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(54) Title: TREATMENT OF STAPHYLOCOCCAL AND ENTEROCOCCAL INFECTIONS USING SUBSTITUTED NITROSTYRENE COMPOUNDS

(57) Abstract: The invention relates generally to the use of substituted nitrostyrene compounds in the treatment of *Staphylococcus aureus* and *Enterococcal* infections. In particular aspects the present invention relates to the use of these compounds in the treatment *S. aureus* infections where the bacteria have a level of vancomycin resistance.

TREATMENT OF STAPHYLOCOCCAL AND ENTEROCOCCAL INFECTIONS USING SUBSTITUTED NITROSTYRENE COMPOUNDS

FILING DATA

[0001] This application is associated with and claims priority from Australian patent application no. 2017903033 filed on 1 August 2017, the entire contents of which is incorporated herein by reference.

TECHNICAL FIELD

[0002] This invention relates generally to the use of substituted nitrostyrene compounds in the treatment of *Staphylococcus aureus* and *Enterococcal* infections. In particular aspects the present invention relates to the use of these compounds in the treatment *S. aureus* infections where the bacteria have a level of vancomycin resistance.

BACKGROUND

[0003] Antibiotics are the most widely used agents in the fight against pathogenic microorganisms. The antibiotic vancomycin is the leading member of the class of clinically important glycopeptide antibiotics used in the fight against life-threating and drug-resistant Gram-positive bacterial infections. In use since the 1950's, vancomycin is on the World Health Organisation's List of Essential Medicines (2013), a list of the most important medications needed in a basic health system.

[0004] However, since 1980 there has been an ever increasing proportion of clinical bacterial isolates resistant to antibiotics. The steady increase of antimicrobial resistance is an evolutionary response to wide-spread antibiotic use. Antibiotic-resistant Gram-positive bacterial pathogens, including vancomycin-resistant enterococci (VRE), *Clostridium difficile* infection (CU) and Methicillin-resistant *S. aureus* (MRSA), have become serious concerns for global public health. In particular, increasing incidences of MRSA and VRE are seen.

[0005] The increasing emergence of vancomycin-resistant enterococci resulted in the development of guidelines for use of vancomycin, by the Centres for Disease Control's

Hospital Infection Control Practices Advisory Committee (HICPAC). The National Institute of Allergy and Infectious Diseases (NIAID's) priority pathogens list includes *Enterococcus faecium, Enterococcus faecalis* (added 2014) and *Staphylococcus aureus* as "additional emerging infectious diseases/pathogens".

[0006] MRSA is particularly problematic clinically as it is multi-drug resistant and refractory to many common clinic antibiotics. MRSA is considered to be any strain of *Staphylococcus aureus* that has developed, through the process of natural selection, resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) and the cephalosporins. The evolution of such resistance does not cause the organism to be more intrinsically virulent than strains of *S. aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous.

[0007] Vancomycin, formerly a gold standard treatment for MRSA, has become increasingly ineffective because of the emergence of vancomycin-resistant and vancomycin-intermediate *Staphylococcus aureus* (VRSA and VISA). An MRSA isolate with decreased susceptibility to vancomycin was first reported in Japan in 1997 (Hiramatsu K. *et al* (1997)). The isolate had only a modestly increased minimum inhibitory concentration (MIC) value for vancomycin, in the range of 3-8 mcg/ml, and became known as vancomycin intermediate-resistant *S. aureus* (VISA). VISA began to be reported with increasing frequency among MRSA isolates identified all over the world. In spite of their moderate increase in MIC value, vancomycin treatment of infections by VISA isolates often ended in treatment failure (Linares J. (2001); Fridkin SK, *et al.* (2003)). In 2002, the first vancomycin-resistant *S. aureus* (VRSA) strain (with a vancomycin MIC value greater than 100 μg/ml) was reported in the United States (Sievert D.M. *et al* (2008)).

[0008] Heteroresistant subpopulations of vancomycin-susceptible *S. aureus* (hVISA) were first described in 1997 (Hiramatsu K. *et al* (1997)), shortly after the initial description of vancomycin intermediate susceptible strains (VISA). hVISA have minimum inhibitory concentrations (MICs) in the intermediately susceptible range and are considered to likely represent a step on the path to the development of a fully VISA population. While high-

level vancomycin resistance has been rarely reported, VISA and hVISA are more common and are associated with clinical failure. Clearly the use of vancomycin therapy against such isolates would be impossible. As a consequence clinicians are continually being challenged by infections caused by *S. aureus* and the treatment of suspected *S. aureus* and other vancomycin-resistant bacterial infections is becoming increasingly more complicated.

[0009] As the prevalence of vancomycin-resistant bacterial strains increases, new antibiotics with the longevity and dependability of vancomycin will be required to contain their impact. This need is arising at the same time that antibiotic discovery efforts are being discontinued at most major pharmaceutical companies. The reasons for this decline in antibiotic development are largely economic, resulting from a combination of patient short term use, the restricted use of new antibiotics with activity against resistant bacteria, and the increased regulatory criteria for approval.

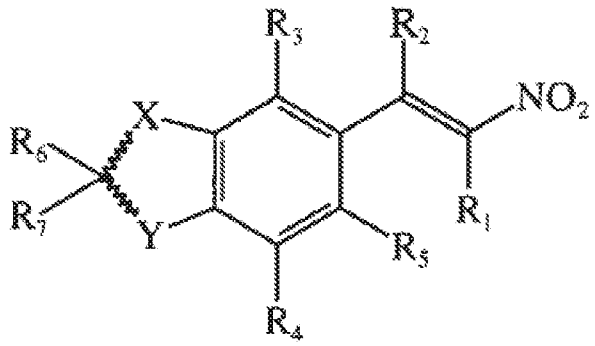
[0010] Protection from infectious disease therefore relies not only on the effective management of current antimicrobial agents, but also on the development of new classes of compounds, and the identification of new microbial targets in order to address the serious problems caused by the emergence of drug-resistant human and animal pathogens (Boucher H.W. (2010)). Unfortunately, developmental drugs have a low probability of reaching and completing clinical development (Payne, D.J. *et al* (2007) so the need to identify new targets and agents remains perpetually urgent. In particular, there is an unmet need for effective treatment and/or prevention of antibiotic resistant *S. aureus, E. faecalis and E. faecium* associated infections, including those caused by MRSA, VRE, VISA and VRSA.

[0011] The antimicrobial properties of several benzyl nitroethenes have been reported (Nicoletti G. *et al* (2013), Schales, O. and Graefe, H.A. (1952), Worthen, L.R. and Bond, H.W. (1970), Milhazes, N. *et al* (2006)). Nicoletti *et al.* have investigated the structure-activity relationships (SAR) of 23 benzyl nitroalkenes against a panel of clinically significant bacterial (Vincent, C. *et al* (2000)) and fungal species (Worthen, L.R. and Bond, H.W. (1970). The SAR study showed the importance of the nitroethenyl and nitropropenyl side chain to anti-microbial activity, proposed by Park and Pei to be essential

for inhibition of protein tyrosine phosphatases (PTP), with the nitropropenyl substituent being the most active (Park, J. and Pei, D. (2004)). The 23 compounds showed broad antimicrobial activity that differed across species, with greatest activity against Gram-positive bacteria and fungi and least against enteric Gram-negative rods. One of the most active compounds was a substituted nitrostyrene compound and a tyrosine mimetic, 3,4-methylenedioxy-β-methyl-β-nitrostyrene. It is broadly microbicidal to many Gram-negative and most Gram-positive bacterial species as well as many fungal species, and is active against strict anaerobes and intracellular pathogens including *Mycobacterium tuberculosis* and *Plasmodium falciparum* (Nicoletti G. *et al* (2013), Denisenko, P.P. (2002), White, K.S. (2008)). It has low oral toxicity for mice and chickens and is poorly absorbed from the intestinal tract (Nicoletti G. *et al* (2013), Nicoletti A. *et al* (2004).

SUMMARY OF THE INVENTION

[0012] In a first aspect the present invention provides a method of treating *S. aureus* infection in a patient wherein the *S. aureus* has at least partial resistance to vancomycin, the method comprising administering to the patent an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof:



(I)

in which

X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

 is a double or single bond depending on the heteroatoms X and Y;

R_1 to R_5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R_6 and R_7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R_6 and R_7 are absent when there is a double bond present.

[0013] In an embodiment, in the compound of formula I, X and Y are either the same or different and selected from O and N, more preferably both X and Y are oxygen.

[0014] In an embodiment, in the compound of formula I R_1 and R_2 are either the same or different and selected from hydrogen, hydroxy, halogen or optionally substituted C_{1-6} alkyl.

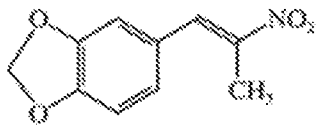
[0015] In the compound of formula I R_3 to R_5 are preferably either the same or different and selected from hydrogen, hydroxy, halogen, nitro, C_{1-6} alkoxy or optionally substituted C_{1-6} alkyl. Preferably halogen is chlorine or bromine.

[0016] The E isomer of the compounds of formula I is preferred.

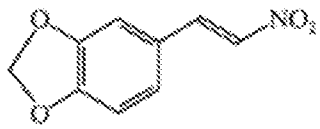
[0017] In an embodiment, are compounds of the formula I in which X, Y, , R_6 and R_7 are as defined above; R_1 and R_2 are either the same or different and selected from hydrogen, hydroxy, Cl, Br and C_{1-4} alkyl; and R_3 to R_5 are either the same or different and selected from hydrogen, hydroxy, Cl, Br, nitro, C_{1-4} alkoxy or C_{1-4} alkyl.

[0018] Specific examples of the compounds of the compounds of formula I are as follows:

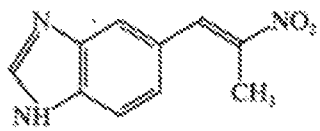
X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)



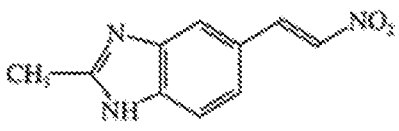
X and Y are O and R_1 to R_3 are hydrogen (3,4-methylenedioxy-β-nitrostyrene)



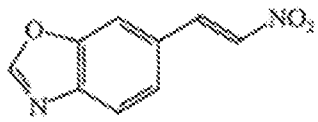
X is N, Y is NH, R_1 is methyl and R_2 and R_3 are hydrogen (benzimidazole-5-β-nitropropylene)



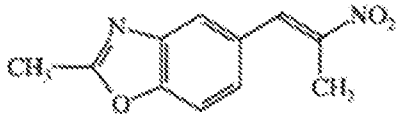
X is N, Y is NH, R_1 is hydrogen, R_2 is methyl and R_3 is absent (2-methyl benzimidazole-5-β-nitroethylene)



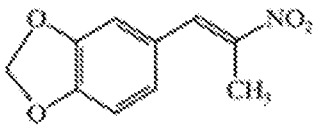
X is O, Y is N, R_1 and R_2 are hydrogen and R_3 is absent (benzoxazole-5-β-nitroethylene)



X is N, Y is O, R_1 and R_2 are methyl and R_3 is absent (2-methyl benzoxazole-5-β-nitropropylene)



[0019] In a preferred embodiment the compound is X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)



[0020] In a second aspect the present invention provides a method of treating *S aureus* infection in a patient wherein the infection has failed to resolve following vancomycin treatment, the method comprising administering to the patient a compound of formula (I) or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0021] In particular embodiments the compound is BDM-I.

[0022] In a third aspect the present invention provides a method of treating *Enterococcal* infection in a patient, the method comprising administering to the patient vancomycin or a derivative thereof and a compound of formula (I) or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0023] In particular embodiments the *Enteroccus* is *vanB* VRE and the compound of Formula I is BDM-I. In certain embodiments the *Enterococcus* is *E. faecalis* or *E. faecium.*

[0024] In a further aspect the present invention provides a composition comprising a combination of vancomycin or a derivative thereof and a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0025] In an embodiment, the composition is a pharmaceutical composition comprising one or more pharmaceutically acceptable carriers, diluents and/or excipients.

[0026] In embodiments, the *S. aureus* is MRSA, VRSA, VISA or hVISA.

[0027] In an embodiment, the compound of formula I or a pharmaceutically acceptable salt or a derivative thereof defined above and vancomycin or a derivative thereof are present in the composition in amounts which together are sufficient to treat a disease or condition in a subject. In an embodiment, the compound of formula I or a pharmaceutically acceptable salt or a derivative thereof defined above and vancomycin or a derivative thereof are present in the composition in synergistically effective amounts. In an embodiment, the compound of formula I or a pharmaceutically acceptable salt or derivative thereof as defined above and/or vancomycin or a derivative thereof are present in the composition in an amount which is less than the minimum inhibitory concentration (MIC) of the agent when used independently.

BRIEF DESCRIPTION OF THE FIGURES

[0028] **Figure 1:** Average BDM-I MIC of triplicate Sa375 cultures serially passaged in the presence of BDM-I over a period of 110 days. Samples were collected for MIC testing when growth could (or could not) be maintained in higher BDM-I concentrations. Error bars denote standard deviation.

[0029] **Figure 2.** BDM-I and vancomycin checkerboard assays (via broth micro dilution method) for *vanB* VRE clinical isolates. Individual MICs are indicated in red and numbers in grey boxes represent FICi values for different BDM-I and vancomycin combinations. X denotes growth and each checkerboard assay was performed in triplicate (identical result each time). Note that *Enterococcus faecium* isolates are considered vancomycin sensitive if the MIC is ≤ 4 mcg/ml.

DETAILED DESCRIPTION

[0030] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which the present disclosure belongs. Any materials and methods similar or equivalent to those described herein can be used to practice the present invention.

[0031] Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

[0032] 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 in the disclosure 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.

[0033] As used herein the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a bacteria" includes a single bacteria, as well as two or more bacterium; reference to "a organism" includes one organism, as well as two or more organisms; and so forth.

[0034] The present disclosure is predicated in part, on the inventors' findings that there is an inverse relationship between MICs for BDM-I and vancomycin MICs in methicillin-resistant *S. aureus*. This "see-saw" effect indicates an advantage for the use of BDM-I in the treatment MRSA infections where there is at least a level of vancomycin

resistance. The present inventors have also noted a synergy between BDM-I and vancomycin against *Enterococcus*.

[0035] In a first aspect the present invention provides a method of treating *S. aureus* infection in a patient wherein the *S. aureus* has at least partial resistance to vancomycin, the method comprising administering to the patent an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

[0036] In a second aspect the present invention provides a method of treating *S aureus* infection in a patient wherein the infection has failed to resolve following vancomycin treatment, the method comprising administering to the patient a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof as defined herein.

[0037] In a third aspect the present invention provides a method of treating *Enterococcal* infection in a patient, the method comprising administering to the patient vancomycin or a derivative thereof and a compound of formula (I) or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0038] In particular embodiments the *Enteroccus* is *vanB* VRE and the compound of Formula I is BDM-I. In certain embodiments the *Enterococcus* is *E. faecalis* or *E. faecium*.

[0039] In a fourth aspect the present invention provides a composition comprising a combination of vancomycin or a derivative thereof and a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0040] In a fifth aspect the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *S. aureus* infection in a patient wherein the *S. aureus* has at least partial resistance to vancomycin.

[0041] In a sixth aspect the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *S aureus* infection in a patient wherein the infection has failed to resolve following vancomycin treatment.

[0042] In a seventh aspect the present invention provides the present invention provides the use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *Enterococcal* infection in a patient, wherein the medicament is administered in combination with vancomycin or a derivative thereof.

[0043] In particular embodiments the compound is BDM-I.

[0044] In compound of formula (I) the term "heteroatom" denotes O, N or S.

[0045] The term "halogen" refers to fluorine, chlorine, bromine and iodine, preferably chlorine and bromine.

[0046] The term "alkoxy" is used herein in its broadest sense and refers to straight chain, branched chain or cyclic oxy-containing radicals each having alkyl portions, preferably C_{1-6} alkyl, more preferably C_{1-4} alkyl. Examples of such alkoxy groups are methoxy, ethoxy, propoxy, butoxy and t-butoxy.

[0047] The terms "C_{1-4} alkyl" or "C_{1-6} alkyl" refer to straight chain, branched chain or cyclic hydrocarbon groups having from 1 to 6 carbon atoms. Illustrative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, neopentyl, hexyl, cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

[0048] The salts of the compound of formula I are preferably pharmaceutically acceptable, but it will be appreciated that non-pharmaceutically acceptable salts also fall within the scope of the present invention, since these are useful as intermediates in the preparation of pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include salts of pharmaceutically acceptable cations such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium; acid addition salts of pharmaceutically acceptable inorganic acids such as hydrochloric, orthophosphoric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic and hydrobromic acids; or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, trihalomethanesulphonic,

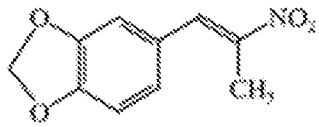
toluenesulphonic, benzenesulphonic, salicylic, sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

[0049] As used herein, the term "pharmaceutically acceptable salt" refers to a compound formulated from a base compound which achieves substantially the same pharmaceutical effect as the base compound.

[0050] In addition, some of the compounds of formula (I) as defined herein may form solvates with water or common organic solvents. Such solvates are encompassed within the scope of the invention.

[0051] The term "derivatives" in relation to the compound of formula I includes but is not limited to ether derivatives, acid derivatives, amide derivatives, ester derivatives and the like. In addition, this invention further includes methods utilizing hydrates of the compound. The term "hydrate" includes but is not limited to hemihydrate, monohydrate, dihydrate, trihydrate and the like.

[0052] In an embodiment, in the compound of formula I, X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) and the compound has the following structure:



BDM-I

[0053] Vancomycin is well known in the art as an antibiotic used to prevent or inhibit the growth of Gram-positive bacteria. In particular vancomycin is used to treat or prevent bacterial infections caused by Gram-positive bacteria.

[0054] The term "vancomycin derivative" as used herein refers to a compound having a structure derived from vancomycin, which exhibits the same or substantially similar biological activity and physicochemical properties as vancomycin. Examples include, but are not limited to, salts, esters, amides, salts of esters or amides, and N-oxides of vancomycin. In the context of the disclosures herein, reference to the biological activity of

vancomycin is reference to its inhibitory or biocidal activity on Gram-positive bacterial growth.

[0055] The present inventors have found that an increasing thickness of the bacterial cell wall which accompanies an increase in vancomycin MIC leads to increasing BDM-I sensitivity. In line with this finding is a demonstration that increasing BDM-I MIC result in decreasing cell wall thickness which correlates with decreasing vancomycin MIC. The present have also noted a synergy between BDM-I and vancomycin in relation to sensitivity of *vanB* vancomycin resistant *Enterococci.*

[0056] Accordingly, in a further aspect there is provided a method of increasing the biocidal activity of vancomycin or a derivative thereof against *Enterococcus,* the method comprising contacting the *Enterococcus* with the vancomycin or a derivative thereof, together with a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

[0057] In an embodiment, the vancomycin or a derivative thereof is used or administered together with a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein to inhibit or prevent antibiotic-resistant bacterial growth or treat, inhibit or prevent an antibiotic-resistant bacterial infection.

[0058] In an embodiment, the antibiotic-resistant bacteria are vancomycin-resistant bacteria.

[0059] It should be understood that reference to "vancomycin-resistant bacteria" includes vancomycin-resistant Gram-positive strains of bacteria that are both completely resistant to inhibition or prevention of growth by vancomycin or which require high levels of vancomycin to prevent or inhibit bacterial growth

[0060] As is known in the art there are three classes of vancomycin-resistant *S. aureus* that differ in vancomycin susceptibility. Vancomycin-intermediate *S. aureus* (VISA), heterogeneous vancomycin-intermediate *S. aureus* (hVISA), and high-level vancomycin-resistant S. aureus (VRSA). VISA has also been termed GISA (glycopeptide-intermediate *S. aureus*), indicating resistance to all glycopeptide antibiotics. MRSA also demonstrates both intermediate and full-resistance to vancomycin. As used herein, reference to

vancomycin-resistant *S. aureus* should be understood as reference to all strains of *S. aureus* which demonstrate a level of resistance to vancomycin, including VISA, hVISA, VRSA, GISA and MRSA.

[0061] Without limiting the present invention to any one theory or mode of application, the present inventor's have found that the combination of the compound of formula I or a pharmaceutically acceptable salt or derivative thereof and vancomycin, significantly decreased bacterial growth of *vanB* VRE in comparison to exposure of these antibiotic-resistant bacterial strains to each compound independently. Accordingly, in an embodiment disclosed herein, VRE comprises the *vanB* resistance gene.

[0062] In an embodiment, the vancomycin or a derivative thereof and a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof are in the form of a pharmaceutical composition.

[0063] Accordingly, in another aspect of the present invention, there is provided a pharmaceutical composition for use in a method of reducing bacterial growth, or for preventing, inhibiting or treating a bacterial infection in a subject, the composition comprising vancomycin or a derivative thereof and a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein, together with one or more pharmaceutically acceptable carriers, diluents or excipients.

[0064] A "pharmaceutically acceptable carrier, diluent and/or excipient" as used herein, is a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering the composition enabled herein to the subject. The carrier may be liquid or solid and is selected with the planned manner of administration in mind. Each carrier must be pharmaceutically "acceptable" in the sense of being compatible with other ingredients of the composition and non injurious to the subject.

[0065] The vancomycin or a derivative thereof and the compound of formula I or a pharmaceutically acceptable salt or derivative thereof are present in the combination or composition in synergistically effective amounts.

[0066] The expression "synergistically effective amounts" of vancomycin or a derivate thereof and the compound of formula (I) or a pharmaceutically acceptable salt or derivative

thereof, refers to an amount of each component of the combination or composition which is effective in producing more than the additive effect of each component on inhibiting or preventing bacterial growth. The person of skill in the art would be well aware of particular methods which can be used to determine the effects of two compounds in an antimicrobial assay. For example, checkerboard, fractional inhibitory concentration (FIC) and time-kill analysis methods are commonly used. Synergy can be defined in terms of the fractional inhibitory concentration (FIC) index, which is the sum of the FIC's for the individual drugs used in the combination, as described by Sande et al., p. 1080-1105 in A. Goodman et al., ed., The Pharmacological Basis of Therapeutics, MacMillan Publishing Co., Inc., New York (1980). Under a strict scientific, and preferred, definition synergy is defined by an FIC index of less than 0.5, i.e., when 50% inhibition results from a combination of one-fourth or less of the concentration of each drug required to elicit the same effect if used individually (i.e., the minimal inhibiting concentration (MIC) of each drug). An FIC index of 0.5 under this strict definition defines an additive response. Under a broader definition used for purposes herein synergistically effective amounts are defined by an FIC index of less than 1.0, i.e., when 50% inhibition results from a combination of one-half or less of the MIC of each drug. An FIC index of 1.0 under this broader definition defines an additive response. Under this test, isobolograms may be prepared from the dose response curves for various combinations of vancomycin or a derivative thereof and compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof on bacterial growth, with synergy indicated by points below the line which line connects the FIC index of 1 for vancomycin or a derivative thereof with the FIC index of 1 for compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof. This standard allows one to determine the MIC's for the combinations tested, so as to provide the MIC of each component needed to achieve a synergistic mixture. The exact amounts will depend, for example, on the particular Gram-positive bacterial strain and the structure of the compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof or vancomycin or derivative thereof employed.

[0067] The practitioner skilled in the art will recognize that actual dosages may vary within a relatively wide range because of inherent limitations in the above calculations and

will be able, according to available skill in the art, to determine more precisely those amounts of the components which will be effective in producing synergistic results.

[0068] Reference to "synergy" as defined herein should also be understood as reference to the anti-bacterial activity of the composition comprising a combination of vancomycin or a derivative thereof and the compound of formula I or a pharmaceutically acceptable salt or a derivative thereof. Without limiting the present invention to any one theory or mode of action, the synergistic anti-bacterial activity includes an enhancement or potentiation of the anti-bacterial activity of vancomycin. The synergistic activity also includes a reduction in the dosage or MIC of each agent required to inhibit, reduce or prevent Gram-positive bacterial growth.

[0069] Suitable methods for determining the MIC of a particular antimicrobial agent would be well known to persons skilled in the art. In an illustrative example, the MIC of both vancomycin or the compound of formula I or a derivative or pharmaceutically salt thereof, can be determined by agar dilution method using CLSI guidelines (Performance standards for antimicrobial susceptibility testing, 17th informational supplement (M100-517) Wayne, Pa: Clinical and Laboratory Standards Institute; 2007. Clinical and Laboratory Standards Institute). However, it will be understood by persons skilled in the art that any suitable method for determining MIC can be used.

[0070] Using the Clinical and Laboratory Standards Institute (CLSI) and the United States Food and Drug Administration (FDA) interpretive criteria, *S. aureus* is considered Vancomycin susceptible at an MIC of ≤2 mcg/ml and vancomycin intermediate if the MIC is 4 to 8 mcg/ml. The European Committee to Harmonize Antimicrobial Breakpoints (EUCAST) considers *S. aureus* resistant to vancomycin if the MIC is ≤2 mcg/ml.

[0071] *E. faecium* isolates are considered vancomycin sensitive if the MIC is ≤4 mcg/ml.

[0072] The specific dosage "amount" will, obviously, vary with such factors as the particular condition being treated, the physical condition of the subject, the type of subject being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulations employed and the structure of the compound of formula (I) or its

derivatives. The dosage amounts of vancomycin which may be utilised are well known in the art.

[0073] The composition of the present invention may additionally be combined with other medicaments to provide an operative combination. It is intended to include any chemically compatible combination of pharmaceutically active agents, as long as the combination does not eliminate the activity of the combination or composition described herein. A non-limiting example of other agents is antimicrobial agents.

[0074] It will be appreciated that the combination or composition described herein and the other medicament may be administered separately, sequentially or simultaneously. Other medicaments which may be used when treating microbial infections include other anti-infective agents such as antibiotics.

[0075] The terms "administer", "administering" or "administration" in reference to a combination or, composition as described herein means introducing the combination or composition into the system of a subject in need of treatment. When a combination or composition described herein is provided in combination with one or more other active agents, "administration" and its variants are each understood to include concurrent and/or sequential introduction of the compound and the other active agents.

[0076] In particular embodiments of any of the methods of the invention, the two components of the combination therapy are administered within 10 days of each other, within five days of each other, within twenty-four hours of each other, or simultaneously. The compounds may be formulated together as a single composition, or may be formulated and administered separately. The duration of the treatment depends on the type of bacterial infection being treated, the age and condition of the subject, the stage and type of the subject's disease, and how the subject responds to the treatment. Additionally, a person having a greater risk of developing a bacterial infection (e.g., a person who is undergoing a surgical procedure) may receive prophylactic treatment.

[0077] Routes of administration for the various embodiments include, but are not limited to, topical, transdermal, and systemic administration (such as, intravenous, intramuscular, subcutaneous, inhalation, rectal, buccal, vaginal, intraperitoneal,

intraarticular, ophthalmic or oral administration). As used herein, "systemic administration" refers to all nondermal routes of administration, and specifically excludes topical and transdermal routes of administration. In an embodiment, the administration is intravenous and/or oral.

[0078] In combination therapy, the dosage and frequency of administration of each component of the combination can be controlled independently. For example, one compound may be administered three times per day, while the second compound may be administered once per day. Combination therapy may be given in on-and-off cycles that include rest periods so that the subject's body has a chance to recover from any as yet unforeseen side effects. The compounds may also be formulated together such that one administration delivers both compounds.

[0079] The administration of a combination or composition as described herein (e.g., compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof/ vancomycin or derivative thereof combination) may be by any suitable means that results in an amount sufficient to treat a Gram-positive bacterial infection or an amount effective to reduce bacterial growth at a target site. A compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for the oral, parenteral (e.g., intravenously, intramuscularly), rectal, cutaneous, nasal, vaginal, inhalant, skin (patch), or ocular administration route. Thus, the composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy, 20th edition, 2000, ed. A.R. Gennaro, Lippincott Williams & Wilkins, Philadelphia, and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

[0080] Each agent of the combination or composition may be formulated in a variety of ways that are known in the art. For example, the first and second agents may be

formulated together or separately. Desirably, the first and second agents are formulated together for the simultaneous or near simultaneous administration of the agents.

[0081] The term "treatment" as used herein covers any treatment of a condition or disease in a subject, preferably a mammal, more preferably a human, and includes: (i) preventing the disease or condition from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (ii) inhibiting the disease or condition, i.e. arresting its development; (iii) relieving the disease or condition, i.e. causing regression of the condition; or (iv) relieving the conditions caused by the disease, i.e. symptoms of the disease.

[0082] The term "preventing" as used herein refers to administering a medicament beforehand to avert or forestall the appearance of one or more symptoms of a disease or disorder. The person of ordinary skill in the medical art recognizes that the term "prevent" is not an absolute term. In the medical art it is understood to refer to the prophylactic administration of a drug to substantially diminish the likelihood or seriousness of a condition, or symptom of the condition and this is the sense intended in this disclosure. As used in a standard text in the field, the Physician's Desk Reference, the terms "prevent", "preventing" and "prevention" with regard to a disorder or disease, refer to averting the cause, effects, symptoms or progression of a disease or disorder prior to the disease or disorder fully manifesting itself.

[0083] All patents, patent applications and publications mentioned herein are hereby incorporated by reference in their entireties.

[0084] Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

EXAMPLES

EXAMPLE 1

Interaction of BDM-I and vancomycin with MRSA

Isolate MIC testing and Statistical Analysis

One-hundred and three MRSA bacteraemia isolates (from Liverpool hospital, Sydney, Australia) were selected for this study. BDM-I MICs were determined by the broth microdilution method (BMD) as described by the CLSI (Clinical Laboratory Standards Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Ninth Edition: Approved Standard M07-A9, CLSI, Wayne, PA, USA, 2012. 2012) using CAMHB (Oxoid-Thermo Fisher, Hampshire, UK). BDM-I concentrations ranged from 1 to 10 mg/L in 1 mg/L increments. All isolates had previously undergone vancomycin MIC testing and hVISA/VISA characterization (van Hal, Barbagiannakos *et al*, 2011; van Hal, Jones *et al*, 2011). Statistical analysis to determine correlation between variables was completed using the Spearman's test; with $P<0.05$ considered significant. Calculations were completed using SPSS statistical software (version 22.0; SPSS Inc, Chicago, IL, USA).

In vitro Mutant Generation

[0085] Induction experiments were completed on two MRSA isolates, Sa057 (VSSA) and Sa375 (VISA) in triplicate series over a period of approximately 110 days. Isolates were passaged daily in Luria Bertani broth (LBB) supplemented with increasing concentrations of BDM-I during this time, with isolates stored for further analysis at -80°C as the LBB BDM-I concentration was increased.

Whole Genome Sequencing

[0086] DNA libraries were constructed using genomic DNA extracted with the ISOLATE Genomic DNA extraction kit (Bioline, London ENG) as per the manufacturer's instructions. Using the NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (New England Biolabs, Ipswich USA) and the Ion Xpress Plus fragment library kit

(Life Technologies, Carlsbad USA), 400 bp barcoded libraries were generated as per the manufacturer's instructions. The barcoded libraries were then amplified using a thermal cycler and subsequently purified using the Agencourt AMPure XP Reagent (Beckman Coulter, Brea USA). The purified libraries were then combined together and bound to Ion Sphere™ Particles for enrichment and clonal amplification using an Ion OneTouch™ 2 System (Life Technologies) as per the manufacturer's instructions. Quantification of the amplified library was completed using a Qubit 2.0 Fluorometer (Life Technologies). The DNA samples were loaded onto an Ion 318™ v2 chip (Life Technologies) and sequenced in accordance to the manufacturer's instructions. Subsequently, whole genome sequencing reads were analysed using CLC Genomics Workbench ver.7.0.3 (CLCbio, N Aarhus DEN).

Electron Microscopy of Sa375 Mutants

[0087] Transmission electron microscopy (TEM) was completed on Sa375 mutants following whole genome sequencing. Overnight cultures of each mutant Sa375 isolate as well as the progenitor Sa375 and Sa057 isolates were centrifuged and re-suspended in a 2.5% Glutaraldehyde solution in 0.1M cacodylate buffer (pH 7.4). Following fixation for at least 4 h, the buffer was exchanged with 2% Osmium tetroxide and rinsed with Sodium acetate, and finally stained with Uranyl acetate for 60 min. The samples were then dehydrated in alcohol then infiltrated with Spurr resin in acetone (1:1) for 30 min, then again in 6:1 resin for 22 h. Polymerisation of samples was then allowed to carry out for 15 h at 70°C before being cut and imaged using a Morgagni 268D transmission electron microscope (FEI, Eindhoven, The Netherlands) used at 80 kV and fitted with a Soft Imaging Systems MegaView III CCD camera (Munster, Germany). In total, ten measurements were taken on ten individual cells.

Results

[0088] The BDM-I MICs for a collection of clinical MRSA isolates, with varying vancomycin susceptibilities, were determined using the BMD method. MICs ranged from 2-5 mg/L and the average values for VSSA (n=43), hVISA (n=54) and VISA isolates were 3.42, 3.28 and 2.5 mg/L, respectively (Table 1). Interestingly, when compared to previously determined vancomycin MICs for these isolates, a 'see-saw' effect was

observed, as BDM-I MICs were inversely correlated (Rho = -0.24; $P = 0.0145$). This is best illustrated in Table 1, where VSSA isolates have a higher average BDM-I MIC in comparison to that for hVISA and VISA isolates.

[0089] **Table 1:** Average BDM-I and vancomycin MICs and MIC_{50} values as determined by BMD, with isolates grouped according to vancomycin phenotype. Of note is the inverse correlation between BDM-I and vancomycin MICs.

Isolate Vancomycin Phenotype	BDM-I MIC (mg/L)		Vancomycin MIC (mg/L)	
	MIC_{50}	MIC	MIC_{50}	MIC
VSSA (n=43)	1.71	3.42	0.58	1.16
hVISA (n=54)	1.64	3.28	0.96	1.92
VISA (n=6)	1.25	2.5	1.1	2.2

[0090] In order to further explore the antimicrobial potential of BDM-I and the observed inverse relationship between BDM-I and vancomycin MICs, the inventors assessed the ability of MRSA isolates (selected from the above collection) to develop increased BDM-I MICs during an extended period of exposure; two MRSA isolates, Sa057 (VSSA) and Sa375 (VISA), were subcultured (in triplicate) in the presence of increasing concentrations of BDM-I for approximately 110 days (see Fig. 1); only the graph for Sa375 is shown. In a clinical context such information is important, as the prolonged use of antibiotics *in vivo* is often associated with treatment failure due to the generation of resistant mutatnts (Mwangi, Wu et al, 104; Pelegi, Miyakis et al, 2012).

[0091] MIC testing of the triplicate day 110 cultures revealed that isolated Sa057 (VSSA) colonies inconsistently displayed small BDM-I MIC increases of 1.5 mg/L (*i.e.*, increased from 3.5 to 5 mg/L); note that only 6 out of 21 colonies tested across the triplicate experiments displayed this small increase. In contrast, all Sa375 (VISA) colonies

tested (21 out of 21) displayed a BDM-I MIC increase of 3 mg/L (*i.e.*, increased from 2 to 5 mg/L).

[0092] Subsequently, in order to identify mutations associated with this MIC increase, whole genome sequencing (WGS) was performed on genomic DNA isolated from 9 (3 from each series) Sa375 mutant colonies. Interestingly, following variant analysis, single nucleotide polymorphisms (SNPs) were identified (for all colonies) within the *walK* gene, which encodes a multi-sensor signal transduction histidine kinase (see Table 2). It is important to note that Sa375 already contained a mutation within *walK* (amino acid change M220I) that resulted in an increase in cell wall thickness (van Hal, Steen et al, 2014), which is the phenotype associated with intermediate vancomycin resistance. As such, due to the observed inverse relationship between BDM-I and vancomycin MICs, it was hypothesized that the newly identified *walK* mutations were compensatory and resulted in a decrease in cell wall thickness and thus increased sensitivity to vancomycin.

[0093] **Table 2:** Mutations identified within sequenced colonies with increased BDM-I MICs. The mutations shown are those which are present within all colonies sequenced for both Sa057 and Sa375

*Mutation identified within all three colonies of the series

Isolate	Series/Colony	Region	Mutation	AA Change	Gene
	1/*	25210	C → T	Leu37Phe	*walK*
Sa375	2/*	26779	G → A	Gly560Ser	*walK*
	3/*	25189	G → T	Gly30Trp	*walK*

[0094] To confirm this, select Sa375 mutants (1 from each series) and controls (Sa057 and Sa375) were subjected to transmission electron microscopy (TEM) and vancomycin MIC testing.

[0095] In summary, each mutant displayed a reduction in cell wall thickness in comparison to the Sa375 progenitor strain. Additional MIC testing also revealed that the mutants were now susceptible to vancomycin. Vancomycin MICs for the mutant isolates were 0.5 mg/L for Sa375-L37F and Sa375-G560S, and 1 mg/L for Sa375-G30W, which are significantly lower than the Sa375 progenitor vancomycin MIC of 4 mg/L. This data suggests that reverting back to a VSSA phenotype with a thinner cell wall is beneficial in the presence of BDM-I, but results in an increased susceptibility to vancomycin.

Conclusion

[0096] Based on the above results, BDM-I shows potential as an option for salvage therapy in the context of reduced-vancomycin-susceptible MRSA infections, as the associated phenotype (*i.e.*, increased cell wall thickness) results in increased BDM-I sensitivity.

EXAMPLE 2

BDM-I Synergy Studies

[0097] BDM-I synergism was determined using the checkerboard method as described in the literature (Orha, Bayram et al, 2005; Sopirala, Mangino et al, 2010). Antibiotic dilutions were prepared in MHB at double the desired concentration and combined in equal volumes (50 µL) into a single well of a sterile 96-well plate. The final concentration range for each antibiotic was as follows; 5-0.25 mg/L BDM-I, and two-fold dilutions of vancomycin from 16-0.0625 mg/L. 10 µL of the prepared bacterial suspension (diluted to $1x10^5$ Cfu) was then inoculated into each well, and the plate then incubated at 37°C for 16-20 hours overnight. MIC values were then determined as described by the CLSI for broth microdilution testing. Determination of the fractional inhibitory concentration index (FICI) was calculated with the equation: FICI = FIC A + FIC B, where FIC A is the MIC of drug A in combination divided by the MIC of drug A alone, and FIC B is the same for drug B. FICI values were then determined as follows: FICI of $\leq$0.5 indicates synergy, FICI of >0.5 to $\leq$4 indicates no interaction, and FICI of >4 indicates antagonism (Odds, 2003).

[0098] Figure 3 shows that vancomycin combined with BDM-I at fixed concentrations demonstrated synergistic activity a *vanB* VRE isolate.

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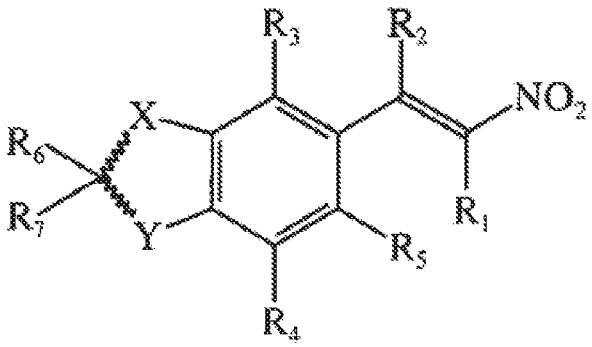
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CLAIMS

1. A method of treating *S. aureus* infection in a patient wherein the *S. aureus* has at least partial resistance to vancomycin, the method comprising administering to the patent an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof:



(I)

in which

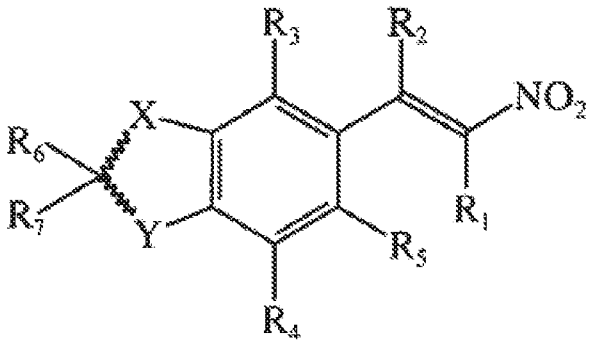
X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

 is a double or single bond depending on the heteroatoms X and Y;

R1 to R5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and

R6 and R7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

2. A method of treating *S aureus* infection in a patient wherein the infection has failed to resolve following vancomycin treatment, the method comprising administering to the patient a compound of formula (I) or a pharmaceutically-acceptable salt or derivative thereof:



(I)

in which

X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

 is a double or single bond depending on the heteroatoms X and Y;

R1 to R5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R6 and R7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

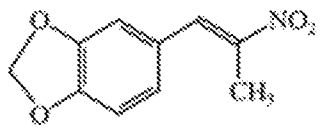
3. A method as claimed in claim 1 or claim 2 in which in the compound of formula I, X and Y are either the same or different and selected from O and N, more preferably both X and Y are oxygen.

4. A method as claimed in any one of claims 1 to 3 in which in the compound of formula I R_1 and R_2 are either the same or different and selected from hydrogen, hydroxy, halogen or optionally substituted C1-6 alkyl.

5. A method as claimed in any one of claims 1 to 4 in which in the compound of formula I R_3 to R_5 are either the same or different and selected from hydrogen, hydroxy, halogen, nitro, C1-6 alkoxy or optionally substituted C1-6 alkyl.

6. A method as claimed in any one of claims 1 to 5 in which the compound of formula I is selected from the group consisting of:

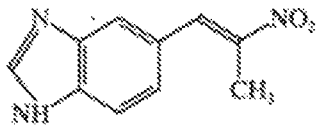
X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)



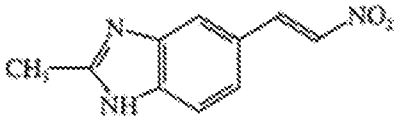
X and Y are O and R_1 to R_3 are hydrogen (3,4-methylenedioxy-β-nitrostyrene)



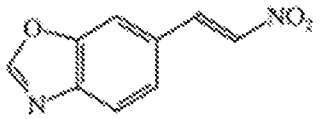
X is N, Y is NH, R_1 is methyl and R_2 and R_3 are hydrogen (benzimidazole-5-β-nitropropylene)



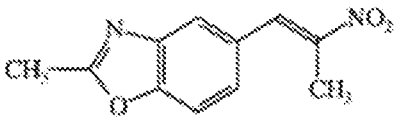
X is N, Y is NH, R_1 is hydrogen, R_2 is methyl and R_3 is absent (2-methyl benzimidazole-5-β-nitroethylene)



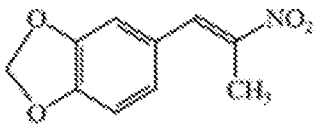
X is O, Y is N, R_1 and R_2 are hydrogen and R_3 is absent (benzoxazole-5-β-nitroethylene)



X is N, Y is O, R_1 and R_2 are methyl and R_3 is absent (2-methyl benzoxazole-5-β-nitropropylene)

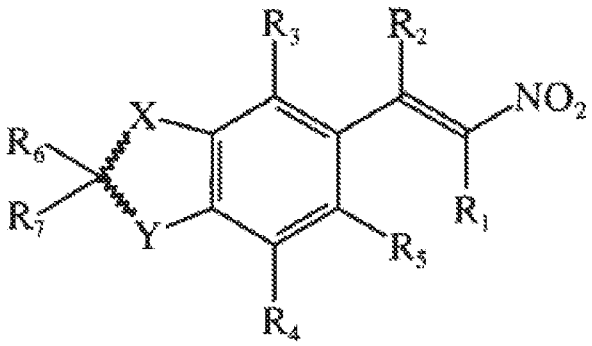


7. The method as claimed in claim 6 in which X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)



8. The method as claimed in any one of claims 1 to 7 in which the *S. aureus* is VRSA, VISA or hVISA.

9. A method of treating *Enterococcal* infection in a patient, the method comprising administering to the patient vancomycin or a derivative thereof and a compound of formula (I) or a pharmaceutically-acceptable salt or derivative thereof:



(I)

in which

X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;



is a double or single bond depending on the heteroatoms X and Y;

R_1 to R_5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl,

hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R_6 and R_7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

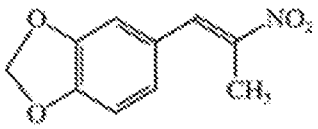
10. A method as claimed in claim 9 in which in the compound of formula I, X and Y are either the same or different and selected from O and N, more preferably both X and Y are oxygen.

11. A method as claimed in claim 9 or claim 10 in which in the compound of formula I R_1 and R_2 are either the same or different and selected from hydrogen, hydroxy, halogen or optionally substituted C1-6 alkyl.

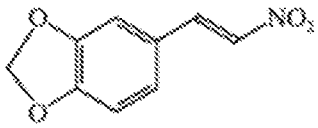
12. A method as claimed in any one of claims 9 to 11 in which in the compound of formula I R_3 to R_5 are either the same or different and selected from hydrogen, hydroxy, halogen, nitro, C1-6 alkoxy or optionally substituted C1-6 alkyl.

13. A method as claimed in any one of claims 9 to 12 in which the compound of formula I is selected from the group consisting of:

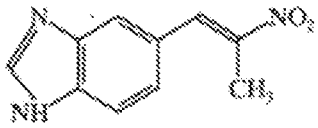
X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)



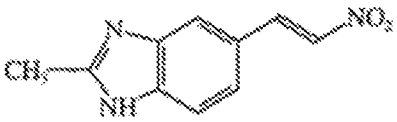
X and Y are O and R_1 to R_3 are hydrogen (3,4-methylenedioxy-β-nitrostyrene)



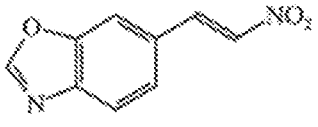
X is N, Y is NH, R_1 is methyl and R_2 and R_3 are hydrogen (benzimidazole-5-β-nitropropylene)



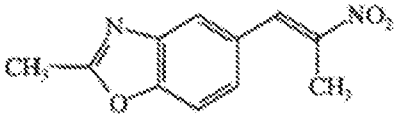
X is N, Y is NH, R_1 is hydrogen, R_2 is methyl and R_3 is absent (2-methyl benzimidazole-5-β-nitroethylene)



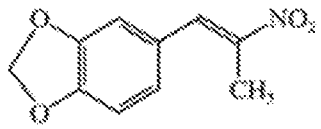
X is O, Y is N, R_1 and R_2 are hydrogen and R_3 is absent (benzoxazole-5-β-nitroethylene)



X is N, Y is O, R_1 and R_2 are methyl and R_3 is absent (2-methyl benzoxazole-5-β-nitropropylene)



14. The method as claimed in claim 13 in which X and Y are O, R_1 is methyl and R_2 and R_3 are hydrogen (3,4-methylenedioxy-β-methyl-β-nitrostyrene) (BDM-I)

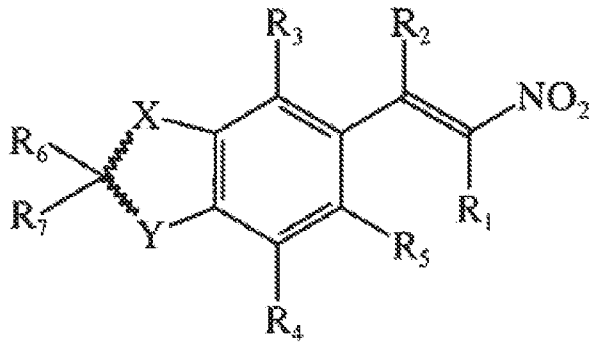


15. The method as claimed in any one of claims 9 to 14 in which the *Enterococcus* is *vanB* VRE.

16. The method as claimed in claim 15 in which the Enterococcus is *E. faecalis* or *E. faecium*.

17. A composition comprising a combination of vancomycin or a derivative thereof and a compound of formula I or a pharmaceutically-acceptable salt or derivative thereof as defined herein.

18. The use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *S. aureus* infection in a patient wherein the *S. aureus* has at least partial resistance to vancomycin:



(I)

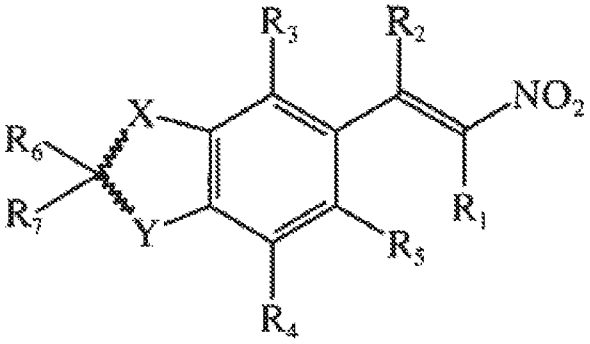
in which

X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

 is a double or single bond depending on the heteroatoms X and Y;

R_1 to R_5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R_6 and R_7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

19. The use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *S aureus* infection in a patient wherein the infection has failed to resolve following vancomycin treatment:



(I)

in which

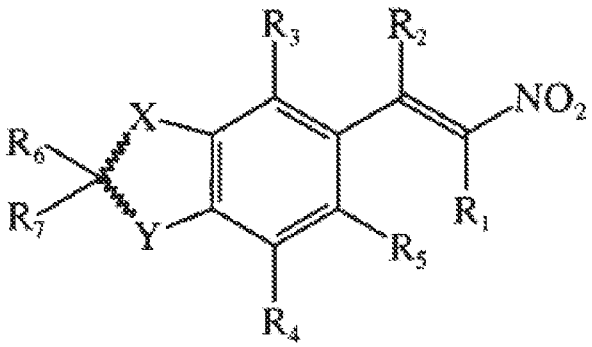
X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

≋ is a double or single bond depending on the heteroatoms X and Y;

R_1 to R_5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R_6 and R_7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy,

arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

20. The use of a compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof in the preparation of a medicament for use in treatment of *Enterococcal* infection in a patient, wherein the medicament is administered in combination with vancomycin or a derivative thereof:



(I)

in which

X and Y are either the same or different and are each a heteroatom selected from the group consisting of O, N, and S;

 is a double or single bond depending on the heteroatoms X and Y;

R_1 to R_5 are either the same or different and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino,

haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds; and R_6 and R_7 are either the same or different, and selected from hydrogen, alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocyclamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, arylthio, acylthio or phosphorus-containing compounds, or one of R6 and R7 are absent when there is a double bond present.

FIGURE 1

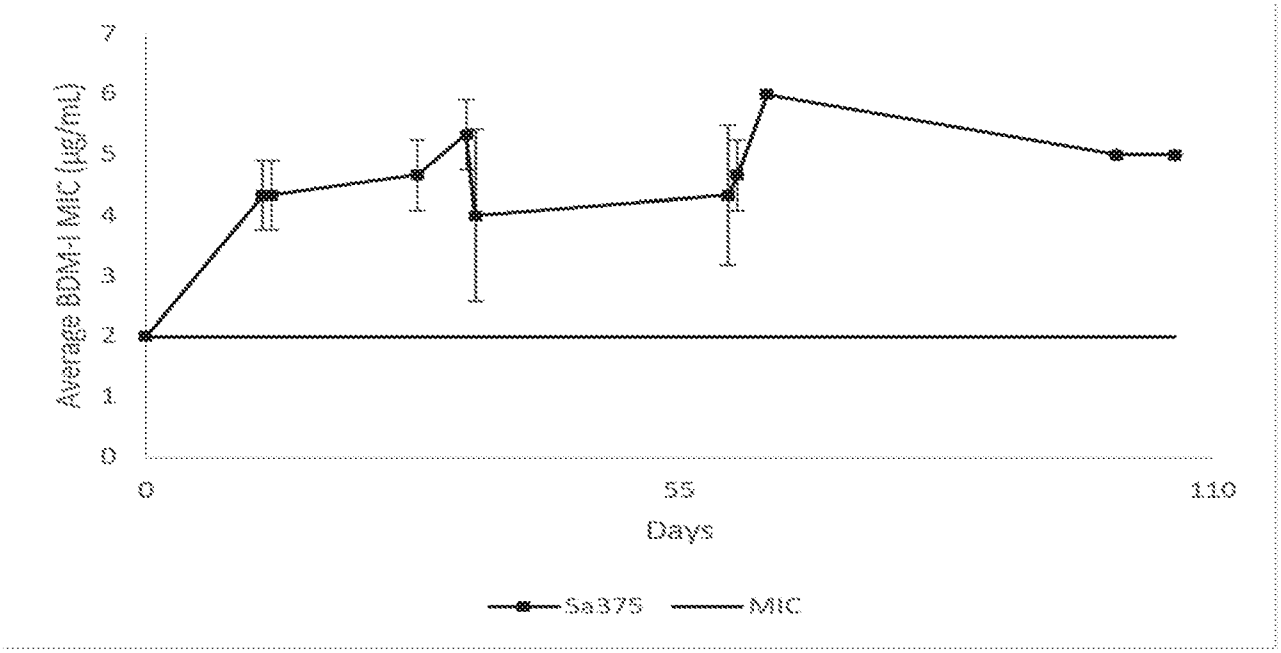
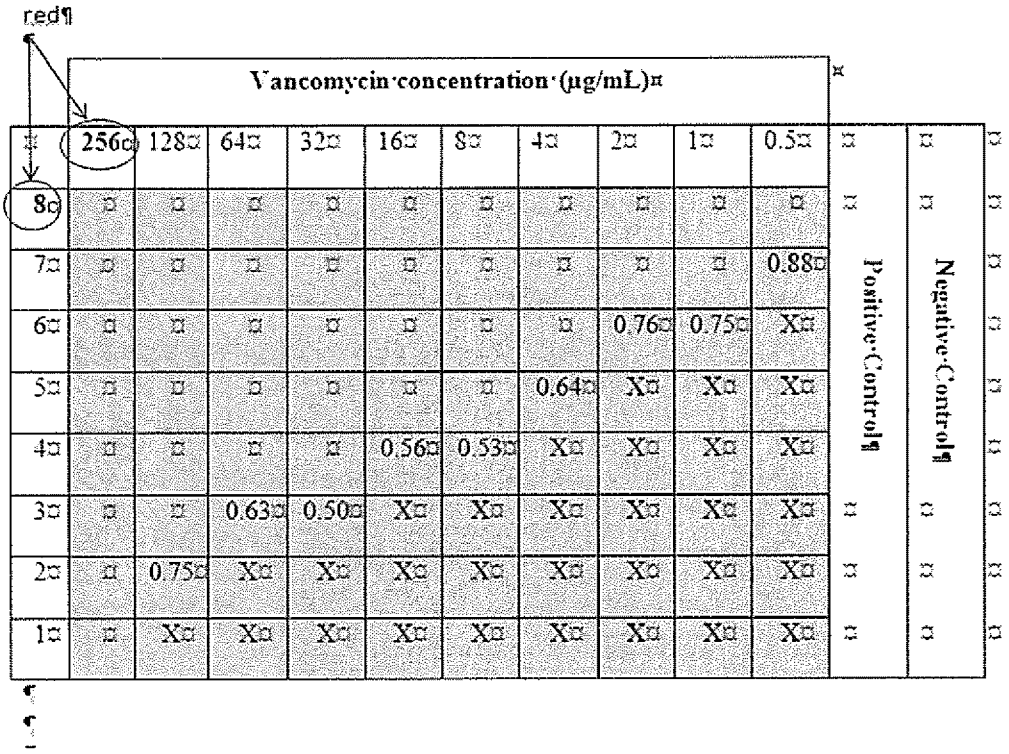


FIGURE 2

FIC Index for *vanB* VRE Isolate



INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050788

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/36 (2006.01) A61K 31/4184 (2006.01) A61K 31/423 (2006.01) A61K 38/14 (2006.01) A61P 31/04 (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)

EPOQUE Databases MEDLINE, PATENW and keywords: nitrostyrene, vancomycin, staphylococcus, enterococcus, VRSA, VRE, VISA, hVISA and related terms

STN Database CAPLUS and structure according to formula I and keywords: vancomycin, staphylococcus, enterococcus, VRSA, VRE, VISA, hVISA and related terms

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:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 August 2018	27 August 2018
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Leah Walker AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256170

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/0266844 A1 (DENISENKO et al.) 30 December 2004 See abstract, paragraphs [0013]-[0038], [0077], [0081], Tables 1, 2, 4 and 5, paragraph [0246]-[0247], Table 9 and Table 11	1-20
X	MILHAZES, N. et al. "β-nitrostyrene derivatives as potential antibacterial agents: A structure-property-activity relationship study" Bioorganic and Medicinal Chemistry (2006) vol. 14, pages 4078-4088 See Table 1 compounds VIa and VIb, abstract, page 4080 right column paragraph 2, page 4081 left column paragraph 2, Tables 2 and 3	1-20
A	CORNELL, H. et al. "Comparisons of halogenated β-nitrostyrenes as antimicrobial agents" Applied Sciences (2014) vol. 4, pages 380-389 See compounds A, A-1 and B in Figure 1, Table 1	
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