against both *S. aureus* and *S. epidermidis* biofilms when compared to comparator antibiotics (vancomycin at 10
15 µg/mL). In the case of *S. epidermidis* biofilm, the dead/live biovolume ratio remained similar for untreated and vancomycin ($p>0.05$)-treated groups (**Fig. 1a** and **Fig. 1c**).

The preformed biofilms were treated with a teixobactin analogue (**3**, **5** and **16**) for 5 h, and the teixobactin analogues displayed potent antibiofilm properties against both *S.*
20 *aureus* and *S. epidermidis* biofilms when compared to comparator clinical antibiotic (vancomycin at 10 µg/mL). In the case of *S. epidermidis* biofilm, the dead/live biovolume ratio remained similar for untreated and vancomycin ($p>0.05$)-treated groups (**Fig. 1a** and **Fig. 1c**). However, the values increased by 2- to 18-fold upon treatment with other teixobactins, suggesting susceptibility and the peptides triggered considerable cell death
25 (more yellow- and red-stained cells). It should be noted that the antibiofilm properties of modified teixobactins were superior to those of the glycopeptide antibiotic, vancomycin. Teixobactin analogues 1 and 8 were also tested and found to be effective (data not shown).

However, an interesting trend was observed when we investigated the activity of peptides
30 against *S. aureus* biofilms (**Fig. 1b** and **Fig. 1d**). At similar concentrations, all the teixobactin analogues displayed higher dead/live cell ratios than the comparator clinically used antibiotic vancomycin ($p<0.001$ for **3**, $p<0.01$ and $p\leq 0.05$ for peptide **16**). These results establish the potent antibiofilm properties of teixobactin analogues against commensal pathogens. To the best of our knowledge, this is the first report demonstrating
35 the antibiofilm properties of synthetic teixobactin analogues against *S. aureus* and *S. epidermidis*. Peptide **3** displayed the optimum antimicrobial/ antibiofilm properties and showed no hemolytic activity for rabbit erythrocytes.

Example 5 – *in vivo* Biofilm Studies

Study: *In vivo* efficacy of teixobactin analogue in a murine non-neutropenic subcutaneous foreign body implant model (Teflon catheter) of *Staphylococcus aureus* ATCC 43300 infection. The teixobactin analogue **17** (as obtained in Example 2) was evaluated for prevention of biofilm formation in subcutaneous implanted Teflon catheters infected with *S aureus* ATCC 43300 in a 7 day murine model. The male CD 1 mice were implanted with a single Teflon catheter. 100 µL of bacterial inoculum was administered into each catheter lumen (1.7×10^6 CFU/catheter implant). A robust foreign body infection model in mice was established with *S. aureus* ATCC 43300 burden reaching 4.15×10^5 CFU/catheter in the vehicle treated mice 7 days post infection, corresponding to a decrease of 0.67 and 1.07 Log₁₀ CFU/catheter from 2h and 48h post infection. Vehicle and teixobactin analogue **17** treated mice showed little or no clinical signs of ill health, with body weight gain in line with mice of similar age. Rifampicin treated mice showed decrease in weight until day 2 post infection and thereafter gained weight. By day 5 the body weight was similar to vehicle treated animals.

Teixobactin analogue **17** administered SC Q8h 25 and 50 mg/kg starting at 2h post infection showed robust efficacy and resulted in a statistically significant burden reduction of 5.25 and 5.11 Log₁₀ CFU/catheter compared to vehicle, corresponding to 5.92 and 5.78 Log₁₀ CFU/catheter below the level of stasis, respectively. 6 out of 8 implants from each treatment group had bacterial burden below the limit of detection.

Rifampicin administered PO Q24h at 10 mg/kg reduced bacterial burden by 5.33 Log₁₀ CFU/catheter compared to vehicle, corresponding to 6.00 Log₁₀ CFU/catheter below the level of stasis. 5 out of 7 implants had burden below the limit of detection. Results are shown in **Fig. 3**.

Example 6: Synthesis of Further Teixobactin Analogues (Compounds 31-34)

The following compounds may be made by methods analogous to those in Examples 1 and 2:

Compound Number	Name
31	D-Arg ₄ -O-acylSer ₇ -Leu ₁₀ -teixobactin
32	D-Arg ₄ -O-acylSer ₃ -Ile ₁₀ -teixobactin
33	D-Arg ₄ -O-acylSer ₃ -O-acylSer ₇ -Leu ₁₀ -teixobactin

Table 5: Compound number and name for compounds **31-33**.

The following compound has been made by a method analogous to that in Example 1:

Compound Number	Name	Chemical formula	Exact mass	Mass found [M + H ⁺]
34	D-Arg ₄ -Nle ₁₀ -teixobactin	C ₅₉ H ₁₀₀ N ₁₄ O ₁₄	1228.7543	1229.58

Table 6: Compound number, name, chemical formula, exact mass and mass found for compound **34**.

5 Example 7: Synthesis of Further Teixobactin Analogues (Compounds 35-38)

The following compounds have been made using methods analogous to those in Examples 1 and 2:

Compound Number	Name	Chemical formula	Exact mass	Mass found [M + H ⁺]
35	Lys ₁₀ (Benzyl)-teixobactin	C ₆₅ H ₁₀₃ N ₁₃ O ₁₅	1305.7697	1307.10
36	Lys ₁₀ (Isobutyl)-teixobactin	C ₆₂ H ₁₀₅ N ₁₃ O ₁₅	1271.7853	1273.21
37	Lys ₁₀ (Cyclohexylmethyl)-teixobactin	C ₆₅ H ₁₀₉ N ₁₃ O ₁₅	1311.8166	1314.27
38	Lys ₁₀ (Isopentyl)-teixobactin	C ₆₃ H ₁₀₇ N ₁₃ O ₁₅	1285.8010	1288.13

Table 7: Compound number, name, chemical formula, exact mass and mass found for compounds **35-38**.

10

Example 8 – MIC Testing

MIC testing for compounds **34** to **38** was performed in accordance with the methods disclosed in Example 3. The results are shown below.

Compound	MIC (ug/mL) MRSA ATCC 33591
34	0.25
35	0.125
36	0.25
37	0.063
38	0.063

Table 8 - MICs (in µg/ml) of compounds **34-38** tested against MRSA.

15

Example 9: MBEC testing

MBEC testing was performed for compounds **35** to **38** using the following assay:

I. Inoculum Preparation and Plating

1. The selected bacterial strain was streaked on Mueller-Hinton Agar (MHA) and incubated at 37 °C overnight.
2. Prepare MHA and pour 50 mL each to square Petri dishes. Set aside and keep in the fridge. (This will be used for biofilm stamping)

20

3. Tryptic soy broth (TSB) was autoclaved, cooled and supplemented with D-glucose sterile filtered solution 1% w/v (TSG) (0 – 2% depending on growth condition).
4. 2-3 colonies of bacterial strain are emulsified in 3 mL of phosphate buffered saline to match the turbidity of 0.5 McFarland standard solution. At least 2 triplicates of inoculum were prepared for each bacterial strain tested.
5. Dilute each inoculum PBS by 1:100 into TSG (3 separate tubes) to achieve a colony count of about 1×10^6 CFU/mL. (i.e. A1 B, A2 B)
6. Nunclon 96-well flat-bottomed plates (Thermo Scientific) were used for biofilm formation assay.
7. Using a multichannel pipette, pipette 100 μ L of sterile dH₂O into each well of all outer wells.
8. Using a multichannel pipette, pipette 100 μ L of TSG into wells B-G11 as negative control.
9. (For biofilm formation and MBEC) Using a multichannel pipette, pipette 100 μ L of inoculum broth to each well.
10. Incubate the plates at 37 C, refer to session II for MBEC.

II. *Minimal Biofilm Eradication Concentration (MBEC) Assay*

1. Remove the incubated microtiter plates from session 1.
2. Read the plates at OD_{600nm} for viable cells prior to treatment.
3. Using a multichannel pipette, remove the solutions from each well.
4. Rinse the wells with 100 μ L of PBS/sterile water, remove the solutions from the wells and repeat this step 2-3 times.
5. Prior to drug treatment, aspirate as much liquid as possible from each biofilm-containing well.
6. Add 50 μ L fresh TSG into each well using a multichannel pipette.
7. Prepare the drug broths as indicated in drug broth preparation. (In this case, the serial dilution of drugs in plate needs to be performed in a separate, non-treated 96-well plate.)
8. Add 50 μ L of drug broth to the wells at the plate.
9. Incubate the plates for 20-24h at 37 C at static conditions.
10. Read OD_{600nm} for viable cells after drug treatment.
11. Refer to session IV to VI for analysis.

III. *OD₆₀₀, Stamping, Assess Biofilm Viable Cells by Colony Count (CFU)*

1. For MBEC CV & CFU plates, read OD_{600nm} of the plates (this represents the OD for the viable cells).

2. Using a replicator (autoclaved and sterile), insert the replicator into the plate until it reaches the bottom of the wells. Lift and gently stamp the replicator on a square MHA plate for each 96 well plate. (*Wash the pins with 70% ethanol and dry in between the plates to avoid contamination)
- 5 3. Label the plates with sample name, MBEC and strain, incubate the agar plates at 37 °C overnight.
4. For the CFU plate, identify 2 positive control wells and 2 wells with 0.0X OD_{600nm} reading, add 100 µL PBS/TSG to each of the two cells.
5. Use a single-channel pipette, strap the biofilm and suspend the biofilm into the media. (CFU count for this well is considered as -2.)
- 10 6. For 1 well for positive and 1 well for MBEC, add this 100 µL to a MHA plate, spread and set aside.
7. For the others, add this 100 µL to Eppendorf tube with 900 µL PBS (-3), perform serial dilution until -9 is achieved. (-3, -4, -5, -6, -7, -8, -9).
- 15 8. Plate the dilutions on separate MHA plates. Incubate all the labelled plates at 37 C for 16-20h.
9. Perform CFU count for each plate.
10. For MBEC, the CFU count for positive control is essential so that a reduction in CFU can be observed after drug treatment.

IV. Assess Biofilm Biomass by CV staining

1. Perform the washing processes (refer to Session 3 Steps 3 to 5).
2. Prepare a solution of 0.1% crystal violet (CV) solution either by diluting 1% CV solution with ethanol or dissolve the required amount of CV powder with ethanol.
- 25 3. Using a multichannel pipette, add 125 µL of 0.1% CV solution to each well containing the biofilm. Incubate the plates at room temperature for 10-15 mins. (*CV solution is fairly volatile, ensure to aspirate the pipette SLOWLY to avoid the solution shooting up to the pipette)
4. Use a multichannel pipette, remove the CV solution from each well (take care with aspiration).
- 30 5. Add 125 µL of PBS/sterile water, remove and repeat the rinsing for 2 to 3 times more until CV dyes have been removed successfully.
6. Turn the microtiter plates upside down and dry in the class 2 cabinet for a few hours and overnight.
- 35 7. For qualitative assays, the CV-stained wells can be photographed when the plates are dry.
8. Prepare a solution of 30% acetic acid in water w/v (for *S. aureus*, depending on bacterial strain 99% ethanol is often used).

9. Add 125 μ L of 30% acetic acid in water to each well with stained biofilm.
10. Incubate the microtiter plate at room temperature, shaken at 600 rpm for 10-15 mins (use the microtitre plate shaker in Molecular Biology lab – can fit 4 plates at a time).
11. Transfer 125 μ L of the solubilised CV to a new flat-bottomed microtiter plate (untreated is ok).
12. Quantify the absorbance in a plate reader at 595 nm using 30% acetic acid in water as blank.

V. Assess Metabolic Activity of Biofilm Cells by Resazurin

1. A solution of resazurin is prepared by dissolving 4 μ g or 8 μ g of resazurin powder in 1 mL of sterile PBS.
2. The solution is sterile filtered and stored at 4 C in the dark (wrap the container with foil completely and labelled) (*only prepare the resazurin solution on the day of the assay)
3. Wash the plate with PBS/sterile water as required.
4. Add 100 μ L of diluted resazurin solution was added to each well (excluding blank outer wells).
5. The plates are incubated at room temperature in the dark for 20 mins.
6. Using Varioskan LUX, measure the relative fluorescence units (RFU) (λ_{Ex} 530nm and λ_{Em} 590nm) after incubation. (*Remember to save this method separately from OD_{600nm} and OD_{595nm} method) This is T=0 reading (time 0).
7. Repeat the readings at 20-min intervals for up to 80 min (cover the plate with foil and leave in the dark in between readings). The experiment was performed twice with three replicates.

VI. Determination of MBEC values

MBEC values were determined by the lowest concentration of antibiotic which displayed biofilm inhibition of >90% based on RFU and CFU determination.

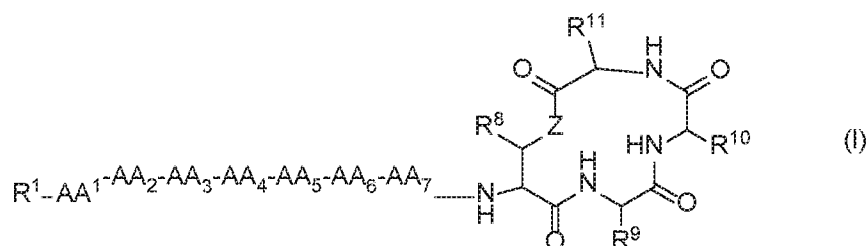
Results

Strain: MRSA ATCC 43300	
Compound No.	Minimum Biofilm Eradication Concentration (MBEC), (μ g/mL)
35	8
36	16
37	16
38	8
Vancomycin	32

Table 9 – MBEC results for compounds **35-38**

Claims

1. A compound of formula (I), or a pharmaceutically-acceptable salt, solvate or clathrate thereof, for use in killing, inhibiting, or preventing the growth of a microbial biofilm,



wherein:

R¹ represents H, C₁₋₆ alkyl, C₁₋₆ acyl, benzyl or benzoyl;

AA¹ represents any hydrophobic proteinogenic or hydrophobic non-proteinogenic amino acid;

AA², AA⁵ and AA⁶ each independently represents an isoleucine or an *allo*-isoleucine residue;

AA³ and AA⁴ each independently represents a proteinogenic or non-proteinogenic amino acid;

AA⁷ represents a serine residue;

R⁸ represents hydrogen or C₁₋₄ alkyl;

R⁹ represents a proteinogenic or non-proteinogenic amino acid side chain;

R¹⁰ represents, a hydrophobic proteinogenic or hydrophobic non-proteinogenic amino acid side chain;

R¹¹ represents a proteinogenic or non-proteinogenic amino acid side chain; and

Z is -O- or -NH-;

provided that the compound of formula (I), or the pharmaceutically-acceptable salt, solvate or clathrate thereof, is not L-Chg₁₀-teixobactin.

2. The compound for use according to Claim 1 wherein R¹⁰ represents a linear or branched C₁₋₆ alkyl group optionally substituted by -NH(R^{10a}) or -NHCH₂(R^{10a}), wherein R^{10a} represents a C₁₋₆ alkyl or phenyl group.

3. The compound for use according to Claim 2 wherein R¹⁰ represents a linear propyl or butyl group.

4. The compound for use according to any one of the preceding claims wherein one or more of R⁹, the side chain of AA³ and the side chain of AA⁴ represents a moiety selected from the group consisting of a positively charged amino acid side chain, -CH₂-NH₂, -(CH₂)₂-NH₂, -(CH₂)₃-NH₂, and a fragment of formula -L¹-L²-L³-X¹; wherein:

L¹ represents a linear or branched C₁₋₁₂ alkylene linker;

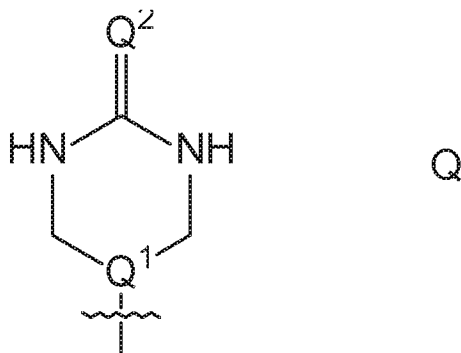
L² is selected from the group consisting of -O-, -N(X^a)-, -[N(X^a)₂]⁺-, -C(O)-, -OC(O)-, -C(O)O-, -NHC(O)-, -C(O)N(X^a)-, -OC(O)N(X^a)-, -NHC(O)O-, or -NHC(O)N(X^a)-;

X^a represents hydrogen or -L³-X¹;

L³ represents a direct bond or a linear or branched C₁₋₁₂ alkylene linker;

each X¹ is independently selected from the group consisting of -C(O)-C₁₋₁₂ alkyl, -C(O)-NH₂, -C(S)-NH₂, a fragment of formula Q, and a C₁₋₁₂ alkyl group optionally substituted by one or more X² substituents;

wherein the fragment of formula Q is:



in which Q¹ represents either CH or N, and Q² represents O, S or NH;

each X^2 independently represents $-NH_2$, $-OH$, $-NHC(O)NH_2$, $-NHC(S)NH_2$, $-NHC(=NH)NH_2$, or a 3- to 12- membered heterocyclyl group;

or $-L^2-L^3-X^1$ together represent a fragment of formula Q.

5

5. The compound for use according to Claim 4 wherein one or more of R^9 , the side chain of AA^3 and the side chain of AA^4 , represents a side chain of an amino acid selected from the group consisting of arginine, lysine, histidine, ornithine, 2,3-diaminopropionic acid, and 2,4-diaminobutyric acid.

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6. The compound for use according to Claim 5 wherein one or more of R^9 , the side chain of AA^3 and the side chain of AA^4 , represents an arginine side chain.

15

7. The compound for use according to Claim 6 wherein AA^4 represents an arginine residue.

8. The compound for use according to any one of the preceding claims wherein AA^1 represents a phenylalanine residue.

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9. The compound for use according to any one of the preceding claims wherein R^8 represents hydrogen or a methyl group.

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10. The compound for use according to any one of the preceding claims wherein R^9 represents $-CH_2-NH_2$, $-(CH_2)_2-NH_2$, $-(CH_2)_3-NH_2$, a hydroxy group, an amide functional group (which latter two groups are bound to the remainder of the molecule via a linear, branched, cyclic or part cyclic C_{1-8} alkylene group), the side chain of an amino acid selected from the group consisting of histidine, lysine, arginine, alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, norvaline, cyclohexylglycine, cyclohexylalanine, phenylglycine, biphenylglycine, biphenylalanine, naphthylglycine, naphthylalanine, serine, threonine, asparagine and glutamine, or a fragment of formula $-L^1-L^2-L^3-X^1$ as defined in Claim 4.

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11. The compound for use according to any one of the preceding claims wherein R^{11} represents a side chain of alanine, valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, norvaline, cyclohexylglycine, cyclohexylalanine, phenylglycine, biphenylglycine, biphenylalanine, naphthylglycine or naphthylalanine.

12. The compound for use according to Claim 11 wherein R¹¹ represents a side chain of isoleucine.

13. A compound as defined in any of Claims 1 to 12 for use in the treatment or prevention of a condition or disorder associated with the presence or growth of a biofilm on or in the body of a living mammal.

14. The compound for use according to any one of the preceding claims wherein the biofilm is on or in the body of a living mammal.

15. The compound for use according to Claim 13 or Claim 14, wherein the biofilm is in the oral cavity, the urinary tract, the sinuses, the ear, the heart, the prostate, the bone, the lungs, the kidneys, or is in or on the skin.

16. The compound for use according to Claim 13 or Claim 14 wherein the biofilm is attached to an inert support within said body.

17. The compound for use according to any one of Claims 13 to 16, wherein the mammal is human.

18. The compound for use according to any one of the preceding claims wherein the microbial biofilm is formed by bacteria.

19. The compound for use according to Claim 18 wherein the bacteria are gram-positive.

20. The compound for use according to Claim 19 wherein the bacteria are Staphylococci or Enterococci.

21. The compound for use according to Claim 18 wherein the bacteria are Staphylococci, preferably *Staphylococcus aureus*, more preferably methicillin-resistant *Staphylococcus aureus*.

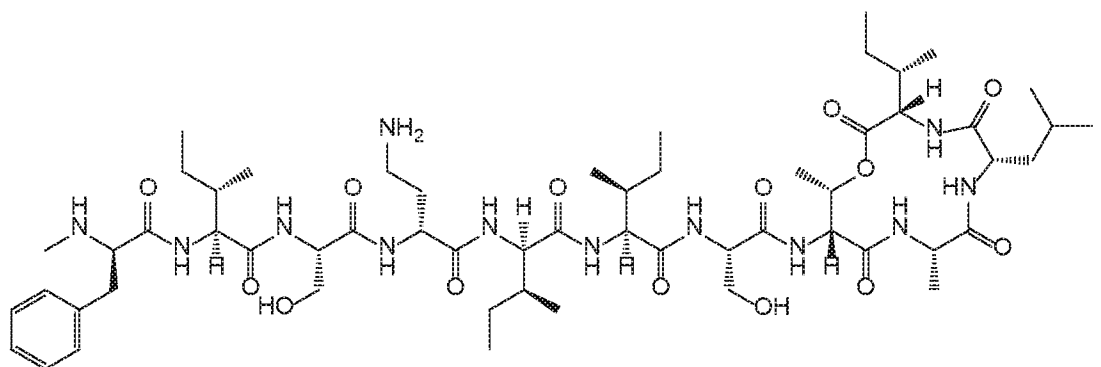
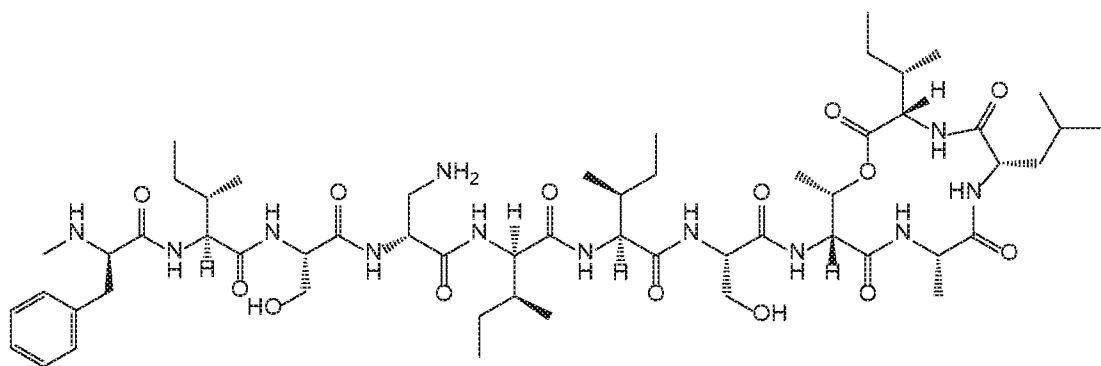
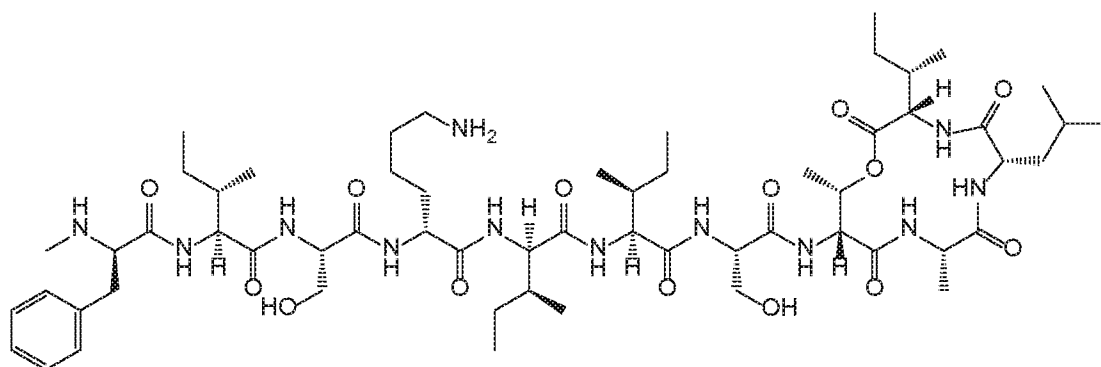
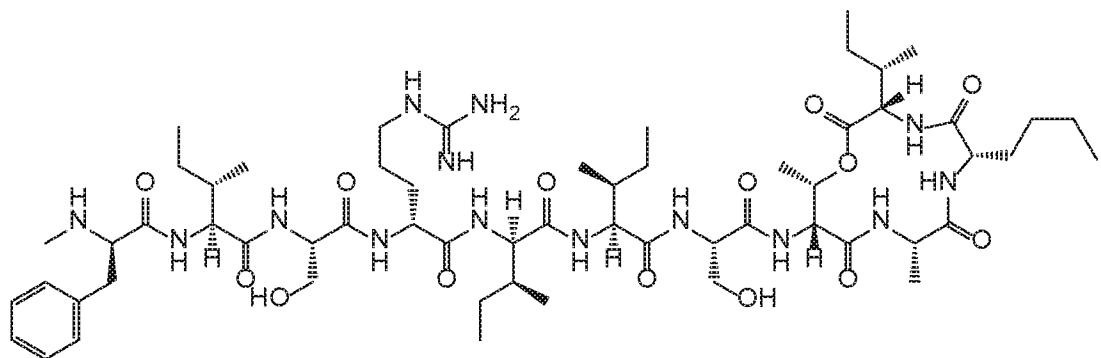
22. The compound for use according to Claim 21 wherein the bacteria are *Staphylococcus epidermidis*.

23. An implantable medical device which is impregnated, coated or otherwise treated with a compound as defined in any one of Claims 1 to 12.

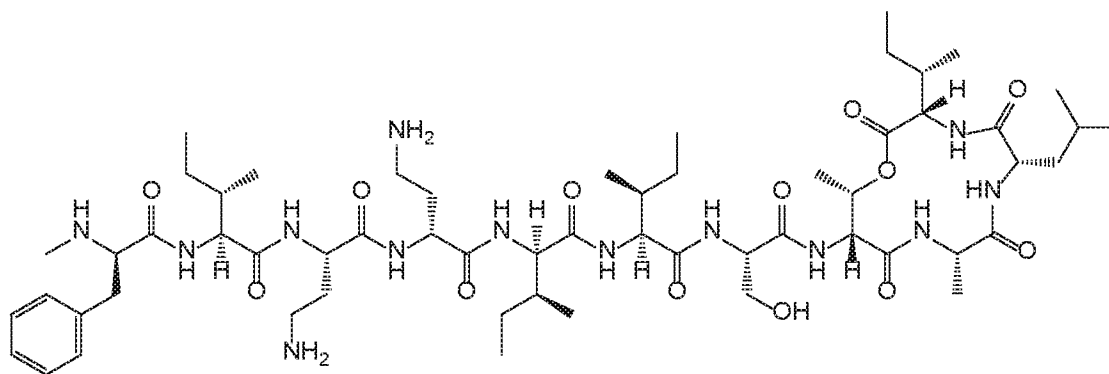
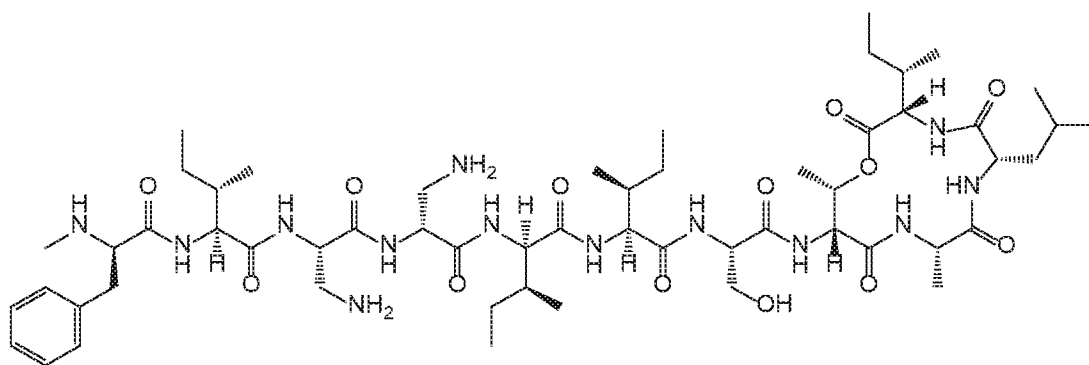
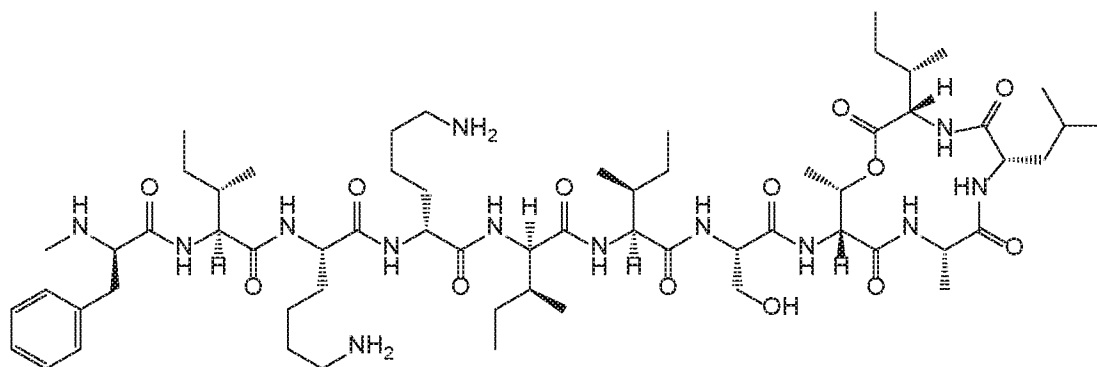
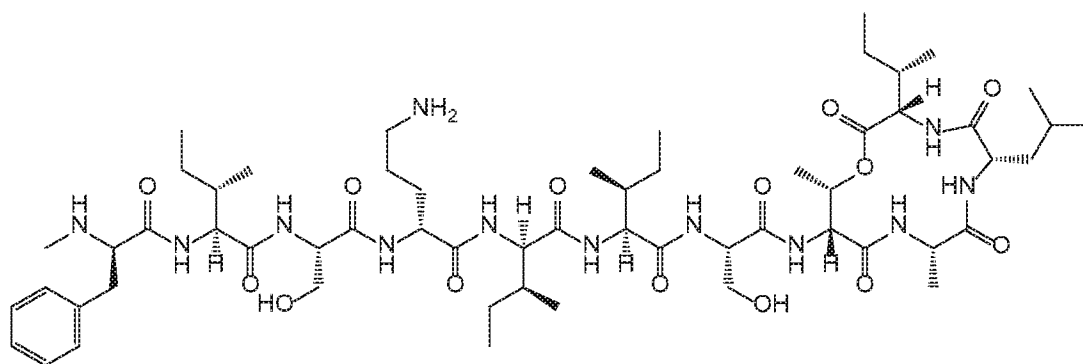
24. An implantable medical device according to Claim 23 selected from the group consisting of an intravascular device, a catheter, a shunt, an intubating and tracheotomy tube, an ophthalmic device, a joint prosthesis, an artificial heart valve and a breast implant.

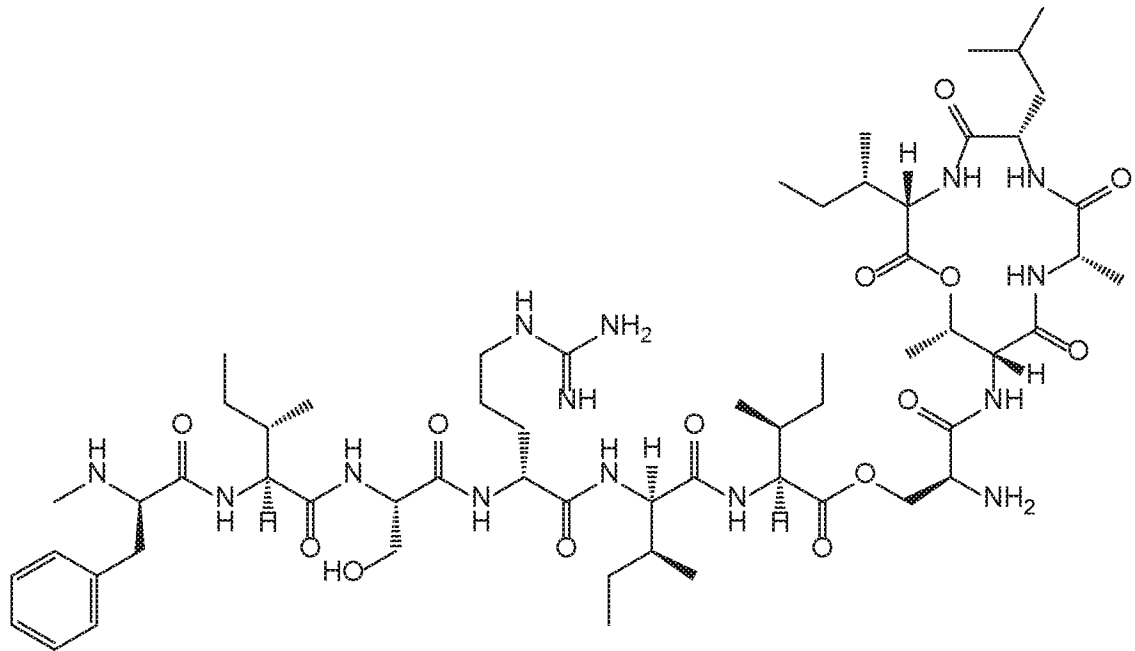
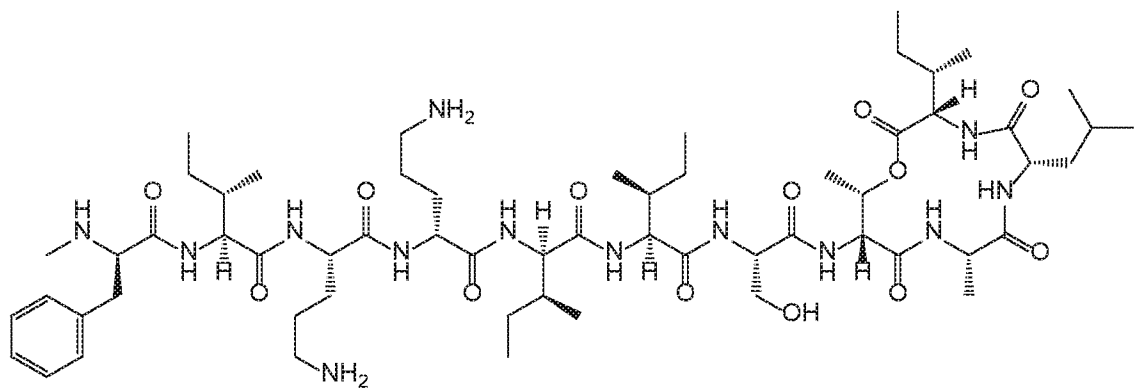
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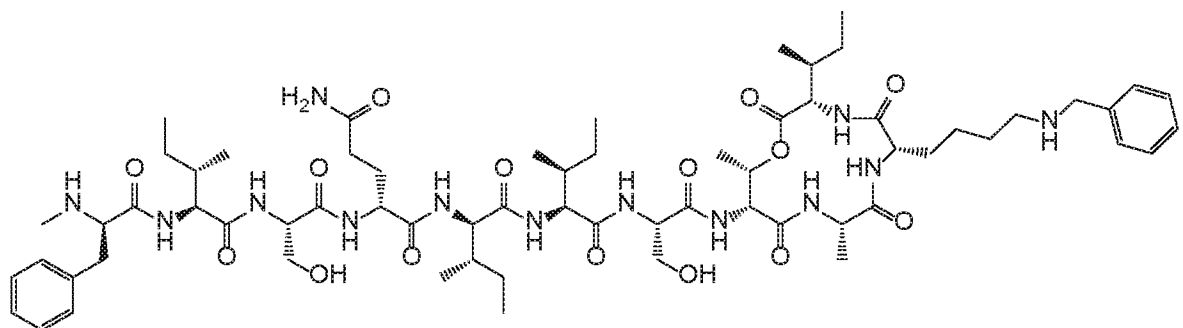
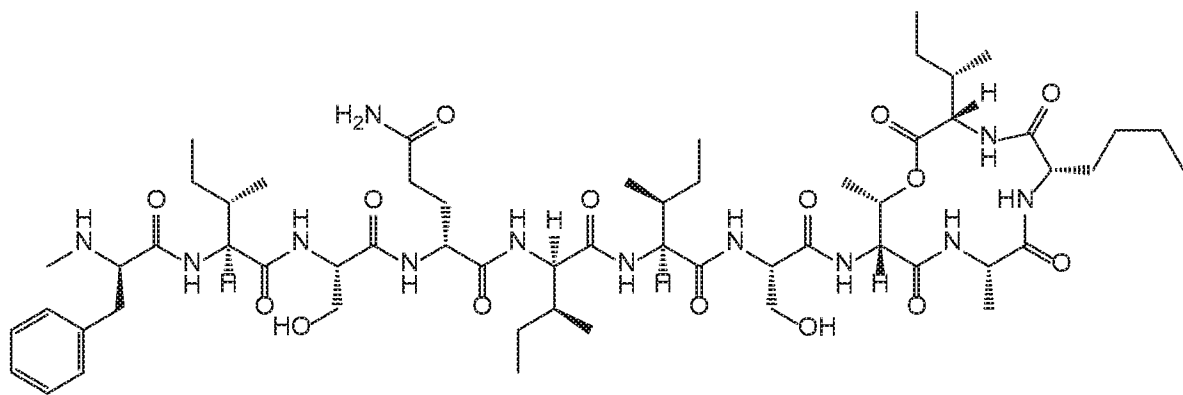
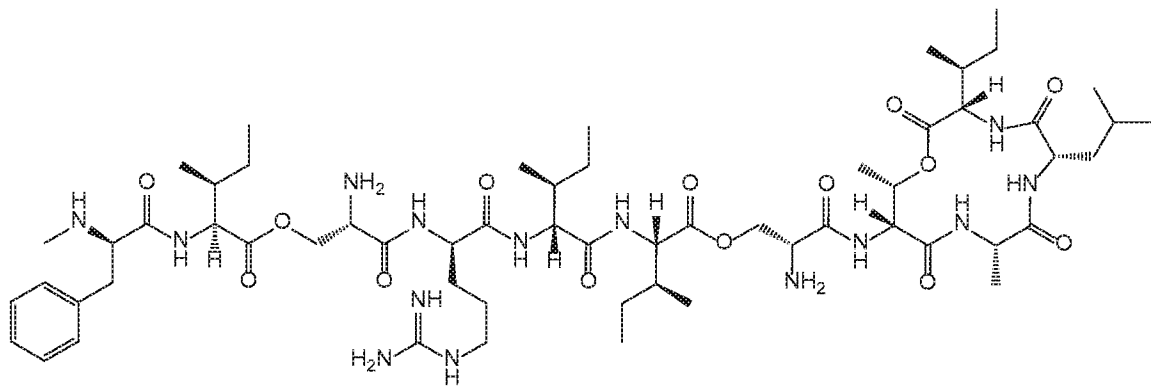
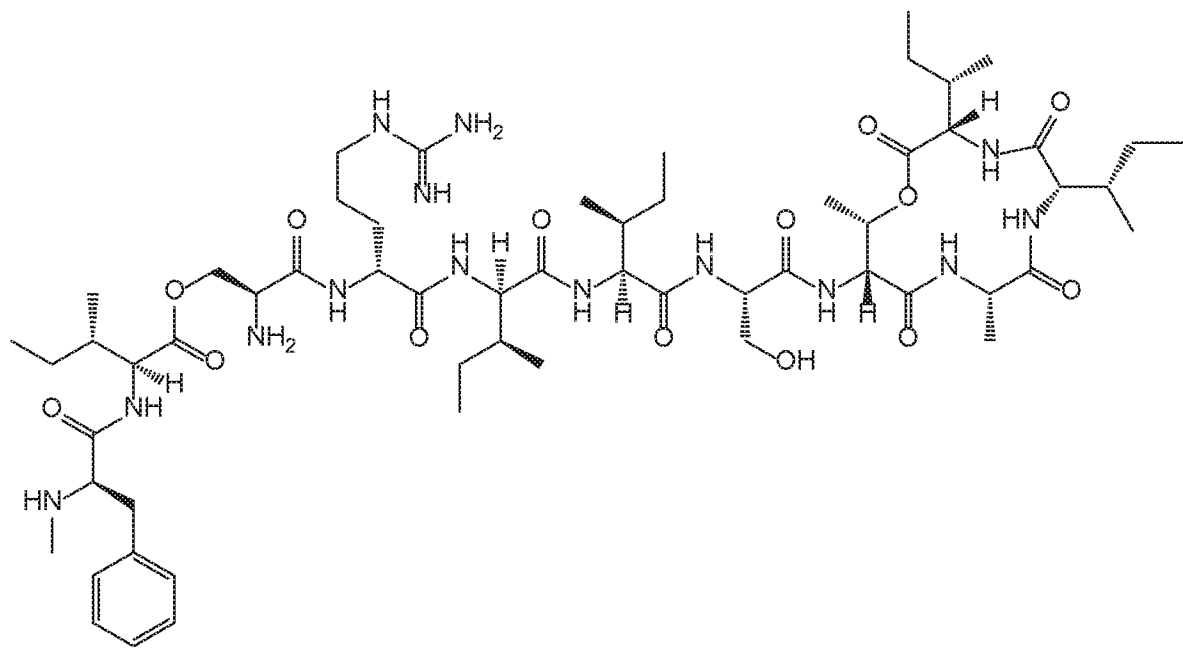
25. A compound selected from the group consisting of

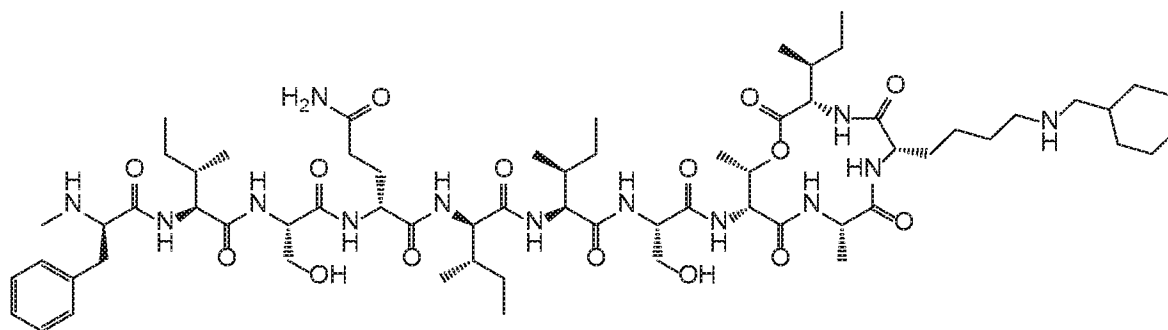
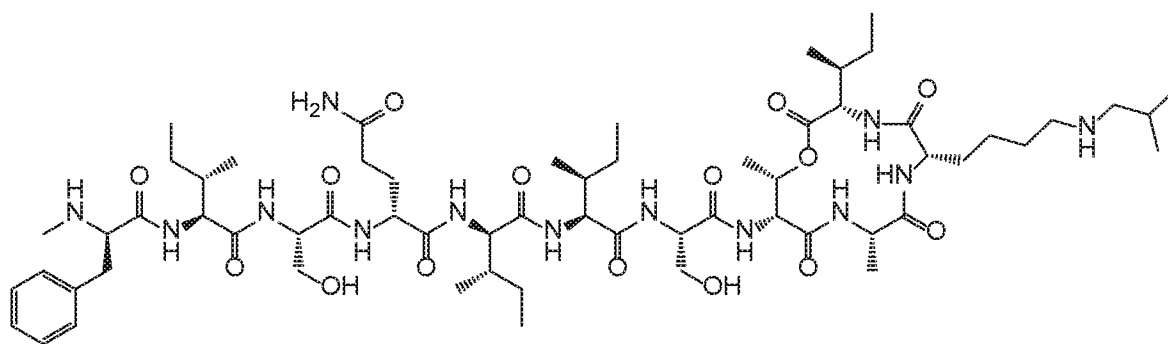


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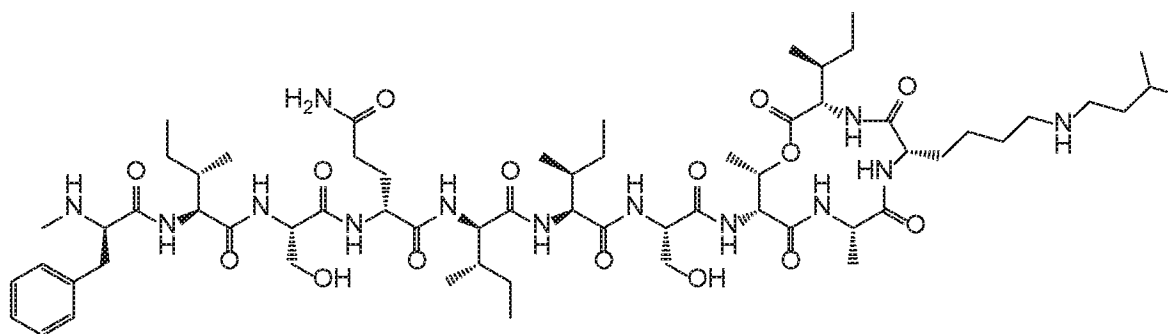








and



or a pharmaceutically-acceptable salt, solvate or clathrate thereof.

Figures 1a and 1b

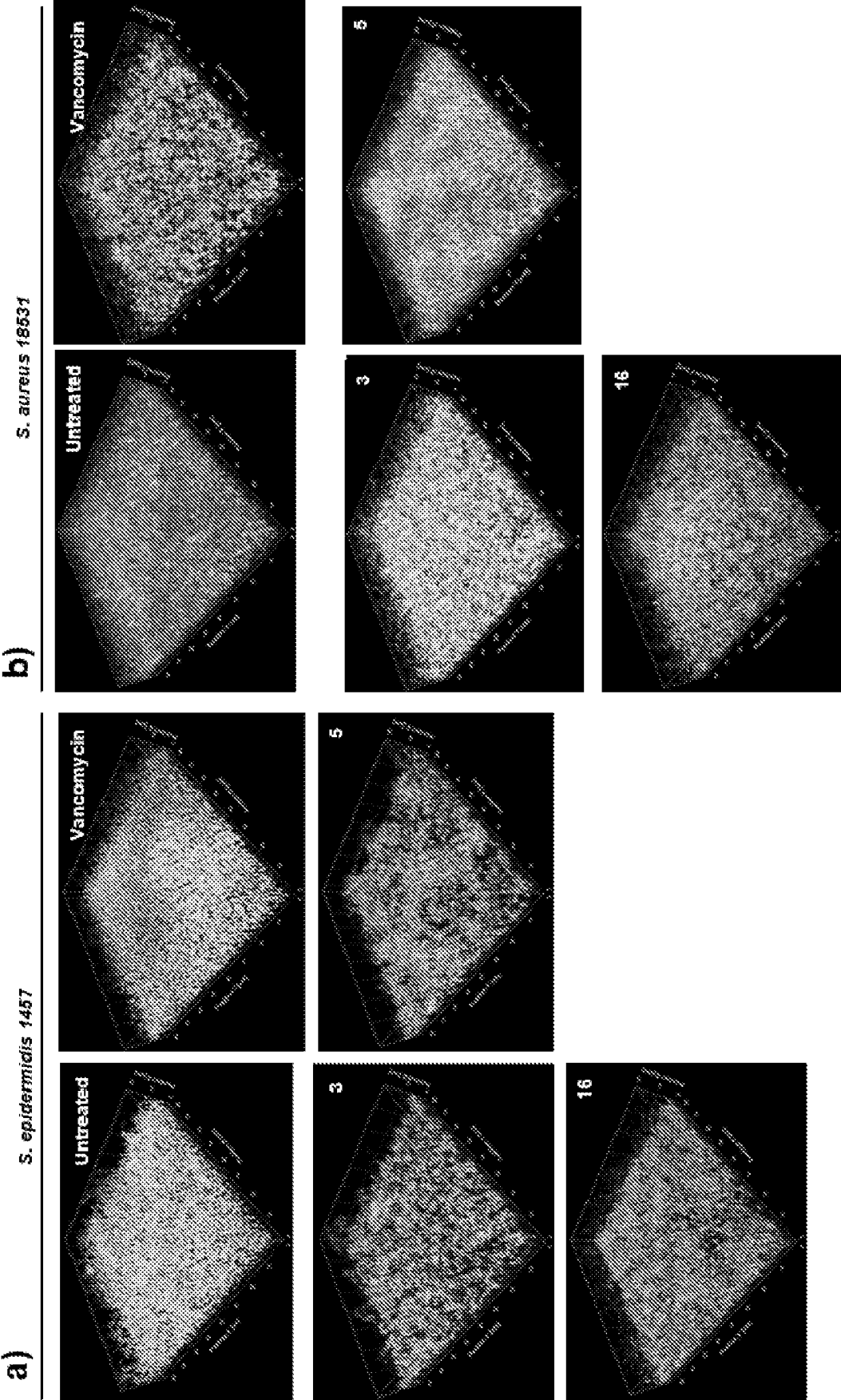


Figure 1c

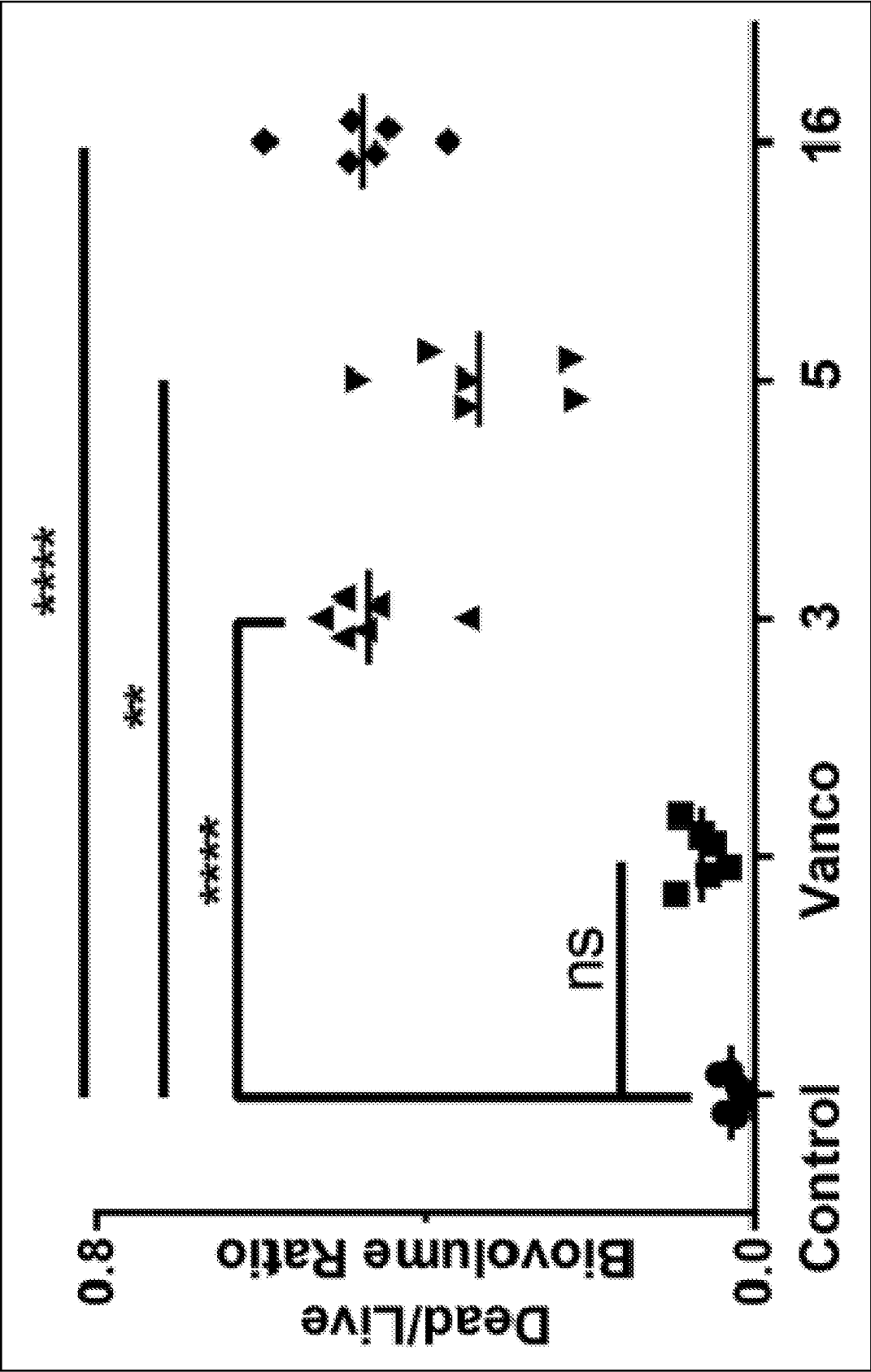


Figure 1d

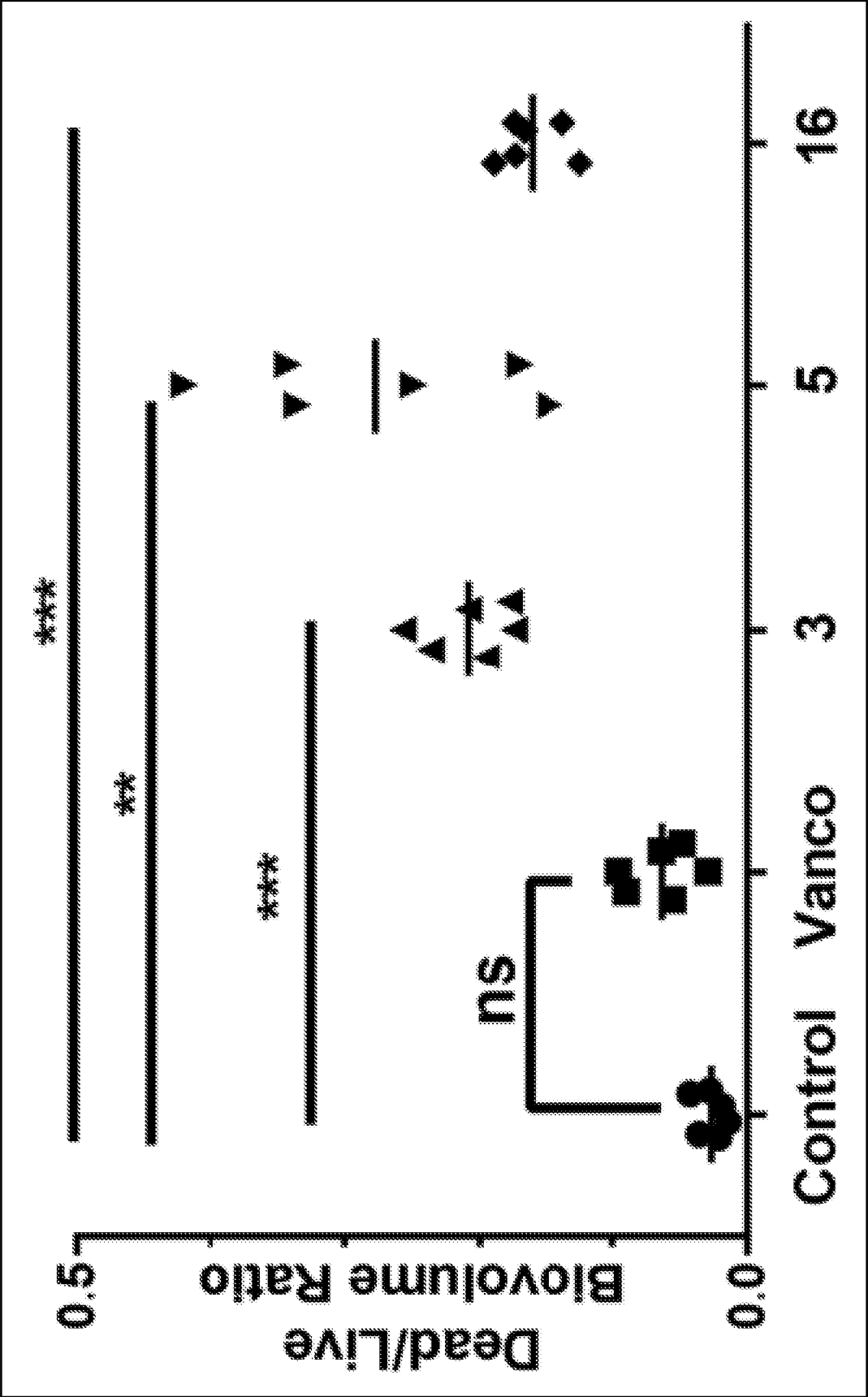


Figure 2a

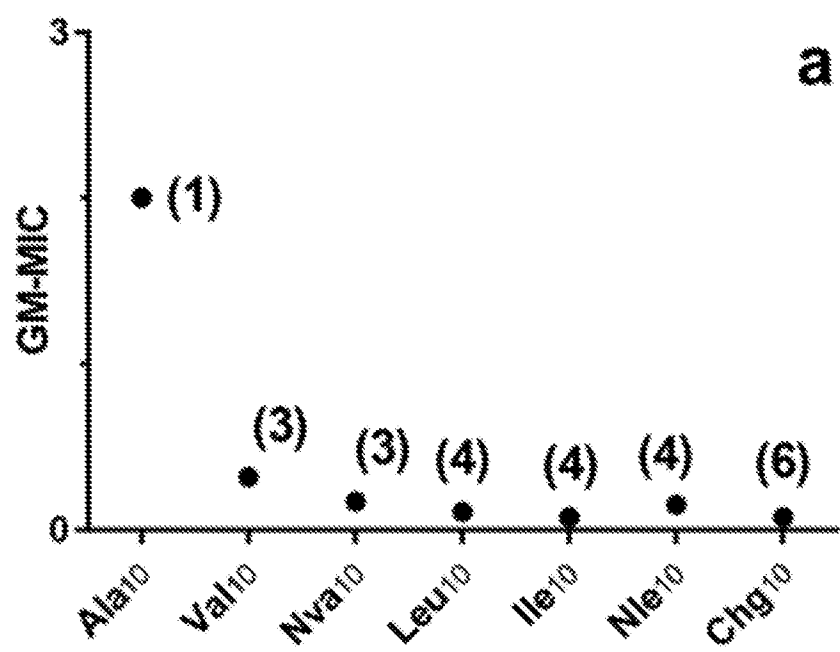


Figure 2b

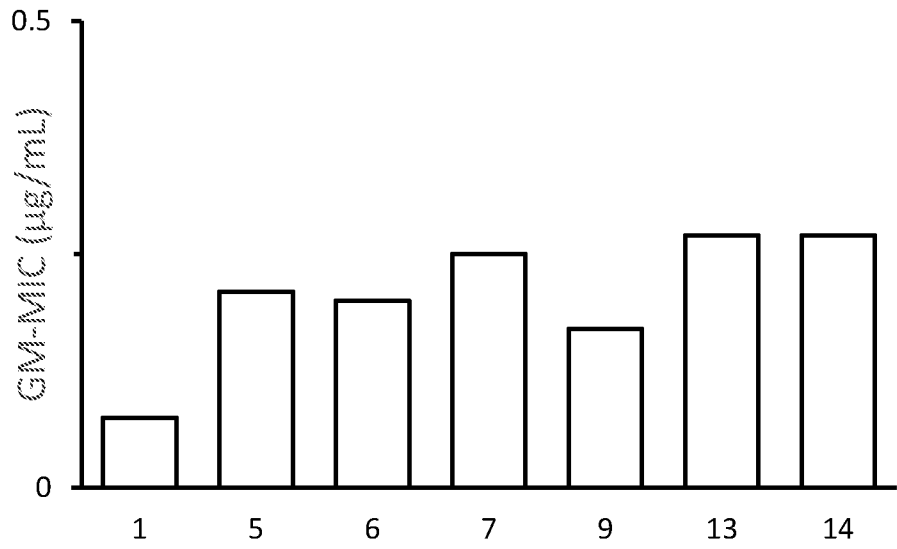


Figure 2c

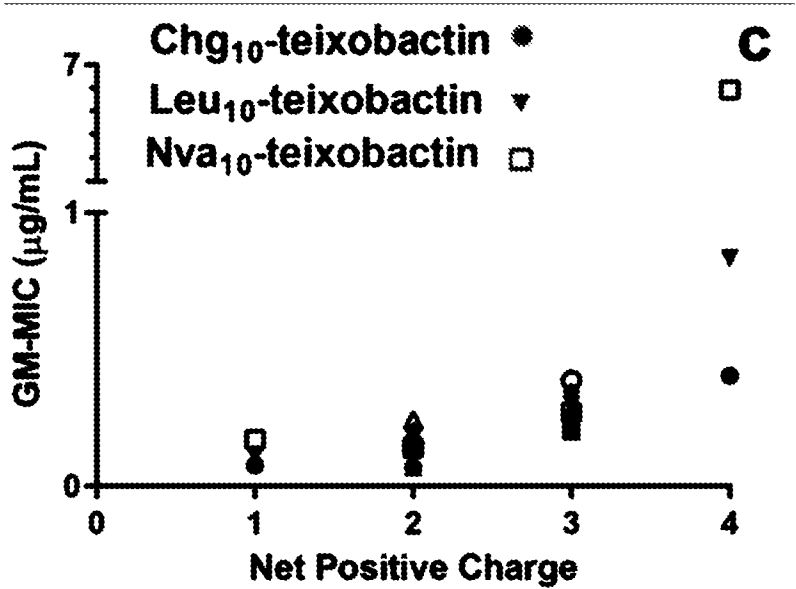


Figure 2d

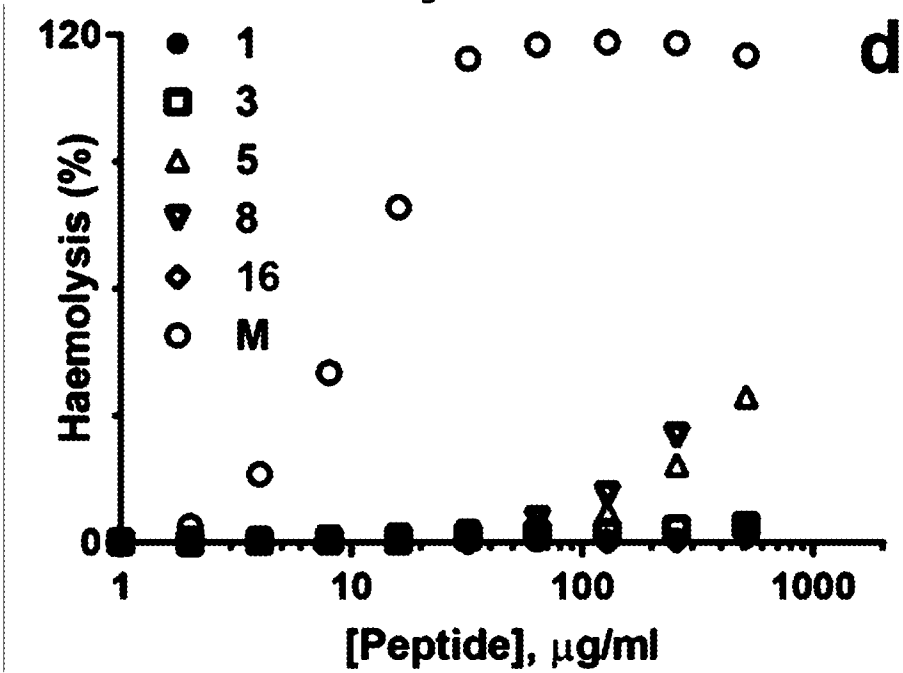
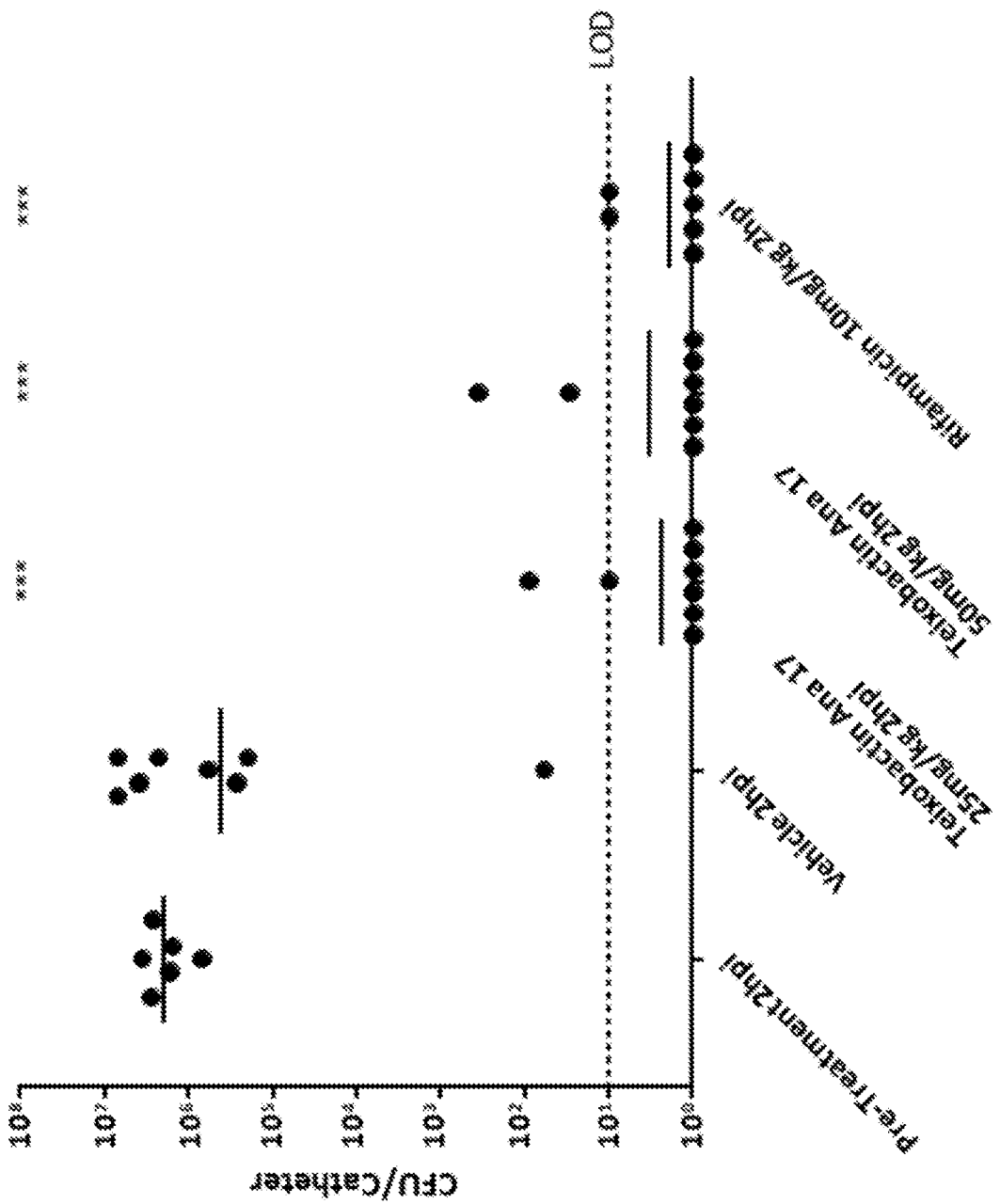


Figure 3



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2024/051527

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K38/12 A61K38/15 A61P31/04 C07K7/56
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P

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

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

EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIN KANG ET AL: "Synthesis and antibacterial studies of teixobactin analogues with non-isostere substitution of enduracididine", BIOORGANIC & MEDICINAL CHEMISTRY, ELSEVIER, AMSTERDAM, NL, vol. 26, no. 5, 1 February 2018 (2018-02-01), pages 1062-1068, XP085351058, ISSN: 0968-0896, DOI: 10.1016/J.BMC.2018.01.016	25
Y	table 1; compound T28 ----- - / - -	1 - 25



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance**"E"** earlier application or patent but published on or after the international filing date**"L"** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)**"O"** document referring to an oral disclosure, use, exhibition or other means**"P"** document published prior to the international filing date but later than the priority date claimed**"T"** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention**"X"** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone**"Y"** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art**"&"** document member of the same patent family

Date of the actual completion of the international search

12 September 2024

Date of mailing of the international search report

23/09/2024

Name and mailing address of the ISA/

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Fax: (+31-70) 340-3016

Authorized officer

Kirsch, Cécile

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051527

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/134530 A1 (VERSITECH LTD [CN]; UNIV HONG KONG POLYTECHNIC [CN]) 11 July 2019 (2019-07-11)	25
Y	Formulas (IV), (IV'); claims; example 4; compounds L-Nle10-teixobactin -----	1-25
X	WO 2018/109042 A2 (FERRING BV [NL]) 21 June 2018 (2018-06-21)	25
Y	Formula (I); claims; example 10; compound 71 -----	1-25
Y	JONES CHELSEA R. ET AL: "Isobactins: O -acyl isopeptide prodrugs of teixobactin and teixobactin derivatives", CHEMICAL SCIENCE, vol. 13, no. 44, 16 November 2022 (2022-11-16), pages 13110-13116, XP093203319, United Kingdom ISSN: 2041-6520, DOI: 10.1039/D2SC02670H Retrieved from the Internet: URL:https://pubs.rsc.org/en/content/articlepdf/2022/sc/d2sc02670h> Prodrugs A, B and C -----	1-25
Y	JARKHI ALAA ET AL: "Antimicrobial Effects of L-Chg10-Teixobactin against Enterococcus faecalis In Vitro", MICROORGANISMS, vol. 10, no. 6, 26 May 2022 (2022-05-26), page 1099, XP093203295, Switzerland ISSN: 2076-2607, DOI: 10.3390/microorganisms10061099 Retrieved from the Internet: URL:https://www.mdpi.com/2076-2607/10/6/1099/pdf> cited in the application the whole document -----	1-25
Y	ANISH PARMAR ET AL: "Design and Syntheses of Highly Potent Teixobactin Analogues against Staphylococcus aureus , Methicillin-Resistant Staphylococcus aureus (MRSA), and Vancomycin-Resistant Enterococci (VRE) in Vitro and in Vivo", JOURNAL OF MEDICINAL CHEMISTRY, vol. 61, no. 5, 24 January 2018 (2018-01-24), pages 2009-2017, XP055459624, US ISSN: 0022-2623, DOI: 10.1021/acs.jmedchem.7b01634 the whole document -----	1-25
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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051527

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	YASIR MUHAMMAD ET AL: "Action of Antimicrobial Peptides against Bacterial Biofilms", MATERIALS, vol. 11, no. 12, 5 December 2018 (2018-12-05), page 2468, XP093203320, CH ISSN: 1996-1944, DOI: 10.3390/ma11122468 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/artic les/PMC6317029/pdf/materials-11-02468.pdf> the whole document -----	1-25
X,P	PARMAR ANISH ET AL: "Development of teixobactin analogues containing hydrophobic, non-proteogenic amino acids that are highly potent against multidrug-resistant bacteria and biofilms", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, vol. 261, 1 December 2023 (2023-12-01), page 115853, XP093202300, ISSN: 0223-5234, DOI: 10.1016/j.ejmech.2023.115853 the whole document -----	1-25
X,P	JONES CHELSEA R. ET AL: "Investigation of Isobactin Analogues of Teixobactin", ACS MEDICINAL CHEMISTRY LETTERS, vol. 15, no. 7, 11 June 2024 (2024-06-11), pages 1136-1142, XP093203321, US ISSN: 1948-5875, DOI: 10.1021/acsmchemlett.4c00215 Retrieved from the Internet: URL:https://pubs.acs.org/doi/pdf/10.1021/a csmedchemlett.4c00215> D-Arg, Leu10-isobactin A and D-Arg, Leu10-isobactin C -----	25

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2024/051527

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			EP 3735415 A1	11-11-2020
			JP 2021510148 A	15-04-2021
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			KR 20190126838 A	12-11-2019
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