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(54) **Title:** DIMERS AND USES THEREFOR

(57) **Abstract:** Disclosed are proteinaceous molecule dimers represented by Formula I, methods for preparing the proteinaceous molecule dimers and methods of use thereof. More specifically, this invention relates to dimers of antimicrobial peptides represented by Formula I, methods for preparing the dimers of antimicrobial peptides and methods of treating an infection, inflammatory disorder and autoimmune disorder, eliciting or enhancing an immune response and disrupting a biofilm. Also provided are bifunctional linkers used in the preparation of the proteinaceous molecule dimers.

TITLE OF THE INVENTION

"DIMERS AND USES THEREFOR"

[0001] This application claims priority to Australian Provisional Patent Application No. 2022900153 entitled "Dimers and uses therefor" filed 28 January 2022, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] This invention relates generally to proteinaceous molecule dimers represented by Formula I, methods for preparing the proteinaceous molecule dimers and methods of use thereof. More specifically, this invention relates to dimers of antimicrobial peptides represented by Formula I, methods for preparing the dimers of antimicrobial peptides and methods of treating an infection, inflammatory disorder and autoimmune disorder, eliciting or enhancing an immune response and disrupting a biofilm. Also provided are bifunctional linkers used in the preparation of the proteinaceous molecule dimers.

BACKGROUND OF THE INVENTION

[0003] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0004] Infections cause a significant economic and social burden to be placed on society. Treatments for infections, where available, are typically specific for the type of infectious agent and, in the case of antibiotics in particular, use is limited due to the development of drug resistance. In this regard, the World Health Organisation has identified antimicrobial resistance as one of the top ten global public health threats facing humanity, and has identified developing new antibiotics against three Gram-negative bacteria, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae*, as of critical priority. It is clear that there is a substantial need for new antimicrobial compounds.

[0005] Antimicrobial peptides have demonstrated significant antimicrobial activity against a wide range of microorganisms, including a range of multi-drug resistant pathogens. Various chemical modifications have been investigated with a view to increasing or enhancing their potency against target microorganisms. A specific class of antimicrobial peptides, proline-rich antimicrobial peptides (such as Chex1-Arg20), are considered a promising class of candidates for rational drug design for targeting Gram-

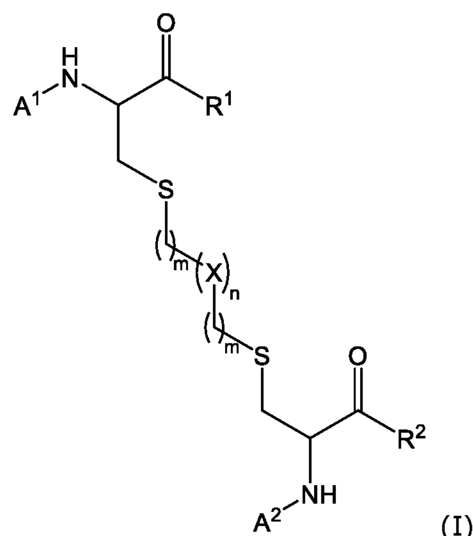
negative bacteria due to their low toxicity and multiple intracellular targets, such as DnaK and the bacterial ribosome.

[0006] Multimerisation of compounds, including antimicrobial peptides, has been previously investigated with a view to increasing their activity and/or stability. In this regard, multimerisation of antimicrobial peptides, such as Chex1-Arg20, has been shown to broaden the spectrum of activity and increase antibacterial activity (e.g. Li *et al.* (2015) *Chem. Biol.*, 22: 1250-1258; and Li *et al.* (2017) *Chem. Eur. J.*, 23: 390-396). However, the synthesis of these multimers requires numerous steps, including multiple purification steps, and substantially increases the duration of peptide synthesis and/or decreases the efficacy of the peptides. Accordingly, improved methods for multimerisation of compounds which increase the synthetic efficiency and efficacy of the compounds are desired.

SUMMARY OF THE INVENTION

[0007] The present invention is predicated in part on the discovery that the efficiency of peptide dimerisation, including dimerisation of antimicrobial peptides, and efficacy of the dimerised peptide may be improved using aryl- or cycloalkyl-containing bifunctional linkers. Accordingly, the inventors have conceived that such linkers may be used to dimerise peptides, especially antimicrobial peptides. In particular, the inventors have conceived that compounds containing such linkers may be used for a variety of uses on the basis of the activity of the dimerised peptide. Specifically, the inventors have conceived that dimerised antimicrobial peptides represented by Formulae I, II, III, IV, V or VI may be useful for treating an infection, inflammatory disorder or autoimmune disorder, eliciting or enhancing an immune response or disrupting a biofilm.

[0008] Accordingly, in one aspect, there is provided a compound represented by Formula I:



(I)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1 and A^2 are each independently a proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween);

each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

R^1 and R^2 are independently selected from the group consisting of -OH, $-NH_2$ and $-NHNH_2$;

n is 1 or 2; and

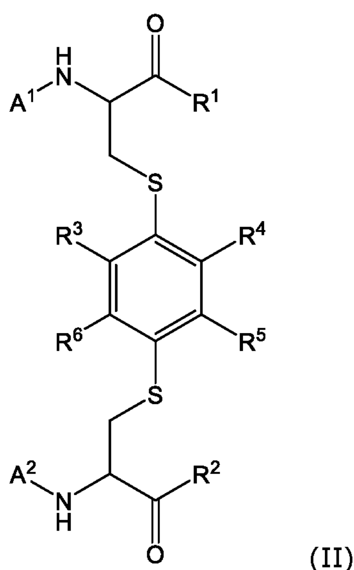
each m is independently 0, 1 or 2.

[0009] In some embodiments, each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene and optionally substituted C_5-C_{12} cycloalkylene; especially optionally substituted C_5-C_{12} arylene, such as optionally substituted C_5 or C_6 arylene. In particular embodiments, each X is optionally substituted phenylene. In specific embodiments, each X is independently phenylene substituted with one or more F, Cl, Br or I, especially one or more F. In particular embodiments, each X is tetrafluorophenylene.

[0010] In some embodiments, n is 1. In alternative embodiments, n is 2.

[0011] In exemplary embodiments, each m is independently 0 or 1, especially wherein each m is 0.

[0012] In further embodiments, the compound is represented by Formula II:



(II)

or a pharmaceutically acceptable salt thereof,

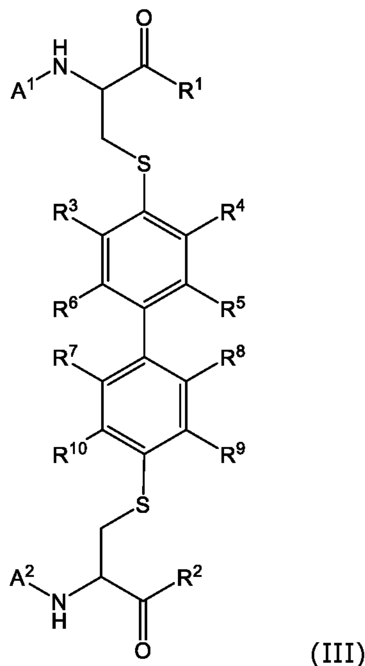
wherein:

A^1, A^2, R^1 and R^2 are as defined for Formula I; and

R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

[0013] In particular embodiments, R^3, R^4, R^5 and R^6 are F.

[0014] In some embodiments, the compound is represented by Formula III:



(III)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1, A^2, R^1 and R^2 are as defined for Formula I; and

R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

[0015] In specific embodiments, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are F.

[0016] In exemplary embodiments, A^1 and A^2 are the same.

[0017] In particular embodiments, A^1 and A^2 are antimicrobial peptides, such as proline-rich antimicrobial peptides. In some embodiments, A^1 and A^2 are independently selected from the group consisting of Chex1-Arg20, oncocin, apidaecin (Api137), drosocin and Bac7(1-35). For example, A^1 and A^2 may independently be a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence selected from the group consisting of:

[Chex]RPDKPRPYLPRPRPPRPVR [SEQ ID NO: 1];

VDKPPYLPRPRPPRRIYNR [SEQ ID NO: 2];
[gu]ONNRPVYIPRPRPPHPRL [SEQ ID NO: 3];
GKPRPYSPRPTSHPRPIRV [SEQ ID NO: 4]; and
RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFP [SEQ ID NO: 5].

[0018] In particular embodiments, A^1 and A^2 are each a proteinaceous molecule comprising the amino acid sequence of SEQ ID NO: 1.

[0019] While A^1 and A^2 may be attached to the linker at any point, in some embodiments, A^1 and A^2 are attached to the adjacent nitrogen atom of the compound of Formula I, II or III via their C-terminus, especially wherein A^1 and A^2 are attached to the adjacent nitrogen atom of the compound of Formula I, II or III via an amide bond.

[0020] In another aspect, there is provided a composition comprising a compound of the invention and a pharmaceutically acceptable carrier or diluent.

[0021] Further provided is a method of treating or inhibiting the development of an infection in a subject, comprising administering a compound of the invention to the subject.

[0022] In particular embodiments, the infection is a bacterial or a viral infection, especially a bacterial infection. In specific embodiments, the infection is caused by a bacterial species that is resistant to one or more antibiotics.

[0023] In some embodiments, the infection is a Gram-negative bacterial infection. In particular embodiments, the infection is by *Escherichia coli*, *Klebsiella pneumoniae* or *Acinetobacter baumannii*, especially *A. baumannii*.

[0024] In alternative embodiments, the infection is by *K. pneumoniae* FADDI-KP028 or *A. baumannii* FADDI-AB156.

[0025] In a further aspect, there is provided a method of treating or inhibiting the development of an inflammatory disorder in a subject, comprising administering a compound of the invention to the subject. In particular embodiments, the inflammatory disorder is selected from the group consisting of inflammatory bowel disease, rheumatoid arthritis, sepsis, atopic dermatitis and acne.

[0026] In another aspect, there is provided a method of eliciting an immune response in a subject, comprising administering a compound of the invention to the subject.

[0027] Further provided herein is a method of enhancing an immune response in a subject to a target antigen by an immune modulating agent, comprising administering a compound of the invention to the subject.

[0028] In particular embodiments, the immune modulating agent is selected from an antigen that corresponds to at least a portion of the target antigen, an antigen-binding molecule that is immuno-interactive with the target antigen and an immune modulating cell that modulates an immune response to the target antigen. In specific embodiments, the target antigen is an antigen from an infectious agent.

[0029] In yet another aspect, there is provided a method of enhancing an innate immune response to an infectious agent in a subject, comprising administering a compound of the invention to the subject.

[0030] Also contemplated is a method of treating or inhibiting the development of an autoimmune disorder in a subject, comprising administering a compound of the invention to the subject.

[0031] In particular embodiments, the autoimmune disorder is type 1 diabetes or multiple sclerosis.

[0032] In a further aspect, there is provided a method of disrupting a biofilm, comprising contacting the biofilm with a compound of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] Exemplary embodiments of the invention are described herein, by way of non-limiting example, with reference to the following drawings.

[0034] Figure 1 is a schematic showing the preparation of homodimers of the Chex1-Arg20 bearing a C-terminal hydrazide moiety. The bioconjugation linkers are shown in the box as disulfide dimer-$NHNH_2$ **2**, p-xylene dimer-$NHNH_2$ **3**, o-xylene dimer-$NHNH_2$ **4**, m-xylene dimer-$NHNH_2$ **5**, tetrafluorobenzene dimer-$NHNH_2$ **6**, and octofluorobiphenyl dimer-$NHNH_2$ **7**.

[0035] Figure 2 is an image showing the characterisation of the dimers. Figure 2A shows the RP-HPLC spectrum for monomer(Chex1-Arg20(C-term Cys)-$NHNH_2$, **1**; Figure 2B shows the RP-HPLC spectrum for disulfide dimer-$NHNH_2$, **2**; Figure 2C shows the RP-HPLC spectrum for p-xylene dimer-$NHNH_2$, **3**; Figure 2D shows the RP-HPLC spectrum for o-xylene dimer-$NHNH_2$, **4**; Figure 2E shows the RP-HPLC spectrum for m-xylene dimer-$NHNH_2$, **5**; Figure 2F shows the RP-HPLC spectrum for tetrafluorobenzene dimer-$NHNH_2$, **6**; and Figure 2G shows the RP-HPLC spectrum for octofluorobiphenyl dimer-$NHNH_2$, **7**.

[0036] Figure 3 is a graphical representation of the cytotoxicity of the test compounds against HEK 293 cells. Figure 3A is a graph showing the lactate dehydrogenase (LDH) cytotoxicity of test compounds at the highest tested concentration (125 μg/mL) and Figure 3B is a graph showing cell proliferation in the presence of the highest tested concentration of test compounds (125 μg/mL). All data are performed twice in duplicate and determined as mean ± standard deviation.

[0037] Figure 4 is a graphical representation of the time killing-kinetic assays for Compounds 6 and 7 against *A. baumannii* and MDR-FADDI-*AB156*. Figure 4A is a graph showing survival of *A. baumannii* and Figure 4C is a graph showing survival of MDR-FADDI-*AB156* incubated with tetrafluorobenzene dimer-$NHNH_2$ **6** at differing times and concentrations. Figure 4B is a graph showing the survival of *A. baumannii* and Figure 4D is a graph showing the survival of MDR-FADDI-*AB156* incubated with octofluorobiphenyl dimer-$NHNH_2$ **7** at differing times and concentrations. Data is representative of two biological replicates with two technical replicates/assay.

[0038] Figure 5 is a series of graphs showing plots of Log(CFU) vs. time which was used to determine the first order death rate constant. Figure 5A is a graph of Log(CFU) vs. time of *A. baumannii* and Figure 5C is a graph of Log(CFU) vs. time of MDR-FADDI-AB156 incubated with tetrafluorobenzene dimer-$NHNH_2$ **6** at differing concentrations with the bacteria only (black squares), 0.25 × MIC compound **6** (gray triangles), 0.5 × MIC compound **6** (golden triangles), 1 × MIC compound **6** (green triangles), 2 × MIC compound **6** (blue diamonds), and 4 × MIC compound **6** (red circles). Figure 5B is a graph of Log(CFU) vs. time of *A. baumannii* and Figure 5D is a graph of Log(CFU) vs. time of MDR-FADDI-AB156 incubated with octofluorobiphenyl dimer-$NHNH_2$ **7** at differing concentrations labelled in colours in accordance with compound **6**.

[0039] Figure 6 is a graph showing the death rate constant of *A. baumannii* and MDR-FADDI-AB156 caused by the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**. The death rate constants were determined by the first order of the linear regression of log(CFU count) vs. various time points (Figure 5).

[0040] Figure 7 is a panel of images of the flow cytometric analysis of *A. baumannii* treated with compounds **6** and **7**. Untreated samples were used as controls to show the untreated membrane population. Blue colour in each panel represents the SYTO 9-labelled bacterial population, while a red colour indicates the inner membrane permeability by test compounds with PI-labelled bacterial population.

[0041] Figure 8 is a panel of images of the flow cytometric analysis of MDR-FADDI-AB156 treated with compounds **6** and **7**. Untreated samples were used as controls to show the untreated membrane population. Blue colour in each panel indicates the SYTO 9-labelled bacterial population, while red colour indicates the inner membrane permeability by test compounds with PI-labelled bacterial population.

[0042] Figure 9 is series of flow cytometry plots of the membrane potential of *A. baumannii* in the presence of compounds **6** and **7**. The red population represents depolarised bacteria and the blue population indicates the untreated stage of the bacteria.

[0043] Figure 10 is a series of flow cytometry plots of the membrane potential of FADDI-AB156 in the presence of compounds **6** and **7**. The red population represents depolarised bacteria and the blue population indicates the untreated stage of the bacteria.

[0044] Figure 11 is a graphical representation of the results of the 1-*N*-phenylnaphthylamine (NPN) assay in the presence of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** for *A. baumannii* (Figure 11A), and MDR-FADDI-*AB156* (Figure 11B). Polymyxin was used as a positive control for its strong NPN permeability. All data are expressed as mean ± standard deviation as indicated by the error bars (n = 4). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Student's t-test, a significant difference from the bacteria only with NPN control to indicate less potent NPN permeability.

[0045] Figure 12 is a graphical representation of the inner membrane permeability of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** for *A. baumannii* (Figure 12A), and MDR-FADDI-AB156 (Figure 12B).

[0046] Figure 13 is a graphical representation of the membrane analysis of *A. baumannii* (Figure 13A), and MDR-FADDI-AB156 (Figure 13B) with treatment of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**. All data are expressed as mean ± standard deviation as indicated by the error bars (n = 4). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Student's t-test, a significant difference from the untreated bacteria control.

[0047] Figure 14 is a graphical representation of the results of the dye leakage assay. Phosphatidylglycerol (PG): cardiolipin (9:1) was used to mimic a Gram-positive bacterial membrane (Figure 14A), and phosphatidylethanolamine (PE): PG (7:3) was used to represent a Gram-negative bacterial membrane (Figure 14B). Positive control was treated with 2% Triton X-100. Assays were performed twice in duplicate and plotted as mean ± standard deviation.

[0048] Figure 15 is a series of flow cytometry plots used to determine the reactive oxygen species (ROS) generated from *A. baumannii* in the presence of compounds **6** and **7**. The untreated samples were used as controls to show normal cells without significant ROS generation. The blue square in the flow cytometry gate indicates the SYTO 9-labelled bacterial population, while the red square indicates the fluorescent dye CellROX deep red reagent labelled bacterial population with ROS generation.

[0049] Figure 16 is a series of flow cytometry plots used to determine the ROS generated from FADDI-AB156 in the presence of compounds **6** and **7**. The untreated samples were used as controls to show normal cells without significant ROS generation. The blue square in the flow cytometry gate indicates the SYTO 9-labelled bacterial

population, while the red square indicates the fluorescent dye CellROX deep red reagent labelled bacterial population with ROS generation.

[0050] Figure 17 is a graph showing the ROS generated from *A. baumannii* (Figure 17A), and MDR-FADDI-AB156 (Figure 17B) following treatment with the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**. All data are expressed as mean ± standard deviation as indicated by the error bars (n = 4). *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Student's t-test, a significant difference from thiourea quenched ROS control.

[0051] Figure 18 is a collection of helium ion microscopy images showing the morphologies of *A. baumannii* and MDR-FADDI-*AB156* under treatment of the tetrafluorobenzene dimer-$NHNH_2$ **6** (Figures 18A and 18C) and octofluorobiphenyl dimer-$NHNH_2$ **7** (Figure 18B and 18D) at different concentrations (2 × MIC, 1 × MIC, 0.5 × MIC, 0.25 × MIC, 0.125 × MIC). Untreated *A. baumannii* and MDR-FADDI-*AB156* were used as controls.

[0052] Figure 19 is a series of graphs showing the eradication of the preformed biofilms of *A. baumannii* (Figure 19A), and MDR-FADDI-AB156 (Figure 19B) by the treatment of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** at different concentrations (0.5 × MBC, 1 × MBC, 2 × MBC, 4 × MBC, 8 × MBC). Crystal violet was used to stain the bacterial biofilm and percentages were calculated with the untreated biofilm as the basis. *P ≤ 0.05, **P ≤ 0.01. Student's t-test, a significant difference from the untreated preformed biofilm control.

[0053] Figure 20 is a collection of helium ion microscopy images showing the preformed biofilms of *A. baumannii* and MDR-FADDI-*AB156* after treatment of the tetrafluorobenzene dimer-$NHNH_2$ **6** (Figures 20A and 20C) and octofluorobiphenyl dimer-$NHNH_2$ **7** (Figures 20B and 20D) at different concentrations (8 × MBC, 4 × MBC, 2 × MBC, 1 × MBC, 0.5 × MBC). The untreated *A. baumannii* and MDR-FADDI-AB156 biofilms were used as controls. The red arrow indicates the EPS in preformed biofilm.

[0054] Figure 21 is a series of graphs showing the stimulated nitric oxide (NO) release from RAW 264.7 cells by different concentrations of purified LPS from *A. baumannii* and MDR-FADDI-AB156.

[0055] Figure 22 is a graph showing the standard curve for NO.

[0056] Figure 23 is a series of graphs showing nitric oxide release from LPS stimulated RAW264.7 cells under treatment of the tetrafluorobenzene dimer-$NHNH_2$ **6** (Figure 23A), and octofluorobiphenyl dimer-$NHNH_2$ **7** (Figure 23B). All data are expressed as mean ± standard deviation as indicated by the error bars (n = 4). *P ≤ 0.05, **P ≤

0.01, ***P ≤ 0.001. Student's t-test, a significant difference from LPS stimulated RAW264.7 cells control.

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0057] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0058] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0059] By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0060] The terms "administration concurrently" or "administering concurrently" or "co-administering" and the like refer to the administration of a single composition containing two or more agents, or the administration of each agent as separate compositions and/or delivered by separate routes either contemporaneously or simultaneously or sequentially within a short enough period of time that the effective result is equivalent to that obtained when all such agents are administered as a single composition. By "simultaneously" is meant that the agents are administered at substantially the same time, and desirably together in the same composition. By "contemporaneously" it is meant that the agents are administered closely in time, e.g., one agent is administered within from about one minute to within about one day before or after another. Any contemporaneous time is useful. However, it will often be the case that when not administered simultaneously, the agents will be administered within about one minute to within about eight hours and suitably within less than about one to about four hours. When administered contemporaneously, the agents are suitably administered at the same site on the subject. The term "same site" includes the exact location, but can be within about 0.5 to about 15 centimeters, preferably from within about 0.5 to about 5 centimeters. The term "separately" as used herein means that the agents are administered at an interval, for example at an interval of about a day to several weeks or months. The agents may be administered in either order. The term "sequentially" as used herein means that the agents are administered in sequence, for example at an interval or intervals of

minutes, hours, days or weeks. If appropriate the agents may be administered in a regular repeating cycle.

[0061] The term "agent" includes a compound that induces a desired pharmacological and/or physiological effect. The term also encompasses pharmaceutically acceptable and pharmacologically active ingredients of those compounds specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the above term is used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites, analogs, etc. The term "agent" is not to be construed narrowly but extends to small molecules, proteinaceous molecules such as peptides, polypeptides and proteins as well as compositions comprising them and genetic molecules such as RNA, DNA and mimetics and chemical analogs thereof as well as cellular agents.

[0062] Amino acid residues are referred to herein interchangeably using their full name or the one or three letter codes standard in the art. Abbreviations used for unnatural or modified amino acid residues or derivatives thereof are defined herein where appropriate.

[0063] As used herein, the term "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0064] As used herein, the term "antigen" and grammatical variants thereof refer to a compound, composition or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (e.g. oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (e.g. polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumour/cancer antigens, antigens involved in autoimmune disease or disorders, allergy and graft rejection, toxins and other miscellaneous antigens. The term "antigen" also encompasses antigens produced by an antigen-encoding nucleic acid molecule introduced into the subject, such as DNA or RNA (e.g. mRNA).

[0065] By "antigen-binding molecule" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity.

[0066] The phrase "antimicrobial peptide" is used herein to refer to a peptide which possesses activity against one or more microorganisms, such as a bacterium, virus, fungi, helminth or protozoan. The antimicrobial peptide may be a naturally-occurring or a synthetic peptide. Antimicrobial peptides are grouped into several subclasses, one of which is proline-rich antimicrobial peptides. The phrase "proline-rich antimicrobial peptide" and grammatical equivalents thereof is used herein to refer to an antimicrobial peptide with at least 25% proline residues, a net charge of at least 1, and at least one intracellular target (e.g. inhibition of DnaK or the 70S ribosome) for their antimicrobial activity. Proline-rich antimicrobial peptides will often have a Pro-Arg-Pro motif in their amino acid sequence, although this is not essential.

[0067] The term "amino acid residue" is used herein to refer to any of the 20 naturally occurring amino acid residues, unnatural amino acid residues and modified versions thereof, including residues with modified side chains, N-methyl amino acids, α-methyl amino acids, residues with acetylated N-termini, beta amino acids, and the like.

[0068] "Aryl" refers to an unsaturated aromatic carbocyclic group having a single ring (e.g. phenyl) or multiple condensed rings (e.g. naphthyl), including from 5 to 12 carbon atoms, especially 6 to 12 carbon atoms. Each ring may be, for example, a 6 to 10 membered ring. Examples of aryl groups include phenyl, naphthyl and the like. "Arylene" is used herein to refer to a divalent aryl group, such as phenylene.

[0069] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. Thus, the use of the term "comprising" and the like indicates that the listed integers are required or mandatory, but that other integers are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0070] "Cycloalkyl" refers to a saturated monocyclic or fused or spiro polycyclic, carbocycle including from 5 to 10 carbons per ring, such as cyclopentyl, cyclohexyl and the like, unless otherwise specified. It includes monocyclic systems such as cyclopentyl and

cyclohexyl, bicyclic systems such as decalin, and polycyclic systems such as adamantane. The term "cycloalkylene" refers to a divalent cycloalkyl group, such as cyclohexylene.

[0071] By "derivative" is meant a molecule, such as a polypeptide or small molecule, that has been derived from the basic molecule by modification, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the art. The term "derivative" also includes within its scope alterations that have been made to a parent molecule including additions or deletions that provide for functionally equivalent molecules.

[0072] As used herein, the term "dosage unit form" refers to physically discrete units suited as unitary dosages for the subject to be treated, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutically acceptable vehicle.

[0073] By "effective amount", in the context of treating or inhibiting the development of a condition is meant the administration of an amount of an agent, compound or composition to an individual in need of such treatment or prophylaxis, either in a single dose or as part of a series, that is effective for the prevention of incurring a symptom, holding in check such symptoms, and/or treating existing symptoms, of that condition. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[0074] The term "enhance" and variants thereof mean to increase a phenomenon. For example, to enhance an immune response, as is well-known in the art, means to increase the animal's capacity to respond to foreign or disease-specific antigens (e.g. antigens of infectious agents) i.e. those cells primed to attack such antigens are increased in number, activity, and ability to detect and destroy the those antigens. Strength of an immune response is measured by standard tests including: direct measurement of peripheral blood lymphocytes by means known to the art; natural killer cell cytotoxicity assays (refer to Provinciali *et al.* (1992) *J. Immunol. Meth.*, 155: 19-24); cell proliferation assays (refer to Vollenweider and Groseurth (1992) *J. Immunol. Meth.*, 149: 133-135); immunoassays of immune cells and subsets (refer to Loeffler *et al.* (1992) *Cytom.*, 13: 169-174; and Rivoltini *et al.* (1992) *Can. Immunol. Immunother.*, 34: 241-251); viral neutralisation assays; bacterial opsonisation assays; or skin tests for cell-mediated immunity (refer to Chang *et al*. (1993) *Cancer Res.*, 53: 1043-1050). Any statistically significant increase in strength of immune response as measured by the foregoing tests is considered an "enhanced immune response" as used herein. An

enhanced immune response is also indicated by physical manifestations such as fever and inflammation, as well as healing of systemic and local infections, and reduction of symptoms of a disease, such as alleviation of symptoms of a disease or condition such as an infection. Such physical manifestations also define an "enhanced immune response" and variants thereof as used herein.

[0075] The phrase "eliciting an immune response" as used herein means to bring about an animal's response to a foreign or disease-specific antigen (e.g. antigens of infectious agents).

[0076] "Heteroaryl" refers to groups containing an aromatic ring (including a 5, 6, 9, 10 or 11 membered aromatic ring) having one or more heteroatoms as ring atoms in the aromatic ring with the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include nitrogen, oxygen and sulfur. Examples of a heteroaryl group include triazole, thiophene, furan, isoindolizine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, tetrazole, indole, isoindole, 1*H*-indazole, purine, quinoline, isoquinoline, phthalazine, naphthyridine, quinoxaline, cinnoline, thiazole, isothiazole, oxazole, isooxazole, furazane, 2-, 3- or 4-pyridyl, 2-, 3-, 4-, 5-, or 8-quinolyl, 1-, 3-, 4-, or 5-isoquinolinyl, 1-, 2-, or 3-indolyl, and 2-, or 3-thienyl, and includes benzofused heteroaryl, such as benzothiophene, benzofuran, benzimidazole, benzoxazole, benzothiazole and benzisothiazole. The term "heteroarylene" is used herein to refer to a divalent heteroaryl group.

[0077] "Heterocycloalkyl" refers to a saturated monocyclic, bicyclic, or polycyclic ring containing at least one heteroatom selected from nitrogen, sulfur and oxygen, including from 1 to 3 heteroatoms in at least one ring. Each ring may be, for example, a 5 to 10 membered ring, including a 5 to 7 membered ring. Examples of suitable heterocyclyl substituents include pyrrolinyl, pyrrolidinyl, pyrazolidinyl, imidazolidinyl, tetrahydrofuryl, tetrahydrothiofuranyl, piperidinyl, thiazolidinyl, piperazinyl, morpholino, thiomorpholinyl, 1,4-oxazepane and 1,4-oxathiapane. The term "heterocycloalkylene" refers to a divalent heterocycloalkyl group.

[0078] An "immune response" as used herein, refers to a response by the immune system of a subject. For example, an immune response may be to an antigen/immunogen that the subject's immune system recognises as foreign (e.g. non-self-antigens) or self (e.g. self-antigens recognised as foreign). Immune responses may be humoral, involving production of immunoglobulins or antibodies, or cellular, involving various types of B and T lymphocytes, dendritic cells, macrophages, antigen presenting cells and the like, or both. Immune responses may also involve the production or elaboration of various effector molecules such as cytokines. The term "immune response" encompasses immunogenic responses that cause, activate, elicit, stimulate, or induce an

immune response against a particular antigen (e.g. a pathogenic antigen or a cancer antigen) or organism (e.g. a pathogenic microorganism) in a subject, as well as immunosuppressive or tolerogenic immune responses that inhibit, suppress, diminish or eliminate an immune response, or render the immune system unresponsive, or delay the occurrence or onset of an immune response, to an allergen, or to a self-antigen or a cell, tissue or organ that expresses such an antigen. In specific embodiments, an immune response is one that includes immunogenic responses that cause, activate, elicit, stimulate, or induce an immune response against a particular antigen or organism in a subject.

[0079] Reference herein to "immuno-interactive" includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0080] As used herein, the phrase "inhibit the development of" refers to a prophylactic treatment which increases the resistance of a subject to developing the disease, disorder or condition or, in other words, decreases the likelihood that the subject will develop the disease, disorder or condition as well as a treatment after the disease, disorder or condition has begun in order to reduce or eliminate it altogether or prevent it from becoming worse. This phrase also includes within its scope preventing the disease, disorder or condition from occurring in a subject which may be predisposed to the disease, disorder or condition but has not yet been diagnosed as having it.

[0081] The term "inhibitor" as used herein refers to an agent that decreases or inhibits at least one function or biological activity of a target molecule.

[0082] By "modulating" is meant increasing or decreasing, either directly or indirectly, the level or functional activity of a target molecule or response. For example, an agent may indirectly modulate the level/activity by interacting with a molecule other than the target molecule.

[0083] The phrase "optionally substituted" as used herein denotes that the group may or may not be further substituted with one or more non-hydrogen substituent groups (such as one, two, three or four non-hydrogen substituent groups). In certain embodiments, the substituent groups are one or more groups independently selected from the group consisting of halogen atoms, including F, Cl, Br or I.

[0084] By "pharmaceutically acceptable carrier" is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, fillers, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives and the like.

[0085] Similarly, a "pharmacologically acceptable" salt, ester, amide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that this not biologically or otherwise undesirable.

[0086] As used herein, the terms "polypeptide", "proteinaceous molecule", "peptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally-occurring amino acid, such as a chemical analogue of a corresponding naturally-occurring amino acid, or a PEG group, as well as to naturally-occurring amino acid polymers. These terms do not exclude modifications, for example, glycosylations, acetylations, phosphorylations and the like. Soluble forms of the subject proteinaceous molecules are particularly useful. Included within the definition are, for example, polypeptides containing one or more analogues of an amino acid including, for example, unnatural amino acids, polypeptides with substituted linkages and polypeptides with PEG groups and lipophilic moieties.

[0087] The terms "reduce", "inhibit", "suppress", "decrease", and grammatical equivalents when used in reference to the level of a substance and/or phenomenon in a first sample relative to a second sample, mean that the quantity of substance and/or phenomenon in the first sample is lower than in the second sample by any amount that is statistically significant using any art-accepted statistical method of analysis. When these terms are used to refer to the action of a compound or agent, the first sample may be a sample in the presence of the compound or agent and the second sample may be a comparative sample without the compound or agent. In one embodiment, the reduction may be determined subjectively, for example when a patient refers to their subjective perception of disease symptoms, such as pain, fatigue, motor symptoms, etc. In another embodiment, the reduction may be determined objectively, for example when the number of bacterial cells in a sample from a patient is lower than in an earlier sample from the patient. In another embodiment, the quantity of substance and/or phenomenon in the first sample is at least 10% lower than the quantity of the same substance and/or phenomenon in a second sample. In another embodiment, the quantity of the substance and/or phenomenon in the first sample is at least 25% lower than the quantity of the same substance and/or phenomenon in a second sample. In yet another embodiment, the quantity of the substance and/or phenomenon in the first sample is at least 50% lower than the quantity of the same substance and/or phenomenon in a second sample. In a further embodiment, the quantity of the substance and/or phenomenon in the first sample is at least 75% lower than the quantity of the same substance and/or phenomenon in a second sample. In yet another embodiment, the quantity of the substance and/or phenomenon in the first sample is at least 90% lower than the quantity of the same

substance and/or phenomenon in a second sample. Alternatively, a difference may be expressed as an "n-fold" difference.

[0088] As used herein, the terms "salts" and "prodrugs" include any pharmaceutically acceptable salt, ester, hydrate or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) a compound of the invention, or an active metabolite or residue thereof. The term "pharmaceutically acceptable salts" refers without limitation to derivatives of the disclosed compounds wherein the parent compound is modified by converting an existing acid or base moiety to its salt form (e.g. by reacting the free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate and valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. The pharmaceutically acceptable salts of the present invention include the conventional non-toxic salts of the parent compound formed, for example, from non-toxic inorganic or organic acids. The pharmaceutically acceptable salts can be synthesised from the parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in, for example, Remington (1985) Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 17th edition; Stahl and Wermuth (2002) Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH; and Berge *et al.* (1977) *Journal of Pharmaceutical Science*, 66: 1-19, each of which is incorporated herein by reference in its entirety.

[0089] The term "sequence identity" as used herein refers to the extent that sequences are identical on an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e. the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0090] "Similarity" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Similarity may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* 12: 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0091] Terms used to describe sequence relationships between two or more polypeptides include "reference sequence," "comparison window", "sequence identity," "percentage of sequence identity" and "substantial identity". A "reference sequence" is at least 8 but frequently 8 to 15 amino acid residues in length. As two amino acid sequences may each comprise (1) a sequence (i.e. only a portion of the complete proteinaceous molecule) that is similar between the two proteinaceous molecules, and (2) a sequence that is divergent between the two proteinaceous molecules, sequence comparisons between two (or more) proteinaceous molecules are typically performed by comparing sequences of the two proteinaceous molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.* (1997) *Nucl. Acids Res.* 25: 3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.* (1994-1998) Current

Protocols in Molecular Biology, John Wiley & Sons Inc, Chapter 15; Lambert *et al.* (2003) *Current Genomics*, 4:131-146; and Bawano *et al.* (2017) *Bioinformatics, Volume 1: Data, Sequence Analysis and Evolution (Methods in Molecular Biology (1525))*, Humana Press, pages 167-189.

[0092] The term "subject" as used herein refers to a vertebrate subject, particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable subjects include, but are not limited to, primates; avians (birds); livestock animals such as sheep, cows, horses, deer, donkeys and pigs; laboratory test animals such as rabbits, mice, rats, guinea pigs and hamsters; companion animals such as cats and dogs; and captive wild animals such as foxes, deer and dingoes. In particular, the subject is a human. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0093] As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be therapeutic in terms of a partial or complete cure for a disease, disorder or condition and/or adverse effect attributable to the disease, disorder or condition. These terms also cover any treatment of a condition, disorder or disease in a subject, particularly in a human, and include: (a) inhibiting the disease, disorder or condition, i.e. arresting its development; or (b) relieving the disease, disorder or condition, i.e. causing regression of the disease, disorder or condition.

[0094] The term "vaccine" is used herein to refer to a composition comprising at least one antigen or antigen-encoding nucleic acid molecule (e.g. DNA or RNA) which, upon inoculation into a subject, induces an immune response specific for that antigen or a cell or organism expressing the antigen and thereby confers protective immunity to the vaccinated subject against the antigen or a cell or organism expressing the antigen. The antigen may be derived for example from a pathogen, such as from bacteria, viruses, fungi, helminths, protozoans etc., or from a tumour or cancerous tissue. Vaccines may induce a complete or partial immunity to the pathogen or tumour, and/or alleviates the symptoms of disease caused by pathogen or tumour. The protective effects of a vaccine against a pathogen or tumour are normally achieved by inducing in the subject an immune response, either a cell-mediated or a humoral immune response, or a combination of both. Generally speaking, abolished or reduced incidence of infection or tumour, amelioration of symptoms, or accelerated elimination of the pathogen or tumour in subjects are indicative of the protective effects of the vaccine.

[0095] Each embodiment described herein is to be applied *mutatis mutandis* to each and every embodiment unless specifically stated otherwise.

2. *Abbreviations*

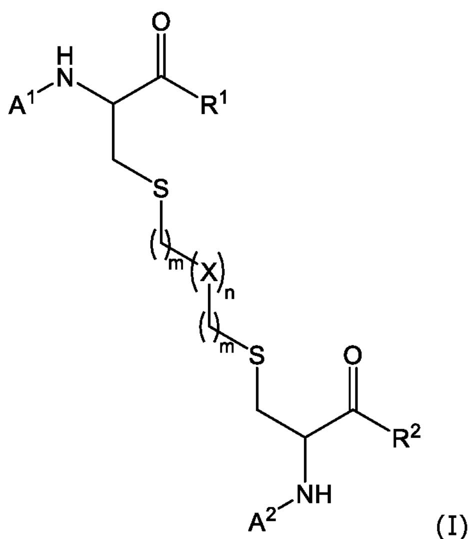
[0096] The following abbreviations are used throughout the application:

Chex = 1-Aminocyclohexanecarboxylic acid
O = Ornithine
gu = *N,N,N′,N′*-tetramethylguanidino
h = hour
min = minutes
MDR = multi-drug resistant
XDR = extensively drug resistant

3. *Compounds*

[0097] The present invention is based on the discovery that the efficiency of peptide dimerisation may be improved using aryl- or cycloalkyl-containing bifunctional linkers. The inventors also found that the efficacy and spectrum of activity of the peptide, especially an antimicrobial peptide, may be improved following dimerisation using such linkers.

[0098] Accordingly, in one aspect there is provided a compound represented by Formula I:



(I)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1 and A^2 are each independently a proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween);

each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

R^1 and R^2 are independently selected from the group consisting of -OH, $-NH_2$ and $-NHNH_2$;

n is 1 or 2; and

each m is independently 0, 1 or 2.

[0099] In some embodiments, each X is independently selected from the group consisting of optionally substituted C_5-C_{10} arylene, especially optionally substituted C_6-C_{10} arylene, optionally substituted C_5-C_{10} heteroarylene, optionally substituted C_5-C_{10} cycloalkylene and optionally substituted C_5-C_{10} heterocycloalkylene. In particular embodiments, each X is independently selected from the group consisting of optionally substituted C_5 or C_6 arylene, especially optionally substituted C_6 arylene, optionally substituted C_5 or C_6 heteroarylene, optionally substituted C_5 or C_6 cycloalkylene and optionally substituted C_5 or C_6 heterocycloalkylene; especially optionally substituted C_6 arylene (optionally substituted phenylene), optionally substituted C_6 heteroarylene, optionally substituted C_6 cycloalkylene (optionally substituted cyclohexylene) and optionally substituted C_6 heterocycloalkylene.

[0100] In alternative embodiments, each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, especially optionally substituted C_6-C_{12} arylene, and optionally substituted C_5-C_{12} cycloalkylene; especially optionally substituted C_5-C_{10} arylene and optionally substituted C_5-C_{10} cycloalkylene. In particular, each X is independently selected from the group consisting of optionally substituted C_5 or C_6 arylene, especially optionally substituted C_6 arylene, and optionally substituted C_5 or C_6 cycloalkylene; especially optionally substituted C_6 arylene (optionally substituted phenylene) and optionally substituted C_6 cycloalkylene (optionally substituted cyclohexylene).

[0101] In particular embodiments, each X is independently optionally substituted C_5-C_{12} arylene, especially optionally substituted C_5-C_{10} arylene, most especially optionally substituted C_5 or C_6 arylene. In particular embodiments, each X is independently optionally substituted C_6-C_{12} arylene, especially optionally substituted C_6-C_{10} arylene, most especially optionally substituted C_6 arylene In specific embodiments, each X is optionally substituted phenylene.

[0102] Each optionally substituted arylene, heteroarylene, cycloalkylene and heterocycloalkylene group may be substituted with one or more optional substituents selected from the group consisting of halogen atoms, including F, Cl, Br or I. In particular embodiments, the optional substituents are selected from the group consisting of F, Cl, Br

and I; especially F. In some embodiments, each optionally substituted arylene, heteroarylene, cycloalkylene and heterocycloalkylene group is substituted with one to four optional substituents (and all integers therebetween), especially four optional substituents.

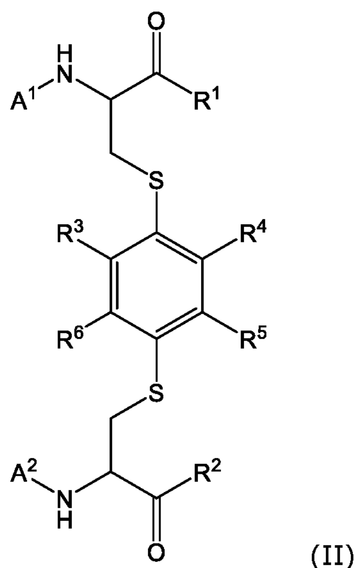
[0103] In particular embodiments, each X is independently phenylene substituted with one or more F, Cl, Br or I; especially one or more F. In specific embodiments, each X is tetrafluorophenylene.

[0104] In alternative embodiments, each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_2-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_2-C_{12} heterocycloalkylene; especially optionally substituted C_6-C_{12} arylene, optionally substituted C_4-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_4-C_{12} heterocycloalkylene. In such embodiments, each arylene and heteroarylene independently contains from 5 to 12 ring atoms, including 5 to 10 ring atoms; especially 5 or 6 ring atoms.

[0105] While n may be 1 or 2, in particular embodiments n is 1. In alternative embodiments, n is 2.

[0106] Each m may independently be 0, 1 or 2; especially independently 0 or 1. In particular embodiments, each m is 0.

[0107] In particular embodiments, the compound is represented by Formula II:



(II)

or a pharmaceutically acceptable salt thereof,

wherein:

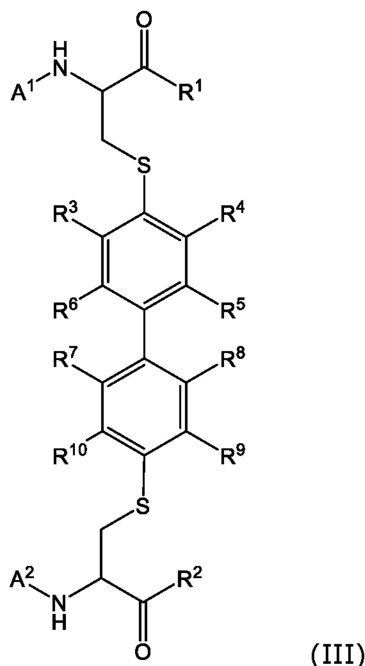
A^1, A^2, R^1 and R^2 are as defined in relation to the compound of Formula I; and

R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

[0108] Suitable embodiments for R^1 and R^2 are discussed *supra.*

[0109] While R^3, R^4, R^5 and R^6 may be H in some embodiments, in preferred embodiments, R^3, R^4, R^5 and R^6 are independently selected from the group consisting of F, Cl, Br and I. In some embodiments, R^3, R^4, R^5 and R^6 are F.

[0110] In alternative embodiments, the compound is represented by Formula III:



(III)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1, A^2, R^1 and R^2 are as defined in relation to the compound represented by Formula I; and

R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

[0111] In some embodiments, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are F.

[0112] Suitable embodiments for R^1 and R^2 are discussed *supra.*

[0113] In any one of the aspects or embodiments described herein, A^1 and A^2 may be any proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween) for which dimerisation is desired. In particular embodiments, the proteinaceous molecule comprises from about 5 to about 50 amino acid residues, about 10 to about 30 amino acid residues or about 15 to about 25 amino acid residues (and all integer residues therebetween); including about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 amino acid residues. A skilled person will readily appreciate

that a number of different proteinaceous molecules can be used. For example, the proteinaceous molecule may be an antimicrobial peptide including a proline-rich antimicrobial peptide, insulin or a fragment or derivative thereof, an amphipathic peptide, a receptor antagonist, a receptor agonist, an ion channel antagonist, an ion channel agonist, an enzyme inhibitor, an enzyme activator, a peptide hormone, a venom peptide, an immunomodulator, an inhibitor of a transporter, an activator of a transporter, an antigen peptide, or a fragment or derivative thereof.

[0114] In particular embodiments, A^1 and A^2 are antimicrobial peptides. Suitable antimicrobial peptides may include, for example, magainins (e.g. magainin-1, magainin-2, magainin-A, pexiganan and PGLa), protegrins (e.g. protegrin-1, protegrin-2, protegrin-3, protegrin-4 and protegrin-5), cathelicidins (e.g. LL-37, hCAP18, SAAP-148, prophenin, PF-18, prophenin-1, and prophenin-2), defensins (including α- and β-defensins, such as human neutrophil peptide 1, 2, 3 and 4, human defensin 5 and 6, HBD1, HBD2, pBD1, DEFb1, HBD3, HBD4 and HBD9, plectasin, eurocin, MGD-1 and copsin), tryptophan- and arginine-rich antimicrobial peptides (e.g. indolicidin, triptrpticin and Octa 2), proline-rich antimicrobial peptides, drosomycin, thionins (including α1-purothionin, α2-purothionin, β-purothionin, type-5 thionin, PR-13, PR-14, Hordothionin α, Barley leaf-thionin DB4, Pyrularia thionin, Viscotoxin A3, Crambin 2, *Arabidopsis thaliana* thionin 2.1, pTTH20 Neutral wheat thionin, *Tulipa gesneriana* thionin 4.1, PsoTHI1.5, PsoTHI1.6 and PsoTHI1.7), histidine-rich antimicrobial peptides (e.g. histatins including histatin-5, histatin-1 and histatin-3, HV2 and L4H4), glycine-rich antimicrobial peptides (such as attacins, diptericins and GG3), cysteine-rich antimicrobial peptides (e.g. gomesin), bactenecin, lactogerricin, cecropins (e.g. cecropin A, cecropin B, CECD, papiliocin and cecropin P1), bacteriocins (e.g. colicins, microcins, tailocins, Class I bacteriocins including lantibiotics such as nisin, Class II bacteriocins including Class IIa, IIb, IIc and IId bacteriocins, Class III bacteriocins including Class IIIa and IIIb bacteriocins, and Class IV bacteriocins), cyclic peptides (such as polymyxin B, Gramicidin S, daptomycin, surfactin, iturin, Dalbavancin and Oritavancin), japonicins (e.g. japonicin-1 and japonicin-2), nigrocins (e.g. nigrocin-1 and nigrocin-2), temporins (e.g. temporin A, temporin B, temporin SHa and temporin L), dermaseptins (such as DRS-S1, DRS-B1, DRS-S4, DRS-CA1, DRS-DU1, DRS-PH, DRS-B2, DRS-B3, DRS-B4, DRS-S2, DRS-S3, DRS-S5, DRS-H3, DRS-L1, DRS-O1, DRS-DI06, DRS-PD1, DRS-PD2 and DRS-PS4), buforins (e.g. buforin I and buforin II), bombinins (including bombinin, BLP-1 and BLP-3), thanatin, melittin, callinectin, penaeidins (e.g. penaeidin-1, penaeidin-2, penaeidin-3, penaeidin-4, Fi-PEN and Mm-PEN), lactoferricin, bacillomycins (e.g. bacillomycin A, bacillomycin C, bacillomycin D, bacillomycin F, bacillomycin Fc, bacillomycin L and bacillomycin S), syringomycins (e.g. syringomycin E), echinocandins (including caspofungin, micafungin, echinocandin B, cilofungin, anidulafungin and rezafungin), aculeacins (e.g. aculeacin A), aureobasidin A,

pardaxin, misgurin, pleurocidin, aprasin I, strongylocins (e.g. strongylocin 1 and strongylocin 2), centrocins (e.g. centrocin 1 and centrocin 2), beta thymosins (e.g. thymosin β4, thymosin β10 and thymosin β15), hyastatin, omiganan and analogues thereof including MBI-226 and MX-594AN, AN5-1, maximin H5, dermicidin derived peptides (e.g. DCD-1L, DCD-1 and SSL25), andropin, moricins (including Bm-Moricin, Bm-moricin A1, Bm-moricin 2, Px-Mor, Px-Mor113/114 and Px-Mor 115), ceratotoxins (e.g. ceratotoxin A, ceratotoxin A 1-29, ceratotoxin B, ceratotoxin C and ceratotoxin D), brevinins (including brevinin-1 and brevinin-2), esculentins (such as esculentin 1, esculentin-1a, esculentin(1-21), esculentin-1a(1-21)NH_2, esculentin(1-21)-1c and esculentin-2Cha(1-30)), tachyplesins (e.g. tachyplesin I, II and III), psoriasin, piscidins (e.g. Rbpisc, piscidin 4, piscidin 1, piscidin 2 and piscidin 3), and fragments, derivatives or analogues thereof.

[0115] In particular embodiments, the antimicrobial peptide is a proline-rich antimicrobial peptide (i.e. A^1 and A^2 are each a proline-rich antimicrobial peptide). Suitable proline-rich antimicrobial peptides include, but are not limited to, Chex1-Arg20, oncocin, apidaecin (Api137), drosocin, Bac7(1-35), abaecin, abaecin (*Bombus pascuorum*), abaecin (*Apis mellifera*), apidaecin 1a, apidaecin 1b, apidaecin Cd3+, arasin 1, Bac5(1-23), BSN-37, formaecin 1, formaecin 2, heliocin, metalnikowin-1, metalnikowin-2A, metalnikowin-2B, metalnikowin-3, metchnikowan, metchnikowin-2, PR-39, pyrrhocoricin, riptocin, Tur1A, Tur1B, alpha-defensin-related sequence 10, alpha-defensin-related sequence 12, alpha-defensin-related sequence 7, antibacterial 6.5 kDa protein, antibacterial napin, apidaecin (*Bombus pascuorum*), apidaecin 2, APO, arasin 2, arasin 1, astacidin 2, attacin-C, bactenecin 5, bactenecin 7, BSN-37, cathelicidin-2 (*Bos taurus*), cathelicidin-2 (*Capra hircus*), cathelicidin-2 (*Ovis aries*), cathelicidin-3 (*Bos taurus*), cathelicidin-3 (*Ovis aries*), cathelicidin-3.4 (*Capra hircus*), Cg-IgPrp, CG-IgPrp P/Q, Dros pro attC, Dros-Pyyr-Dros, heliocin, lebocin-1/2, OaBac6, oncopeltus antibacterial peptide-4, P9, PP30 and PR-bombesin. In particular embodiments, the antimicrobial peptide (i.e. A^1 and A^2) is selected from the group consisting of Chex1-Arg20 (SEQ ID NO: 1), oncocin (SEQ ID NO: 2), apidaecin (Api137) (SEQ ID NO: 3), drosocin (SEQ ID NO: 4) and Bac7(1-35) (SEQ ID NO: 5):

[Chex]RPDKPRPYLPRPRPPRPVR	[SEQ ID NO: 1];
VDKPPYLPRPRPPRRIYNR	[SEQ ID NO: 2];
[gu]ONNRPVYIPRPRPPHPRL	[SEQ ID NO: 3];
GKPRPYSPRPTSHPRPIRV	[SEQ ID NO: 4]; and
RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFP	[SEQ ID NO: 5].

[0116] In specific embodiments, the antimicrobial peptide (i.e. each of A^1 and A^2) is a proteinaceous molecule comprising, consisting or consisting essentially of an amino acid sequence of SEQ ID NO: 1.

[0117] In some embodiments, the proteinaceous molecule is other than a cyclic proteinaceous molecule (i.e. the proteinaceous molecule comprises an N- and/or C-terminus).

[0118] In alternative embodiments, A^1 and A^2 are amphipathic peptides, representative examples of which include ApoA-I mimetic peptides such as 18A, D-4F, L-4F, 5A and FAMP; ApoE mimetic peptides such as AEM28, Ac-[R]hE18A-NH_2, Myr-[R]hE18A-NH_2, mR18L, ATI-5261, CS-6253, EpK, hEp and CN-105; and ApoC-II mimetic peptides such as 18A-CII and D6PV.

[0119] Also contemplated are embodiments wherein A^1 and A^2 are receptor or ion channel antagonists or agonists. For example, in some embodiments, A^1 and A^2 may be independently selected from a sodium channel (such as μ-conotoxins and spider venom peptides e.g. GpTx-1), calcium channel (e.g. ω-conotoxins such as ziconotide), potassium channel (e.g. ShK and α-DTx), transient receptor potential channel (e.g. protoxin-1), acid-sensing ion channel (e.g. PcTx1 and APETx2), thrombin receptor such as a protease-activated receptor (e.g. 2-furoyl-LIGRLO-NH_2), natriuretic peptide receptor (e.g. ANP, BNP, nesiritide, ularitide or vosoritide), G-protein coupled receptor (including those listed herein), opioid receptor (e.g. acetalin 3, enkephalin and analogues thereof such as [D-Ala^2, *N*-$MePhe^4$, Gly-ol]-enkephalin, [D-Ala^2, D-Leu^5]-enkephalin and [D-Pen^2,D-Pen^5]-enkephalin, dynorphins, endorphins, nociceptin, zyklophin and morphiceptin), hepatocyte growth factor receptor including cMet (e.g. C7, CM 7 and eNK1), endothelin receptor (e.g. BQ123, TAK-044, sarafotoxin S6C, BQ3020, IRL1620, FR139317 and BQ788), vasopressin receptor (e.g. desmopressin), oxytocin receptor (e.g. basusiban and atosiban) or interleukin receptor (e.g. IL-1, IL-6 or IL-23 receptor) agonist or antagonist; a glucagon-like peptide-1 receptor (e.g. exenatide, liraglutide, lixisenatide, albiglutide, dulaglutide, exendin-4 and the peptides disclosed in WO 2015067716 A1), glucose dependent insulinotropic peptide receptor, melanocortin 1 receptor (e.g. bremelanotide and setmelanotide), motilin receptor (e.g. atilmotin), somatostatin receptor (e.g. pasireotide, octreotide, BIM-355, BIM-071 and BIM-839), angiotensin II receptor (e.g. LJPC-501) or erythropoietin receptor (e.g. hematide) agonist; or a calcitonin gene-related peptide receptor, androgen receptor, growth hormone receptor (e.g. S1H), prolactin receptor, chemokine receptor such as CXCR4 (e.g. LY2510924), T-cell receptor, gastrin-releasing peptide receptor, estrogen receptor (e.g. the peptides disclosed in Phillips *et al.* (2011) *J. Am. Chem. Soc.*, 133(25): 9696–9699), insulin receptor (e.g. S597, S661 and S961), bradykinin receptor (e.g. icatibant, B9958 and B10324), gastrin receptor, gastrin-releasing peptide receptor (e.g. RC-3095), integrin such as α4β7, αvβ3 and α5β1 integrin (such as cilengitide, PN-943, ATN-161 and AXT-107) or vascular endothelial growth factor receptor (e.g. peptide HRH, VGB4 and VGB3) antagonist.

[0120] The proteinaceous molecule may also be an enzyme inhibitor or activator. For example, the enzyme inhibitor may be an ACE inhibitor (e.g. RXPA380, RXP407 and the peptides disclosed in WO 2004082709 A1, WO 2008108649 A2 and WO 2012104462 A1), a kinase inhibitor such as a c-Jun N-terminal kinase inhibitor (e.g. JIP^{10}-△-TAT^{i}, JIP^{10}-△-R^{9}, JNK3-N-TAT, Tat-SabKIM1, pepJIP1, pepSAB, FRATtide, PKI-(6-22)-amide, L803-mts, SAPK inhibitor I, KRX-014 H151, KRX-702 H105, p21 WAF1, GID, εV1-2, βC2-4, βC2-2, MLCK inhibitor peptide 18, αV5-3-TAT peptide, S-Ht31 and the peptides disclosed in WO 2016145234 A2, WO 2009021137 A2, WO 2009144037 A1 and WO 2008154004 A2), a renin inhibitor (e.g. pepstatin, renin prosegment or angiotensinogen N-terminus analogues or fragments thereof and CGP2928), a HIV protease inhibitor (such as G12, p27 and the peptides disclosed in WO 1998052970 A9), a dipeptidyl peptidase-4 inhibitor (e.g. the peptides disclosed in US 2014/0309401 A1 and WO 2008066070 A1, and dipeptide derivatives or dipeptide mimetics such as alanine-pyrrolidide, isoleucine-thiazolidide and N-valyl prolyl, O-benzoyl hydroxylamine), a serine protease inhibitor (e.g. the peptides disclosed in WO 2003070770 A2, aprotinin, mupain-1 and analogues thereof) or a matrix metalloproteinase inhibitor (e.g. CTT, M204C4, M205C4, APP-IP, Cltx, P713, TCTP-1, IVS4, IS4, P3a, cyclic LRSG, regaseptin 1, STX-S4-CT, Peptide G, CTT1, CTT2 and RXP470.1). Suitable enzyme activators include, but are not limited to, a kinase activator (such as PI 3-kinase activator and an S-tide such as S1.1, S1.2, S1.4, S1.5, S1.9 and S1.10) and an agonist of endothelial cell receptor tyrosine kinases, including Tie2/Tek (e.g. vasculotide and ATX107).

[0121] In some embodiments, A^1 and A^2 are immunomodulators, representative examples of which include thymopentin or thymosin alpha 1; inhibitors of a transporter, such as ferroportin, PEPT1 (e.g. Lys[Z(NO(2))]-Pro) and ABCB1 (e.g. HX-12C); or an antigenic peptide, such as a tumour antigen peptide.

[0122] Also contemplated is wherein A^1 and A^2 are variants of any one of the proteinaceous molecules described above, especially a variant of Chex1-Arg20 (SEQ ID NO: 1). In some embodiments, the variants are distinguished from the parent sequence by the addition, deletion, or substitution of one or more amino acid residues. In general, variants will display at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence similarity to a parent or reference proteinaceous molecule sequence as, for example, set forth in SEQ ID NO: 1, as determined by sequence alignment programs described elsewhere herein using default parameters. Desirably, variants will have at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to a parent or reference proteinaceous molecule sequence as, for example, set forth in SEQ ID NO: 1, as determined by sequence alignment programs described herein using default parameters. Variants of the proteinaceous molecule,

including SEQ ID NO: 1, which fall within the scope of a variant proteinaceous molecule, may differ from the parent molecule generally by at least 1, but by less than 5, 4, 3, 2 or 1 amino acid residue(s). In some embodiments, a variant proteinaceous molecule of the invention differs from the corresponding sequence in SEQ ID NO: 1, by at least 1, but by less than 5, 4, 3, 2 or 1 amino acid residue(s).

[0123] Variant proteinaceous molecules also include proteinaceous molecules derived from SEQ ID NO: 1 by deletion or addition of one or more amino acids (such as from 1-50 amino acid residues and all integer amino acids therebetween, including from 1-10 or 1-5 amino acid residues) to the N-terminal and/or C-terminal end of the proteinaceous molecule, deletion or addition of one or more amino acids (such as from 1-5 amino acid residues and all integer amino acids therebetween) at one or more sites in the proteinaceous molecule, or substitution of one or more amino acids at one or more sites in the proteinaceous molecule. For example, in some embodiments, the variant proteinaceous molecule comprises an addition of one amino acid residue or deletion of one amino acid residue.

[0124] Variant proteinaceous molecules encompassed by the present invention are biologically active, that is, they continue to possess the desired biological activity of the parent proteinaceous molecule, for example, antimicrobial activity.

[0125] In suitable embodiments, the proteinaceous molecules described above may comprise a linking moiety. For example, the proteinaceous molecule may comprise a linking moiety between its C-terminus and the adjacent nitrogen atom of the compound of Formula I, II or III. Exemplary linking moieties include a peptide linker. Variation within the peptide sequence of the linking moiety is possible, such that the linking moiety may be modified to alter the physicochemical properties of the compound and potentially reduce side effects of the compound of the invention or otherwise improve the therapeutic use of the compound, for example, by improving stability. The peptidic linking moiety may be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues in length.

[0126] The proteinaceous molecules described above may also contain modified amino acid residues. Modified amino acid residues may include residues with modified side chains, N-methyl amino acids, α-methyl amino acids, residues with acetylated N-termini, beta amino acids, and the like. The proteinaceous molecules may also comprise unnatural amino acid residues and/or their derivatives and the use of cross-linkers and other methods which impose conformational constraints on the proteinaceous molecules.

[0127] The use of proteinaceous molecules (e.g. of A^1 and A^2) with high levels of stability may be desired, for example, to increase the half-life of the compound in a subject. Thus, in some embodiments, the proteinaceous molecules comprise a stabilising or protecting moiety. The stabilising or protecting moiety may be conjugated at any point on

the proteinaceous molecule. The stabilising or protecting moiety may be any moiety which delays or prevents substantial degradation of the compound. A skilled person will be well aware of suitable stabilising or protecting moieties which may be used. Exemplary stabilising or protecting moieties include, but are not limited to, a peptide or protein such as an albumin including human serum albumin or a fragment or variant thereof, a glycine-rich homo-amino-acid polymer, a PAS sequence comprising a combination of alanine, serine and proline residues, the C-terminal peptide (CTP) of the β subunit of human chorionic gonadotropin or fragment or variant thereof, transferrin or a fragment or variant thereof, an albumin binding moiety, which comprises an albumin binding peptide, a bacterial albumin binding domain, an albumin-binding antibody fragment, or any combinations thereof, or an XTEN polypeptide (an extended length polypeptide with a non-naturally occurring, substantially non-repetitive sequence that is composed mainly of small hydrophilic amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions); an Fc region or single chain Fc region comprising a functional neonatal Fc receptor (FcRn) binding partner comprising an Fc domain, variant, or fragment thereof; a polymer such as a polyethylene glycol (PEG), a polysialic acid or a derivative thereof, hydroxyethyl starch or a derivative thereof, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran or polyvinyl alcohol; a glycan or polysaccharide; a lipid moiety for example, a C_6-C_{20} fatty acyl group; or a capping moiety, including an acetyl group, pyroglutamate or an amino group.

[0128] In some embodiments, the protecting or stabilising moiety is a PEG. The PEG can be of any molecular weight, and can be branched or unbranched. In one embodiment, the molecular weight is between about 1 kDa and about 100 kDa for ease in handling and manufacturing. Other sizes can be used, depending on the desired profile (e.g. the duration of sustained release desired, the effects, if any on biological activity, the ease in handling and other known effects of the polyethylene glycol to a peptide or protein). For example, the polyethylene glycol can have an average molecular weight of about 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500 or 5000 kDa.

[0129] In some embodiments, the polyethylene glycol has a branched structure. Branched polyethylene glycols are described, for example, in U.S. Pat. No. 5,643,575; Morpurgo *et al.* (1996, *Appl. Biochem. Biotechnol.,* 56: 59-72); Vorobjev *et al.* (1999, *Nucleosides Nucleotides,* 18: 2745-2750); and Caliceti *et al.* (1999, *Bioconjug. Chem.,* 10: 638-646).

[0130] In some embodiments, the protecting or stabilising moiety is a lipid moiety. The lipid moiety may be a lipid moiety comprising 6 to 24 carbon atoms in the alkyl chain (and all integers therebetween); especially 8 to 22 carbon atoms; most

especially 10 to 20 carbon atoms (e.g. a C_6-C_{20} fatty acyl group). For example, the lipid moiety may be hexanoyl (C_6), heptanoyl (C_7), octanoyl (C_8), nonanoyk (C_9), decanoyl (C_{10}), undecanoyl (C_{11}), dodecanoyl (C_{12}), tridecanoyl (C_{13}), tetradecanoyl (C_{14}), pentadecanoyl (C_{15}), hexadecanoyl (C_{16}), heptadecanoyl (C_{17}) or octadecanoyl (C_{18}). In particular embodiments, the lipid moiety is hexanoyl (C_6), octanoyl (C_8), decanoyl (C_{10}), dodecanoyl (C_{12}), tetradecanoyl (C_{14}), hexadecanoyl (C_{16}) or octadecanoyl (C_{18}); especially tetradecanoyl, hexadecanoyl or octadecanoyl. While the lipid moiety may be directly conjugated to the proteinaceous molecule, in some embodiments, the lipid moiety is conjugated via a linker to the proteinaceous molecule, such as a PEG linker (e.g. a PEG containing from 4 to 12 ethylene glycol groups).

[0131] When used, a capping moiety such as an acetyl group and/or pyroglutamate are conjugated to the N-terminal amino acid residue of the proteinaceous molecule, e.g. the N-terminus of the proteinaceous molecule is a pyroglutamide or acetamide (e.g. in compounds of Formulae I, II and III). The amino group, for example, may be conjugated to the C-terminal amino acid residue of the proteinaceous molecule, e.g. the proteinaceous molecule has a primary amide at the C-terminus (e.g. in compounds of Formulae IV, V and VI described herein).

[0132] When present, a PEG or lipid moiety may be, for example, conjugated to the N-terminal or C-terminal amino acid residue of the proteinaceous molecule or through the amine of an amino acid side chain, such as a lysine side chain, especially through the N-terminal amino acid residue (e.g. in compounds of Formulae I, II and III), such as through the α-amino group, or through the amino group of a lysine side chain (i.e. the ε-amino group).

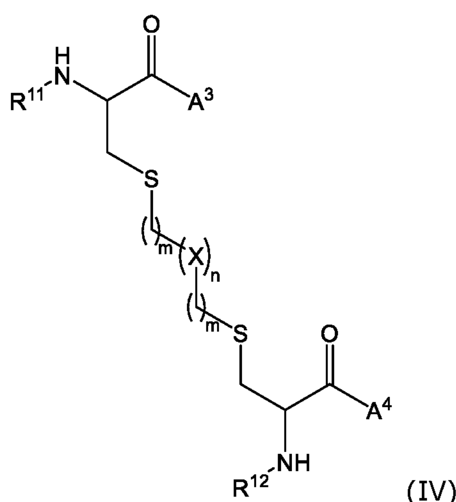
[0133] In particular embodiments, the proteinaceous molecule has a primary amide or a free carboxyl group (acid) at the C-terminus and a primary amine or acetamide at the N-terminus; especially a C-terminal acid, and an N-terminal amine.

[0134] While the protecting or stabilising moiety may be attached to the N- and/or C-terminus of the proteinaceous molecule, the moiety may also be attached to the proteinaceous molecule through a side-chain of an amino acid residue, such as through the amino group in the side chain of an amine- or amide-containing amino acid residue, such as lysine, arginine, glutamine and asparagine or other suitably modified side chain, especially through a lysine side chain.

[0135] While compounds wherein A^1 and A^2 are different are contemplated, in preferred embodiments, A^1 and A^2 are the same. That is, in particular embodiments, the compound of the invention is a homodimer.

[0136] A^1 and A^2 may be attached to the adjacent nitrogen atom of the compound of Formula I, II or III at any point on the proteinaceous molecule. In particular embodiments, A^1 and A^2 are attached to the adjacent nitrogen atom of the compound of Formula I, II or III via their C-terminus. A skilled person will be well aware of suitable means for attachment of the proteinaceous molecule to the adjacent nitrogen atom of Formula I, such as via an amide bond.

[0137] While the above aspects focused on dimers wherein the proteinaceous molecules are dimerised via their C-termini, the inventors have conceived that the proteinaceous molecules may alternatively be dimerised via their N-termini. Accordingly, in another aspect, there is provided a compound represented by Formula IV:



(IV)

or a pharmaceutically acceptable salt thereof,

wherein:

A^3 and A^4 are each independently a proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween);

each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

R^{11} and R^{12} are independently selected from the group consisting of -H and -C(O)CH_3;

n is 1 or 2; and

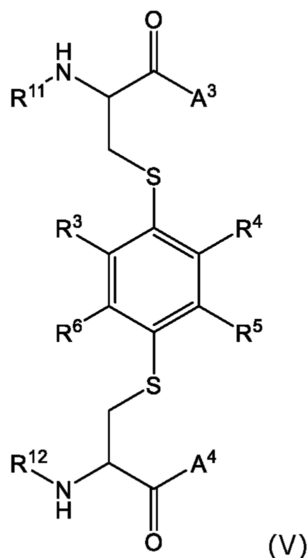
each m is independently 0, 1 or 2.

[0138] Suitable embodiments of X, n and m are discussed *supra* in relation to the compound represented by Formula I.

[0139] In particular embodiments, R^{11} and R^{12} are H.

[0140] A^3 and A^4 are independently a proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween). Suitable embodiments of A^3 and A^4 are as discussed above for A^1 and A^2.

[0141] In some embodiments, the compound is a compound represented by Formula V:



(V)

or a pharmaceutically acceptable salt thereof,

wherein:

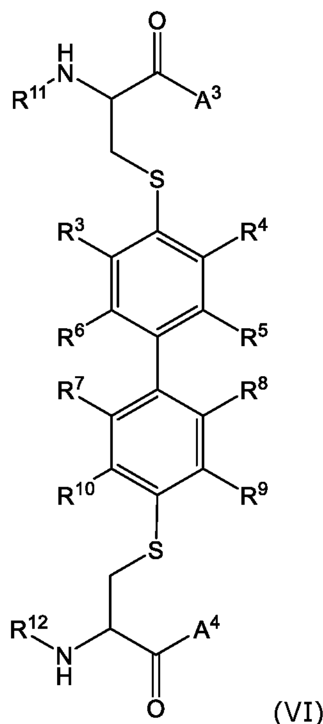
A^3, A^4, R^{11} and R^{12} are as defined for the compound represented by Formula IV; and

R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

[0142] Suitable embodiments of R^3, R^4, R^5 and R^6 are discussed *supra* in relation to the compound represented by Formula II.

[0143] Suitable embodiments for A^3, A^4, R^{11} and R^{12} are discussed *supra.*

[0144] In alternative embodiments, the compound is represented by Formula VI:



(VI)

or a pharmaceutically acceptable salt thereof,

wherein:

A^3, A^4, R^{11} and R^{12} are as defined for the compound represented by Formula IV; and

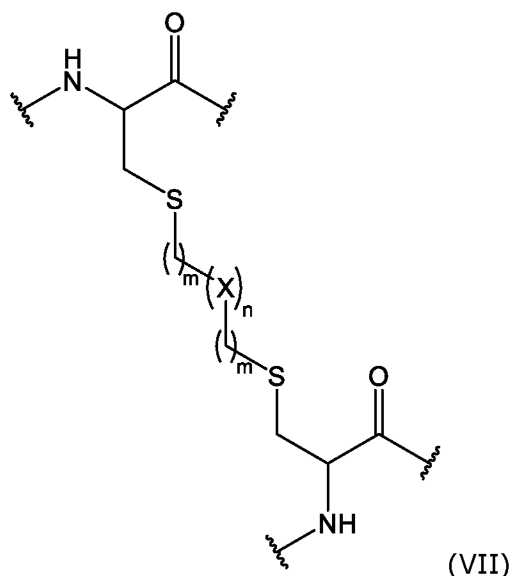
R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

[0145] Suitable embodiments of R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are discussed above in relation to the compound represented by Formula III.

[0146] Suitable embodiments for A^3, A^4, R^{11} and R^{12} are discussed *supra.*

[0147] While A^3 and A^4 may be attached to the adjacent carbon atom at any point in their amino acid sequence, in some embodiments A^3 and A^4 are attached to the adjacent carbon atom of the compound of Formula IV, V or VI via their N-terminus. A skilled person will be aware of suitable chemical methods for attachment of A^3 and A^4 to the adjacent carbon atom, such as via an amide bond.

[0148] In another aspect, there is provided a linker represented by Formula VII:



(VII)

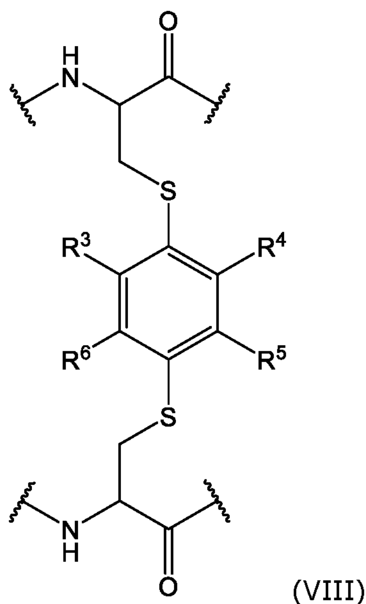
wherein each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

n is 1 or 2; and

each m is independently 0, 1 or 2.

[0149] Suitable embodiments of X, n and m are discussed *supra*.

[0150] In some embodiments, the linker is represented by Formula VIII:

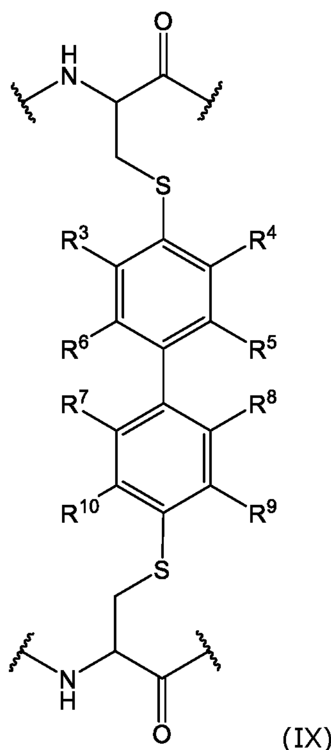


(VIII)

wherein R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

[0151] Suitable embodiments of R^3, R^4, R^5 and R^6 are discussed *supra*.

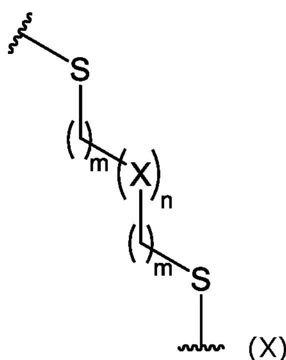
[0152] In alternative embodiments, the linker is represented by Formula IX:



wherein R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

[0153] Suitable embodiments of R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are discussed *supra*.

[0154] In yet another aspect, there is provided a linker represented by Formula X:



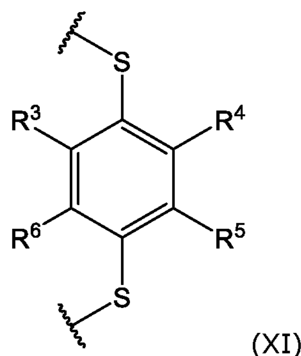
wherein each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

n is 1 or 2; and

each m is independently 0, 1 or 2.

[0155] Suitable embodiments of X, n and m are discussed *supra*.

[0156] In some embodiments, the linker is represented by Formula XI:

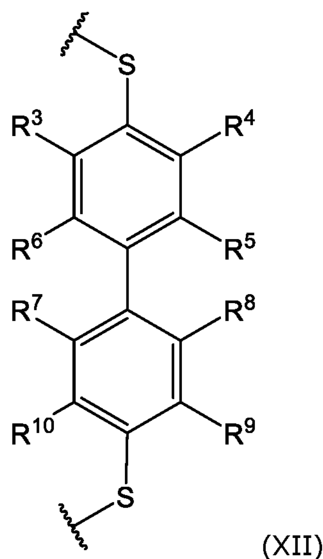


(XI)

wherein R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

[0157] Suitable embodiments of R^3, R^4, R^5 and R^6 are discussed *supra*.

[0158] In alternative embodiments, the linker is represented by Formula XII:



(XII)

wherein R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

[0159] Suitable embodiments of R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are discussed *supra*.

[0160] Compounds comprising the linkers represented by Formulae VII, VIII, IX, X, XI or XII are also contemplated, especially proteinaceous molecule dimers. For example, a proteinaceous molecule (e.g. A^1, A^2, A^3 and/or A^4) may be attached to the linker at two or more of the available positions (i.e. the positions represented by ⟋). Suitable proteinaceous molecules are as discussed herein in relation to A^1, A^2, A^3 and A^4.

[0161] The use of the linkers represented by Formulae VII, VIII, IX, X, XI or XII for dimerising a proteinaceous molecule or for preparing a proteinaceous molecule dimer

is also encompassed by the present invention. Suitable proteinaceous molecules are as discussed herein in relation to A^1, A^2, A^3 and A^4.

[0162] The compounds of the invention may be in the form of salts or prodrugs. The salts of the compounds of the invention are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention.

[0163] The compounds may be in crystalline form and/or in the form of solvates, for example, hydrates. Solvation may be performed using methods known in the art.

[0164] The compounds may be prepared using any suitable method, such as chemical synthesis or a combination of recombinant DNA techniques and chemical synthesis. In some embodiments, the compounds are prepared using standard peptide synthesis methods, such as solution synthesis or solid phase synthesis. The chemical synthesis may be performed manually or using an automated synthesiser. For example, the linear peptides may be synthesised using solid phase peptide synthesis using either Boc or Fmoc chemistry, as described in Merrifield (1963) *J Am Chem* Soc, 85(14): 2149-2154; Schnolzer, *et al.* (1992) *Int J Pept Protein Res*, 40: 180-193; Cardoso, *et al.* (2015) *Mol Pharmacol*, 88(2): 291-303; and Kumar *et al.* (2020) *ACS Omega*, 5: 2345-2354, the entire contents of which are incorporated by reference. For compounds of Formulae I, II and III, a C-terminal cysteine residue may be added to the linear peptide sequence, and for compounds of Formulae IV, V and VI, an N-terminal cysteine residue may be added to the linear peptide sequence. Following deprotection and cleavage from the solid support, the linear peptides are purified using suitable methods, such as preparative chromatography, and disulfide bonds are formed using oxidation where appropriate. Suitable conditions for oxidation of the peptide will be readily determined by a person skilled in the art. For dimerisation, the purified peptide is then reacted with a linker solution (e.g. 1,2-dibromomethyl-benzene, 1,3-dibromomethyl-benzene, 1,4-dibromomethyl-benzene, hexafluorobenzene or decafluorobiphenyl) in a suitable solvent such as dimethylformamide (DMF) for a time sufficient for dimerisation to occur, such as from about 1 hour to about 48 hours (and all integer minutes therebetween), especially about 12 hours. A skilled person will readily be able to determine a suitable temperature for the reaction, such as from about 10-35 °C, including about room temperature.

[0165] In some embodiments, the proteinaceous molecules of the invention may be cyclised, for example, prior to dimerisation. Cyclisation may be performed using several techniques, for example, as described in Davies (2003) *J Pept Sci*, 9: 471-501; or Thongyoo *et al.* (2006) *Chem Commun (Camb)*, 27: 2848-2850. The cyclised peptide may then be deprotected (i.e. the side chain protecting groups may then be removed) using standard techniques, followed by purification using suitable methods, such as preparative

chromatography. Alternatively, cyclisation may be achieved on resin using a suitable coupling agent and a suitable resin, such as a Kaiser oxime resin, and/or linker (e.g. a safety catch linker), or via native chemical ligation as described in Thongyoo *et al.* (2006) *Chem Commun (Camb)*, 27: 2848-2850.

[0166] In some embodiments, the proteinaceous molecules are prepared using recombinant DNA techniques prior to dimerisation using the procedure described above. For example, the proteinaceous molecules may be prepared by a procedure including the steps of: (a) preparing a construct comprising a polynucleotide sequence that encodes the proteinaceous molecule and that is operably linked to a regulatory element; (b) introducing the construct into a host cell; (c) culturing the host cell to express the polynucleotide sequence to thereby produce the encoded proteinaceous molecule; and (d) isolating the proteinaceous molecule from the host cell. The proteinaceous molecule may be prepared recombinantly using standard protocols, for example, as described in Klint, *et al.* (2013) *PLOS One*, 8(5): e63865; Sambrook, *et al.* (1989) Molecular Cloning: A Laboratory Manual (Cold Spring Harbour Press), in particular Sections 16 and 17; Ausubel, *et al.* (1998) Current Protocols in Molecular Biology (John Wiley and Sons, Inc.), in particular Chapters 10 and 16; and Coligan, *et al.* (1997) Current Protocols in Protein Science (John Wiley and Sons, Inc.), in particular Chapters 1, 5 and 6. Under some circumstances it may be desirable to undertake oxidative disulfide bond formation of the expressed peptide after peptide expression. This may be preceded by a reductive step to provide the linear peptide. Suitable conditions for reduction and oxidation of the peptide will be readily determined by a person skilled in the art.

4. Compositions

[0167] In accordance with the present invention, the compounds of the invention are also useful in compositions and methods for treating or inhibiting the progression or development of a disease, disorder or condition. In particular, the compounds represented by Formulae I, II, III, IV, V or VI may be useful for treating an infection, inflammatory disorder or autoimmune disorder, eliciting or enhancing an immune response or disrupting a biofilm. Thus, in some embodiments, the compounds may be in the form of a pharmaceutical composition, wherein the pharmaceutical composition comprises a compound of the invention and a pharmaceutically acceptable carrier or diluent.

[0168] The compound may be formulated into the pharmaceutical composition as a neutral or salt form.

[0169] As will be appreciated by those skilled in the art, the choice of pharmaceutically acceptable carrier or diluent will be dependent on the route of administration and on the nature of the condition and subject to be treated. The particular carrier or delivery system and route of administration may be readily determined by a

person skilled in the art. The carrier or delivery system and route of administration should be carefully selected to ensure that the activity of the compound is not depleted during preparation of the formulation and the compound is able to reach the site of action intact. The pharmaceutical compositions of the invention may be administered through a variety of routes including, but not limited to, oral, rectal, topical, intranasal, intraocular, transmucosal, intestinal, enteral, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intracerebral, intravaginal, intravesical, intravenous or intraperitoneal administration; especially oral, intravenous, intramuscular, subcutaneous, intrathecal, or intraperitoneal; most especially oral or intravenous administration.

[0170] The pharmaceutical forms suitable for injectable use include sterile injectable solutions or dispersions and sterile powders for the preparation of sterile injectable solutions. Such forms should be stable under the conditions of manufacture and storage and may be preserved against reduction, oxidation and microbial contamination.

[0171] A person skilled in the art will readily be able to determine appropriate formulations for the compounds using conventional approaches. Techniques for formulation and administration may be found in, for example, *Remington: The Science and Practice of Pharmacy*, Loyd V. Allen, Jr (Ed), The Pharmaceutical Press, London, 22nd Edition, September 2012.

[0172] Identification of preferred pH ranges and suitable excipients, such as antioxidants, is routine in the art, for example, as described in Katdare and Chaubel (2006) Excipient Development for Pharmaceutical, Biotechnology and Drug Delivery Systems (CRC Press). Buffer systems are routinely used to provide pH values of a desired range and may include, but are not limited to, carboxylic acid buffers, such as acetate, citrate, lactate, tartrate and succinate; glycine; histidine; phosphate; tris(hydroxymethyl)aminomethane (Tris); arginine; sodium hydroxide; glutamate; and carbonate buffers. Suitable antioxidants may include, but are not limited to, phenolic compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole; vitamin E; ascorbic acid; reducing agents such as methionine or sulphite; metal chelators such as ethylene diamine tetraacetic acid (EDTA); cysteine hydrochloride; sodium bisulfite; sodium metabisulfite; sodium sulfite; ascorbyl palmitate; lecithin; propyl gallate; and alpha-tocopherol.

[0173] For injection, the compound may be formulated in an aqueous solution, suitably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, dextrose solution or physiological saline buffer, such as phosphate buffered saline (PBS). For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0174] The compositions of the present invention may be formulated for administration in the form of liquids, containing acceptable diluents (such as saline and

sterile water), or may be in the form of lotions, creams or gels containing acceptable diluents or carriers to impart the desired texture, consistency, viscosity and appearance. Acceptable diluents and carriers are familiar to those skilled in the art and include, but are not restricted to, ethoxylated and nonethoxylated surfactants, fatty alcohols, fatty acids, hydrocarbon oils (such as palm oil, coconut oil, and mineral oil), cocoa butter waxes, silicon oils, pH balancers, cellulose derivatives, emulsifying agents such as non-ionic organic and inorganic bases, preserving agents, wax esters, steroid alcohols, triglyceride esters, phospholipids such as lecithin and cephalin, polyhydric alcohol esters, fatty alcohol esters, hydrophilic lanolin derivatives and hydrophilic beeswax derivatives.

[0175] Alternatively, the compound can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration, which is also contemplated for the practice of the present invention. Such carriers enable the compounds of the invention to be formulated in dosage forms such as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. These carriers may be selected from sugars, chitosan, starches, cellulose and its derivatives, malt, gelatin, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and pyrogen-free water.

[0176] Pharmaceutical formulations for parenteral administration include aqueous solutions of the compound in water-soluble form. Additionally, suspensions of the compound may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilisers or agents that increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[0177] Sterile solutions may be prepared by combining the compound in the required amount in the appropriate solvent with other excipients as described above as required, followed by sterilization, such as filtration. Generally, dispersions are prepared by incorporating the various sterilized active compounds into a sterile vehicle which contains the basic dispersion medium and the required excipients as described above. Sterile dry powders may be prepared by vacuum- or freeze-drying a sterile solution comprising the active compounds and other required excipients as described above.

[0178] Pharmaceutical preparations for oral use can be obtained by combining the compounds with solid excipients and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in

particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatine, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association one or more compounds of the invention with the carrier which constitutes one or more necessary ingredients. In general, the pharmaceutical compositions of the invention may be manufactured in a manner that is itself known, e.g. by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilising processes.

[0179] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterise different combinations of particle doses.

[0180] Pharmaceuticals which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilisers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilisers may be added.

[0181] The compounds may be incorporated into modified-release preparations and formulations, for example, polymeric microsphere formulations, and oil- or gel-based formulations.

[0182] The compounds may be administered in a local rather than systemic manner, such as by injection directly into a tissue, which is preferably subcutaneous or omental tissue, often in a depot or sustained release formulation. In other embodiments, the compound is systemically administered.

[0183] Furthermore, the compound may be administered in a targeted drug delivery system, such as in a particle which is suitable targeted to and taken up selectively by a cell or tissue. In some embodiments, the compound is contained or otherwise associated with a vehicle selected from liposomes, micelles, dendrimers, biodegradable particles, artificial DNA nanostructure, lipid-based nanoparticles and carbon or old

nanoparticles. In illustrative examples of this type, the vehicle is selected from poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), PLA-PEG copolymers and combinations thereof.

[0184] In cases of local administration or selective uptake, the effective local concentration of the agent may not be related to plasma concentration.

[0185] It is advantageous to formulate the compositions in dosage unit form for ease of administration and uniformity of dosage. The determination of the novel dosage unit forms of the present invention is dictated by and directly dependent on the unique characteristics of the active material, the particular therapeutic effect to be achieved and the limitations inherent in the art of compounding active materials for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

[0186] While the compound of the invention may be the sole active agent administered to the subject, the administration of other active agents concurrently with said compound is within the scope of the invention. For example, in some embodiments, the compound may be administered concurrently with one or more anti-inflammatory agents, immunosuppressants, antibiotics, anti-virals, anti-protozoals, anti-fungals or anthelmintics. The compound may be therapeutically used after the other active agent or may be therapeutically used together with the other active agent. The compound may be administered separately, simultaneously or sequentially with the other active agent.

[0187] Accordingly, in another aspect of the invention, there is provided a composition comprising a compound of the invention and an anti-inflammatory agent, immunosuppressant, antibiotic, anti-viral, anti-protozoal, anti-fungal or anthelmintic.

[0188] Suitable immunosuppressants or immunomodulatory agents include prednisone, methylprednisolone, dexamethasone, hydrocortisone, budesonide, prednisolone, tofacitinib, cyclosporine, cyclophosphamide, nitrosoureas, platinum compounds, methotrexate, azathioprine, mercaptopurine, fluorouracil, dactinomycin, anthracyclines, mitomycin C, bleomycin, mithramycin, antithymocyte globulin, thymoglobulin, muromonab-CD3, basiliximab, daclizumab, tacrolimus, sirolimus, everolimus, infliximab, etanercept, IFN-β, mycophenolic acid or mycophenolate, fingolimod, azathioprine, leflunomide, abatacept, adalimumab, anakinra, certolizumab, golimumab, ixekizumab, natalizumab, rituximab, secukinumab, toclizumab, ustekinumab, vedolizumab, glatiramer acetate, dimethyl fumarate, diroximel fumarate, teriflunomide, siponimod, cladribine, ocrelizumab, natalizumab, alemtuzumab and myriocin.

[0189] Exemplary anti-inflammatory agents include NSAIDs (e.g. acetylsalicylic acid (aspirin), diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen,

ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, meloxicam, nabumetone, naproxen, nimesulide, nitroflurbiprofen, olsalazine, oxaprozin, phenylbutazone, piroxicam, sulfasalazine, sulindac, tolmetin, zomepirac, celecoxib, deracoxib, etoricoxib, mavacoxib or parecoxib), disease-modifying antirheumatic drugs (DMARDs) (e.g. methotrexate, leflunomide, sulfasalazine, hydroxychloroquinone, penicillamine, anatacept, baricitinib, cetolizumab, sarilumab, tocilizumab or tofacitinib), prednisone, methylprednisolone, dexamethasone, hydrocortisone, budesonide, prednisolone, etanercept, golimumab, infliximab, adalimumab, anakinra, rituximab, natalizumab and abatacept.

[0190] Exemplary antibiotics include, but are not limited to, quinolones (e.g. amifloxacin, cinoxacin, ciprofloxacin, enoxacin, fleroxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, levofloxacin, lomefloxacin, oxolinic acid, pefloxacin, rosoxacin, temafloxacin, tosufloxacin, sparfloxacin, clinafloxacin, gatifloxacin, moxifloxacin, gemifloxacin, or garenoxacin), tetracyclines, glycylcyclines or oxazolidinones (e.g. chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, tetracycline, tigecycline, linezolide or eperezolid), aminoglycosides (e.g. amikacin, arbekacin, butirosin, dibekacin, fortimicins, gentamicin, kanamycin, menomycin, netilmicin, ribostamycin, sisomicin, spectinomycin, streptomycin or tobramycin), β-lactams (e.g. imipenem, meropenem, biapenem, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefixime, cefmenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefpimizole, cefpiramide, cefpodoxime, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cefinetazole, cefoxitin, cefotetan, azthreonam, carumonam, flomoxef, moxalactam, amdinocillin, amoxicillin, ampicillin, azlocillin, carbenicillin, benzylpenicillin, carfecillin, cloxacillin, dicloxacillin, methicillin, mezlocillin, nafcillin, oxacillin, penicillin G, piperacillin, sulbenicillin, temocillin, ticarcillin, cefditoren, cefdinir, ceftibuten or cefozopran), rifamycins, macrolides (e.g. azithromycin, clarithromycin, erythromycin, oleandomycin, rokitamycin, rosaramicin, roxithromycin or troleandomycin), ketolides (e.g. telithromycin or cethromycin), coumermycins, lincosamides (e.g. clindamycin or lincomycin) or chloramphenicol.

[0191] Suitable anti-virals include, but are not limited to, abacavir sulfate, acyclovir sodium, amantadine hydrochloride, amprenavir, cidofovir, delavirdine mesylate, didanosine, efavirenz, famciclovir, fomivirsen sodium, foscarnet sodium, ganciclovir, indinavir sulfate, lamivudine, lamivudine/zidovudine, nelfinavir mesylate, nevirapine, oseltamivir phosphate, ribavirin, rimantadine hydrochloride, ritonavir, saquinavir, saquinavir mesylate, stavudine, valacyclovir hydrochloride, zalcitabine, zanamivir or zidovudine.

[0192] Exemplary anti-protozoals include atovaquone, chloroquine hydrochloride, chloroquine phosphate, doxycycline, hydroxychloroquine sulfate, mefloquine hydrochloride, primaquine phosphate, pyrimethamine, metronidazole, metronidazole hydrochloride and pentamidine isethionate.

[0193] Illustrative anti-fungals include, but are not limited to, amphotericin B, amphotericin B cholesteryl sulfate complex, amphotericin B lipid complex, amphotericin B liposomal, anidulafungin, caspofungin, clotrimazole, fluconazole, flucytosine, griseofulvin, griseofulvin microsize, griseofulvin ultramicrosize, isavuconazonium, itraconazole, ketoconazole, micafungin, miconazole, nystatin, posaconazole, terbinafine, voriconazole, fosfluconazole, isavuconazole, candicidin, hamycin, natamycin, bifonazole, butoconazole, econazole, fenticonazole, isoconazole, luliconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, albaconazole, efinaconazole, terconazole, abafungin, amofolfin, butenafine, naftifine, ciclopirox, tolnafate, orotomide, oteseconazole (VT-1161, (2R)-2-(2,4-difluorophenyl)-1,1-difluoro-3-(tetrazol-1-yl)-1-[5-[4-(2,2,2-trifluoroethoxy)phenyl]pyridin-2-yl]propan-2-ol), ibrexafungerp (SCY-078, 2-(2-amino-2,3,3-trimethylbutoxy)-1,6a,8,10a-tetramethyl-8-(3-methylbutan-2-yl)-3-(5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl)-1,3,4,6,6a,7,8,9,10,10a,10b,11,12,12a-tetradecahydro-2H-1,4a-(methanooxymethano)chrysene-7-carboxylic acid), rezafungin (CD-101, 2-(((2R,9S,11R,12R,14aS,15S,16S,20S,25aS)-23-((1S,2S)-1,2-dihydroxy-2-(4-hydroxyphenyl)ethyl)-2,11,15-trihydroxy-6,20-bis((R)-1-hydroxyethyl)-16-methyl-5,8,14,19,22,25-hexaoxo-9-(4''-(pentyloxy)-[1,1':4',1''-terphenyl]-4-carboxamido)tetracosahydro-1H-dipyrrolo[2,1-c:2',1'-l][1,4,7,10,13,16]hexaazacyclohenicosin-12-yl)oxy)-N,N,N-trimethylethan-1-aminium acetate), and salts and combinations thereof.

[0194] Anthelmintics contemplated include, but are not limited to, a benzimidazole (e.g. albendazole, mebendazole, thiabendazole, fenbendazole, triclabendazole and flubendazole), abamectin, ivermectin, diethylcarbamazine, pyrantel pamoate, levamisole, salicylanilide, niclosamide, oxyclozanide, rafoxanide, nitazoxanide, oxamniquine, praziquantel, an octadepsipeptide (e.g. emodepside), monepantel, a spiroindole (e.g. derquantel), antemisinin, piperazine, morantel, pyrantel, tribendimidine, and salts and combinations thereof.

[0195] In some embodiments, the compound of the invention is used for enhancing an immune response in a subject to a target antigen by an immune modulating agent. In such embodiments, the compound may be administered concurrently with one or more immune modulating agents. The compound may be therapeutically used after the one or more immune modulating agents or may be therapeutically used together with the

one or more immune modulating agents. The compound may be administered separately, simultaneously or sequentially with the one or more immune modulating agents.

[0196] Accordingly, in another aspect of the invention, there is provided a composition comprising a compound of the invention and an immune modulating agent. Such compositions may be, for example, a vaccine composition, which may also comprise a pharmaceutically acceptable carrier or diluent as discussed *supra*.

[0197] Suitable immune modulating agents are as described in further detail herein.

[0198] The invention also provides a composition comprising a compound of the invention and an antigen-encoding nucleic acid molecule (e.g. DNA or RNA, especially mRNA). These vaccine compositions may also comprise a pharmaceutically acceptable carrier or diluent as discussed *supra*. Suitable antigens are described in further detail herein.

[0199] As previously described, the compound may be compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. In some embodiments, a unit dosage form may comprise the compound in an amount in the range of from about 0.25 µg to about 2000 mg. The compound may be present in an amount of from about 0.25 µg to about 2000 mg/mL of carrier. In embodiments where the pharmaceutical composition comprises one or more additional active agents, the dosages are determined by reference to the usual dose and manner of administration of the said agents.

5. Methods of Use

[0200] The compounds of the invention can be used for a range of purposes depending on the identity and activity of the proteinaceous molecules that form the dimer. In particular, the inventors have conceived that compounds represented by Formulae I, II, III, IV, V or VI may be useful for treating an infection, inflammatory disorder or autoimmune disorder, eliciting or enhancing an immune response or disrupting a biofilm, for example, when the proteinaceous molecule is an antimicrobial peptide. Accordingly, a compound of the invention for use in therapy, or as a medicament is provided.

[0201] In another aspect, there is provided a method of treating or inhibiting the development of an infection in a subject, comprising administering a compound of the invention to the subject. Also provided is a compound of the invention for use in treating or inhibiting the development of an infection in a subject, a use of a compound of the invention for treating or inhibiting the development of an infection in a subject, and a use of a compound of the invention in the manufacture of a medicament for treating or inhibiting the development of an infection in a subject.

[0202] Suitable embodiments of the compound are discussed in Sections 3 and 4 *supra*.

[0203] Suitable infections include, but are not limited to, a bacterial, viral, protozoan, fungal or helminth infection; especially a bacterial or a viral infection; most especially a bacterial infection.

[0204] Exemplary bacterial infections include those caused by *Neisseria* species (e.g. *N. meningitidis* and *N. gonorrhoeae*), *Salmonella* species (e.g. *S. enterica* and *S. bongori*), *Streptococcus* species (e.g. *S. pyogenes, S. pneumoniae, S. mitis, S. agalactiae, S. dysgalactiae, S. gallolyticus, S. anginosus, S. sanguinis and S. mutans*), *Legionella* species (e.g. *L. pneumophila*), *Mycoplasma* species (e.g. *M. pneumoniae, M. hominis* and *M. genitalum)*, *Bacillus* species (e.g. *B. anthracis* and *B. cereus*), *Staphylococcus* species (e.g. *S. aureus* and *S. epidermis*), *Chlamydia* species (e.g. *C. trachomatis, C. pneumoniae* and *C. psittacci*), *Acinetobacter* species (e.g. *A. baumannii*), *Actinomadura* species, *Actinomyces* species (e.g. *A. israelii*), *Anabaena* species, *Anaplasma* species, *Arcanobacterium* species (e.g. *A. haemolyticum*), *Bacteroides* species (e.g. *B. fragilis*), *Bdellovibrio* species (e.g. *B. bacteriovorus*), *Bordetella* species (e.g. *B. pertussis*), *Borrelia* species (e.g. *B. burgdorferi*), *Brucella* species (e.g. *B. melitensis*), *Burkholderia* species (e.g. *B. cepacia, B. pseudomallei, B. xenovorans* and *B. mallei*), *Campylobacter* species (e.g. *C. jejuni* and *C. coli*), *Caulobacter* species (e.g. *C. crescentus*), *Chlrorbium* species, *Chromatium* species, *Clostridium* species (e.g. *C. difficile, C. botulinum, C. perfringens, C. tetani* and *C. sordellii*), *Corynebacterium* species (e.g. *C. diptheriae* and *C. pseudotuberculosis*), *Coxiella* species (e.g. *C. burnetti*), *Cytophaga* species, *Deinococcus* species, *Ehrlichia* species (e.g. *E. chaffeensis* and *E. ewingii*), *Enterococcus* species (e.g. *E. faecalis* and *E. faecium*), *Escherichia* species (e.g. *E. coli*), *Francisella* species (e.g. *F. tularensis, F. novicida* and *F. philomiragia*), *Fusobacterium* species, *Helicobacter* species (e.g. *H. pylori*), *Haemophilus* species (e.g. *H. influenzae* and *H. ducreyi*), *Hyphomicrobium* species, *Kingella* species (e.g. *K. kingae*), *Klebsiella* species (e.g. *K. pneumoniae*), *Leptospira* species (e.g. *L. interrogans*), *Listeria* species (e.g. *L. monocytogenes*), *Micrococcus* species (e.g. *M. luteus*), *Myxococcus* species, *Nitrobacter* species, *Nocardia* species (e.g. *N. asteroids, N. brasiliensis* and *N. caviae*), *Oscillatoria* species, *Pasteurella* species (e.g. *P. multocida*), *Prochloron* species, *Prevotella* species (e.g. *P. intermedia, P. nigrescens* and *P. copri*), *Proteus* species (e.g. *P. mirabilis*), *Pseudomonas* species (e.g. *P. aeruginosa*, *P. oryzihabitans* and *P. plecoglossicida*), *Rhodospirillum* species, *Rickettsia* species (e.g. *R. typhi, R. rickettsia, R. akari, R. conorii, R. sibirica, R. australis, R. felis, R. japonica, R. africae* and *R. prowazekii*), *Shigella* species (e.g. *S. dysenteriaw, S. flexneri, S. boydii* and *S. sonnei*), *Spirillum* species, *Spirochaeta* species, *Streptobacillus* species (e.g. *S. moniliformis* and *S. minus*), *Streptomyces* species, *Thiobacillus* species, *Treponema* species (e.g. *T. pallidum*), *Ureaplasma* species (e.g. *U. urealyticum*), *Vibrio*

species (e.g. *V. cholera, V. parahaemolyticus* and *V. vulnificus*), *Yersinia* species (e.g. *Y. pestis* and *Y. enterocolitica*), and *Mycobacterium* species (e.g. *M. tuberculosis* and *M. leprae*).

[0205] In some embodiments, the bacterial infection is a gram-negative bacterial infection. Exemplary gram-negative bacterial species include, but are not limited to, *Neisseria* species, *Haemophilus* species, *Salmonella* species, *Legionella* species, *Chlamydia* species, *Acinetobacter* species, *Anabaena* species, *Anaplasma* species, *Bacteroides* species, *Bdellovibrio* species, *Bordetella* species, *Borrelia* species, *Brucella* species, *Burkholderia* species, *Campylobacter* species, *Caulobacter* species, *Chlrorbium* species, *Chromatium* species, *Coxiella* species, *Cytophaga* species, *Ehrlichia* species, *Escherichia* species, *Francisella* species, *Fusobacterium* species, *Helicobacter* species, *Hyphomicrobium* species, *Kingella* species, *Klebsiella* species, *Leptospira* species, *Myxococcus* species, *Nitrobacter* species, *Oscillatoria* species, *Pasteurella* species, *Prochloron* species, *Prevotella* species, *Proteus* species, *Pseudomonas* species, *Rhodospirillum* species, *Rickettsia* species, *Shigella* species, *Spirillum* species, *Spirochaeta* species, *Streptobacillus* species, *Thiobacillus* species, *Treponema* species, *Ureaplasma* species, *Vibrio* species and *Yersinia* species. In some embodiments, the bacterial infection is an infection caused by an *Escherichia* species, a *Klebsiella* species or an *Acinetobacter* species. In particular embodiments, the infection is caused by *E. coli*, *K. pneumoniae* or *A. baumannii*, especially *A. baumannii*.

[0206] In particular embodiments, the infection is caused by a bacterial species that is resistant to one or more antibiotics (e.g. colistin, polymyxin B, oxacillin, ampicillin, erythromycin, tetracycline, penicillin, ampicillin, methicillin, chloramphenicol, vancomycin, tigecycline, doxycycline and/or a carbapanem such as imipenem, doripenem and meropenem), including a multi-drug resistant bacterium. For example, the infection may be caused by drug resistant *E. coli*, *K. pneumoniae*, *A. baumannii*, including *K. pneumoniae* FADDI-KP028 or *A. baumannii* FADDI-AB156. Other exemplary strains include *K. pneumoniae* MS6771, MCR1-positive *E. coli* strain MS8345 and *A. baumannii* strain 42-A.

[0207] Suitable viral infections include, but are not limited to, infections caused by Picornaviruses (e.g. hepatitis A virus, enteroviruses such as poliovirus, enterovirus 71, 70, 69, and 68, Coxsackieviruses, echoviruses, foot and mouth disease virus, and rhinoviruses), Caliciviruses (e.g. hepatitis E virus, noroviruses such as Norwalk virus, feline calicivirus), Arteriviruses (e.g. equine arteritis virus), Togaviruses (e.g. sindbis virus, the equine encephalitis viruses, chikungunya virus, rubella virus, Ross River virus, bovine diarrhea virus, hog cholera virus, Semliki forest virus), Flaviviruses (e.g. dengue virus, West Nile virus, yellow fever virus, Japanese encephalitis virus, St. Louis encephalitis virus, tick-borne encephalitis virus, bovine viral diarrhea virus, classical swine fever virus),

Coronaviruses (e.g. human coronaviruses, including betacoronaviruses such OC43 and HKU1 of the A lineage, SARS-CoV and SARS-CoV-2 of the B lineage and MERS-CoV of the C lineage, swine gastroenteritis virus), Rhabdoviruses (e.g. rabies virus, Australian bat lyssavirus, vesicular stomatitis viruses), Filoviruses (e.g. Marburg virus, Ebola virus), Paramyxoviruses (e.g. measles virus, canine distemper virus, mumps virus, parainfluenza viruses, respiratory syncytial virus, Newcastle disease virus, rinderpest virus, Nipah virus, Hendra virus), Orthomyxoviruses (e.g. human influenza viruses, including human influenza virus types A, B and C, avian influenza viruses, equine influenza viruses), Bunyaviruses (e.g. hantavirus, LaCrosse virus, Rift Valley fever virus), Arenaviruses (e.g. Lassa virus, Machupo virus), Reoviruses (e.g. human and animal reoviruses, such as rotaviruses, bluetongue virus), Birnaviruses (e.g. infectious bursal virus, fish pancreatic necrosis virus), Retroviruses (e.g. HIV 1, HIV 2, HTLV-1, HTLV-2, bovine leukemia virus, feline immunodeficiency virus, feline sarcoma virus, mouse mammary tumor virus), Hepadnaviruses (e.g. hepatitis B virus), Parvoviruses (e.g. B19 virus, canine parvovirus, feline panleukopenia virus), Papovaviruses (e.g. human papillomaviruses, SV40, bovine papillomaviruses), Adenoviruses (e.g. human, canine, bovine, and porcine adenoviruses), Herpesviruses (e.g. herpes simplex viruses, varicella-zoster virus, infectious bovine rhinotracheitis virus, cytomegalovirus, human herpesvirus 6, human herpesvirus 7, human herpesvirus 8, Epstein-Barr virus), Poxviruses (e.g. vaccinia, fowlpoxviruses, raccoon poxvirus, skunkpox virus, monkeypoxvirus, cowpox virus, buffalopox virus, musculum contagiosum virus), human T-cell lymphotropic virus, small pox virus, polyoma virus, junin virus, an astrovirus, BK virus, machupo virus, sabia virus, sapovirus, a coltivirus, bocavirus, human metapneumovirus, lymphocytic choriomeningitis mammarenavirus, guanarito mammarenavirus, molluscum contagiosum virus and JC virus; especially infections caused by a coronavirus, such as SARS-CoV-2.

[0208] Suitable helminth infections include, but are not limited to, infections caused by *Ascaris* species, *Baylisascaris* species, *Clonorchis* species, *Taenia* species, *Diphyllobothrium* species, *Dracunculus* species, *Echinococcus* species, *Enterobius* species, *Fasciolopsiasis* species, *Fasciola* species, *Wuchereria* species, *Brugia* species, *Gnathostoma* species, *Ancylostoma* species, *Hymenolepis* species, *Metagonimiasis* species, *Onchocerciasis* species, *Paragonimus* species, *Schistosoma* species, *Strongyloides* species, *Toxocara* apecies, *Trichinella* species and *Trichuris* species.

[0209] The protozoan infection may include, but is not limited to, malaria (e.g. an infection caused by *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* or *Plasmodium knowlesi*), or an infection caused by *Entamoeba histolytica*, *Balantidium coli*, *Blastocystis* species, *Cyclospora cayetanensis*, *Acanthamoeba* species, *Balamuthia* species, *Naegleria* species, *Leishmania* species, *Sappinia* species, *Giardia* species, *Isospora* species, *Rhinosporidium* species, *Toxoplasma* species,

Trichomonas species, *Trypanosoma* species (e.g. *T. brucei* or *T. cruzi*), *Babesia* species, *Cryptosporidium* species or *Theileria* species.

[0210] Exemplary fungal infections include, but are not limited to, infections caused by *Candida* species (e.g. *C. parapsilosis, C. famata, C. krusei, C. albicans, C. glabrata* and *C. tropicalis*), *Aspergillus* species, *Blastomyces* species, *Coccidioides* species, *Cryptococcus* species, *Trichophyton* species, *Microsporum* species, *Geotrichum* species, *Histoplasma* species, *Madurella* species, *Paracoccidioides* species, *Pneumocystis* species, *Sporothrix* species, *Nannizzia* species, *Rhizopus* species, *Mucor* species, *Rhizomucor* species and *Syncephalastrum* species.

[0211] In view of the antimicrobial activity of the compounds of the invention (e.g. when A^1, A^2, A^3 and/or A^4 are antimicrobial peptides), the compounds are also useful for disrupting biofilms and killing or inhibiting the growth of an infectious agent. Accordingly, further provided is a method of disrupting a biofilm, comprising contacting the biofilm with a compound of the invention. The use of the compound of the invention for disrupting a biofilm, and a compound of the invention for use in disrupting a biofilm are also provided.

[0212] The biofilm may be on any suitable surface, such as a mammalian surface (e.g. a human), a surface of a medical device (e.g. a catheter, a valve, a prosthetic device, a drug pump, a stent or an orthopaedic material), plastic, glass, a membrane, a medical surface and the like.

[0213] The compound may be maintained in contact with the biofilm for a suitable amount of time to disrupt the biofilm, which may include, for example, from about 1 minute to about 2 hours (and all integer minutes therebetween).

[0214] Suitable biofilm-forming species include one or more of the microbial species listed *supra*, especially a gram-negative bacterial species such as *E. coli* or *A. baumannii*.

[0215] The compound may also be used for treating or inhibiting the development of a biofilm.

[0216] Further provided is a method of killing or inhibiting the growth of an infectious agent, comprising contacting the infectious agent with a compound of the invention; a compound of the invention for use in killing or inhibiting the growth of an infectious agent, wherein the infectious agent is contacted with the compound of the invention; a use of a compound of the invention for killing or inhibiting the growth of an infectious agent, wherein the infectious agent is contacted with the compound of the invention; and a use of a compound of the invention in the manufacture of a medicament

for killing or inhibiting the growth of an infectious agent, wherein the infectious agent is contacted with the compound of the invention.

[0217] In particular embodiments, the infectious agent has infected a subject, such as a human subject.

[0218] Suitable infectious agents include a bacteria, virus, protozoa, fungus or helminth. Representative bacteria, viruses, protozoans, fungi and helminths include those discussed above.

[0219] Antimicrobial peptides, such as Chex1-Arg20 have also been shown to have a wide range of uses in addition to antimicrobial activity, including anti-inflammatory and immunomodulatory activity. Accordingly, the use of the compound of the invention for treating or inhibiting the development of an inflammatory disorder or an autoimmune disorder and for eliciting an immune response and enhancing an immune response to a target antigen by an immune modulating agent are also encompassed herein.

[0220] In another aspect, there is provided a method of treating or inhibiting the development of an inflammatory disorder in a subject, comprising administering a compound of the invention to the subject. Also provided is a compound of the invention for use in treating or inhibiting the development of an inflammatory disorder in a subject, a use of a compound of the invention for treating or inhibiting the development of an inflammatory disorder in a subject, and a use of a compound of the invention in the manufacture of a medicament for treating or inhibiting the development of an inflammatory disorder in a subject.

[0221] Suitable inflammatory disorders include, but are not limited to, rheumatoid arthritis, inflammatory bowel disease (e.g. Crohn's disease or ulcerative colitis), sepsis, septic shock, chronic obstructive pulmonary disorder, lung injury, asthma, fibrosis, multiple organ dysfunction syndrome (e.g. sepsis-induced multiple organ dysfunction syndrome), atopic dermatitis, acne (e.g. acne vulgaris), endometriosis, Alzheimer's disease and Parkinson's disease; especially rheumatoid arthritis, inflammatory bowel disease, sepsis, atopic dermatitis and acne.

[0222] In some embodiments, the inflammatory disorder is associated with an infection, suitable examples of which are discussed *supra*.

[0223] In another aspect, there is provided a method of treating or inhibiting the development of an autoimmune disorder in a subject, comprising administering a compound of the invention to the subject.

[0224] Suitable autoimmune disorders include, but are not limited to type 1 diabetes, multiple sclerosis, lupus, Crohn's disease, psoriasis and celiac disease; especially type 1 diabetes or multiple sclerosis.

[0225] The compounds of the invention have been shown to have immunomodulatory effects, for example, when A^1, A^2, A^3 and/or A^4 are an antimicrobial peptide such as Chex1-Arg20. Accordingly, the inventors have conceived that the compounds of the invention can be used to elicit and/or enhance an immune response in a subject.

[0226] In another aspect, there is provided a method of eliciting an immune response in a subject, comprising administering a compound of the invention to the subject. Also contemplated is a use of a compound of the invention for eliciting an immune response in a subject, a compound of the invention for use in eliciting an immune response in a subject, and a use of a compound of the invention in the manufacture of a medicament for eliciting an immune response in a subject.

[0227] While any subject is contemplated, in some embodiments, the subject is suffering from an infection or a cancer, especially an infection, suitable examples of which are discussed *supra*. In such embodiments, the method may comprise promoting or enhancing the subject's immune response to the infection or cancer. In some embodiments, the subject is immunocompromised, has an impaired immune response, and/or is immunosenescent.

[0228] Also provided is a method of enhancing an immune response in a subject to a target antigen by an immune modulating agent, comprising administering a compound of the invention to the subject. A use of a compound of the invention for enhancing an immune response in a subject to a target antigen by an immune modulating agent, a compound of the invention for use in enhancing an immune response in a subject to a target antigen by an immune modulating agent, and a use of a compound of the invention in the manufacture of a medicament or preparation for enhancing an immune response in a subject to a target antigen by an immune modulating agent is also provided in further aspects.

[0229] In particular embodiments, the immune modulating agent is selected from an antigen that corresponds to at least a portion of the target antigen, an antigen-binding molecule that is immuno-interactive with the target antigen and an immune modulating cell that modulates an immune response to the target antigen.

[0230] In some embodiments, the immune modulating agent is an antigen that corresponds to at least a portion of the target antigen, which is produced following administration of an antigen-encoding nucleic acid molecule, such as DNA or RNA, especially mRNA.

[0231] The target antigen may be any substance that will elicit a desired immune response, which is typically associated with a disease or condition of interest, such as an

infection or a cancer. For example, the target antigen may be any substance, such as a peptide or protein, that reacts with antibodies or T-cells.

[0232] Target antigens may be selected from endogenous antigens produced by a host or exogenous antigens that are foreign to the host, preferably exogenous antigens.

[0233] In some embodiments, the target antigen may be a component of an infectious agent, such as a bacterium, virus, protozoan, fungi or helminth (e.g. the infectious agent produces the target antigen). Suitable examples of such infectious agents are described *supra*.

[0234] Exemplary bacterial antigens include, but are not limited to, pertussis bacterial antigens such as pertussis toxin, filamentous hemagglutinin, pertactin, F M2, FIM3, adenylate cyclase and other pertussis bacterial antigen components; diphtheria bacterial antigens such as diphtheria toxin or toxoid and other diphtheria bacterial antigen components; tetanus bacterial antigens such as tetanus toxin or toxoid and other tetanus bacterial antigen components; streptococcal bacterial antigens such as M proteins and other streptococcal bacterial antigen components; gram-negative bacilli bacterial antigens such as lipopolysaccharides and other gram-negative bacterial antigen components; *Mycobacterium tuberculosis* bacterial antigens such as mycolic acid, heat shock protein 65 (HSP65), the 30kDa major secreted protein, antigen 85 A and other mycobacterial antigen components; *Helicobacter pylori* bacterial antigen components; pneumococcal bacterial antigens such as pneumolysin, pneumococcal capsular polysaccharides and other pnermiococcal bacterial antigen components; *Haemophilus influenzae* bacterial antigens such as capsular polysaccharides and other *H. influenzae* bacterial antigen components; anthrax bacterial antigens such as anthrax protective antigen and other anthrax bacterial antigen components; and rickettsiae bacterial antigens such as rompA and other rickettsiae bacterial antigen component. Also included with the bacterial antigens described herein are any other bacterial, mycobacterial, mycoplasmal, rickettsial, or chlamydial antigens.

[0235] Exemplary viral antigens include, but are not limited to, retroviral antigens derived from HIV such as gene products of the gag, pol and env genes, the Nef protein, reverse transcriptase, and other HIV components; hepatitis viral antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, e.g., hepatitis A, B, and C, viral components such as hepatitis C viral RNA; influenza viral antigens such as hemagglutinin and neuraminidase and other influenza viral components; measles viral antigens such as the measles virus fusion protein and other measles virus components; rubella viral antigens such as proteins E1 and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components; cytomegaloviral antigens such as envelope glycoprotein B and other cytomegaloviral antigen components; respiratory syncytial viral antigens such as the RSV

fusion protein, the M2 protein and other respiratory syncytial viral antigen components; herpes simplex viral antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components; varicella zoster viral antigens include antigens such as 9PI, gpll, and other varicella zoster viral antigen components; Japanese encephalitis viral antigens include antigens such as proteins E, M-E, M-E-NS 1, NS 1, NS 1-NS2A, 80%E, and other Japanese encephalitis viral antigen components; rabies viral antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components; papillomavirus antigens such as the LI and L2 capsid proteins as well as the E6/E7 antigens associated with cervical cancers; and coronavirus antigens such as SARS-CoV-2 spike protein, spike RBD, nucleocapsid protein, envelope protein, membrane protein, and other coronavirus antigen components.

[0236] Non-limiting exemplary fungal antigens include candida fungal antigen components; histoplasma fungal antigens such as heat shock protein 60 (HSP60) and other histoplasma fungal antigen components; cryptococcal fungal antigens such as capsular polysaccharides and other cryptococcal fungal antigen components; coccidiodes fungal antigens such as spherule antigens and other coccidiodes fungal antigen components; and tinea fungal antigens such as trichophytin and other coccidiodes fungal antigen components.

[0237] Exemplary protozoal and helminth antigens include, but are not limited to, *Plasmodium falciparum* antigens such as merozoite surface antigens, sporozoite surface antigens, circumsporozoite antigens, gametocyte/gamete surface antigens, blood-stage antigen pf 155/RESA and other plasmodial antigen components; toxoplasma antigens such as SAG-1, p30 and other toxoplasmal antigen components; schistosomae antigens such as glutathione-S-transferase, paramyosin, and other schistosomal antigen components; leishmania major and other leishmaniae antigens such as gp63, lipophosphoglycan and its associated protein and other leishmanial antigen components; and *Trypanosoma cruzi* antigens such as the 75-77kDa antigen, the 56kDa antigen and other trypanosomal antigen components.

[0238] The present invention also contemplates toxin components as antigens. Illustrative examples of toxins include, but are not limited to, staphylococcal enterotoxins, toxic shock syndrome toxin, retroviral antigens (e.g., antigens derived from HIV), streptococcal antigens, staphylococcal enterotoxin-A (SEA), staphylococcal enterotoxin-B (SEB), staphylococcal enterotoxin$_{1-3}$ (SE$_{1-3}$), staphylococcal enterotoxin-D (SED), staphylococcal enterotoxin-E (SEE) as well as toxins derived from mycoplasma, mycobacterium and herpes viruses.

[0239] The target antigen may also be a component of a cancer cell (e.g. the cancer cell produces the target antigen). Suitable cancers include, but are not limited to,

breast (including invasive breast carcinoma), gastric (including stomach adenocarcinoma), prostate, lung (including non-small cell lung cancer, lung squamous carcinoma and lung adenocarcinoma), bladder, pancreatic, colon, liver (including hepatocellular carcinoma), ovarian (including ovarian serous cystadenocarcinoma), kidney (including kidney renal clear cell carcinoma and kidney renal papillary cell carcinoma), brain (e.g. glioma or glioblastoma), urothelial, renal, bone, bowel, cervical, thyroid, testis, endometrial, skin and head and neck cancer (including head and neck squamous cell carcinoma, and lymphoma (e.g. diffuse large B cell lymphoma), melanoma, mesothelioma and sarcoma, such as a bone or soft tissue sarcoma. For example, in some embodiments, the target antigen is a carbonic anhydrase IX, alpha-fetoprotein (AFP), α-actinin-4, PlGF, ILGF, ILGF-1R, IL-6, IL-25, RS5, RANTES, T101, SAGE, S100, survivin, survivin-2B, A3, antigen specific for A33 antibody, ART-4, B7, Ba 733, BAGE, BrE3-antigen, CA125, CAMEL, CAP-1, CASP-8/m, CCL19, CCL21, CD1, CD1a, CD2, CD3, CD4, CD5, CD8, CD11A, CD14, CD15, CD16, CD18, CD19, CD20, CD21, CD22, CD23, CD25, CD29, CD30, CD32b, CD33, CD37, CD38, CD40, CD40L, CD44, CD45, CD46, CD52, CD54, CD55, CD59, CD64, CD66a-e, CD67, CD70, CD70L, CD74, CD79a, CD80, CD83, CD95, CD126, CD132, CD133, CD138, CD147, CD154, CDC27, hypoxia inducible factor (HIF-1), HSP70-2M, HST-2, Ia, IGF-1R, IFN-γ, IFN-α, IFN-β, IFN-λ, IL-4R, IL-6R, IL-13R, IL-15R, IL-17R, IL-18R, IL-2, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, IL-23, IL-25, insulin-like growth factor-1 (IGF-1), CDK-4/m, CDKN2A, CTLA-4, CXCR4, CXCR7, CXCL12, HIF-1α, PLAGL2, prostatic acid phosphatase, PSA, PRAME, PSMA, colon-specific antigen-p (CSAp), CEACAM5, CEACAM6, c-Met, DAM, EGFR, EGFRvIII, EGP-1 (TROP-2), EGP-2, ELF2-M, Ep-CAM, fibroblast growth factor (FGF), Flt-1, Flt-3, folate receptor, G250 antigen, GAGE, gp100, GRO-β, HLA-DR, HM1.24, human chorionic gonadotropin (HCG) and its subunits, HER2/neu, histone H2B, histone H3, histone H4, HMGB-1, KC4-antigen, KS-1-antigen, KS1-4, Le-Y, LDR/FUT, macrophage migration inhibitory factor (MIF), MAGE, MAGE-3, MART-1, MART-2, NY-ESO-1, TRAG-3, mCRP, MCP-1, MIP-1A, MIP-1B, MIF, MUC1, MUC2, MUC3, MUC4, MUC5ac, MUC13, MUC16, MUM-1/2, MUM-3, NCA66, NCA95, NCA90, pancreatic cancer mucin, PD-1, PD-L1, PD-1 receptor, placental growth factor, p53, TAC, TAG-72, tenascin, TRAIL receptors, TNF-α, Tn antigen, Thomson-Friedenreich antigens, tumor necrosis antigens, VEGFR, ED-B fibronectin, WT-1, 17-1A-antigen, complement factors C3, C3a, C3b, C5a, C5, an angiogenesis marker, bcl-2, bcl-6, Kras, an oncogene marker or an oncogene product.

[0240] In other embodiments, the target antigen is associated with an unwanted immune response including, for example, transplant rejection, graft versus host disease, allergies, parasitic diseases, inflammatory diseases or disorders and autoimmune diseases or disorders. In these embodiments, the methods may involve inducing a tolerogenic response including the induction of an anergic response, and the suppression of a future or existing immune response, to the target antigen.

[0241] The antigen that corresponds to at least a portion of a target antigen may be in soluble form (e.g. peptide, polypeptide or an antigen-encoding nucleic acid molecule from which a peptide or polypeptide is expressible) or in the form of whole cells or attenuated pathogen preparations (e.g. attenuated virus or bacteria) or it may be presented by antigen-presenting cells as described in more detail below.

[0242] The present invention also contemplates the use of antigen-presenting cells as the immune modulating cells, which present an antigen corresponding to at least a portion of the target antigen. Such antigen-presenting cells include professional or facultative antigen-presenting cells. Professional antigen-presenting cells function physiologically to present antigen in a form that is recognised by specific T cell receptors so as to stimulate or anergise a T lymphocyte or B lymphocyte mediated immune response. Professional antigen-presenting cells not only process and present antigens in the context of the major histocompatability complex (MHC), but also possess the additional immunoregulatory molecules required to complete T cell activation or induce a tolerogenic response. Professional antigen-presenting cells include, but are not limited to, macrophages, monocytes, B lymphocytes, cells of myeloid lineage, including monocytic- or granulocytic-DC precursors, marginal zone Kupffer cells, microglia, T cells, Langerhans cells and dendritic cells including interdigitating dendritic cells and follicular dendritic cells. Non-professional or facultative antigen-presenting cells typically lack one or more of the immunoregulatory molecules required to complete T lymphocyte activation or anergy. Examples of non-professional or facultative antigen-presenting cells include, but are not limited to, activated T lymphocytes, eosinophils, keratinocytes, astrocytes, follicular cells, microglial cells, thymic cortical cells, endothelial cells, Schwann cells, retinal pigment epithelial cells, myoblasts, vascular smooth muscle cells, chondrocytes, enterocytes, thymocytes, kidney tubule cells and fibroblasts.

[0243] In some embodiments, the antigen-presenting cells described above are useful for producing primed T lymphocytes to an antigen or group of antigens. In other embodiments, the antigen-specific antigen-presenting cells are useful for producing T lymphocytes that exhibit tolerance/anergy to an antigen or group of antigens.

[0244] Accordingly, the immune modulating cells may also be antigen-specific B or T lymphocytes, which respond in an antigen-specific fashion to representation of the antigen. Antigen-specific T lymphocytes may be produced by contacting an antigen-presenting cell as defined above with a population of T lymphocytes, which may be obtained from any suitable source such as spleen or tonsil/lymph nodes but is preferably obtained from peripheral blood. The T lymphocytes can be used as crude preparations or as partially purified or substantially purified preparations.

[0245] The invention also contemplates the use of antigen-binding molecules that are specifically immuno-interactive with a selected target antigen as immune modulating agents. The antigen-binding molecule is suitably interactive with a target antigen as described *supra*. Numerous antigen-binding molecules useful as immune modulating agents are known in the art. In some embodiments, the antigen-binding molecule is a whole polyclonal antibody. Such antibodies may be prepared, for example, by injecting an antigen that corresponds to at least a portion of the target antigen into a production species, which may include mice or rabbits, to obtain polyclonal antisera. Methods of producing polyclonal antibodies are well known to those skilled in the art. In lieu of polyclonal antisera obtained in a production species, monoclonal antibodies may be produced using the standard method, for example, by immortalising spleen or other antibody producing cells derived from a production species which has been inoculated with one or more antigens as described above.

[0246] The invention also contemplates as antigen-binding molecules Fv, Fab, Fab' and $F(ab')_2$ immunoglobulin fragments. Alternatively, the antigen-binding molecule may comprise a synthetic stabilised Fv fragment. Exemplary fragments of this type include single chain Fv fragments (sFv, frequently termed scFv) in which a peptide linker is used to bridge the N-terminus or C-terminus of a V_H domain with the C-terminus or N-terminus, respectively, of a V_L domain. ScFv lack all constant parts of whole antibodies and are not able to activate complement. In another embodiment, the synthetic stabilised Fv fragment comprises a disulfide stabilised Fv (dsFv) in which cysteine residues are introduced into the V_H and V_L domains such that in the fully folded Fv molecule the two residues will form a disulfide bond between them.

[0247] Accordingly, the compound of the invention may be useful as a component of a vaccine, such as an adjuvant. In addition, the invention contemplates co-administration of antigenic compositions, including immunogenic compositions and vaccines, with a compound of the invention to a subject.

[0248] Representative antigenic compositions include but are not limited to killed or attenuated live viral vaccines, live-vectored vaccines, subunit vaccines, virus-like particle vaccines, and DNA or RNA vaccines (refer to Roth *et al.* (2011) *Veterinary Clinics North America: Food Animal Practice*, 17: 585-597). The antigenic compositions may comprise one or more immune modulating agents as discussed herein, or a nucleic acid molecule which encodes one or more immune modulating agents, such as an antigen-encoding nucleic acid molecule (e.g. DNA or RNA).

[0249] Also provided is a method of enhancing an innate immune response to an infectious agent in a subject, comprising administering a compound of the invention to the subject. Further aspects also provide a compound of the invention for use in enhancing

an innate immune response to an infectious agent in a subject, a use of a compound of the invention for enhancing an innate immune response to an infectious agent in a subject, and a use of a compound of the invention in the manufacture of a medicament for enhancing an innate immune response to an infectious agent in a subject.

[0250] Suitable infectious agents are discussed *supra*.

[0251] The compound of the invention may also be used to enhance an immune response. The immune response may be in response to an infectious agent, an antigen or a fragment thereof or a component of a vaccine. Suitable infectious agents and antigens are discussed elsewhere herein.

[0252] The compound of the invention may be used in methods of treating or inhibiting the development of a range of different diseases, disorders and conditions and/or in non-therapeutic uses depending on the identity, molecular target or mechanism of action of the proteinaceous molecule of A^1, A^2, A^3 and/or A^4. A skilled person will readily be able to identify suitable uses.

[0253] Any one of the aspects described above may involve administration of an effective amount of the compound of the invention as described in Section 4 *supra*. The compound of the invention may be administered via any suitable route of administration, such as oral, rectal, topical, intranasal, intraocular, transmucosal, intestinal, enteral, intramuscular, subcutaneous, intramedullary, intrathecal, intraventricular, intracerebral, intravaginal, intravesical, intravenous or intraperitoneal administration. In particular embodiments, the compound is administered via oral or intravenous administration.

[0254] The dosage and frequency will depend on the subject, the condition, disease or disorder to be treated and the route of administration. A skilled person will readily be able to determine suitable dosages and frequency of such dosages. For example, the compound may be administered in an amount in the range of from about 0.25 μg to about 2000 mg, and may be administered at a frequency of, for example, once daily, or twice or three times daily. The compound may also be administered via an infusion. The treatment may be continued for multiple days, weeks, months or years. In embodiments where the pharmaceutical composition comprises one or more additional active agents, the dosages and frequency of administration are determined by reference to the usual dose and manner of administration of the said agents.

[0255] Any one of the methods described above may, in some embodiments, involve the administration of one or more further active agents as described in Section 4 *supra*, such as an anti-inflammatory agent, immunosuppressant, antibiotic, anti-viral, anti-protozoal, anti-fungal or anthelmintic.

[0256] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

[0257] All amino acids, chemicals and reagents used are commercially available from, for example, Iris Biotech GmbH (Marktredwitz, Germany) or Sigma Aldrich, Inc. (Merck KGaA, Darmstadt, Germany) and were obtained from commercial sources unless otherwise specified. 9-Fluorenylmethoxylcarbonyl (Fmoc)-L-amino acids, 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylamonium hexafluorophosphate (HCTU), and 2-chlorotrityl chloride resin were purchased from GL Biochem (Shanghai, China). TentaGel-MB-RAM-resin was from Rapp Polymere (Tubingen, Germany). N,N-Diisopropylethylamine (DIPEA), dimethylformamide (DMF), colistin (Aldrich), gentamicin (Aldrich), thiourea (>99%, Aldrich) and trifluoroacetic acid (TFA) were obtained from Auspep (Melbourne, Australia). 1,6-Bimaleimidohexane was obtained from TCL (Adelaide, Australia). Isobutyl chloroformate (IBCF), α,α′-dibromo-*p*-xylene, piperidine, triisopropylsilane (TIPS), anisole and acetonitrile (CH_3CN) were all obtained from Sigma (Sydney, Australia). Dulbecco's Modified Eagle Medium (DMEM, GIBCO Cat. No. 11995), fetal bovine serum (FBS, GIBCO Cat. No. 10099), SYTO® 9 green fluorescent nucleic acid stain and propidium iodide (PI) were purchased from Invitrogen and used as received. Mueller-Hinton Broth (MHB) (CM0405), and Yeast Extract (LP0021) were purchased from Oxoid. BactoTM Tryptone and BactoTM Agar were purchased from BD Biosciences. BacLight Bacterial Membrane Potential Kit (Invitrogen) was used to conduct the membrane potential assay. CellROX® Orange Reagent (Invitrogen) was used to perform the reactive oxygen species (ROS) production assay. 96-well cell culture plates were used for cell culture. Microscope Coverglass (ProSciTech) was used to contain samples for imaging with helium ion microscopy (HIM).

EXAMPLE 1 – DIMER SYNTHESIS

Materials and Methods

[0258] The monomeric Chex1-Arg20 with C-terminal Cys and hydrazide (-$NHNH_2$) modification (SEQ ID NO: 6; refer to Table 1) was synthesised using chloro-(2′-chloro)trityl polystyrene resin by Fmoc/tBu solid-phase methods as previously described in Li *et al.* (2017) *Chem. Eur. J.*, 23: 390-396. Standard Fmoc-chemistry was used throughout with a 4-fold molar excess of the Fmoc-protected amino acids in the presence of 3.9-fold HCTU and 10-fold DIPEA. The peptide was cleaved from the solid support resin with TFA in the presence of anisole, TIPS and 3,6-dioxa-1,8-octanedithiol (DODT) as the scavenger (ratio 95:2:2:1) for 1 h at room temperature. After filtration to remove the resin, the filtrate was concentrated under a stream of nitrogen, and the peptide products were precipitated in ice-cold diethyl ether and washed three times. The peptides were

then purified with C18 column (Shimadzu Shim-Pack C18 3.0x75mm) by reversed-phase high performance liquid chromatography (RP-HPLC) in water and acetonitrile containing 0.1% TFA with the gradient 0-80% of buffer B (acetonitrile) at 1.2 mL/min. The final products were characterised by both Shimadzu RP-HPLC and Shimadzu matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

TABLE 1

MONOMER PEPTIDE SEQUENCE

Name	SEQ ID NO:	Sequence
Chex1-Arg20 with C-terminal Cys and hydrazide	6	[Chex]RPDKPRPYLPRPRPPRPVRC-$NHNH_2$

[0259] To facilitate the thiol chemistry, a single Cys residue was incorporated onto the C-terminus of monomeric Chex1-Arg20. A control, monomeric Chex1-Arg20 with Cys, was thiol-capped via the treatment of iodoacetamide to form compound **1**. Each monomeric Chex1-Arg20 analogue bearing a C-terminal Cys (Chex1-Arg20(C-term Cys)-$NHNH_2$) was subjected to bioconjugation using different bifunctional linkers in biological buffer. The structure of the linkers is provided in Figure 1.

[0260] For peptide conjugation with (dibromomethyl)benzene linkers, a 20 mM solution of linker (1,2-dibromomethyl-benzene, 1,3-dibromomethyl-benzene, or 1,4-dibromomethyl-benzene) that was dissolved in acetonitrile (3.5 μmol, 175 μL) was added dropwise to a solution of monomeric Chex1-Arg20(C-term Cys)-$NHNH_2$ (7.7 μmol) in 0.1%TFA (40 μL) and 1 M zinc acetate (36.8 μmol, 36.8 μL). The mixture was reacted for 2-6 hours at room temperature and the crude compounds were purified by RP-HPLC in water and acetonitrile containing 0.1% TFA in overall moderate yield (refer to Table 2). The final products were characterised by both RP-HPLC and MALDI-TOF MS (refer to Figure 2).

[0261] For peptide conjugation with hexafluorobenzene and decafluorobiphenyl linkers, a 20 mM solution of linker (hexafluorobenzene, decafluorobiphenyl) that was dissolved in DMF (2.2 μmol, 110 μL) was added dropwise to a solution of monomeric Chex1-Arg20(C-term Cys)-$NHNH_2$ (5.4 μmol) and 4-(dimethylamino)pyridine (53 μmol, 6.5 mg) in DMF (30 μL). The mixture was reacted overnight at room temperature and the crude peptides were purified by RP-HPLC in water and acetonitrile containing 0.1% TFA in overall moderate yield (refer to Table 2). The final products were characterised by both RP-HPLC and MALDI-TOF MS (refer to Figure 2).

[0262] All test compounds in the following examples were prepared according to this example.

TABLE 2

COMPOUNDS, MOLECULAR WEIGHT AND YIELDS

Compound No.	Name	Linker	SEQ ID NO:	MW (Da)	Yield (%)
1	Monomer (Chex1-Arg20(C-term Cys)-$NHNH_2$)	N/A	6	2650.96	56.3
2	Disulfide dimer-$NHNH_2$	disulfide bond	7	5185.92	45.6
3	p-Xylene dimer-$NHNH_2$	p-(1,4)-dibromomethyl-benzene	8	5289.08	46.8
4	o-Xylene dimer-$NHNH_2$	o-(1,3)-dibromomethyl-benzene	9	5289.08	22.2
5	m-Xylene dimer-$NHNH_2$	m-(1,2)-dibromomethyl-benzene	10	5289.08	40.5
6	Tetrafluorobenzene dimer-$NHNH_2$	hexafluorobenzene	11	5333.97	22.1
7	Octofluorobiphenyl dimer-$NHNH_2$	decafluorobiphenyl	12	5482.03	27.9

EXAMPLE 2 – ANTIMICROBIAL ACTIVITY OF DIMERS

Materials and Methods

[0263] Each test compound was tested for antibacterial activity in 100% Mueller Hinton broth (MHB) against a panel of Gram-negative bacteria, *E. coli ATCC 25922, K. pneumoniae ATCC 13883, A. baumannii ATCC 19606,* as well as the multi-drug resistant FADDI-*KP028* (MDR) and FADDI-*AB156 (*colistin-resistant, rifampin-resistant & MDR, XDR). A series of 100 µL solutions diluted from 250 µg/mL to 4 µg/mL test compounds, antibiotics or bifunctional linkers in MHB were added to a 96-well plate followed by the addition of 100 µl of 2 x 10^6 cells/mL with 90 min incubation at 37°C. The minimum inhibitory concentration (MIC) was determined by plotting maximal growth versus test compound concentration, according to the Lambert and Pearson analysis method (refer to Lam *et al.* (2016) *Nat. Microbiol.*, 1: 16162), while minimum bactericidal concentration (MBC) was confirmed via colony-forming unit (CFU) measuring assay.

[0264] To determine the membrane integrity, the DNA DNAgescent dyes propidium iodide (PI) and SYTO 9 were applied to monitor the effects of the test compounds using microbial MPs to monitor as previously described (refer to Li *et al.* (2015) *Chem. Biol.*, 22: 1250-1258 and O'Brien-Simpson *et al.* (2016) *PLoS One*, 11: e0151694). Briefly, a series of 100 µL test compound solutions diluted from 250 µg/mL to 4 µg/mL were added to a 96-well plate with prefilled 100 µL of 2 x 10^6 cells/mL. After 90 min incubation at 37° C, 50 µL of the culture mixture was transferred to a new 96-well plate and mixed with 50 µL mixture of PI (1.67 µM) and SYTO 9 (0.83 µM) in phosphate buffered saline (PBS). Then

the plate was subjected to flow cytometric analysis via CytoFLEX LX Flow Cytometer (Beckman Coulter) with the channel setting of SYTO 9 (Blue channel 525-40 nm, bacterial population with intact membrane) and PI (Yellow/Green channel 610-20 nm, bacterial population without intact membrane). The MDC values were determined by the concentration of the test compound resulting in over 90% PI positive labelling of bacterial population under CytoFLEX LX Flow Cytometer.

[0265] The proliferation and LDH of HEK-293 (ATCC® CRL-1573™) cells were determined by using CellTiter 96 AQ$_{ueous}$ Non-Radioactive Cell Proliferation Assay and CytoTox 96® Non-Radioactive Cytotoxicity Assay (Promega) as described in Li *et al.* (2015) *Aust. J. Chem.,* 68: 1373-1378. Briefly, 5×10^3 cells (100 µL) and test compounds at different concentrations (from 125 µg/mL to 0.2 µg/mL) were seeded into 96-well plates and cultured overnight at 37°C, 5% CO_2. After incubation, 50 µL of the supernatant from each well was transferred to a new 96 well flat bottom plate, followed by the addition of 50 µL LDH solution for 30 min incubation at room temperature. After the addition of 50 µL stop solution, the plate was subjected to record absorbance at 490 nm. The LDH generation from the cell samples were calculated as follows:

$$\text{Cytotoxicity}\ \% = \frac{\text{Experimental LDH Release (OD490)}}{\text{Maximum LDH Release (OD490)}} \times 100$$

[0266] After the LDH test in 96 well plate, 20 µL of the solution with tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent (phenazine methosulfate) PMS were added to each well followed by 1 h incubation at 37°C, 5% CO_2. The plate was then subjected to record absorbance at 490 nm. The proliferation effects of the test compounds on HEK cells were calculated as follows:

$$\text{Viable cells}\ \% = \frac{\text{Experimental viable cells (OD490)}}{\text{Maximum viable cells (OD490)}} \times 100$$

[0267] The safety profile of the test compounds was determined using the calculated therapeutic index (TI). As the MIC (refer to Table 3) and cytotoxicity (refer to Figure 3) were determined, the TI was calculated with the formula as IC_{50}/MIC_{50}. Since all the test compounds did not show any significant toxicity towards HEK-293 (ATCC® CRL-1573™) cells, the TI can only be calculated as the minimum index (refer to Table 5). The weight-of-evidence approach and bioactivity parameters suggest that the test compounds display a more balanced safety–efficacy profile.

Results

[0268] Each test compound (compounds **1**-**7**) was subjected to antibacterial testing against a panel of Gram-negative pathogens, including *E. coli ATCC 25922, K. pneumoniae ATCC 13883, A. baumannii ATCC 19606,* as well as FADDI-*KP028* (MDR) *and* FADDI-*AB156 (*colistin-resistant, rifampin-resistant & MDR, XDR*),* in undiluted Mueller-

Hinton broth (MHB). The minimum inhibitory concentration (MIC) that measures their efficacy in inhibiting bacterial growth showed that the dimeric Chex1-Arg20 compounds (compounds **2**-**7**) had significantly improved activity against MDR/XDR pathogens (refer to Table 3). The tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** had particularly low MIC values (5-13 μg/mL) on both *A. baumannii* and MDR/XDR-FADDI-*AB156*, bacterial species that are associated with a high mortality rate in hospital-acquired infections and are WHO priority critical listed pathogens. Both the hexafluorobenzene and decafluorobiphenyl linkers showed no antibacterial activity themselves.

TABLE 3

ANTIBACTERIAL ACTIVITY OF THE TEST COMPOUNDS AGAINST A PANEL OF GRAM-NEGATIVE PATHOGENS

Compound	**MIC (μg/mL)**				
	***E. coli* 25922**	***K. pneumoniae* 13883**	***A. baumannii* 19606**	**FADDI-*KP028***	**FADDI-*AB156***
1	2.5±1.1 (0.9)	3.2±0.1 (1.2)	>250 (>94.3)	199.8±0.2 (75.4)	>250 (>94.3)
2	3.1±0.1 (0.6)	5.1±0.8 (1.0)	30.8±3.3 (5.9)	111.3±0.3 (21.5)	59.2±0.2 (11.4)
3	8.1±0.4 (1.5)	3.5±0.6 (0.7)	13.1±0.7 (2.5)	59.6±0.3 (11.3)	54.4±1.4 (10.3)
4	8.5±0.7 (1.6)	3.7±0.6 (0.7)	16.2±2.8 (3.1)	67.5±4.1 (12.8)	55.4±0.9 (10.5)
5	8.1±0.6 (1.5)	3.6±0.7 (0.7)	13.1±0.6 (2.5)	70.1±3.9 (13.3)	53.9±2.1 (10.2)
6	6.7±0.9 (1.3)	3.6±0.1 (0.7)	6.5±0.1 (1.2)	120.9±2.9 (22.7)	13.4±0.9 (2.5)
7	2.7±0.7 (1.5)	2.8±0.9 (0.5)	4.9±1.3 (0.9)	51.8±1.5 (9.4)	13.7±0.1 (2.5)
Gentamicin	0.9±0.1 (1.88)	0.1±0.05 (0.2)	2.7±0.2 (5.7)	>250 (>523.5)	>250 (>523.5)
Colistin	N/A	N/A	0.5±0.04 (0.4)	N/A	12.6±1.6 (10.9)
Hexafluorobenzene linker alone	>250	>250	>250	>250	>250
Decafluorobiphenyl linker alone	>250	>250	>250	>250	>250

All assays were performed twice in duplicate and determined as mean ± standard deviation. Conventional antibiotics, gentamicin and colistin, were used as controls. Calculated μM values in brackets.

[0269] The membrane disruption assay further showed the potent membrane activity against Gram-negative bacteria (refer to Table 4) by the lead candidates, tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**, and absence of cytotoxicity (refer to Figure 3). Their high therapeutic index (refer to Table 5) reflects their significant potential for future therapeutic investigation. Additionally, a trimeric PrAMP *via* a 1,3,5-tris(methyl)benzene linkage was prepared but did not confer any improvement in activity (data not shown).

TABLE 4

MINIMUM MEMBRANE DISRUPTION CONCENTRATION (MDC)

Compound	*E. coli 25922*	*K. pneumoniae 13883*	*A. baumannii 19606*
1	>250	>250	>250
2	30.9±1.4	8.3±0.3	43.9±1.0
3	11.8±0.6	17.5±0.1	21.1±0.6
4	19.1±0.2	18.1±2.0	25.7±1.0
5	19.8±0.8	32.7±1.4	23.7±0.7
6	6.2±0.5	6.9±0.2	10.6±0.5
7	4.1±0.1	4.2±0.1	4.5±0.1

All data are performed twice in duplicate and determined as mean ± standard deviation.

TABLE 5

THERAPEUTIC INDEX (TI) FOR A. BAUMANNII 19606 AND FADDI-AB156

Compound	*A. baumannii 19606*			*FADDI-AB156*		
	MIC_{50}	IC_{50}	$TI=IC_{50}/MIC_{50}$	MIC_{50}	IC_{50}	$TI=IC_{50}/MIC_{50}$
1	125	>125	>1	200	>125	>0.6
2	25.9±1.8	>125	>4.8	16.6±3.1	>125	>7.5
3	7.4±0.8	>125	>16.8	31.3±1.8	>125	>4
4	10.0±1.4	>125	>12.5	31.1±0.1	>125	>4
5	7.4±0.9	>125	>16.9	31.6±0.3	>125	>4
6	3.3±0.1	>125	>37.9	7.6±0.2	>125	>16
7	3.5±0.3	>125	>35.7	4.1±0.2	>125	>30

[0270] The tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** with their potent antibacterial inhibition activity against both *A. baumannii* and MDR/XDR-FADDI-AB156, were further assessed for minimum bactericidal concentration (MBC) as previously reported (refer to Lam *et al.* (2016) *Nat. Microbiol.*, 1: 16162). In comparison with the conventional antibiotics, gentamicin and the last resort antibiotic, colistin, both compounds **6** and **7** showed very strong killing activity against *A. baumannii and MDR/XDR-FADDI*-AB156 (refer to Table 6).

TABLE 6

BACTERICIDAL MBC OF THE TETRAFLUOROBENZENE DIMER-$NHNH_2$ COMPOUND 6 AND OCTOFLUOROBIPHENYL DIMER-$NHNH_2$ COMPOUND 7

Compound	MBC (μg/mL)	
	A. baumannii **19606**	**MDR-FADDI-AB156**
6	14.1±0.9 (2.6)	22.5±2.5 (4.2)
7	30.6±0.6 (5.6)	32.5±2.5 (5.9)
Gentamicin	4.6±0.3 (9.6)	n.d.*
Colistin	0.8±0.02 (0.7)	>31.25 (> 27.1)

*n.d. = no measurable bactericidal activity

All data were performed twice in duplicate and determined as mean ± standard deviation. Conventional antibiotics, gentamicin and colistin, were used as control. Calculated μM values shown in brackets.

EXAMPLE 3 – KILLING EFFICIENCY OF COMPOUNDS 6 AND 7

Materials and Methods

[0271] Bactericidal time killing efficiency of compounds **6** and **7** was determined against *A. baumannii* and MDR-FADDI-*AB156*. The mid-logarithmic phase bacterial cells were re-suspended into MHB of 2×10^6 cells/mL and then 100 μL bacteria were incubated with test compounds at different concentrations (4×MIC, 2×MIC, 1×MIC, 0.5×MIC, 0.25×MIC) at 37°C. A 10 μL aliquot was removed from the treated bacterial suspension at specific time intervals (0, 5, 10, 20, 30, 45, 60, 75 and 90 min) and plated on LB-agar plates to determine the colony-forming units (CFU) at each time point after overnight incubation (refer to Figure 4).

[0272] Based on the CFU count at various time points, the first order death rate constant was determined by plotting Log(CFU) vs. time range from 5-60 min (refer to Figure 5). The regression of the linear fit was obtained with Graphpad and the slope of linear fit provided the death rate constant (refer to Figure 5). Then the death rate constants obtained from Figure 5 were subject of the plot for death rate constant vs. peptide concentration in Figure 6.

Results

[0273] To determine the killing efficiency of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** against *A. baumannii* and MDR-FADDI-AB156 a time-killing kinetic assay was designed by measuring the death rate constant under treatment with compounds **6** and **7**. By choosing different concentrations (4 × MIC, 2 × MIC, 1 × MIC, 0.5 × MIC, 0.25 × MIC) and a 3-log (99.9%) reduction in survival and zero colony forming unit (CFU) as a basis, both compounds **6** and **7** (4 × MIC ~ MBC) clearly showed potent bactericidal activity and sterility, respectively, to kill *A. baumannii* and MDR-

FADDI-*AB156* at the 75 min to 90 min incubation time points (refer to Figure 4). These results are consistent with the MBC killing of all the bacteria after the full-course incubation (refer to Table 6). More specifically, at the 4 × MIC concentration, compound **6** reduced *A. baumannii* and MDR-FADDI-*AB156* survival within 60 minutes by 2.38 log (>99%) and 2.78 log (>99%), respectively (refer to Figures 4A and 4C), while compound **7** reduced the survival by 1.81 log (>98%) and 2.72 log (>99%) (refer to Figures 4B and 4D). At 2 × MIC of 1 hour incubation, compound **6** still displayed strong bactericidal activity by survival reduction of 1.18 log (>93%) and 2.09 log (>99%) towards *A. baumannii* and MDR-FADDI-*AB156* (refer to Figures 4A and 4C). In contrast, compound **7** at 2 × MIC showed less killing activity within 60 minutes with a CFU reduction of 0.68 log (>79%) and 0.94 log (>88%) (refer to Figures 4B and 4D).

[0274] Based on the CFU count at various time points, the first order death rate constant was determined by plotting log(CFU) vs. time ranging from 5-60 min, the slope of linear fit providing the death rate constant (refer to Figure 5). On comparing the death rate constant at various concentrations (refer to Figure 6), a rapid killing rate was observed for both compounds **6** and **7**. However, the activity of compound **7** at 1 × MIC was significantly ($P<0.05$) weaker than that of compound **6** (1 × MIC), reducing survival by 0.74 log (>81%) and 0.19 log (>35%), respectively (refer to Figure 4). Taken together, the tetrafluorobenzene dimer-$NHNH_2$ **6** possesses a faster killing rate for both *A. baumannii* and MDR-FADDI-*AB156* compared to the octofluorobiphenyl dimer-$NHNH_2$ **7**.

<u>EXAMPLE 4 – MEMBRANE PERMEABILITY OF COMPOUNDS 6 AND 7</u>

<u>Materials and Methods</u>

[0275] Based on the N-phenylnaphthylamine (NPN) fluorescence property while in contact with a phospholipid bilayer, the outer membrane permeability was determined at different concentrations of the test compounds (compounds 6 and 7). Briefly, the bacterial culture at exponential phase (OD = 0.5) was harvested at 3500 rpm (10 min, r.t.) and resuspended into HEPES buffer (1 mM, pH 7.2) into OD_{600} of 0.5. 50 µL of a series concentration of the test compounds (2×MIC, 1×MIC, 0.5×MIC, 0.25×MIC, 0.1×MIC) were added to a 96-well plate prefilled with 100 µL bacterial culture (in HEPES buffer), followed by the addition of 50 µL of NPN (40 µM, final concentration of 10 µM). Meanwhile, due to the potent permeability of polymyxin B, a triple well with polymyxin B (6.4 µg/mL) was prepared as the positive control. After 10 min incubation, the plate was measured at 355/405 nm using a fluorescence spectrophotometer (PerkinElmer 1420 Multilabel Counter VICTOR). The fluorescence intensity of each treated sample was compared with the positive control of polymyxin B.

[0276] Since the inner membrane is impermeable to PI, it has been used to differentiate the membrane integrity under different treatments, while SYTO 9 penetrates

all cell membranes. In this assay, 100 µL of diluted test compounds from 62.5 µg/mL to 0.9 µg/mL were added to 100 µL of 2×10^6 cells/mL in a 96-well plate. After 60 min incubation at 37°C, 50 µL of the culture mixture was transferred to a new 96-well plate and mixed with 50 µL mixture of PI (1.67 µM) and SYTO 9 (0.83 µM) in PBS. Then the plates were subjected to flow cytometric analysis via CytoFLEX LX Flow Cytometer (Beckman Coulter), with the SYTO 9 labelled bacterial population with the intact membrane (blue coloured) and PI-labelled bacterial population for PrAMPs with inner membrane penetration (red coloured) (refer to Figures 7 and 8).

[0277] For the dye leakage assay, large unilamellar vesicles (LUVs) were prepared to mimic bacterial membranes. Phosphatidylglycerol (PG): cardiolipin (9:1) were used to mimic a Gram-positive bacterial membrane and phosphatidylethanolamine (PE): PG (7:3) was used to represent a Gram-negative bacterial membrane, as previously described (refer to Sani *et al.* (2013) *Antimicrob Agents Chemother*, 57: 3593-3600 and O'Brien-Simpson (2017) *Aust. J. Chem.*, 70: 220-228). Using an Avanti Mini-Extruder with 100 nm diameter pore-size polycarbonate filter, LUVs with encapsulated calcein for dye leakage experiments were produced in 20 mM phosphate buffer (with 5 mM NaCl and 0.5 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4) by extrusion at room temperature. Calcein leakage was measured using a 96-well plate with a fluorescence plate reader at wavelength 485/535 nm with 3 cycles at 25°C. The fluorescence intensities were averaged and percentage calcein fluorescence calculated *via* the following equation:

$$Fluorescence\ \% = \frac{(I - In)}{Ip - In} \times 100\%$$

in which I represents the fluorescence of LUVs with test compounds, In represents the fluorescence of the LUVs only, as the negative control, and Ip represents the fluorescence of the LUVs with 2% Triton X-100 as the positive control. Then, the normalised intensities were plotted against the lipid to peptide molar ratio (L/P) (refer to Figure 10).

[0278] The effects of the test compounds on membrane potential were assessed using a BacLight Bacterial Membrane Potential Kit (Invitrogen) by flow cytometric analysis (CytoFLEX Flow Cytometer, Beckman Coulter). Briefly, 100 µL of bacterial culture (2×10^6 cells/mL) in MHB was mixed with 100 µL serial dilution of test compounds (0.125 × MBC, 0.25 × MBC, 0.5 × MBC, 1 × MBC, 2 × MBC) in a 96-well plate. After 60 min incubation at 37°C, the 3,3'-diethyloxa-carbocyanine iodide ($diOC_23$) (30 µM) was added to the tested samples and then the plate was subjected to flow cytometric analysis via CytoFLEX LX Flow Cytometer (Beckman Coulter). Meanwhile, the protonophore, carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP, final concentration 5 µM), treated sample acted as a depolarised population control. Based on the CCCP control and normal cell samples, gates

were drawn to present normal cell population or depolarized regions (refer to Figures 9 and 10).

Results

[0279] The membrane permeability of compounds **6** and **7** was further determined by using 1-*N*-phenylnaphthylamine (NPN). NPN is a small molecule that can become strongly fluorescent in contact with a phospholipid bilayer and is widely used to indicate the outer membrane permeabilization of Gram- negative bacteria. Polymyxin B, as a positive control, is well known to permeabilise the outer membrane, as reflected by a high fluorescent intensity of NPN signal in Figure 11. As shown in Figure 11, both compounds **6** and **7** displayed a similar trend to promote NPN uptake across the outer membrane of *A. baumannii* (Figure 11A) and MDR-FADDI-*AB156* (Figure 11B) as the concentration of compound increased. Compared to the positive control of polymyxin treated bacterial samples, tetrafluorobenzene dimer-$NHNH_2$ **6** showed significant NPN permeability over the concentration of 0.1 × MIC or 0.25 × MIC. Notably at the 1 × MIC or 2 × MIC, compound **6** treatment increased significantly (***P ≤ 0.001) 1.5-fold to 2-fold NPN fluorescence intensity over the NPN bacteria sample in the absence of compound/polymyxin treatment (refer to Figures 11A and 11B). The octofluorobiphenyl dimer-$NHNH_2$ **7** showed slightly lower permeability than the tetrafluorobenzene dimer-$NHNH_2$ **6**, with compound **7** at 1 × MIC or 2 × MIC able to significantly induce 1.3-1.7-fold (***P ≤ 0.001) NPN fluorescence intensity over the NPN bacteria sample without compound/polymyxin treatment.

[0280] Given the observation that the effective concentration for outer membrane permeability (refer to Figure 11) caused by the compounds is much lower than their bacterial inhibition or killing activity (refer to Table 3), the inner membrane permeability was further assessed by using the DNA binding dyes, propidium iodide (PI) and SYTO 9 *via* flow cytometry. SYTO 9, being a membrane permeable dye will label every bacterium (thus discriminating bacteria from background), while PI, a membrane impermeable dye, can only enter the cytoplasm when the inner membrane has been disrupted by compound treatment. By measuring the percentage of PI-labelled bacteria via flow cytometry, the ability of the test compounds to permeabilise the inner membrane can be assessed. As shown in Figure 12, both the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** gradually increased the portion of PI-labelled bacteria to > 80% of the whole bacterial population. At the bacterial inhibition (MIC) or killing concentration (MBC) (refer to Table 6), it was observed that 50-60% of *A. baumannii* were PI-positive labelled bacteria population at 1 × MBC (refer to Figure 12A), and that 50-60% of MDR-FADDI-AB156 are PI+ at 2 × MBC (refer to Figure 12B) for both dimers, **6** and **7**. The results strongly correlated their inner membrane permeability with their bacterial inhibition or killing activity.

[0281] A major energy source for nutrient uptake, waste material excretion and micro-environment upregulation is the trans-cytoplasmic membrane ion motive force driven by an electrical potential gradient. Based on the NPN and PI/SYTO 9 assays (refer to Figures 11 and 12), the observed membrane interaction with the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** may further modulate the membrane potential. Thus, ion permeability was then examined by using a membrane potential-sensitive fluorescent probe, $DiOC_2(3)$, which can be taken up through the inner membrane by the electrical potential gradient and fluoresces green in the cytoplasm under normal membrane potential conditions. When any compound interacts and depolarises the inner membrane, $DiOC_2(3)$ self assembles and produces a red shift in fluorescence, with the strength in the shift indicating the strength of the change in membrane potential. As Figure 13 shows, both the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** caused almost 100% depolarisation at their 1 × MBC concentration, the same as the positive control sample treated with CCCP. Even at 0.1 × MBC (*A. baumannii*) or 0.5 × MBC (MDR-FADDI-*AB156*) compound treatment resulted in more than 50% depolarisation ($p^{**}<0.01$). Bacterial membranes are composed of roughly equal proportions of membrane-associated proteins and phospholipids forming a classical bilayer structure. To further investigate the phospholipid bilayer membrane interaction, large unilamellar vesicles (LUVs) were further used to examine pore formation property and showed no leakage of calcein (refer to Figure 14). These results suggest both the compounds can permeabilise the outer and inner membranes at low concentrations (0.25 × MIC or 0.5 × MIC) and induce smaller pore formation enabling PI or ions exchange but not calcein (larger molecules). The surprising weaker PI-positive labelling (60-80%) at higher concentration (> 31.3 µg/ml, refer to Figure 12) further indicated the small pore size formed by compounds **6** and **7**, with only some PI getting into the bacteria. Together with the capability of strong membrane depolarisation, this contributes to their potent antibacterial activity towards *A. baumannii* and MDR-FADDI-*AB156.*

EXAMPLE 5 – STRESS RESPONSE BY GENERATION OF REACTIVE OXYGEN SPECIES (ROS) FOLLOWING TREATMENT WITH COMPOUNDS 6 AND 7

Materials and Methods

[0282] ROS production from compounds **6** and **7** was determined by CellROX deep red reagent by flow cytometric analysis. Briefly, 100 µL of bacterial culture (2 × 10^6 cells/mL) in DMEM was mixed with 100 µL serial diluted of test compounds (0.125 × MBC, 0.25 × MBC, 0.5 × MBC, 1 × MBC, 2 × MBC) in a 96-well plate. After 90 min incubation at 37°C, the bacterial culture was then stained with a mixture of CellROX deep red reagent (5 µM) and SYTO 9 reagent (0.83 µM), which was then subjected to CytoFLEX Flow Cytometer (Beckman Coulter) analysis. Additionally, the ROS scavenger, thiourea, was added to the test compound-treated samples as the confirmation for the ROS generation.

Based on the untreated bacteria, gates were determined during the flow cytometric analysis (refer to Figures 15 and 16). The level of ROS production was plotted at different concentrations (refer to Figure 17).

Results

[0283] Disturbance of membrane integrity and potential can further lead to a stress response and the generation of ROS, thus resulting in oxidative damage then cell death *via* the oxidative damage-mediated pathway. To investigate the effect of our lead PrAMPs for ROS production, the microbial flow cytometry method was applied to determine the ROS level for *A. baumannii* and MDR-FADDI-*AB156* treated with the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**, using CellROX® Deep Red fluorescence. The addition of thiourea, a ROS scavenger, was used to quench ROS to a level of the untreated bacterial sample, which further verified the induction of ROS production under PrAMP treatment. As shown in Figure 17, both compounds **6** and **7** induced significant ($p^{**}<0.01$) amounts of ROS as compared to the untreated bacteria and thiourea-quenched ROS control. At 2 × MBC, tetrafluorobenzene dimer-$NHNH_2$ **6** triggered a 4.8-fold and 3.9-fold increase of ROS generation for *A. baumannii* and MDR-FADDI-AB156, respectively, while the octofluorobiphenyl dimer-$NHNH_2$ **7** induced 2.2-fold and 2.7-fold increase (refer to Figure 17). At the lethal dose level (1 × MBC), both compounds displayed very similar effects on the ROS generation with a 2.4-3.0-fold difference over the control samples (refer to Figure 17). Compared to the octofluorobiphenyl dimer-$NHNH_2$ **7,** the tetrafluorobenzene dimer-$NHNH_2$ **6** could trigger a high level of ROS at the lower concentrations (0.25-0.5 × MBC) with a 1.5-fold and 2.7-fold increase for *A. baumannii* and MDR-FADDI-AB156, respectively. Taken together, both compounds **6** and **7**, significantly induced the generation of ROS at the lethal and sublethal doses, which contributed to the death of *A. baumannii* and MDR-FADDI-AB156.

EXAMPLE 6 – MORPHOLOGICAL CHANGES FOLLOWING TREATMENT WITH COMPOUNDS 6 AND 7

Materials and Methods

[0284] The morphologies of planktonic bacteria *A. baumannii ATCC 19606* and FADDI-*AB156* was assessed after treatment with the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** at different concentrations (2 × MIC, 1 × MIC, 0.5 × MIC, 0.25 × MIC, 0.125 × MIC) using helium ion microscopy (HIM, Zeiss, Germany). The samples incubated with the test compounds (90 min) and the PBS treated control sample were added to clean glass cover slides, followed by evaporation at 37 °C for 20-30 min. The dried samples on glass cover slides were then transferred to a 12-well plate for fixation with 2.5% glutaraldehyde (v/v) for 1 h. The glass slides were then stepwise washed with PBS gently, then gradient ethanol solution (30%, 50%, 60%, 70%, 80%,

90%, 95% and 100%) for dehydration for 10 mins each time. The prepared samples were dried in a fume hood overnight and then subjected to an ORION NanoFab (Zeiss, Peabody MA) using a 25 keV He^+ ion beam with a typical beam current of 0.8-1.0 pA.

Results

[0285] Although fluorescence imaging methods are widely used to visualise the interaction between antibacterial agents and bacteria, the addition of the fluorescent dyes can reduce the bioactivity of the target compounds. The advanced development of surface scanning microscopy, such as the helium ion microscope (HIM), enables direct analysis of the biological samples at super-high resolution. To directly observe the morphological changes of bacterial cells due to the test compounds, HIM was used to investigate the morphologies of *A. baumannii* and MDR-FADDI-*AB156* under the treatment of tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** at different concentrations (2 × MIC, 1 × MIC, 0.5 × MIC, 0.25 × MIC, 0.125 × MIC). The HIM images showed clear visualisation of the morphological changes of *A. baumannii* and MDR-FADDI-*AB156* with test compound treatment (refer to Figure 18). The multiple morphological changes with the test compounds at the lethal dose (2 × MIC) can be explained by destabilisation of the outer membrane, followed by membrane fragmentation and disruption (refer to Figure 18). Interestingly, the tetrafluorobenzene dimer-$NHNH_2$ **6** treated bacteria led to compaction or shrinkage (refer to Figures 18A and 18C), while the octofluorobiphenyl dimer-$NHNH_2$ **7** caused the membrane to burst (refer to Figures 18B and 18D). Such observations of the morphological charges clearly showed the mechanism for membrane interaction via destabilisation in the outer membrane, followed by disruption of the inner membrane.

EXAMPLE 7 – EFFECT OF COMPOUNDS 6 AND 7 ON BIOFILMS

Materials and Methods

[0286] The microtiter plate preformed biofilms were prepared by adding 100 µL of the diluted bacterial culture of *A. baumannii ATCC 19606* or FADDI-*AB156* (OD_{600} = 0.5-0.6, 10 µL into 8 mL MHB) into each well of a 96-well plate. After incubating for 24 h at 37°C, the biofilm formation was confirmed by crystal violet staining under microscopy. At 24 h incubation, the media and planktonic bacteria were removed gently followed by two washing steps (200 µlL phosphate buffered saline, PBS). Then 100 µL of the test compounds (compounds **6** and **7**) in MHB of different concentration (0.5 × MBC, 1 × MBC, 2 × MBC, 4 × MBC, 8 × MBC) in triplicate and 100 µL MHB as control were added to each well. After 90 min incubation at 37°C, the peptides were removed and washed with 200 µL PBS (two times), followed by crystal violet (100 µL, 2% w/v in 20% v/v ethanol/water) staining for 30 min. The crystal violet was removed and washed with 200 µL PBS (two times). The crystal violet stained samples were dissolved with 100 µL acetic acid (30% in

MilliQ water) and absorption measured at 540 nm. In comparison to the untreated control biofilms, the absorption of each sample indicated the efficiency of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** to eradicate preformed biofilms.

[0287] HIM was performed as described in Example 6.

Results

[0288] Biofilms are an accumulation of microbes forming a self-produced matrix of extracellular polymeric substances (EPS) which are more resistant to abrupt environmental changes and antibiotics than planktonic bacteria. Due to biofilms comprising over 65% of human infections, antibiotics used to treat planktonic bacterial infections are commonly prescribed to combat bacterial biofilms. The biofilm disruption properties of compounds **6** and **7** were investigated. In the microtiter plate preformed biofilm assay, the biofilm biomass was measured after crystal violet staining which quantified detaching bacteria after test compound treatment at various concentrations (0.5 × MBC, 1 × MBC, 2 × MBC, 4 × MBC, 8 × MBC). Both compounds **6** and **7** eradicated the preformed biofilm with a similar trend, that generally destroyed the biofilm with increased compound concentration (refer to Figure 19). With 0.5 × MBC and 1 × MBC compound treatment, both test compounds had little effect on eradicating the preformed biofilm with only 10-15% reduction in the crystal violet absorption (refer to Figure 19). At a higher lethal dose for the planktonic bacteria (2 × MBC), almost 50% of the preformed biofilm was significantly ($P < 0.05$) destroyed during the 90 min peptide treatment and suggested potent antibiofilm activity (refer to Figure 19). However, a higher dose of the test compounds (4 × MBC, 8 × MBC) did not further eradicate higher percentage (over 50%) of biofilm (refer to Figure 19), which reflects the difficulty in combating biofilms.

[0289] To further visualise their action on the preformed biofilms, we used HIM to assess the effect of tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** on the biofilm formed on glass coverslips after 90 min peptide treatment (refer to Figure 20). The untreated performed bacterial biofilm sample clearly showed the EPS matrix and the connections among bacteria. With the increased concentration of compounds **6** and **7** (0.5 × MBC, 1 × MBC, 2 × MBC, 4 × MBC, 8 × MBC), the EPS matrix was gradually disrupted or demolished, especially at the higher concentrations (4 × MBC, 8 × MBC) with destroyed EPS matrix and disrupted bacteria both observed (refer to Figure 20). In addition to the EPS matrix disturbance, the bacteria underwent a similar trend as the planktonic bacteria with destabilisation of the outer membrane, followed by membrane fragmentation and disruption (refer to Figure 20).

EXAMPLE 8 – IMMUNOMODULATORY ACTIVITY OF COMPOUNDS 6 AND 7

Materials and Methods

[0290] Although the the commercially available E. coli LPS is commonly used to stimulate macrophage cells, the LPS originating from *A. baumannii* ATCC 19606 and FADDI-*AB156* from the laboratory cultured bacteria were pursued with adaption from the Tri-Reagent method (refer to Yi and Hackett (2000) *Analyst*, 125: 651-656). Briefly, the bacterial culture was centrifuged to pellet at 5000 g, 15 min at 4°C. The pellet was suspended into Tri-Reagent (5 ml) for 10 min at room temperature, followed by chloroform (500 µL) extraction. The organic phase was further extracted two times with MilliQ water. Then all three aqueous extracts were combined into a 50 mL Falcon tube, followed by the addition of 40 µL DNase/RNase/Lipase (stock 10 mg/mL) and incubation for 2 h at room temperature. To precipitate the LPS, two equivalents of the volume of $MgCl_2$ (0.375 M in ethanol) was added and kept at -20°C overnight. The precipitates of *A. baumannii* ATCC 19606 and FADDI-*AB156* LPS were obtained after centrifugation and lyophilisation for further usage.

[0291] Using the extracted LPS from *A. baumannii* ATCC 19606 and FADDI-*AB156,* the NO released from RAW264.7 cells was determined with a diluted LPS series. In brief, 100 µL of 1×10^6 RAW264.7 cells/mL was seeded into a flat-bottom 96-well plate for 24 h at 37°C with 5% CO_2 in DMEM. Then, a series of 50µL/well diluted LPS in DMEM, from 200 µg/mL to 0.05 µg/mL, was added followed by the addition of 100 µL DMEM to give a total of 250 µL in each well. After overnight incubation at 37°C, the culture supernatant (50 µL) was collected for NO concentration determination using a Promega Griess Reagent Kit to measure the absorption with a filter of 540 nm (refer to Figure 21). The concentration of NO in µM (refer to Figure 21) was calculated via the nitrate standard curve (refer to Figure 22).

[0292] As shown in Figure 21, 12.5 µg/mL of LPS originated from *A. baumannii* ATCC 19606 and FADDI-*AB156* can fully stimulate RAW264.7 cells for nitric oxide (NO) release. To assess the effect of the test compounds on suppressing LPS stimulation, the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7**, were incubated with RAW264.7 cells at different concentrations in the presence of 12.5 µg/mL of the extracted LPS. In brief, 100 µL of 1×10^6 RAW264.7 cells/mL was seeded into a flat-bottom 96-well plate for 24 h at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM). Then, a series of 50 µL/well of test compounds diluted from 100 µg/mL to 3 µg/mL in DMEM were added into each well of RAW264.7 cells, followed by the addition of 50 µL extracted LPS (12.5 µg/mL in DMEM) and 50 µL DMEM. After overnight incubation at 37°C, the culture supernatant (50 µL) was collected for NO concentration determination

using a Promega Griess Reagent Kit to measure the absorption with a 540 nm filter. The concentration of NO in µM was further converted via a nitrate standard curve.

Results

[0293] Antimicrobial peptides can modulate the host innate immunity by promoting protective responses, such as cytokine tumour necrosis factor-α (TNF-α), and suppressing inflammation, like nitric oxide (NO) and proline-rich antimicrobial peptides have been found to have potent immunomodulatory properties that modulate cell function and cytokine secretion to up-regulate the recovery from bacterial infections. Accordingly, the immunomodulatory activity of the tetrafluorobenzene dimer-$NHNH_2$ **6** and octofluorobiphenyl dimer-$NHNH_2$ **7** was assessed. Using freshly purified lipopolysaccharide (LPS) from *A. baumannii* and MDR-FADDI-*AB156*, the potential anti-inflammation effect of the test compounds on LPS-stimulated NO production from macrophages was assessed using the Griess assay. As shown in Figure 21, the NO concentration gradually increased in RAW 264.7 cell cultures as the purified LPS concentration increased, while the NO production was substantially suppressed by 2-3-fold at the higher concentration of the tested compounds (refer to Figure 23). Through their dose-dependent manner to neutralize the endotoxin (bacterial LPS) and NO release in macrophages (via well-recognised TLR4-dependant pathway), compounds **6** and **7** indicate a synergistic approach resulting in enhanced direct killing and modulation of the adaptive immune response.

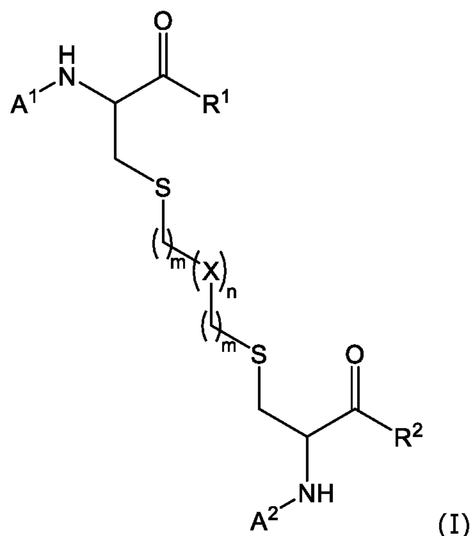
[0294] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0295] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0296] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A compound represented by Formula I:



(I)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1 and A^2 are each independently a proteinaceous molecule comprising from about 5 to about 100 amino acid residues (and all integer residues therebetween);

each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene, optionally substituted C_5-C_{12} heteroarylene, optionally substituted C_5-C_{12} cycloalkylene and optionally substituted C_5-C_{12} heterocycloalkylene;

R^1 and R^2 are independently selected from the group consisting of -OH, $-NH_2$ and $-NHNH_2$;

n is 1 or 2; and

each m is independently 0, 1 or 2.

2. The compound according to claim 1, wherein each X is independently selected from the group consisting of optionally substituted C_5-C_{12} arylene and optionally substituted C_5-C_{12} cycloalkylene.

3. The compound according to claim 2, wherein each X is independently optionally substituted C_5-C_{12} arylene.

4. The compound according to claim 3, wherein each X is independently optionally substituted C_5 or C_6 arylene.

5. The compound according to claim 4, wherein each X is optionally substituted phenylene.

6. The compound according to claim 5, wherein each X is independently phenylene substituted with one or more F, Cl, Br or I.

7. The compound according to claim 6, wherein each X is independently phenylene substituted with one or more F.

8. The compound according to claim 7, wherein each X is tetrafluorophenylene.

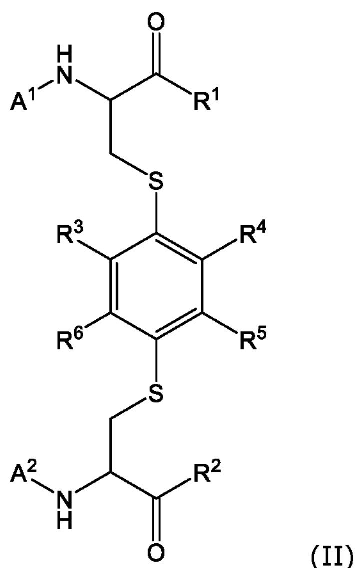
9. The compound according to any one of claims 1-8, wherein n is 1.

10. The compound according to any one of claims 1-8, wherein n is 2.

11. The compound according to any one of claims 1-10, wherein each m is independently 0 or 1.

12. The compound according to any one of claims 1-10, wherein each m is 0.

13. The compound according to claim 1, wherein the compound is represented by Formula II:



(II)

or a pharmaceutically acceptable salt thereof,

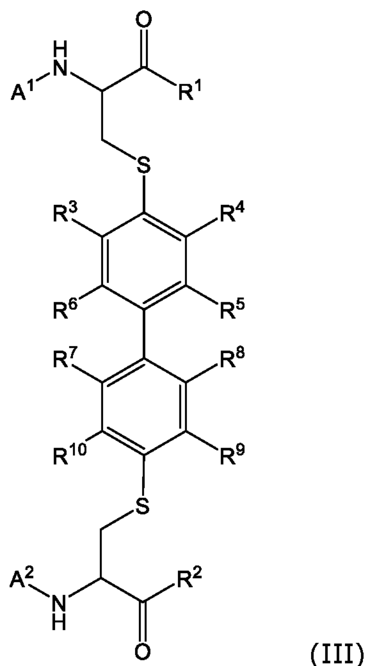
wherein:

A^1, A^2, R^1 and R^2 are as defined in claim 1; and

R^3, R^4, R^5 and R^6 are independently selected from the group consisting of H, F, Cl, Br and I.

14. The compound according to claim 13, wherein R^3, R^4, R^5 and R^6 are F.

15. The compound according to claim 1, wherein the compound is represented by Formula III:



(III)

or a pharmaceutically acceptable salt thereof,

wherein:

A^1, A^2, R^1 and R^2 are as defined in claim 1; and

R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are independently selected from the group consisting of H, F, Cl, Br and I.

16. The compound according to claim 15, wherein R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are F.

17. The compound according to any one of claims 1-16, wherein A^1 and A^2 are the same.

18. The compound according to any one of claims 1-17, wherein A^1 and A^2 are antimicrobial peptides.

19. The compound according to claim 18, wherein A^1 and A^2 are proline-rich antimicrobial peptides.

20. The compound according to claim 19, wherein A^1 and A^2 are independently selected from the group consisting of Chex1-Arg20, oncocin, apidaecin (Api137), drosocin and Bac7(1-35).

21. The compound according to claim 19, wherein A^1 and A^2 are independently proteinaceous molecules comprising, consisting or consisting essentially of an amino acid sequence selected from the group consisting of:

[Chex]RPDKPRPYLPRPRPPRPVR [SEQ ID NO: 1];

VDKPPYLPRPRPPRRIYNR [SEQ ID NO: 2];

[gu]ONNRPVYIPRPRPPHPRL [SEQ ID NO: 3];

GKPRPYSPRPTSHPRPIRV [SEQ ID NO: 4]; and

RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFP [SEQ ID NO: 5].

22. The compound according to claim 21, wherein A^1 and A^2 are each a proteinaceous molecule comprising an amino acid sequence of SEQ ID NO: 1:

[Chex]RPDKPRPYLPRPRPPRPVR [SEQ ID NO: 1].

23. The compound according to any one of claims 1-22, wherein A^1 and A^2 are attached to the adjacent nitrogen atom of the compound of Formula I, II or III via their C-terminus.

24. The compound according to any one of claims 1-23, wherein A^1 and A^2 are attached to the adjacent nitrogen atom of the compound of Formula I via an amide bond.

25. A composition comprising a compound according to any one of claims 1-24 and a pharmaceutically acceptable carrier or diluent.

26. A method of treating or inhibiting the development of an infection in a subject, comprising administering a compound according to any one of claims 1-24 to the subject.

27. The method according to claim 26, wherein the infection is a bacterial or a viral infection.

28. The method according to claim 27, wherein the infection is a bacterial infection.

29. The method according to claim 28, wherein the infection is caused by a bacterial species that is resistant to one or more antibiotics.

30. The method according to claim 28 or claim 29, wherein the infection is a gram-negative bacterial infection.

31. The method according to claim 30, wherein the infection is by *Escherichia coli*, *Klebsiella pneumoniae* or *Acinetobacter baumannii*.

32. The method according to claim 31, wherein the infection is by *A. baumannii*.

33. The method according to claim 30, wherein the infection is by *K. pneumoniae* FADDI-KP028 or *A. baumannii* FADDI-AB156.

34. A method of treating or inhibiting the development of an inflammatory disorder in a subject, comprising administering a compound according to any one of claims 1-24 to the subject.

35. The method according to claim 34, wherein the inflammatory disorder is selected from the group consisting of inflammatory bowel disease, rheumatoid arthritis, sepsis, atopic dermatitis and acne.

36. A method of eliciting an immune response in a subject, comprising administering a compound according to any one of claims 1-24 to the subject.

37. A method of enhancing an immune response in a subject to a target antigen by an immune modulating agent, comprising administering a compound according to any one of claims 1-24 to the subject.

38. The method according to claim 37, wherein the immune modulating agent is selected from an antigen that corresponds to at least a portion of the target antigen, an antigen-binding molecule that is immuno-interactive with the target antigen and an immune modulating cell that modulates an immune response to the target antigen.

39. The method according to claim 37 or claim 38, wherein the target antigen is an antigen from an infectious agent.

40. A method of enhancing an innate immune response to an infectious agent in a subject, comprising administering a compound according to any one of claims 1-24 to the subject.

41. A method of treating or inhibiting the development of an autoimmune disorder in a subject, comprising administering a compound according to any one of claims 1-24 to the subject.

42. The method according to claim 41, wherein the autoimmune disorder is type 1 diabetes or multiple sclerosis.

43. A method of disrupting a biofilm, comprising contacting the biofilm with a compound according to any one of claims 1-24.

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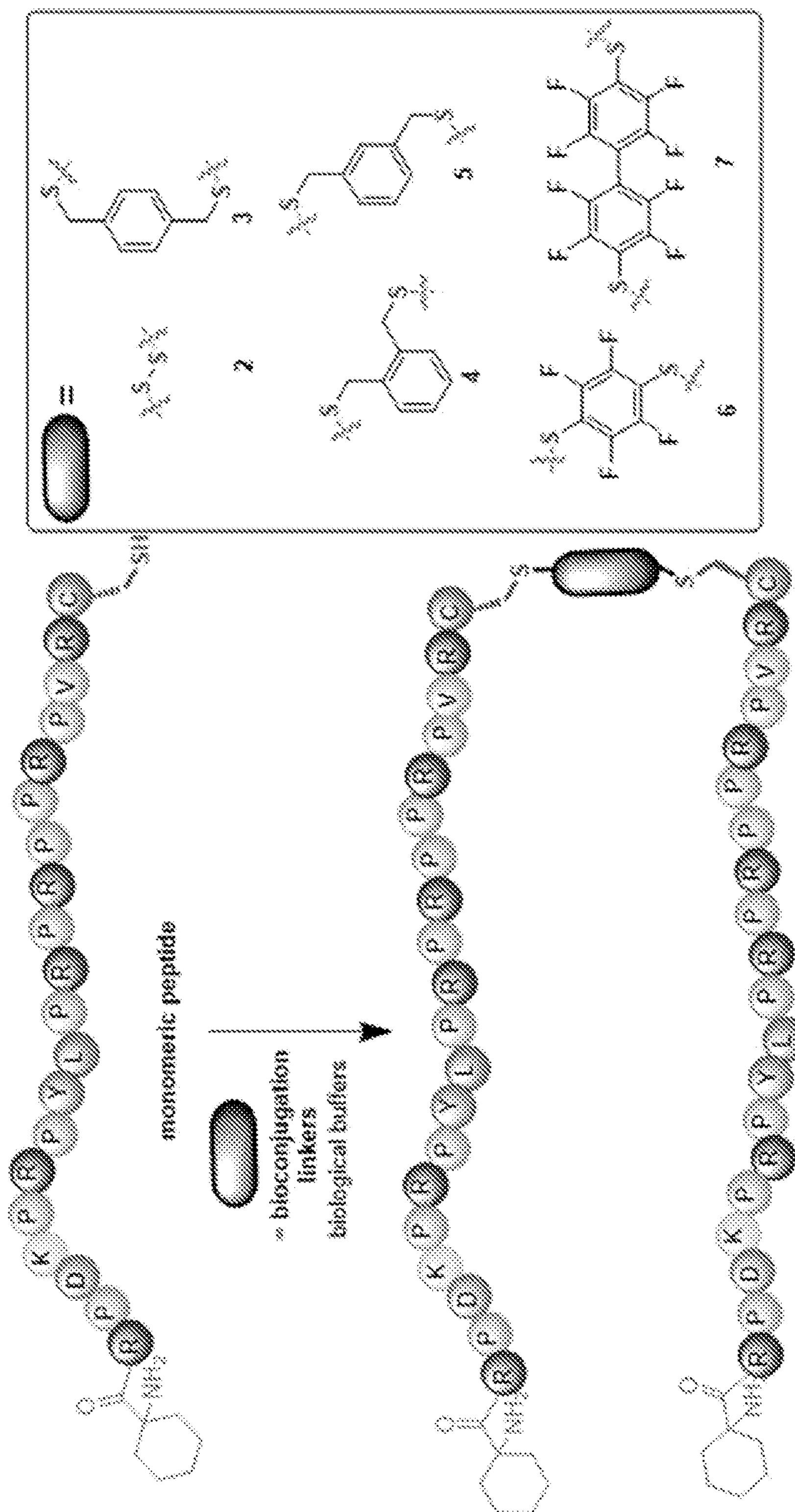


FIGURE 1

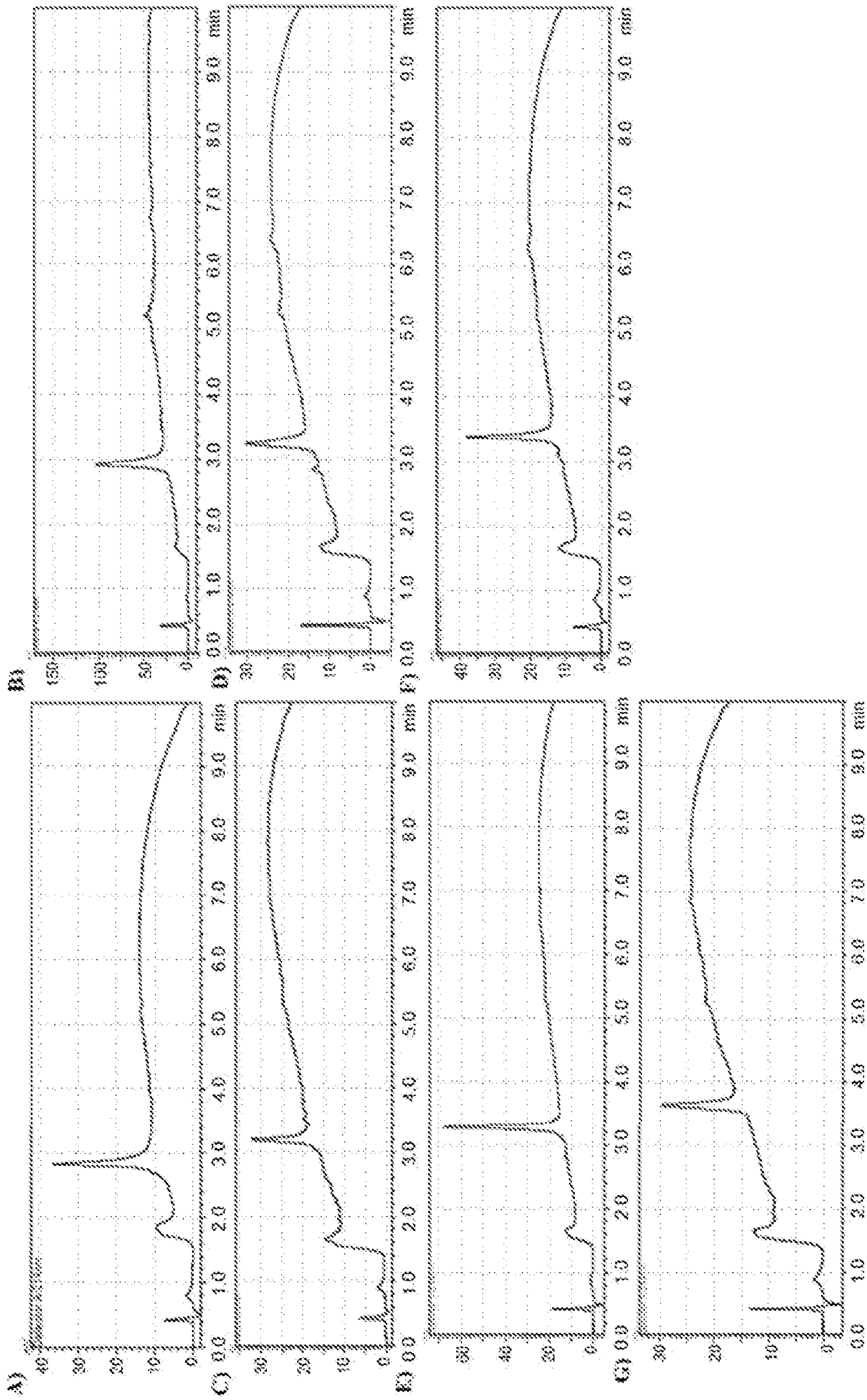


FIGURE 2

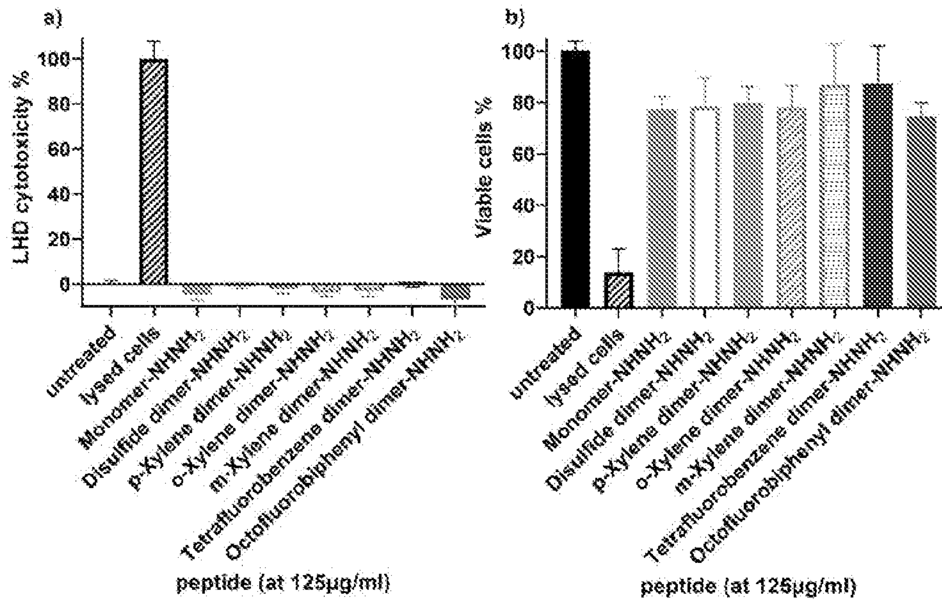
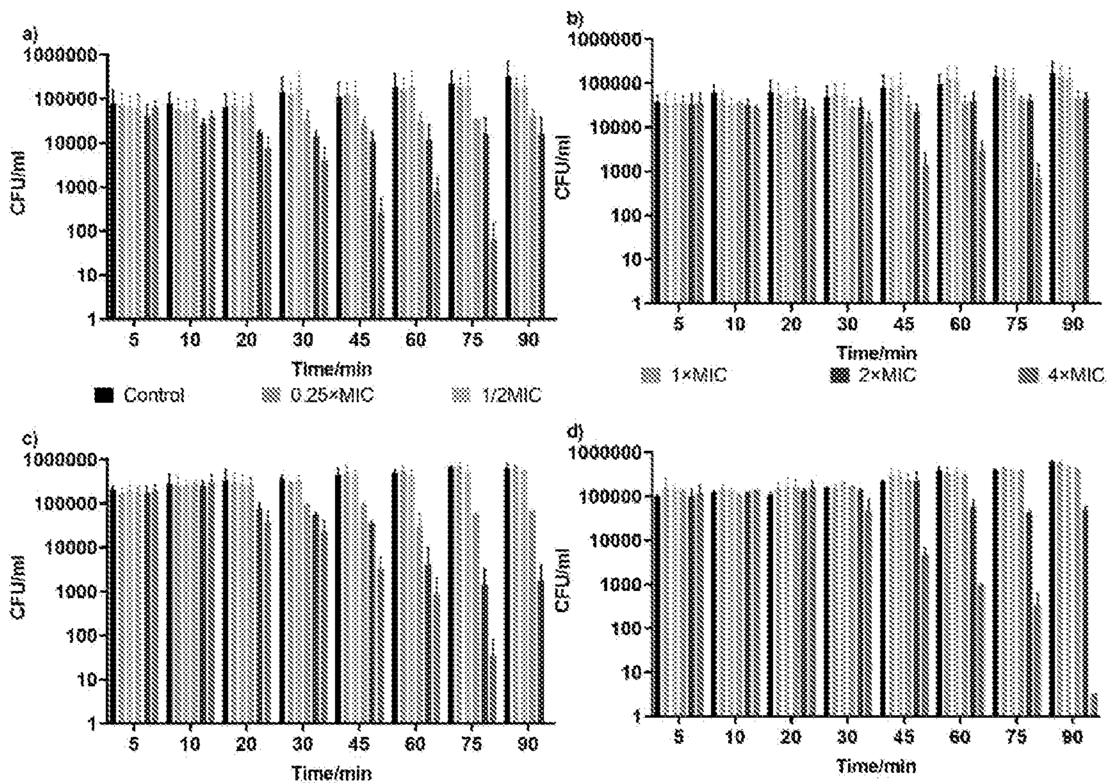


FIGURE 3



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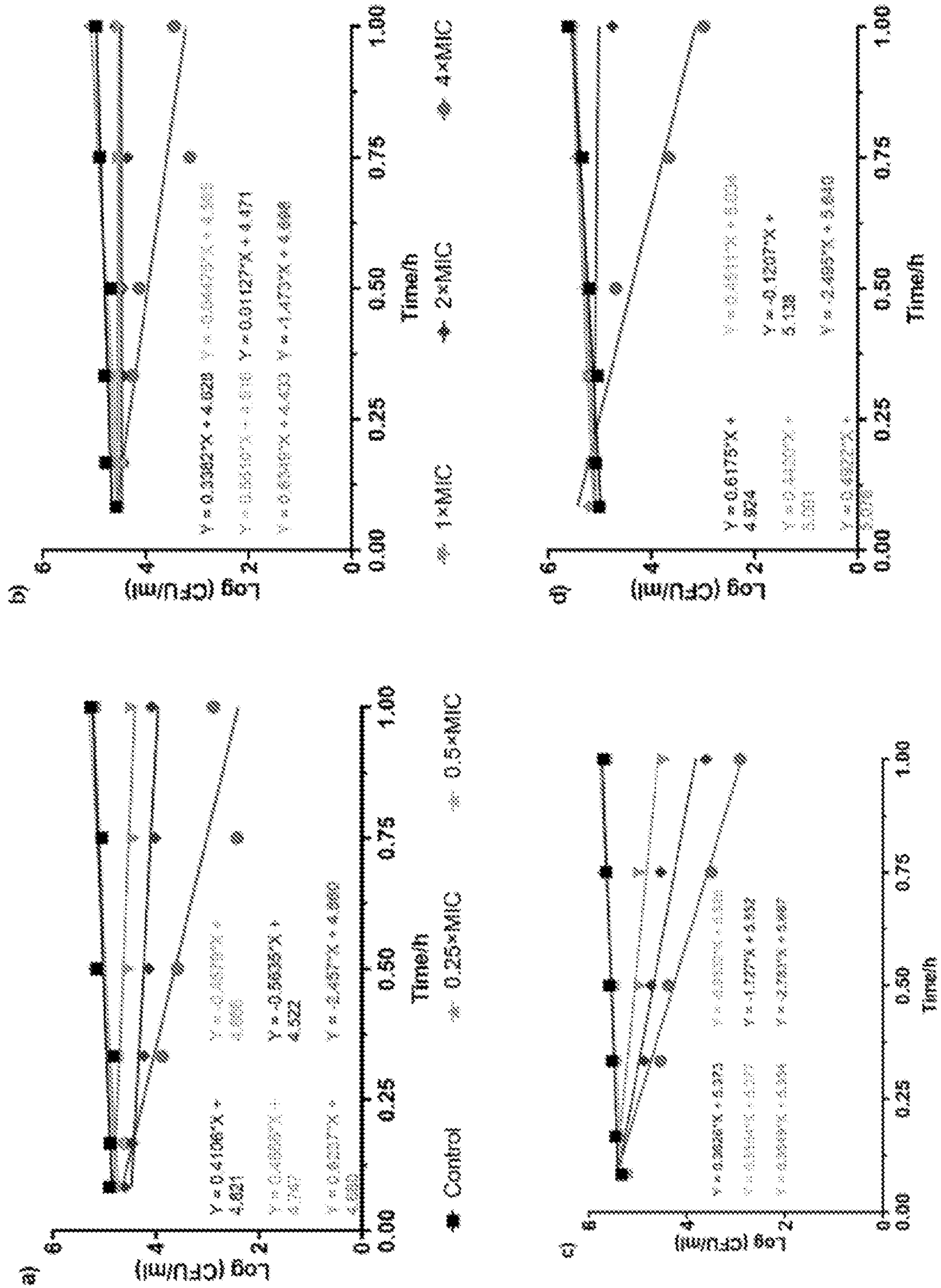

FIGURE 5

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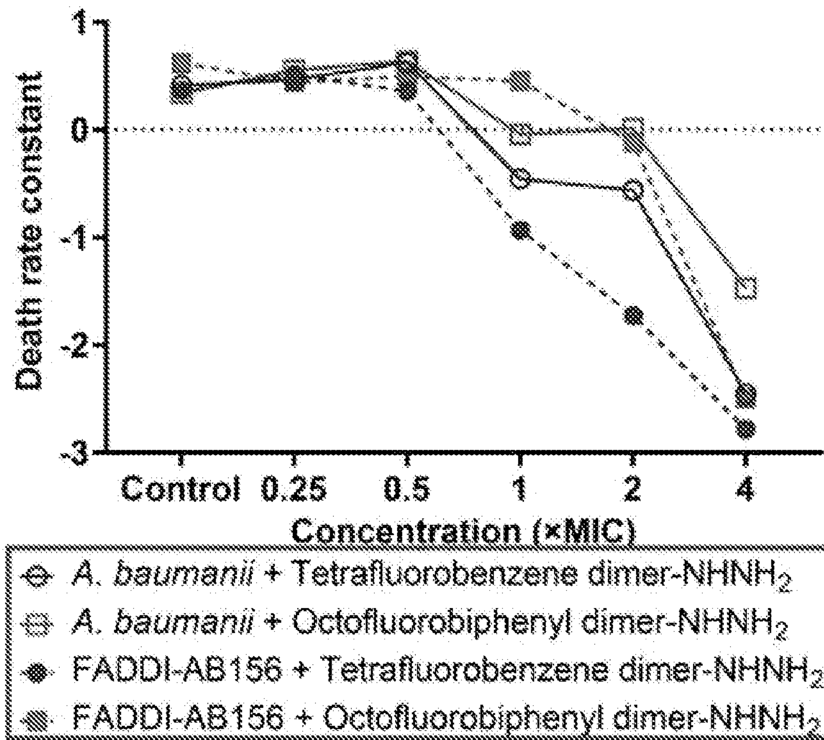
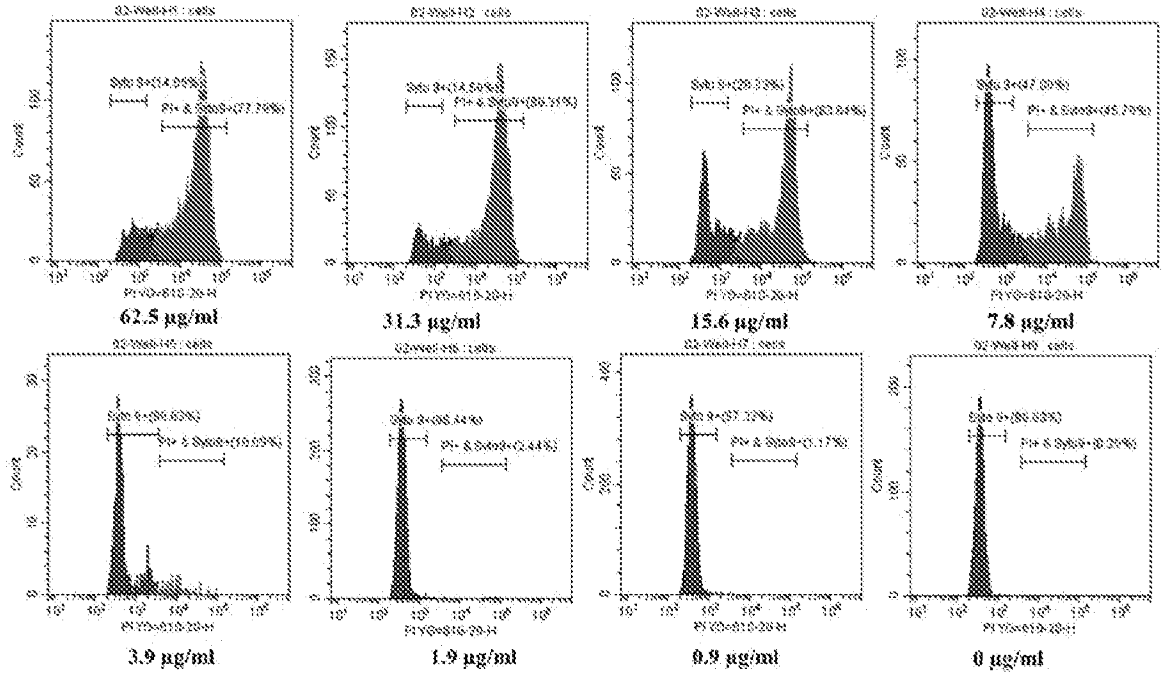


FIGURE 6

Tetrafluorobenzene dimer-hydrazide against A. baumannii



Octofluorobiphenyl dimer-hydrazide against A. baumannii

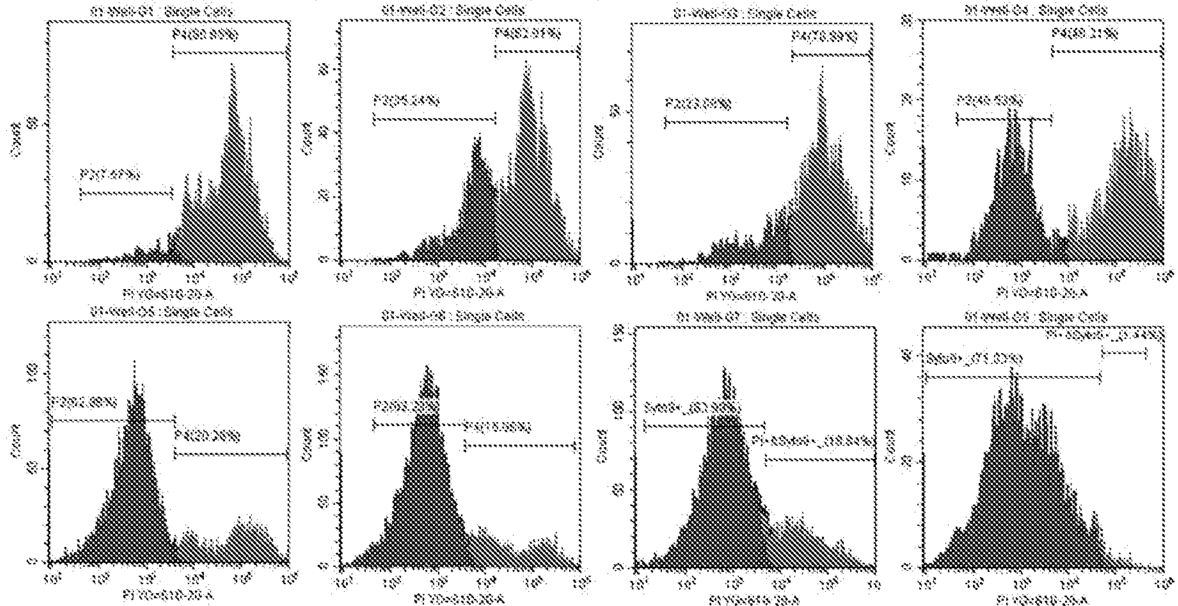


FIGURE 7

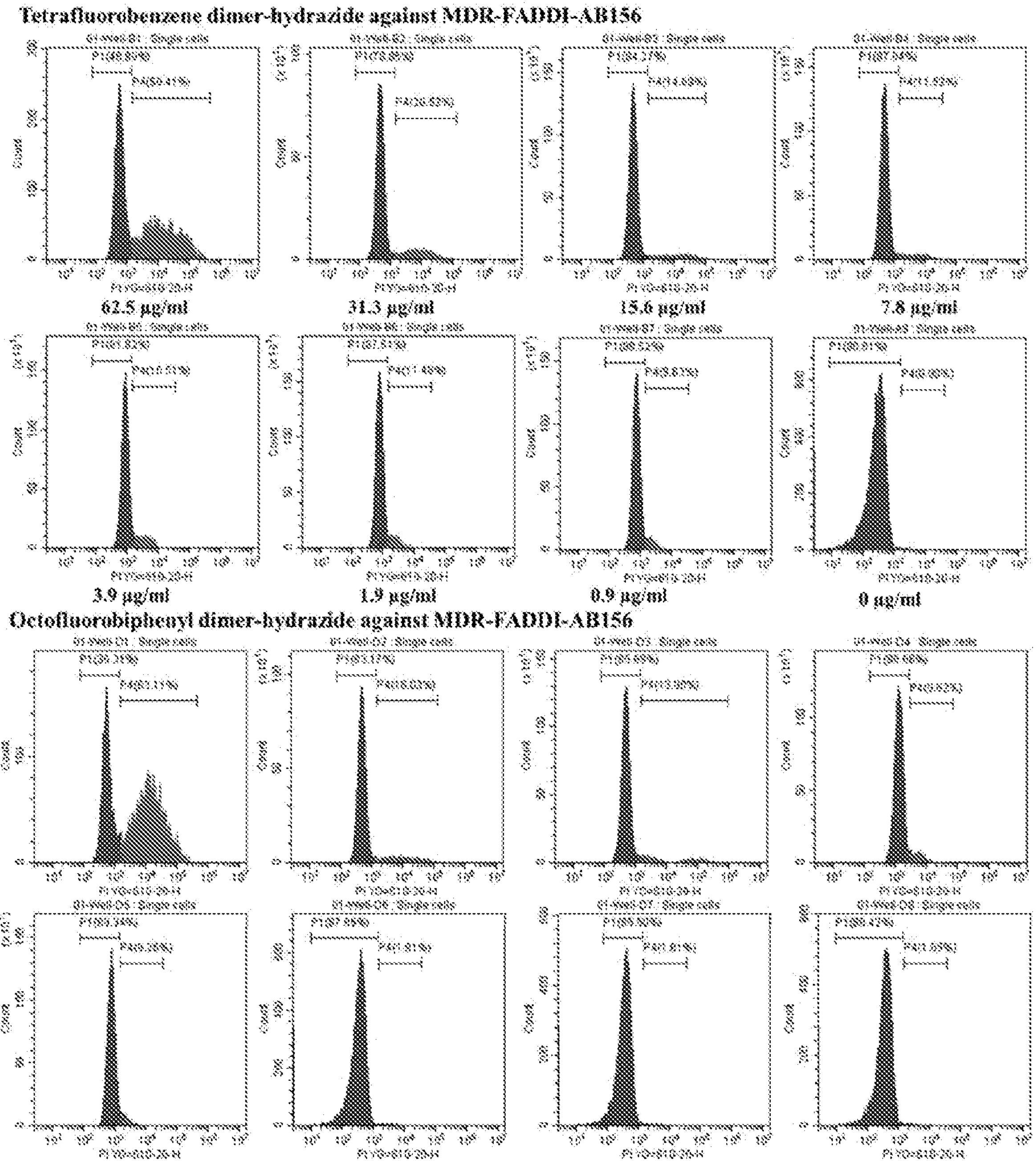


FIGURE 8

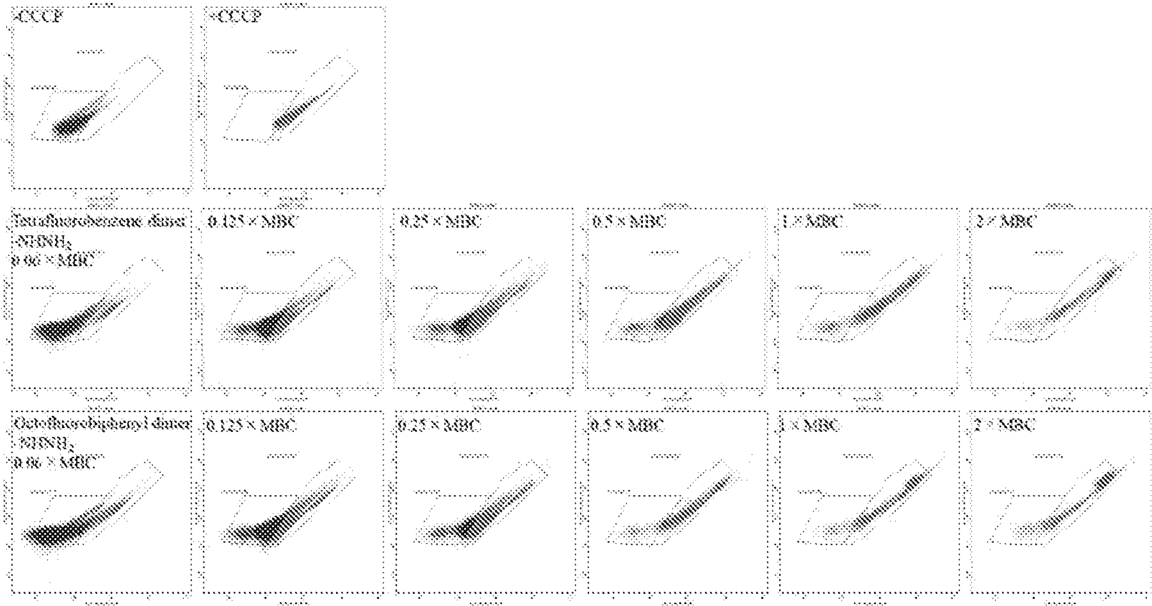


FIGURE 9

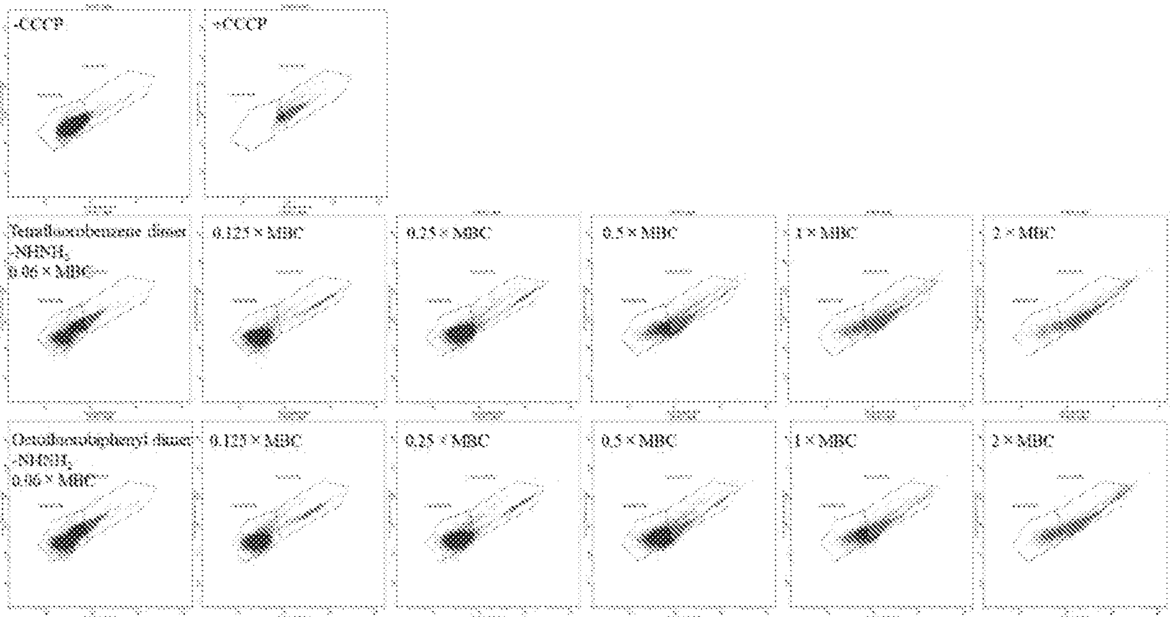


FIGURE 10

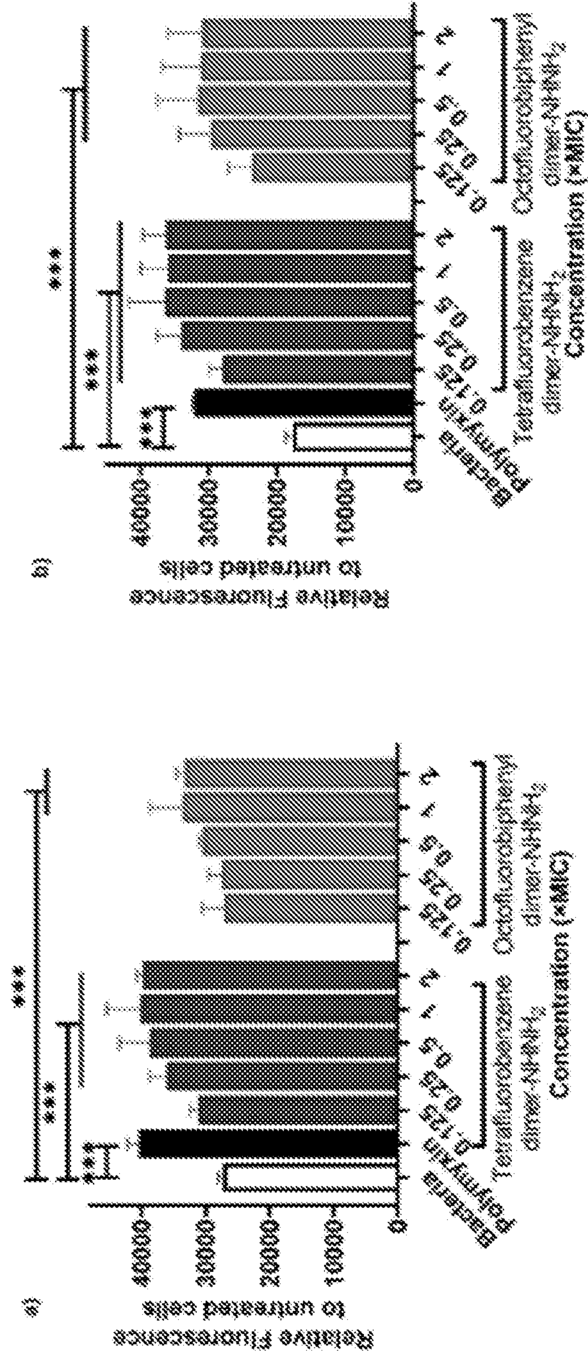


FIGURE 11

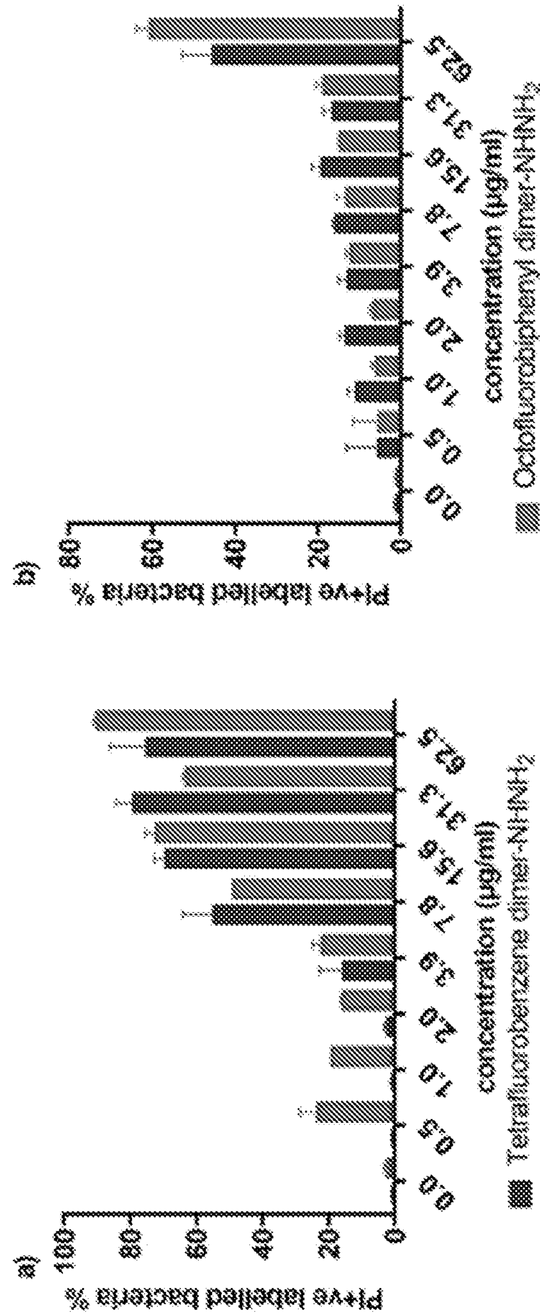


FIGURE 12

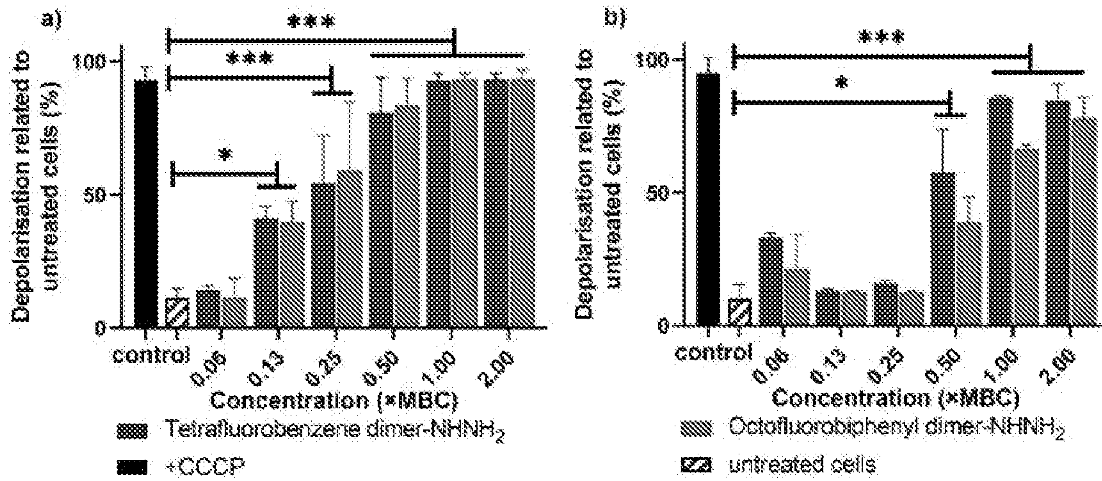


FIGURE 13

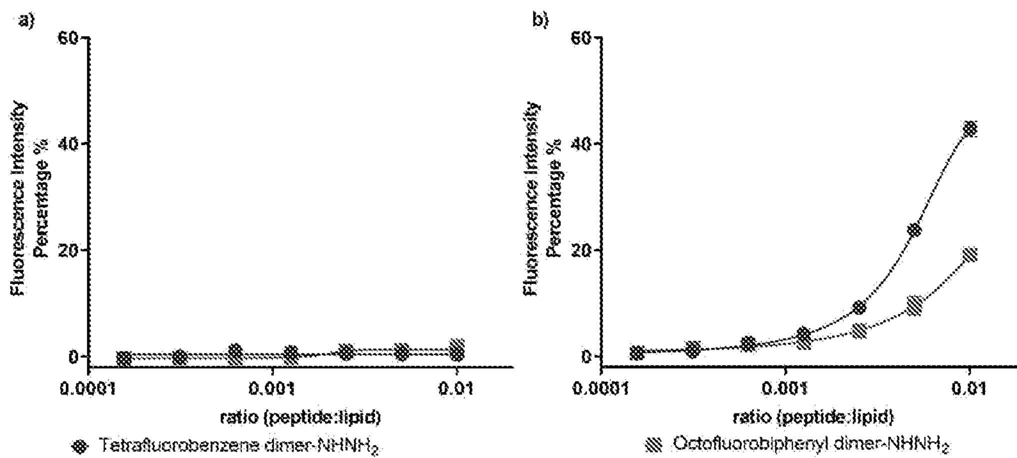


FIGURE 14

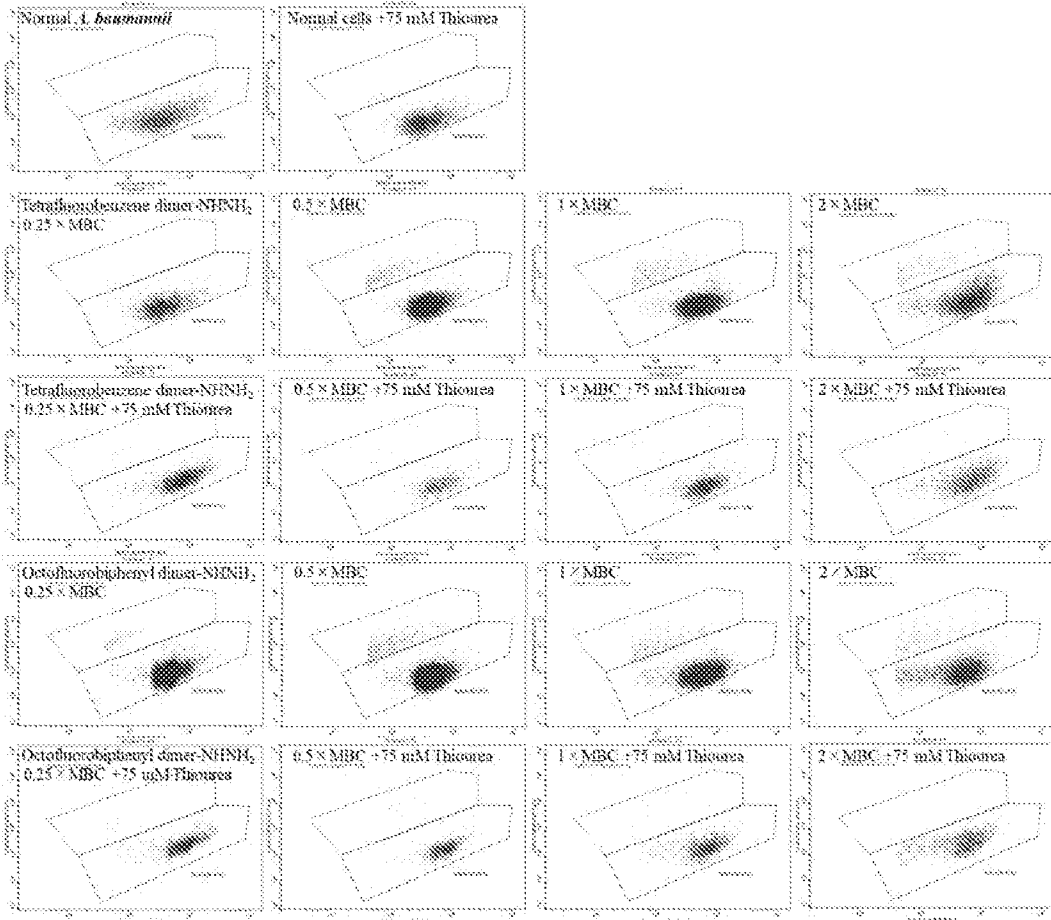


FIGURE 15

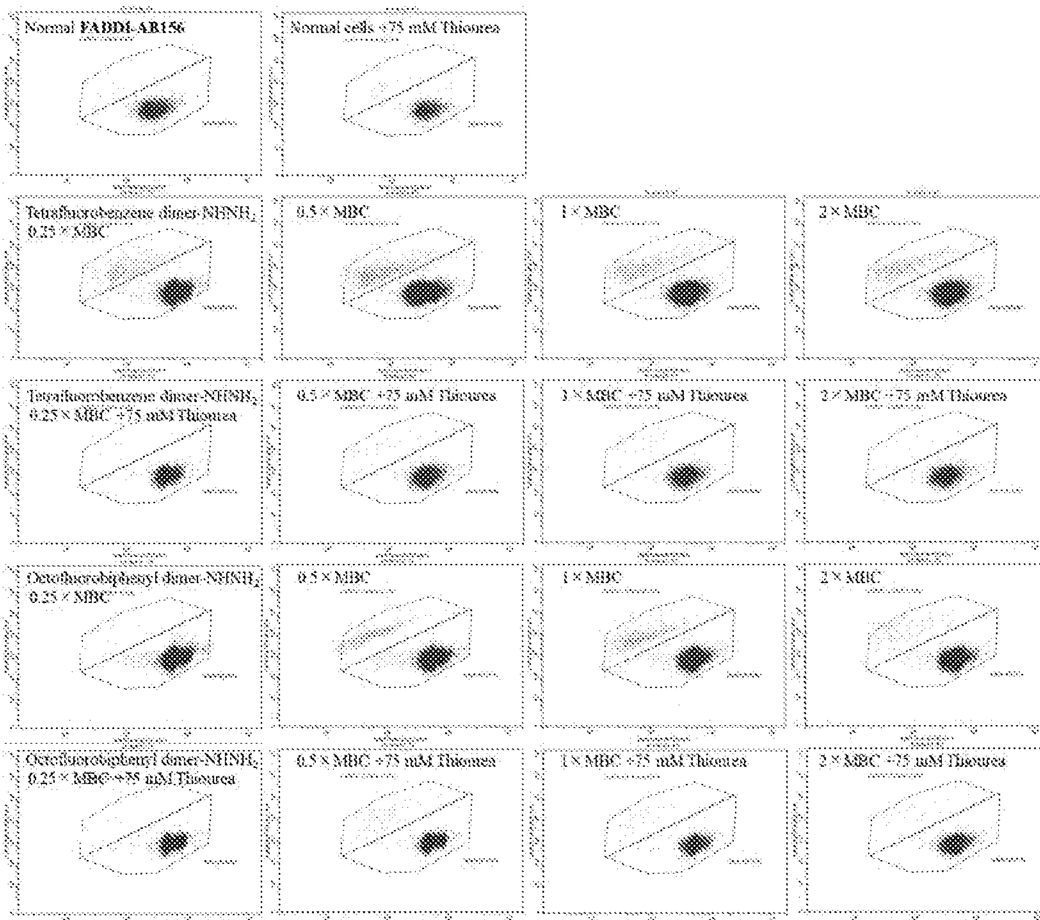


FIGURE 16

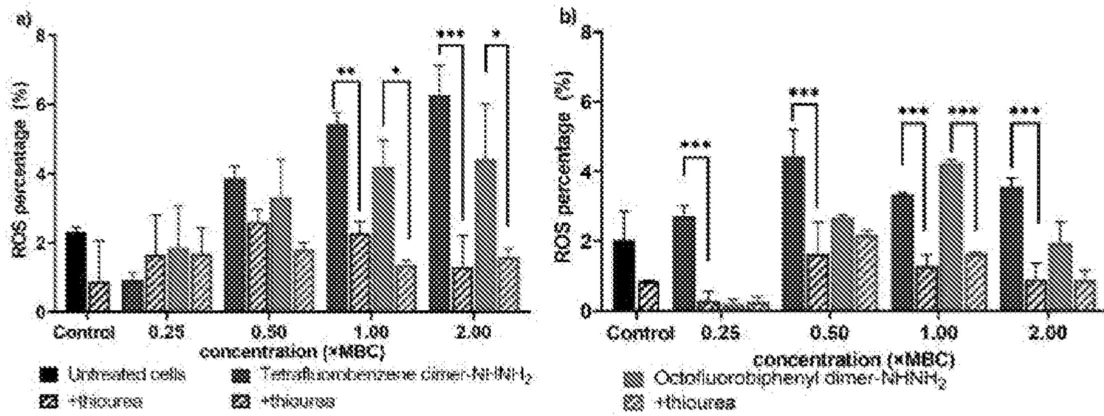


FIGURE 17

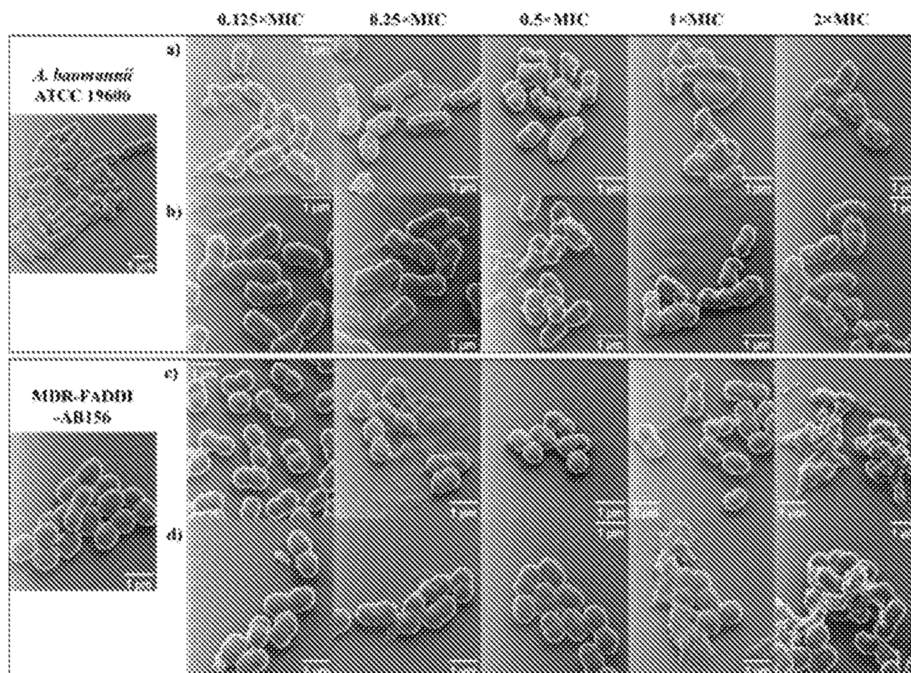


FIGURE 18

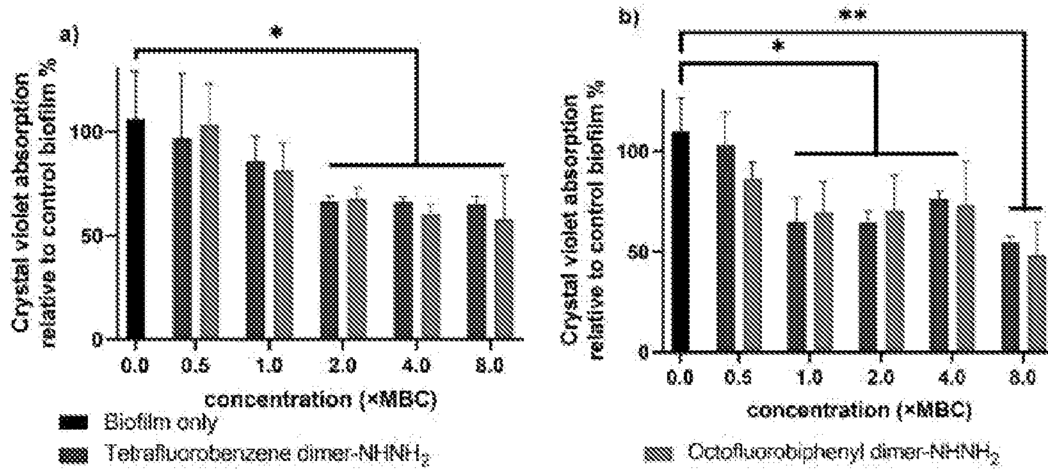


FIGURE 19

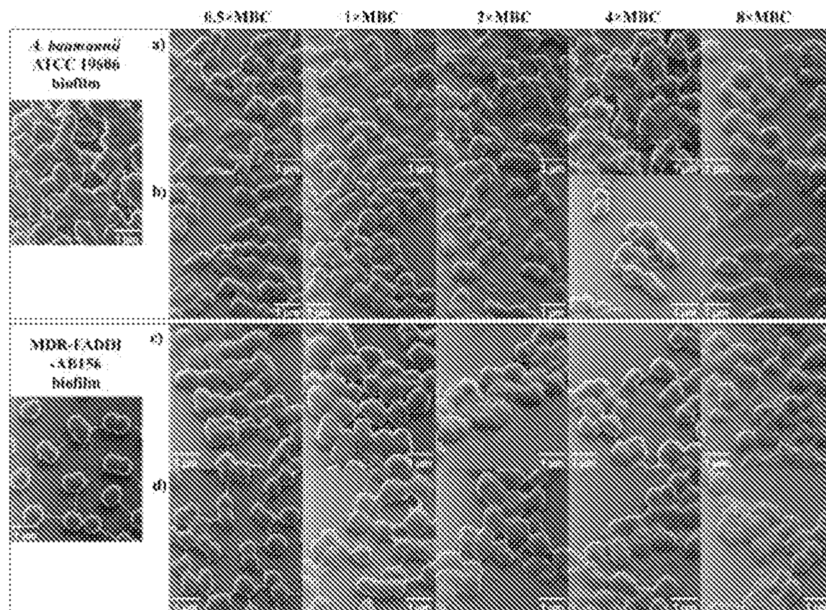


FIGURE 20

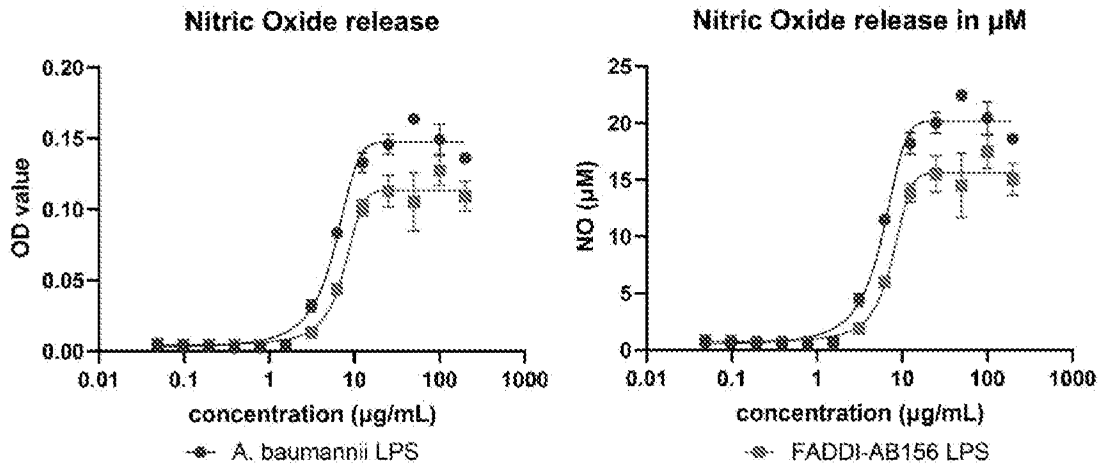


FIGURE 21

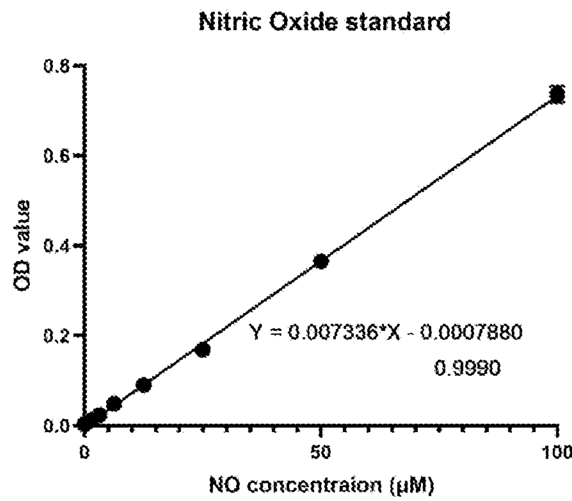


FIGURE 22

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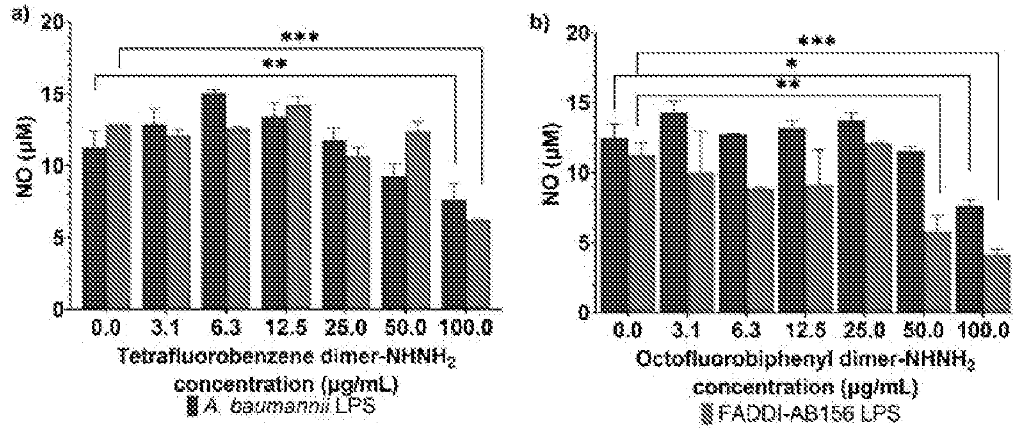

FIGURE 23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2023/050051

A. CLASSIFICATION OF SUBJECT MATTER

C07K 7/08 (2006.01) A61K 38/00 (2006.01) A61K 38/10 (2006.01) A61P 31/04 (2006.01) C07K 14/435 (2006.01)

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)

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)

STN Registry Caplus: Substructure search based on formula (I) and subsequence search PDKPRPYLPRPRPPRPVR OR VDKPPYLPRPRPPRRIYNR OR ONNRPVYIPRPRPPHPRL OR GKPRPYSPRPTSHPRPIRV OR RRIRPRPPRLPRPRPRPLPFPRPGPRPIPRPLPFP)/SQSP & Applicants and Inventor search in Internal databases provided by IP Australia and external databases: THE NEAR FLOREY NEAR INSTITUTE NEAR OF NEAR NEUROSCIENCE NEAR2 MENTAL NEAR HEALTH) OR (THE NEAR UNIVERSITY NEAR OF NEAR MELBOURNE)) AND inn=(WADE NEAR John NEAR Desmond).

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

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- "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	Date of mailing of the international search report
9 March 2023	09 March 2023

Name and mailing address of the ISA/AU

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Authorised officer

Ricky Fung
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No. +61 2 6222 3648

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ramesh, S., et al.,"6-(Bromomaleimido) hexanoic acid as a connector for the construction of multiple branched peptide platforms." Organic letters, 2015, 17(3), pp.464-467. see compound in Figure 1A on page 465 and introduction first paragraph on page 464)	1, 9, 11, 12, 17, 24, 25
A	CN 113637048 A (TECHNICAL INSITUTE OF PHYSICS AND CHEMISTRY OF CAS) 12 November 2021 See compound IB-5 in paragraph [0075] on page 10 and abstract	1-43
A	Frost, J.R., et al., "Proline-to-cysteine cyclization for generating conformationally constrained cyclic peptides." Peptide Science, 2020, 112(4), p.e24160. CAS RN 2416654-44-7	1-43
A	US 20080216256 A1 (FREEMAN ET AL) 11 September 2008 See reactive dye compounds in paragrapha [0060] on page 4 (CAS RN 1055320-18-7 and 1055320-18-7 STN Entry Dates 30 Sep 2008)	1-43
A	WO 2019/057964 A1 (HEIDELBERG PHARMA RESEARCH GMBH) 28 March 2019 See compound HDP 30.2448 [CAS RN 2304957-28-4 STN Entry: 19 Apr 2019] on page 129 and paragraph [00133]	1-43

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2023/050051**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
CN 113637048 A	12 November 2021	CN 113637048 A	12 Nov 2021
US 20080216256 A1	11 September 2008	US 2008216256 A1	11 Sep 2008
WO 2019/057964 A1	28 March 2019	WO 2019057964 A1	28 Mar 2019
		AU 2018335378 A1	09 Apr 2020
		BR 112020005605 A2	13 Oct 2020
		CA 3076289 A1	28 Mar 2019
		CN 111432844 A	17 Jul 2020
		EP 3684416 A1	29 Jul 2020
		JP 2020534319 A	26 Nov 2020
		KR 20200056403 A	22 May 2020
		US 2020345807 A1	05 Nov 2020
		US 11583569 B2	21 Feb 2023

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.