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[Continued on next page]

(54) Title: STEROID ALKALOIDS AND USES THEREOF AS ANTIMICROBIAL AGENTS AGAINST ELECTRON TRANSPORT-DEFICIENT MICROBES AND AS POTENTIATORS FOR ANTIMICROBIAL AGENTS AGAINST PATHOGENIC BACTERIA

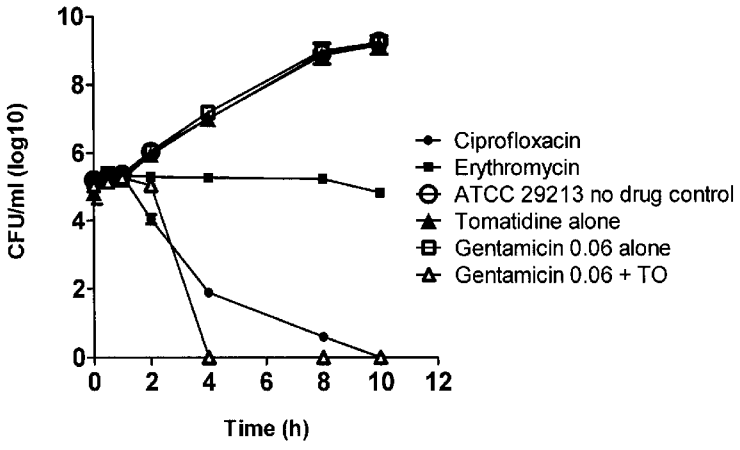

FIG 8

(57) Abstract: The present invention includes novel compounds based on the tomatidine skeleton as well as composition comprising these compounds alone and in combination with known compounds, which exhibit antimicrobial activity against extracellular or intracellular electron transport-deficient microbes and/or increase the antimicrobial activity of aminoglycoside antibiotics against their targets, and which are useful as antibacterial agents for treatment or prophylaxis of monomicrobiotic or polymicrobic bacterial infections or for the reduction of antibiotic resistance development in animals or in humans, or for use as antiseptics or agents for sterilization or disinfection.

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TITLE OF THE INVENTION

Steroid alkaloids and uses thereof as antimicrobial agents against electron transport-deficient microbes and as potentiators for antimicrobial agents against pathogenic bacteria

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a PCT application no. PCT/CA2012/* filed on February 15 2012 and published in English under PCT Article 21(2), claiming benefit of U.S. Provisional applications Serial No. 61/442,948, filed on February 15, 2011. All documents above are incorporated herein in their entirety by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to novel antimicrobial compounds and potentiators for antimicrobial compounds. More specifically, the present invention is concerned with the use of steroid alkaloids as antimicrobial agents, and potentiators of the antimicrobials activity of aminoglycosides against pathogenic bacterial strains, methods of manufacturing same, disinfection, sterilization or antisepsis methods using the same.

BACKGROUND OF THE INVENTION

Staphylococci

[0003] Staphylococci are widely disseminated Gram-positive opportunistic bacterial pathogens responsible for many medical problems in humans, including skin and soft-tissue infections, surgical infections, endocarditis and hospital-acquired bacteriemia (Casey *et al.*, 2007; Kloos and Bannerman, 1994). These bacteria are also the cause of several diseases in animals such as birds, cows, dogs, poultries, rabbits and others (Jacques *et al.*, 2010; Pyorala and Taponen, 2009; Stepan *et al.*, 2004). Staphylococci are divided in coagulase-positive species, *Staphylococcus aureus* (*S. aureus*) being the most clinically relevant of this group, and coagulase-negative species, such as *Staphylococcus epidermidis* (*S. epidermidis*), the most prevalent pathogen associated with infections of implanted medical devices (Vuong and Otto, 2002). The emergence and spread of resistance to multiple antibiotics in staphylococci is now considered a real health treat and impaired therapeutic endeavor to combat these bacteria (Witte et *al.*, 2008).

[0004] *S. aureus* is an opportunistic pathogen that has extraordinary versatility. Diseases caused by this pathogen can affect several hosts, organs and body sites and may become both life threatening as well as chronic (Archer, 1998; Goerke and Wolz, 2004). For example, *S. aureus*

is associated with significant mortality rates in hospitals and increased health costs (Talbot et al., 2006), but is also the most common cause of difficult-to-treat bovine mastitis (Sears and McCarthy, 2003). The ability of *S. aureus* to cause a broad spectrum of diseases is related to its numerous virulence factors (Archer, 1998) and it is likely that the coordinated or selected expression of specific groups of virulence factors contribute to the development of specific types of infections. For example, the formation of biofilms and the persistence within non-phagocytic host cells seem to facilitate the development of chronic infections by offering the bacterium protection against the host immune system and the action of antibiotics (Alexander and Hudson, 2001; Brouillette *et al.*, 2004; Galli *et al.*, 2007; Stewart, 2002).

Bacterial small-colony variants

[0005] Bacterial small-colony variants (SCVs) are derived from normal bacterial strains and show a slow-growth phenotype (i.e., they produce small colonies when cultivated on solid media). *S. aureus* SCVs are known to form biofilms (Mitchell *et al.*, 2010a; Mitchell *et al.*, 2010b) and persist within non-phagocytic host cells (Sendi and Proctor, 2009). SCVs are bacteria with a dysfunctional oxidative metabolism causing an alteration in the expression of virulence factors, a slow growth and a loss of colony pigmentation (Proctor *et al.*, 2006). This dysfunctional oxidative metabolism causes a decreased susceptibility to aminoglycosides because these antibiotics require the proton-motive force in order to penetrate the bacterium (Bryan and Kwan, 1981). In *S. aureus*, the SCV phenotype results from mutations affecting the electron-transport system and several SCV isolates are auxotrophic for either hemin or menadione, which are needed to synthesize electron-transport system components. SCVs can also be auxotrophic for thiamine because thiamine is required for the biosynthesis of menadione. Other SCVs are no longer able to synthesize thymidine due to mutations in the folate pathway and this also results in a defect in electron transport although the fundamental basis of this is not well understood (Proctor *et al.*, 2006). Some SCVs present yet unknown auxotrophy but still have in common electron transport deficiency which may result, for example, from a defect in the bacterial F_0F_1-ATPase (Proctor *et al.*, 2006). *S. aureus* SCVs are isolated from chronic infections, such as lung infections in cystic fibrosis (CF) patients and from osteomyelitis, septic arthritis, bovine mastitis and infection of orthopedic devices (Atalla *et al.*, 2008; Moisan *et al.*, 2006; Proctor *et al.*, 2006). SCVs that are MRSA (methicillin-resistant *S. aureus*) and multiresistant to several class of antibiotics have also been reported (Vergison *et al*, 2007). It is now thought that switching from the normal to the SCV phenotype is an integral part of the pathogenesis of *S. aureus* and that novel therapeutic strategies targeting SCVs are needed to combat infections caused by bacterial species capable of generating electron transport-deficient SCVs (Tuchscherr *et al.*, 2011).

[0006] The SCV phenotype is widespread among microbes. SCVs have been

described for several bacterial species and have been recovered from many different clinical specimens such as abscesses, blood, bones and joints, the respiratory tract and soft tissues (Proctor *et al.*, 2006). For examples, SCVs were detected among the staphylococci such as *S. aureus*, *S. epidermidis*, *Staphylococcus lugdunensis* and *Staphylococcus capitis*, among the enteric-disease causing bacteria such as *Salmonella* serovars, *Shigella* spp., *Escherichia coli* and *Vibrio cholerae*, among the nosocomial pathogens such as *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Escherichia coli*, *Serratia marcescens*, *Stenotrophomonas maltophilia* and *Enterococcus faecalis*, among the respiratory tract pathogens such as *Streptococcus pneumoniae* and *Corynebacterium* spp., among uro-genital pathogens such as *Neisseria gonorrhoeae* and also in a variety of other species such as *Brucella melitensis* and *Lactobacillus lactophilus* (Allegrucci and Sauer, 2008; Melter and Radojevic, 2010; Proctor *et al.*, 2006; Wellinghausen *et al.*, 2009). In most of these cases, the SCV phenotype is consequent to a defect in the electron transport chain either caused by alteration of electron transport proteins, restriction in necessary coenzymes, cofactors or precursors or an overall reduction of some metabolic pathways such as the tricarboxilic cycle that ultimately affect and reduce electron transport (Chatterjee *et al.*, 2007; Proctor *et al.*, 2006).

Anaerobic bacteria

[0007] Anaerobic bacteria predominantly constitute the indigenous flora of human and are the source of infections affecting virtually all organs (Nagy, 2010), and the prevalence of antibiotic resistance in several anaerobic pathogens is increasing (Hetch *et al.*, 2006; Nagy, 2010). Among the numerous anaerobic bacteria causing human diseases are the clostridia (Hetch *et al.*, 2006; Nagy, 2010), also known to be sources of infections in animals (Songer, 2010). The better example is probably *Clostridium difficile*, now considered to be an important cause of infections associated with health-care (Rupnik *et al.*, 2009). Another good example is *Clostridium perfringens*, which is the third in incidence among pathogen causing food-borne illness in the USA (Mead *et al.*, 1999; Songer, 2010) and diseases in pigs and chickens (Van Immerseel et al., 2004; Songer and Uzal, 2005).

Cystic fibrosis

[0008] Although cystic fibrosis (CF) is fundamentally a genetic disorder, the majority of patients afflicted by this disease will ultimately succumb from respiratory failure subsequent to chronic bacterial infections (Lyczak *et al.*, 2002). More recent investigations reveal that the CF airways are colonized by complex polymicrobial communities constituted of numerous microorganisms, encompassing more bacterial species than originally thought, and suggest that interactions between these microorganisms influence the course of the disease (Sibley and Surette, 2011). Some focus has

been directed toward understanding the outcome of the interactions between *P. aeruginosa* and *S. aureus* because they are often co-isolated from the CF airways (Harrison, 2007; Hoffman *et al.*, 2006; Mitchell *et al.*, 2010b). The polymicrobial nature of CF lung infections needs to be considered in the development of novel therapeutic approaches (Sibley *et al.*, 2009; Sibley and Surette, 2011).

[0009] *Staphylococcus aureus* is one of the most common pulmonary pathogens recovered from North American CF patients (Canadian Cystic Fibrosis Foundation, 2007; Cystic Fibrosis Foundation, 2008). While it is well accepted that antibiotic therapy leads to improvement of lung function and may reduce morbidity associated with CF, decisions regarding which antibiotics to use and when to treat remain largely empirical (Lyczak *et al.*, 2002; Parkins and Elborn, 2010). Consequently, many antibiotics are currently used to treat CF patients infected with bacteria, including aminoglycoside antibiotics (Gibson *et al.*, 2003; Lyczak *et al.*, 2002). A major problem encountered by CF patients is the emergence of bacteria resistant to antibiotics. For example, the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), most often multi-resistant to antibiotics (Chambers and Deleo, 2009), is increasing among CF patients (Parkins and Elborn, 2010). MRSA infections has been associated with a decline of lung function in CF patients (Dasenbrook *et al.*, 2010).

Bovine mastitis

[0010] Bovine mastitis is the most frequently occurring and costly disease affecting dairy producers. The transmittable bacterium *Staphylococcus aureus*, the coagulase-negative staphylococci and also many streptococci (*S. agalactiae*, *S. dysgalactiae*, *S. uberis* and others) are amongst the most common causes of intramammary infections leading to bovine mastitis (Tenhagen *et al.*, 2006) and current antibiotic therapies usually fail to eliminate the infection from dairy herds (Sears, P. M. and K. K. McCarthy, 2003). Both the normal and SCV phenotypes of pathogenic bacteria were recovered from mastitis cases (Atalla *et al.*, 2008).

Antibiotic-resistant bacteria

[0011] Infections caused by antibiotic-resistant bacteria represent an overwhelming growing problem both in human and veterinary medicine. One reason explaining this widespread of drug resistances is that the currently available antibiotics have been largely designed on a limited number of chemical scaffolds, which allowed pathogens to adapt and circumvent common antibiotic action mechanisms (Shah, 2005; Talbot *et al.*, 2006).

Foodborne bacteria and illnesses

[0012] A number of bacterial species such as *Listeria* spp. and *Bacillus* spp. can contaminate food and cause infections in humans. To name a few, *Listeria monocytogenes*, *L. ivanovii*, and *Bacillus cereus* can cause listeriosis (Guillet et al, 2010) and food poisoining (Bad Bug Book, FDA). *Bacillus subtilis*, *B. coagulans*, *B. licheniformis* and *B. sphaericus* are also known to cause illnesses. *Bacillus anthracis* causes anthrax and can often be acquired by contact with food producing animals and cattle (beef cattle, sheeps, etc.) and this bacterium is also well-known for its endospores that have been used as biological weapons (Beierlein and Anderson, 2011).

[0013] It would be highly desirable to identify antibiotic compounds targeting electron transport-deficient microbes (*e.g.*, SCVs and anaerobe bacteria) and/or potentiating the growth inhibitory activity of aminoglycosides against pathogenic bacteria (*e.g.*, antibiotic-resistant bacteria and/or those causing chronic infections) and/or reducing bacterial resistance development toward aminoglycosides. It would also be highly desirable to identify antibiotic compounds that can be used to reduce bacterial colonization in food, preserve food or treat infections caused by foodborne pathogens.

[0014] The present description refers to a number of documents, the content of which is herein incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0015] The present invention relates in part to the discovery that steroid alkaloids specifically and selectively inhibit the growth of electron transport-deficient microbes.

[0016] In accordance with one aspect, the present invention provides steroid alkaloids for use as antibiotic-like compounds with antimicrobial activity against pathogenic electron transport-deficient microbes (*e.g.*, SCVs, anaerobe bacteria, bacteria affected by another organism producing inhibitors of the electron transport chain).

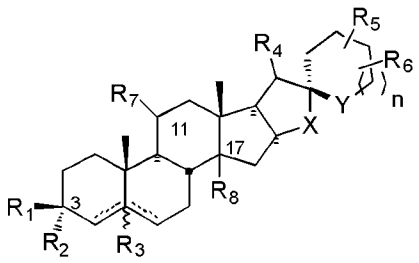
[0017] In accordance with another aspect, the present invention provides steroid alkaloids for use as agents potentiating the antimicrobial activity of aminoglycosides against a variety of bacteria that do not have electron-transport deficiency.

[0018] In accordance with another aspect, the present invention provides steroid alkaloids for use as agents reducing the development of bacterial resistance toward aminoglycosides.

Compounds

[0019] More specifically, in accordance with one aspect, the present invention provides a

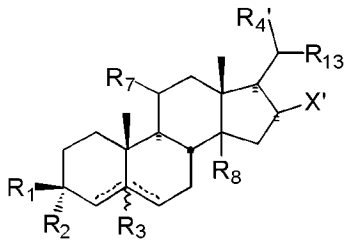
compound of formula:



(1.0)

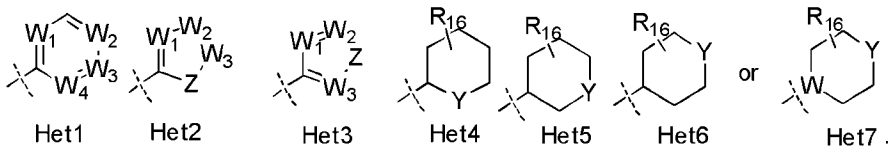
[0020] wherein, (1) R1 is H, OH, NH_2, NHR12, $N(R12)_2$ or OR12; and R2=H; or (2) R2 is H, OH, NH_2, NHR12, $N(R12)_2$ or OR12; and R1=H; or (3) R1 and R2 together form =O or =NR12; R3 is α-H, β-H, α-alkyl, β-alkyl, α-OH or β-OH, or is absent when the double bond is present either in C4=C5, or in C5=C6; - - - - - is an optional double bond; R4-R6 are identical or different and are H, alkyl, OH, OR12, NHR12 or $N(R12)_2$; R7 is H, α-OH or β-OH; R8 is α-H, β-H, α-OH or β-OH; X and Y are identical or different and are O, NR12 or CH_2; R12 is H, alkyl, aryl, COalkyl, COaryl,CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R12)_p$, PO_3H_2, CO-amino-acid, CH_2-NH-R14, C(=NH)NHR4, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$ $(CH_2)_mNHC(=NH)NH_2$, or $(CH_2)_m$-C(=NH)NH_2; NHalkyl or NHaryl; R14 is H, alkyl, aryl, COalkyl, COOalkyl, COaryl, CO_2aryl, SO_2alkyl, SO_2aryl, $SO_2N(R12)p$ or CO-amino-acid; n is 0-5; m is 1-5; p=1-2, wherein the compound of formula 1.0 is not tomatidine or solasodine;

or



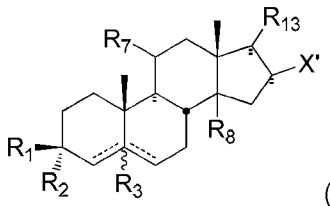
(2.0)

[0021] X' is H, OR14 or NHR14, wherein R14 is H, alkyl, aryl, COalkyl, COaryl,CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R12)_p$, PO_3H_2, CO-amino-acid, CH_2-NH-R14, C(=NH)NHR4, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$ $(CH_2)_mNHC(=NH)NH_2$, or $(CH_2)_m$-C(=NH)NH_2; R1, R2, R3, R7 and R8 are as defined above; R4' is H, alkyl or aryl; R13 is NHR15, wherein R15 is H, alkyl, aryl, COalkyl, COaryl,CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R12)_p$, PO_3H_2, CO-amino-acid, CH_2-NH-R14, C(=NH)NHR4, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$ $(CH_2)_mNHC(=NH)NH_2$, or $(CH_2)_m$-C(=NH)NH_2; or



[0022] wherein W1, W2, W3, W4 are identical or different and are independently N or CH; R16 is H, alkyl or aryl; Y is CH_2, NH, N-alkyl, N-COalkyl, N-COaryl, N-SO_2alkyl, N-SO_2aryl, NH-C(=NH)NH_2, N-CO_2alkyl or N-CO_2aryl; and Z is NH, NR17, S or O, where R17 is H, alkyl, aryl, COalkyl, COaryl,CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R12)_p$, PO_3H_2, CO-amino-acid, CH_2-NH-R14, C(=NH)NHR4, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$, $(CH_2)_mNHC(=NH)NH_2$, or $(CH_2)_m$-C(=NH)NH_2; wherein the compound of formula 2.0 is not dihydrosolacongestidine;

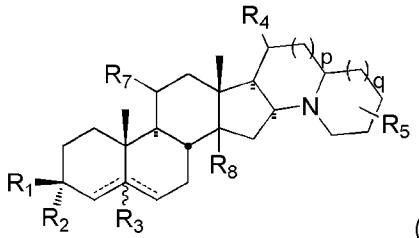
or



(3.0)

[0023] wherein R1, R2, R3, R7, R8, R13 and X' are as defined above;

or

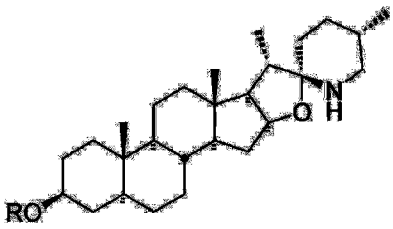


(4.0)

[0024] wherein R1, R2, R3, R4, R5, R7 and R8 are as defined above; p is 0-5; and q is 0-5, wherein the compound of formula 4.0 is not demissidine.

[0025] In a specific embodiment of the compound, the compound is of formula 1.0 and (i) R1 is OR12 or H; (ii) R2 is OR12 or H; (iii) R3 is H; (iv) R4 is CH_3; (v) R5 is H; (vi) R6 is CH_3; (vii) R7 is H; (ix) R8 is H; (x) n is 1; (xi) X is O; (xii) Y is NH.; or (xiii) any combination of (i) to (xii).

[0026] In another specific embodiment of the compound, the compound is of formula 1.1:



(1.1),

[0027] wherein R is alkyl, aryl, COalkyl, COaryl,CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R12)_p$, PO_3H_2, CO-amino-acid, CH_2-NH-R14, C(=NH)NHR4, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$ $(CH_2)_mNHC(=NH)NH_2$, or $(CH_2)_m$-C(=NH)NH_2; NHalkyl or NHaryl.

[0028] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is SO_3H, n is 1, X is O and Y is NH.

[0029] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is PO_3H_2, n is 1, X is O and Y is NH.

[0030] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is $(CH_2)_mCO_2H$, n is 1, m is 1, X is O and Y is NH.

[0031] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is $(CH_2)_mNH_2$, n is 1, m is 2, X is O and Y is NH.

[0032] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is $(CH_2)_mCH_2$, n is 1, m is 1, X is O and Y is NH.

[0033] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is $(CH_2)_mNHC(=NH)NH_2$, n is 1, m is 2, X is O and Y is NH.

[0034] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is H, R2 is OR12, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, R12 is H, n is 1, X is O and Y is NH.

[0035] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 and R2 together form =O, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, n is 1, X is O and

Y is NH.

[0036] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is NH2, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is CH_3, R7 is H, R8 is H, n is 1, X is O and Y is NH.

[0037] In another specific embodiment of the compound, the compound is of formula 1.0 and R1 is OR12, R2 is H, R3 is H, R4 is CH_3, R5 is H, R6 is as defined above, R7 is H, R8 is H, R12 is a protective group, n is 1, X is O and Y is NH.

[0038] In another specific embodiment of the compound, the compound is of formula 2.0 and (i) R1 is OH; (ii) R2 is H; (iii) R3 is H; (iv) R4' is CH_3;(v) R7 is H; (vi) R8 is H; or (vii) any combination of (i) to (vi).

[0039] In another specific embodiment of the compound, the compound is of formula 2.0 and R1 is OR12, R2 is H, R3 is H, R4' is CH_3, R7 is H, R8 is H, R12 is H, X' is H, and R13 is of formula Het1, wherein W1, W2 and W3 are CH and W4 is N.

[0040] In another specific embodiment of the compound, the compound is of formula 2.0 and wherein R1 is OR12, R2 is H, R3 is H, R4' is CH_3, R7 is H, R8 is H, R12 is H, X' is H, and R13 is of formula Het2, wherein W1 is N, W2 and W3 are CH and Z is S.

[0041] In another specific embodiment of the compound, the compound is of formula 2.0 and wherein R1 is OR12, R2 is H, R3 is H, R4' is CH_3, R7 is H, R8 is H, R12 is H, X' is H, and R13 is of formula Het4, wherein Y is NH and R16 is H.

[0042] In another specific embodiment of the compound, the compound is of formula 2.0 and wherein R1 is OR12, R2 is H, R3 is H, R4' is CH_3, R7 is H, R8 is H, R12 is H, X' is OR14, R14 is CH_3, and R13 is of formula Het4, wherein Y is NH and R16 is H.

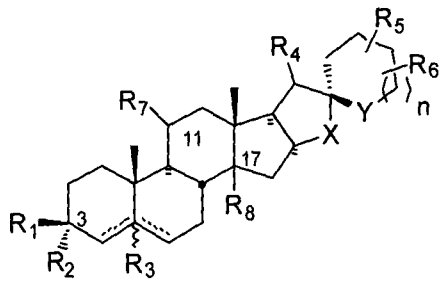
[0043] In another specific embodiment of the compound, the compound is of formula 3.0 and (i) R1 is OR12; (ii) R2 is H; (iii) R3 is H; (iv) R7 is H; (v) R8 is H; (vi) R12 is H; or (vii) any combination of (i) to (vi).

[0044] In another specific embodiment of the compound, the compound is of formula 3.0 and R1 is OR12, R2 is H, R3 is H, R7 is H, R8 is H, R12 is H, X' is H, and R13 is of formula Het3, wherein W1 is N, W2 and W3 are CH and Z is S.

[0045] In another specific embodiment of the compound, the compound is of formula 3.0 and wherein R1 is OR12, R2 is H, R3 is H, R7 is H, R8 is H, R12 is H, X' is H, and R13 is of formula Het3, wherein with W1 is N, W2 and W3 are CH and Z is NH.

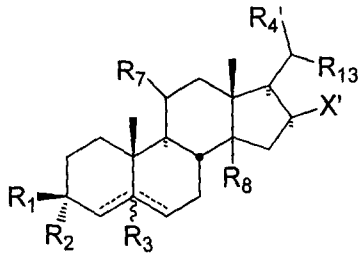
[0046] In another specific embodiment of the compound, the compound is of formula 4.0 and wherein R1 is OR12, R2 is H, R3 is H, R7 is H, R8 is H and R12 is H.

[0047] In accordance with another aspect, the present invention provides a compound of formula:



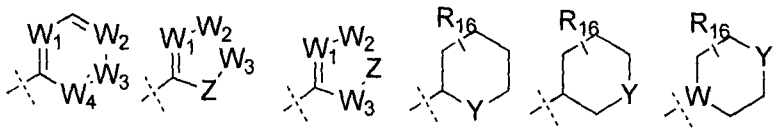
(1.0)

[0048] wherein, (1) R1 is H, OH, NH_2, NHR12, $N(R12)_2$, N(R12)(R12'), OR12 or SR12; and R2=H; or (2) R2 is H, OH, NH_2, NHR12, $N(R12)_2$, N(R12)(R12'), OR12 or SR12; and R1=H; or (3) R1 and R2 together form =O or =NR12; R3 is α-H, β-H, α-alkyl, β-alkyl, α-OH or β-OH, or is absent when the double bond is present either in C4=C5, or in C5=C6; - - - - - is an optional double bond; R4-R6 are identical or different and are H, alkyl, OH, OR18, NHR18 or N(R18)(R18'); R7 is H, α-OH or β-OH; R8 is α-H, β-H, α-OH or β-OH; X and Y are identical or different and are O, NR19, or CH_2; R12 and R12' are identical or different and are H, alkyl, aryl, COalkyl, COaryl, CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R14)_p$, PO_3H_2, CO-CH(R20)NH_2, $(CH_2)_{n'}$-NH-R14, C(=NH)NHR21, CH_3OCH_2 ,Silylalkyl, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$, $(CH_2)_mNHC(=NH)NH_2$, $(CH_2)_m$-C(=NH)NH_2, NHalkyl or NHaryl; R14, R22 and R22' are identical or different and are H, alkyl, aryl, COalkyl, CO_2alkyl, COaryl, CO_2aryl, SO_2alkyl, SO_2aryl, $SO_2N(alkyl)p'$ or CO-CH(R20)NH_2; R18 and R18' are identical or different and are H, alkyl, aryl, COalkyl, COaryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(alkyl)_{p''}$, PO_3H_2, CO-CH(R20')NH_2, $(CH_2)_{n''}$-NH-R22, C(=NH)NHR21', $(CH_2)_{m'}CO_2H$, $(CH_2)_{m'}SO_3H$, $(CH_2)_{m'}NH_2$, $(CH_2)_{m'}NHC(=NH)NH_2$, $(CH_2)_{m'}$-C(=NH)NH_2, NHalkyl or NHaryl; R19 is H, alkyl, aryl, COH, COalkyl, COaryl, CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(Ralkyl)_{p'''}$, PO_3H_2, CO-CH(R20'')NH_2, $(CH_2)_{n'''}$-NH-R22', C(=NH)NHR21'', $(CH_2)_{m''}CO_2H$, $(CH_2)_{m''}SO_3H$, $(CH_2)_{m''}NH_2$, $(CH_2)_{m''}NHC(=NH)NH_2$, $(CH_2)_{m''}$-C(=NH)NH_2, NHalkyl or NHaryl; R20, R20' and R20'' are identical or different and correspond to the side chain of any L- and D- amino acid; R21, R21' and R21'' are identical or different are are H, alkyl, OH, Oalkyl, Oaryl, NHalkyl, NHaryl, $N(alkyl)_2$, $N(aryl)_2$, or N(alkyl)(aryl);n, n', n'' and n''' are identical or different and re 0-5; m, m' and m'' are identical or different and are 1-5; and p, p', p'' and p''' are identical or different and are 1-2; wherein the compound of formula 1.0 is not tomatidine, solasodine, 3α-hydroxytomatidine or 3-oxo-tomatidine; or



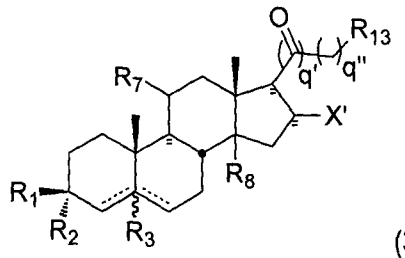
(2.0)

[0049] wherein, R1, R2, R3, R7 and R8 are as defined above; - - - - - is an optional double bond; X' is H, OR15 or NHR15, wherein R15 is H, alkyl, aryl, COalkyl, COaryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R14)_p$, PO_3H_2, $COCH(R20)NH_2$, $(CH_2)_{n'}$-NH-R14, C(=NH)NHR21, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$, $(CH_2)_mNHC(=NH)NH_2$, $(CH_2)_m$-C(=NH)NH_2, alkylNHalkyl, alkylNalkyl, alkylN(alkyl)$_2$, alkylNH_2, alkyl$NHCO_2$alkyl or Silylalkyl; wherein p, n', R14, R21 and m are as defined above; R4' is H, alkyl or aryl; R13 is halogen, $N(CH_3)_2$, OR15', NHR15' or COR15', wherein R15' is defined as is R15 and is identical or different from R15; or



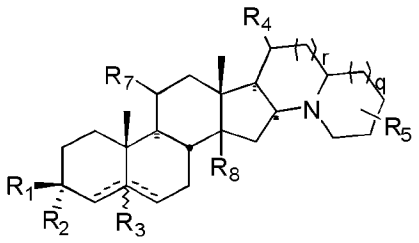
Het1 Het2 Het3 Het4 Het5 Het6

[0050] wherein W, W1, W2, W3, W4 are identical or different and are N or CH or CR16; R16 is H, alkyl, aryl, NHR15' or OR15', wherein R15' is as defined above; Y is CH_2, NH, N-alkyl, N-COalkyl, N-COaryl, N-SO_2alkyl, N-SO_2aryl, NH-C(=NH)NH_2, N-CO_2alkyl or N-CO_2aryl; and Z is NH, NR15', S or O, wherein R15' is as defined above; wherein the compound of formula 2.0 is not dihydrosolacongestidine, pregnan-3β-ol-20-amine, pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine or pregnane -3,20-diol; or



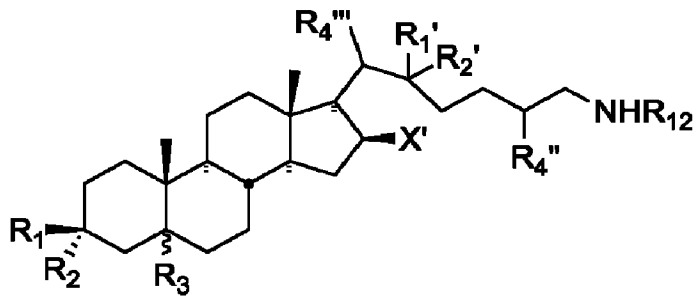
(3.0)

[0051] wherein R1, R2, R3, R7, R8, R13 and X' are as defined above; - - - - is an optional double bond; and q' and q" are identical or different and are 0-1; wherein the compound of formula 3.0 is not pregnanolone, pregnan-3β-ol-20-(aminopropyl)amine, pregnan-3β-ol-20-(aminobutyl)amine or O-*t*-butyldimethylsilylpregnanolone; or



(4.0)

[0052] wherein R1, R2, R3, R4, R5, R7, R8 are as defined above; - - - - - is an optional double bond; r is 0-5; and q is 0-5, wherein the compound of formula 4.0 is not demissidine; or



(5.0)

[0053] wherein R1, R2, R3 and R12 are as defined above; R4'' and R4''' are identifical or different and are H or CH_3; R1' and R2' are identical or different and are H, OH, Oalkyl or NHalkyl; and X' is as defined above; or a salt, stereoisomer or any mixture of stereoisomers of the compound of formula 1.0. 2.0, 3.0, 4.0 or 5.0.

[0054] In a specific embodiment, the compound is of formula 1.0 and

(i) R1 is OR12 or H;
(ii) R2 is OR12 or H;
(iii) R3 is H;
(iv) R4 is an alkyl;
(v) R5 is H;
(vi) R6 is an alkyl;
(vii) R7 is H;
(viii) R8 is H;
(ix) n is 1;
(x) X is O;
(xi) Y is NR19;
(xii) there is no double bond; or

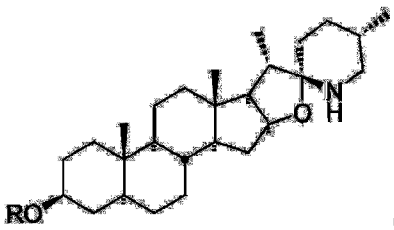
(xiii) any combination of (i) to (xii).

[0055] In another specific embodiment, the compound is of formula 1.0 and :

(xiv) R1 is OR12 and R2 is H;
(xv) R3 is H;
(xvi) R4 is CH3;
(xvii) R5 is H;
(xviii) R6 is CH3;
(xix) R7 is H;
(xx) R8 is H;
(xxi) n is 1;
(xxii) X is O;
(xxiii) Y is NR19 ;
(xxiv) there is no double bond; or
(xxv) any combination of (xiv) to (xxiv).

[0056] In another specific embodiment, the compound is of formula 1.0 and R3 is H, R4 is alkyl, R5 is H, R6 is alkyl, R7 is H, R8 is H, n is 1, X is O, Y is NR19 or $N^{+}R(19)(R19')$ and there is no double bond. In another specific embodiment, Y is NR19. In another specific embodiment, R1 is H, R2 is OR12, R4 is CH_3 and R6 is CH_3. In another specific embodiment, R1 is OR12, R2 is H, R4 is CH_3 and R6 is CH_3. In another specific embodiment, R12 is SO_3H and R19 is H. In another specific embodiment, R12 is PO_3H_2 and R19 is H. In another specific embodiment, R12 is $(CH_2)_m$-CO_2H, m is 1 and R19 is H. In another specific embodiment, R12 is $(CH_2)_mNH_2$, m is 2 and R19 is H. In another specific embodiment, R12 is alkyl, and R19 is H. In another specific embodiment, R12 is $(CH_2)_mNHC(=NH)NH_2$, m is 2 and R19 is H. In another specific embodiment, 1 is NH2 and R2 is H or R1 is H and R2 is NH2 R4 is CH_3,R6 is CH_3, and R19 is H. In another specific embodiment, R12 is a CH_3OCH_2 and R19 is H. In another specific embodiment, R12 is H and R19 is COH. In another specific embodiment, R12 is COalkyl, and R19 is COH. In another specific embodiment, COalkyl is $COCH_3$. In another specific embodiment, there is provided a methanesulfonate salt of a compound of the present inventionwherein R12 is H and R19 is H. In another specific embodiment, there is provided a citrate salt of a compound of the present invention, wherein R12 is H and R19 is H. In another specific embodiment, R1 and R2 together form =O, R4 is CH_3 and R6 is CH_3 and R19 is (C=O)H. In another specific embodiment, R1 and R2 together form =O, R4 is CH_3, R6 is CH_3 and R19 is H. In another specific embodiment, there is provided a hydrochloride salt of a compound of the present invention. In another specific embodiment, R12 is an alkyl and R19 is COH. In another specific embodiment, the alkyl is $-CH_2-CH=CH_2$. In another

specific embodiment, 12 is an alkyl and R19 is H. In another specific embodiment, In another specific embodiment, the alkyl is $-CH_2-CH=CH_2$. In another specific embodiment, there is provided a hydrochloride salt of a compound of the present invention. In another specific embodiment, there is provided the compound is of formula 1.1:



(1.1),

[0057] wherein R is defined as R12 in claim 1.

[0058] In another specific embodiment, the compound is of formula 2.0 and :

(xxvi) R1 is OR12;
(xxvii) R2 is H;
(xxviii) R3 is H or absent;
(xxix) R7 is H;
(xxx) R8 is H;
(xxxi) X' is H or OR15;
(xxxii) there is no double bond; or
(xxxiii) any combination of (xxvi) to (xxxii).

[0059] In another specific embodiment, the compound is of formula 2.0 and:

(xxxiv) R1 is OR12;
(xxxv) R2 is H;
(xxxvi) R3 is H or absent;
(xxxvii) R4' is alkyl or aralkyl
(xxxviii) R7 is H;
(xxxix) R8 is H;
(xl) X' is H or OR15;
(xli) there is no double bond; or
(xlii) any combination of (xxxiv) to (xli).

[0060] In another specific embodiment, the compound is of formula 2.0 and R1 is OR12, R2 is

H, R3 is H or absent, R7 is H, R8 is H, X' is H or OR15. In another specific embodiment, R3 is H. In another specific embodiment, R4' is alkyl. In another specific embodiment, R4' is CH_3. In another specific embodiment, R12 is H. In another specific embodiment, X' is H, there is no double bond and R13 is of formula Het1. In another specific embodiment, W1, W2 and W3 are CH, W4 is N and R16 is H. In another specific embodiment, X' is H, there is no double bond and R13 is of formula Het2. In another specific embodiment, W1 is N, W2 and W3 are CH, Z is S and R16 is H. In another specific embodiment, X' is H, there is no double bond and R13 is of formula Het4. In another specific embodiment, Y is NH and R16 is H. In another specific embodiment, X' is OR15, there is no double bond and R13 is of formula Het4. In another specific embodiment, R15 is CH_3, Y is NH and R16 is H. In another specific embodiment, R3 is absent, R4' is alkyl, X' is H, R12 is COalkyl, R13 is NHR15, and there is a double bond. In another specific embodiment, R3 is H, R4' is alkyl, X' is H, R12 is H, R13 is NHR15, and there is no double bond. In another specific embodiment, R4' is CH_3, R12 is $COCH_3$ and R15 is aryl. In another specific embodiment, the aryl is benzyl. In another specific embodiment, R4' is CH_3, R12 is $COCH_3$ and R15 is alkylN(alkyl)$_2$. In another specific embodiment, R15 is $(CH_2)_3$-N$(CH_3)_2$. In another specific embodiment, R4' is CH_3. In another specific embodiment, R3 is H, R4' is alkyl, X' is H, R12 is COalkyl, R13 is NHR15, and there is no double bond. In another specific embodiment, R3 is H, R4' is alkyl, X' is H, R12 is H, R13 is NHR15, and there is no double bond. In another specific embodiment, R4' is CH_3, R12 is $COCH_3$. In another specific embodiment, R15 is alkylNHCO_2alkyl. In another specific embodiment, R15 is $(CH_2)_2$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is $(CH_2)_3$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is $(CH_2)_4$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is alkylNHCO_2alkyl. In another specific embodiment, R15 is$(CH_2)_2$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is $(CH_2)_3$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is $(CH_2)_4$-NH$CO_2C(CH_3)_3$. In another specific embodiment, R15 is $(CH_2)_m$NH_2. In another specific embodiment, R15 is $(CH_2)_2$NH_2. In another specific embodiment, there is provided a hydrochloride salt of the compound of of the present invention. In another specific embodiment, X' is H, R13 is OR15 and there is no double bond. In another specific embodiment, R15 is H.

[0061] In another specific embodiment, the compound is of formula 3.0 and:

(xliii) R1 is OR12;

(xliv) R2 is H;

(xlv) R3 is H;

(xlvi) R7 is H;

(xlvii) R8 is H;

(xlviii) X' is H; or

(xlix) any combination of (xliii) to (xlviii).

[0062] In another specific embodiment, R1 is OR12, R2 is H, R3 is H, R7 is H, R8 is H, X' is H and there is no double bond. In another specific embodiment, R12 is H. In another specific embodiment, q' and q" are 0. In another specific embodiment, R12 is $Si(CH_3)_2C(CH_3)_3$. In another specific embodiment, q' and q" are 0. In another specific embodiment, R13 is of formula Het3. In another specific embodiment, R13 is of formula Het3. In another specific embodiment, W1 is N, W2 and W3 are CH and Z is S. In another specific embodiment, W1 is N, W2 and W3 are CH and Z is NH. In another specific embodiment, W1 is N, W2 is CR16, W3 is CH and Z is S. In another specific embodiment, R16 is NH_2.In another specific embodiment, there is provided a hydrochloride salt of the compound of the present invention. In another specific embodiment, R12 is $Si(CH_3)_2C(CH_3)_3$. In another specific embodiment, W1 is N, W2 is CR16, W3 is CH and Z is S. In another specific embodiment, R16 is NH_2. In another specific embodiment, q' and q" are 1. In another specific embodiment, q' and q" are 1. In another specific embodiment, R13 is $N(CH_3)_2$. In another specific embodiment, R13 is Het6. In another specific embodiment, W is N, Y is CH and R16 is H. In another specific embodiment, W is N, Y is NH and R16 is H. In another specific embodiment, there is provided a hydrochloride salt of a compound of of the present invention. In another specific embodiment, R13 is $NHCH_3$. In another specific embodiment, there is provided a hydrochloride salt of the compound of the present invention. In another specific embodiment, R13 is halogen. In another specific embodiment, the halogen is bromium.

[0063] In another specific embodiment, the compound is of formula 4.0 and :

(l) R1 is OR12;
(li) R2 is H;
(lii) R3 is H;
(liii) R7 is H;
(liv) R8 is H;
(lv) R12 is H;
(lvi) there is no double bond; or
(lvii) any combination of (l) to (lvi).

[0064] In another specific embodiment, the compound is of formula 5.0 and:

(lviii) R1 is OR12;
(lix) R2 is H;
(lx) R3 is H;
(lxi) R4" is H or CH_3;
(lxii) R4"' is H or CH_3;
(lxiii) X' is H or OR15;

(lxiv) any combination of (lviii) to (lxiii).

[0065] In another specific embodiment, the compound is of formula 5.0 and R1 is OR12, R2 is H, R3 is H, R4'' is H or CH_3, R4''' is H or CH_3, and X' is H or OR15.

[0066] In another specific embodiment,wherein R4'' and R4''' are CH_3. In another specific embodiment, R12 is H. In another specific embodiment, X' is OH and R1' and R2' are H.

[0067] In accordance with another aspect, there is provided a compound of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound.

[0068] In accordance with another aspect, there is provided a compound as listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with another embodiment, there is provided a compound as listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

Compositions

[0069] In accordance with another aspect of the present invention, there is provided a composition comprising the compound as defined above, and (a) an antibiotic; (b) an antiseptic; (c) a disinfectant; (d) a diluent; (e) an excipient; (f) a pharmaceutically acceptable carrier; or (g) any combination of (a)-(f).

[0070] In accordance with another aspect of the present invention, there is provided a composition comprising (A) (i) the compound of formula 1.0, 2.0, 3.0, 4.0 or 5.0 as defined herein; (ii) tomatidine; (iii) demissidine; (iv) solasodine; (v) 3α-hydroxytomatidine; (vi) 3-oxo-tomatidine; (vii) pregnanolone; (viii) pregnan-3β-ol-20-amine; (ix) pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine; (x) pregnan-3β-ol-20-(aminopropyl)amine; (xi) pregnan-3β-ol-20-(aminobutyl)amine; (xii) O-t-butyldimethylsilylpregnanolone; (xiii) pregnane -3,20-diol; (xiv) dihydrosolacongestidine; or (xv) a salt, stereoisomer or any mixture of stereoisomers of any one of (ii) to (xiv);and (B) (a) an antibiotic; (b) an antiseptic; (c) a disinfectant; (d) any combination of (a)-(c).

[0071] In a specific embodiment of the composition, said composition is a pharmaceutical composition.

[0072] In accordance with another aspect of the present invention, there is provided a composition comprising a combination of: (i) the compound as defined above; and (ii) an

aminoglycoside antimicrobial agent. In a specific embodiment of the composition, the composition further comprises (iii) an antiseptic; (iv) a disinfectant; (v) a diluent; (vi) an excipient; (vii) a pharmaceutically acceptable carrier; or (viii) any combination of (iii)-(viii).

[0073] In a specific embodiment of the composition, the aminoglycoside antimicrobial agent is amikacin, gentamicin, kanamycin, streptomycin or tobramycin. In a specific embodiment of the composition, the composition further comprises a beta-lactam antimicrobial agent. In a specific embodiment of the composition, the composition comprises a compound of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound. In another specific embodiment of the composition, the composition comprises a compound as listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with yet another embodiment,the composition comprises a compound as listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

Methods

[0074] In accordance with another aspect of the present invention, there is provided a method of preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe, said method comprising administering to said subject a therapeutically effective amount of a compound or a composition comprising the compound and a pharmaceutically acceptable carrier, the compound being: (i) of formula 1.0, 2.0, 3.0, 4.0 or 5.0 as defined herein; (ii) tomatidine; (iii) demissidine; (iv) solasodine; (v) 3α-hydroxytomatidine; (vi) 3-oxo-tomatidine; (vii) pregnanolone; (viii) pregnan-3β-ol-20-amine; (ix) pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine; (x) pregnan-3β-ol-20-(aminopropyl)amine; (xi) pregnan-3β-ol-20-(aminobutyl)amine; (xii) O-*t*-butyldimethylsilylpregnanolone; (xiii) pregnane -3,20-diol; (xiv) dihydrosolacongestidine; or (xv) a salt, stereoisomer or any mixture of stereoisomers of any one of (ii) to (xiv), whereby said bacterial infection is prevented or treated.

[0075] In accordance with another aspect of the present invention, there is provided a method of preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe, said method comprising administering to said subject a therapeutically effective amount of a compound or a composition comprising the compound and a pharmaceutically acceptable carrier, the compound being: (i) of formula 1.0, 2.0 or 3.0 as defined above; (ii) tomatidine; (iii) demissidine; (iv) solasodine; or (v) dihydrosolacongestidine, whereby said bacterial infection is prevented or treated.

[0076] In accordance with another aspect of the present invention, there is provided a method

of disinfecting and/or sterilizing an object of an electron transport-deficient microbe, said method comprising applying an effective amount of the compound as defined above or of a composition comprising said compound to said object. In a specific embodiment of the method, said object is an animal, an animal tissue, animal cells, a synthetic material or a natural material.

[0077] In a specific embodiment of the methods, the electron transport-deficient microbe is an electron transport-deficient bacterium.

[0078] In another specific embodiment of the methods, the electron transport-deficient microbe is an intracellular bacteria. In another specific embodiment of the methods, the electron transport-deficient microbe is a bacterial small-colony variant (SCV). In another specific embodiment of the methods, the SCV is a coagulase-positive or -negative staphylococci, an enterococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus* spp., a *Listeria* spp., a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the methods, SCV is of the Firmicutes phylum. In another specific embodiment of the methods, the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the methods, the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.* In another specific embodiment of the methods, the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the methods, the electron transport-deficient microbe is a staphylococci. In another specific embodiment of the methods, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the methods, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis,* a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the methods, the staphylococci is a *Staphylococcus aureus*. In another specific embodiment of the methods, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the methods, the electron transport-deficient microbe is an anaerobe bacterium. In another specific embodiment of the methods, the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium*. In another specific

embodiment of the methods, the electron transport-deficient microbe is a *Clostridium*. In another specific embodiment of the methods, the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*. In another specific embodiment of the methods, the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen. In another specific embodiment of the methods, the electron transport-deficient microbe is a bacterium that is affected by another microorganism producing at least one electron transport inhibitor.In another specific embodiment of the methods, the electron transport-deficient microbe is a bacterium that is affected by another organism producing inhibitors of the electron transport chain. In another specific embodiment of the methods, the organism producing inhibitors of the electron transport chain is *Pseudomonas aeruginosa* or any other microorganism found in polymicrobic infections and producing electron transport inhibitors. In another specific embodiment of the methods, polymicrobic infections are infections of the airways in cystic fibrosis patients, hospital-acquired pneumonia, and infections associated with burns, catheters, and endotracheal tubes.

[0079] In accordance with another aspect of the present invention, there is provided a method of preventing or treating a microbial infection caused by a bacterial pathogen in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or composition as defined above, in combination with an aminoglycoside antimicrobial agent.

[0080] In accordance with another aspect of the present invention, there is provided a method of disinfecting and/or sterilizing an object of a bacterial pathogen, said method comprising applying an effective amount of the compound as defined above or of a composition comprising the compound, in combination with an aminoglycoside antimicrobial agent to said object.

[0081] In accordance with another aspect of the present invention, there is provided a method of preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or composition as defined herein, whereby said polymicrobial infection is prevented or treated. In a specific embodiment, the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*. In another specific embodiment, the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof. In another specific embodiment, the subject has cystic fibrosis. In another specific embodiment, the subject has an polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

[0082] In another specific embodiment of the methods, said object is an animal, an animal tissue, animal cells, food (*e.g.*, packaged food preparation, meat, milk, milk products, etc.), a synthetic

material or a natural material. In another specific embodiment of the methods, the bacterial pathogen is an intracellular bacterium. In another specific embodiment of the methods, the bacterial pathogen is a coagulase-positive or -negative staphylococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium*, a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the methods, the bacterial pathogen is of the Firmicutes phylum. In another specific embodiment of the methods, the bacterial pathogen of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the methods, the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes*. In another specific embodiment of the methods, the bacterial pathogen is a *Staphylococcus aureus, Stfaphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis, Streptococcus intermedius, Bacillus subtilis*, *Bacillus anthracis, Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the methods, the bacterial pathogen is a staphylococci. In another specific embodiment of the methods, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the methods, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis,* a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the methods, the staphylococci is a *Staphylococcus aureus*. In another specific embodiment of the methods, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the methods, the aminoglycoside antimicrobial agent is amikacin, gentamicin, kanamycin, streptomycin or tobramycin. In another specific embodiment of the methods, the methods further comprise a beta-lactam antibiotic. In another specific embodiment of the methods, said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

[0083] In accordance with yet another aspect of the present invention there is provided a method for reducing the development of resistance toward aminoglycosides in a bacteria, or treating a bacteria resistant to aminoglycoside in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or composition as defined herein, whereby said development of resistance toward aminoglycosides in a bacteria is prevented or said bacteria resistant

to aminoglycoside is treated. In a specific embodiment, said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

[0084] In another specific embodiment of the methods, said subject or object is food, a cow or a human. In another specific embodiment of the methods, said subject is a human.

[0085] In a specific embodiment of the methods above, the compound is of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound. In another specific embodiment of the methods, the compound is one listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with yet another embodiment of the method, the compound is one listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

Uses

[0086] In accordance with another aspect of the present invention, there is provided a use of the compound as defined above or of a composition comprising the compound, for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from a an electron transport-deficient microbe.

[0087] In accordance with another aspect of the present invention, there is provided a use of the compound as defined above or of a composition comprising the compound, in the manufacture of a medicament for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from a an electron transport-deficient microbe.

[0088] In a specific embodiment of the uses, said object is an animal, an animal tissue, animal cells, food (*e.g.*, packaged food preparation, meat, milk, milk products, etc.), a synthetic material or a natural material. In another specific embodiment of the uses, the electron transport-deficient microbe is an electron transport-deficient bacterium. In another specific embodiment of the uses, the electron transport-deficient microbe is an intracellular bacterium. In another specific embodiment of the uses, electron transport-deficient microbe is a bacterial small-colony variant (SCV). In another specific embodiment of the uses, the SCV is a coagulase-positive or -negative staphylococci, an enterococci, a

streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium*, a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the uses, the SCV is of the Firmicutes phylum. In another specific embodiment of the uses, the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the uses, the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes*. In another specific embodiment of the uses, the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the uses, the electron transport-deficient microbe is a staphylococci. In another specific embodiment of the uses, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the uses, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the uses, the staphylococcus is a *Staphylococcus aureus*. In another specific embodiment of the uses, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the uses, the electron transport-deficient microbe is an anaerobe bacterium. In another specific embodiment of the uses, the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium*. In another specific embodiment of the uses, the electron transport-deficient microbe is a *Clostridium*. In another specific embodiment of the uses, the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*. In another specific embodiment of the uses, the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen. In another specific embodiment of the uses, the electron transport-deficient microbe is a bacterium that is affected by another microorganism producing at least one electron transport inhibitor. In another specific embodiment of the uses, the electron transport-deficient microbe is a bacterium that is affected by another organism producing inhibitors of the electron transport chain. In another specific embodiment of the uses, the organism producing inhibitors of the electron transport chain is *Pseudomonas aeruginosa* or any other microorganism found in polymicrobic infections and producing electron transport inhibitors. In another specific embodiment of the uses, polymicrobic infections is an infection of the airways of a cystic fibrosis subject, hospital-acquired pneumonia, and

infections associated with burns, catheters, and endotracheal tubes.

[0089] In accordance with another aspect of the present invention, there is provided a use of the compound as defined above or of a composition comprising the compound, in combination with an aminoglycoside antimicrobial agent, for: (a) preventing or treating a bacterial pathogen infection in a subject; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

[0090] In accordance with another aspect of the present invention, there is provided a use of the compound as defined above or of a composition comprising the compound, in combination with an aminoglycoside antimicrobial agent, in the manufacture of a medicament for: (a) preventing or treating a bacterial pathogen infection in a subject; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

[0091] In accordance with another aspect of the present invention, there is provided a use of the compound as defined herein or of a composition comprising the compound, for: (a) preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor; or (b) the disinfection, sterilization and/or antisepsis of an object from a the polymicrobial infection. In a specific embodiment, the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*. In another specific embodiment, the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof. In another specific embodiment, the polymicrobial infection is an infection of the airways of a cystic fibrosis subject. In another specific embodiment, the polymicrobial infection is a polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

[0092] In another specific embodiment of the uses, said object is an animal, an animal tissue, animal cells, a synthetic material or a natural material. In another specific embodiment of the uses, the bacterial pathogen is an intracellular bacterium. In another specific embodiment of the uses, the bacterial pathogen is a coagulase-positive or -negative staphylococci, streptococci of group A, streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the uses, the bacterial pathogen is of the Firmicutes phylum. In another specific embodiment of the uses, the bacterial pathogen of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the uses, the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.* In another specific embodiment of the uses, the bacterial pathogen is a *Staphylococcus aureus, Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*,

Staphylococcus saprophyticus, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the uses, the bacterial pathogen is a staphylococci. In another specific embodiment of the uses, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the uses, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the uses, the staphylococci is a *Staphylococcus aureus*. In another specific embodiment of the uses, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the uses, the aminoglycoside antimicrobial agent is amikacin, gentamicin, kanamycin, streptomycin or tobramycin. In another specific embodiment of the uses, the uses further comprise a beta-lactam antibiotic.

[0093] In accordance with yet another aspect of the present invention there is provided a use use of the compound as defined herein or of a composition comprising the compound, for: (a) for reducing the development of resistance toward aminoglycosides in a bacteria, or treating a bacteria resistant to aminoglycoside in a subject.

[0094] In another specific embodiment of the uses, said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube. In another specific embodiment of the uses, said subject or object is food, a cow or a human. In another specific embodiment of the uses, said subject is a human.

[0095] In a specific embodiment of the uses above, the compound is of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound. In another specific embodiment of the uses, the compound is one listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with yet another embodiment of the uses, the compound is one listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

Compounds for use

[0096] In accordance with another aspect of the present invention, there is provided a compound as defined above or of a composition comprising the compound, for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from an electron transport-deficient microbe.

[0097] In accordance with another aspect of the present invention, there is provided a compound use of the compound as defined herein or of a composition comprising the compound, for: (a) preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor; or (b) the disinfection, sterilization and/or antisepsis of an object from a the polymicrobial infection. In a specific embodiment, the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*. In another specific embodiment, the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof. In another specific embodiment, the polymicrobial infection is an infection of the airways of a cystic fibrosis subject. In another specific embodiment, the polymicrobial infection is a polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

[0098] In a specific embodiment of the compound for use, said object is an animal, an animal tissue, animal cells, food (*e.g.*, packaged food preparation, meat, milk, milk products, etc.), a synthetic material or a natural material. In another specific embodiment of the compound for use, the electron transport-deficient microbe is an electron transport-deficient bacterium. In another specific embodiment of the compound for use, the electron transport-deficient microbe is an intracellular bacterium. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a bacterial small-colony variant (SCV). In another specific embodiment of the compound for use, the SCV is a coagulase-positive or -negative staphylococci, an enterococci , a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the compounds for use, the SCV is of the Firmicutes phylum. In another specific embodiment of the compounds for use, the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the compounds for use, the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.* In another specific embodiment of the compound for use, the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*,

Streptococcus dysgalactiae, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a staphylococci. In another specific embodiment of the compound for use, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the compound for use, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the compound for use, the staphylococcus is a *Staphylococcus aureus*. In another specific embodiment of the compound for use, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the compound for use, the electron transport-deficient microbe is an anaerobe bacterium. In another specific embodiment of the compound for use, the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium*. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a *Clostridium*. In another specific embodiment of the compound for use, the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a bacterium that is affected by another microorganism producing at least one electron transport inhibitor. In another specific embodiment of the compound for use, the electron transport-deficient microbe is a bacterium that is affected by another organism producing inhibitors of the electron transport chain. In another specific embodiment of the compound for use, the organism producing inhibitors of the electron transport chain is *Pseudomonas aeruginosa* or any other microorganism found in polymicrobic infections and producing electron transport inhibitors. In another specific embodiment of the compound for use, polymicrobic infections are infections of the airways in a cystic fibrosis patient, hospital-acquired pneumonia, and infections associated with burns, catheters, and endotracheal tubes.

[0099] In accordance with another aspect of the present invention, there is provided a compound as defined in above or of a composition comprising the compound, in combination with an aminoglycoside antimicrobial agent for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by a bacterial pathogen; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

[00100] In another specific embodiment of the compound for use, said object is an animal, an animal tissue, animal cells, food, a synthetic material or a natural material. In another specific

embodiment of the compound for use, the bacterial pathogen is an intracellular bacterium. In another specific embodiment of the compound for use, the bacterial pathogen is a coagulase-positive or -negative staphylococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium*, a *Lactobacillus* or a *Gardnerella*. In another specific embodiment of the compounds for use, the bacterial pathogen is of the Firmicutes phylum. In another specific embodiment of the compounds for use, the bacterial pathogen of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.* In another specific embodiment of the compounds for use, the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes*. In another specific embodiment of the compound for use, the bacterial pathogen is a *Staphylococcus aureus, Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes, Streptococcus mitis, Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis, Streptococcus bovis, Streptococcus intermedius, Bacillus subtilis*, *Bacillus anthracis, Bacillus cereus, Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*. In another specific embodiment of the compound for use, the bacterial pathogen is a staphylococci. In another specific embodiment of the compound for use, the staphylococci is an antibiotic-resistant *Staphylococcus*. In another specific embodiment of the compound for use, the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis,* a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*. In another specific embodiment of the compound for use, the staphylococci is a *Staphylococcus aureus*. In another specific embodiment of the compound for use, said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA). In another specific embodiment of the compound for use, the aminoglycoside antimicrobial agent is amikacin, gentamicin, kanamycin, streptomycin or tobramycin. In another specific embodiment of the compound for use, the composition further comprises a beta-lactam antibiotic.

[00101] In accordance with yet another aspect of the present invention there is provided a compound for use or a composition comprising the compound for use, for: (a) for reducing the development of resistance toward aminoglycosides in a bacteria, or treating a bacteria resistant to aminoglycoside in a subject.

[00102] In another specific embodiment of the compound for use, said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an

endotracheal tube.In another specific embodiment of the compound for use, said subject or object is food, a cow or a human. In another specific embodiment of the compound for use, said subject is a human.

[00103] In a specific embodiment of the compounds for use above, the compound is of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound. In another specific embodiment of the compounds for use, the compound is one listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with yet another embodiment of thecompounds for use, the compound is one listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

Screening methods

[00104] In accordance with another aspect of the present invention, there is provided a method of identifying a pathogen, the microbial infection of which is treatable by the compound as defined above or a composition comprising the compound, said method comprising contacting said bacterial pathogen with said compound or composition and determining the effect of said compound or composition on the growth or survival of said pathogen, wherein a decrease in the growth or survival of said pathogen in the presence as compared to in the absence of said compound or composition is an indication that said bacterial pathogen is treatable by said compound or composition.

Kits

[00105] In accordance with another aspect of the present invention, there is provided a kit comprising the compound defined above or the above-mentioned composition, and instructions to use same in the prevention or treatment of a bacterial infection.

[00106] In a specific embodiment of the kit, the kit comprises: (i) one or more compounds defined above; and/or (ii) one or more compositions defined above, and instructions to use same in the prevention or treatment of a microbial infection. In another specific embodiment of the kit, the kit further comprises (iii) an antiseptic; (iv) a disinfectant; (v) a diluent; (vi) an excipient; (vii) a pharmaceutically acceptable carrier; or (viii) any combination of (iii)-(vii). In another specific embodiment of the kit, the kit comprises: (a) an antibiotic; (b) an antiseptic; (c) a disinfectant; (d) any combination of (a)-(c).

[00107] More specifically, in accordance with another aspect of the present invention, there is provided a kit comprising the compound as defined above, and instructions to use same in (a) the prevention or treatment of a microbial infection; or (b) the disinfection, sterilization and/or antisepsis of an object.

[00108] In a specific embodiment of the kit, the kit further comprises an aminoglycoside antimicrobial agent. In another specific embodiment of the kit, the aminoglycoside antimicrobial agent is amikacin, gentamicin, kanamycin, streptomycin or tobramycin. In another specific embodiment of the kit, the kit further comprises a beta-lactam antimicrobial agent.

[00109] In a specific embodiment of the kits above, the compound is of the formula1.0, 2.0, 3.0 or 5.0 as defined herein or a salt, stereoisomer or any mixture of stereoisomers of such compound. In another specific embodiment of the kits, the compound is one listed in Table 11 below or a salt, stereoisomer or any mixture of stereoisomers of such compound. In accordance with yet another embodiment of the kits, the compound is one listed in Table 11 below which has a moderate to strong potentiation activity and or a moderate to strong antibacterial activity or a salt, stereoisomer or any mixture of stereoisomers of such compound.

[00110] In a specific embodiment of the method, use and compositions for uses of the present invention, said subject is an animal (*e.g.*, cattle such as cow; goat, ewe, ass, horse, pig, cat, dog, etc.). In another specific embodiment, said subject is a cow. In another specific embodiment, said subject is a human.

[00111] Other advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of specific embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[00112] In the appended drawings:

[00113] Figures 1A-C show the effect of various compounds, including tomatidine, on the growth of *S. aureus*. Figure 1A displays pictures of samples (10 µl) from bacterial cultures treated with various concentrations of tomatidine for 48h in the Brain Hearth Infusion (BHI) medium at 35°C and spotted on agar plated. Figures 1B and 1C show the effect of tomatidine (TO), tomatine (TN) (for CF07-S only), erythromycin (ERY) or ciprofloxacin (CIP) on the growth and viability of the normal non electron transport-deficient strain CF07-L and of the electron transport-deficient SCV strain CF07-S, respectively. Concentrations of 16 µg/ml of TO (n=3), 0.5 µg/ml of ERY (n=3) and 1.0 µg/ml of CIP (n=3) were used against CF07-L (Figure 1B), whereas concentrations of 0.25 µg/ml of TO (n=4), 16 µg/ml of TN (n=3), 0.25 µg/ml of ERY (n=3) and 0.5 µg/ml of CIP (n=2) were used against the SCV strain CF07-S (Figure 1C). The no drug control experiments (Ctrl) are from 4 independent experiments (n=4).

[00114] Figure 2 shows the effect of TO and the inhibitor of the electron transport system 4-hydroxy-2-heptylquinoline-N-oxide (HQNO), each alone or in combination, on a culture of the normal *S.*

aureus strain CF07-L. TO and HQNO were used at a concentration of 8 and 20 µg/ml, respectively.

[00115] Figures 3A-C show the effect of various compounds on the biosynthesis of macromolecules of *S. aureus* in absence or presence of HQNO. In Figure 3A, the effect of control antibiotics at approximately 4XMIC (four times their minimal inhibitory concentration (MIC)) on the biosynthesis of proteins (chloramphenicol (CHL), DNA (Norfloxacin (NOR)), RNA (Rifampicin (RIF)) and cell wall peptidoglycan synthesis (Vancomycin (VAN)) was evaluated for the normal strain ATCC 29213. Figure 3B shows the effect of TO at 125 µg/ml on the biosynthesis of the same four macromolecules in ATCC 29213. Figure 3C shows the effect of different concentrations of TO on the biosynthesis of the same four macromolecules in ATCC 29213 in the presence of HQNO at 20 µg/ml. Significant decreases of the biosynthesis of proteins in comparison to the three others are indicated (*, $P<0.05$, one-way ANOVA with Dunnett's post test for A and B and two-way ANOVA with a Bonferroni's post test for C). Results are from three independent experiments and are expressed as percentages of incorporation of radiolabeled molecules by untreated (Figure 3A), DMSO-treated (Figure 3B) or HQNO-treated bacteria (Figure 3C). Data are presented as means with standard deviations.

[00116] Figures 4A-B show the effect of tomatidine on the intracellular replication of a clinical SCV strain of *S. aureus* in polarized cystic fibrosis (CF) airway epithelial cells. Figure 4A presents infection levels of polarized CF airway epithelial cells with the normal strain CF07-L and the SCV strain CF07-S, 24 and 48 h post-internalization (*, $P<0.05$; two-way ANOVA with the Bonferroni's post test). Results are from 2 to 3 independent experiments performed in duplicate. In Figure 4B, CF07-S cells treated with 1.25 and 12.5 µg/ml of tomatidine contained significantly less SCVs than DMSO-treated cells 48 h post-internalization. Data are from 3 independent experiments performed in duplicate. Significant differences in comparison to the control are shown (**, $P<0.01$; ***, $P<0.001$; one-way ANOVA with a Dunnett's post test). Data are presented as means with standard deviations.

[00117] Figures 5A-C show the effect of tomatidine and gentamicin alone or in combination on both pure and mixed cultures of normal and SCV *S. aureus* strains. Figure 5A shows a broth inoculated with the normal strain CF07-L grown in absence (–) or presence (+) of 4 µg/ml of gentamicin or 0.12 µg/ml of TO. Figure 5B shows a broth inoculated with the SCV strain CF07-S grown in absence (–) or presence (+) of 4 µg/ml of gentamicin or 0.12 µg/ml of TO. Figure 5C shows a broth inoculated with both the normal strain CF07-L and the SCV CF07-S grown in absence (–) or presence (+) of 4 µg/ml of gentamicin and/or 0.12 µg/ml of TO.

[00118] Figure 6A displays the MIC of the aminoglycoside gentamicin in absence (0 µg/ml) or presence of tomatidine (8 µg/ml) for several normal *S. aureus* strains (i.e. 8325-4, SHY-3906, CF4B-L, Sa220c, ATCC 29213, Newman, Newbould, MRSA COL, CF1A-L, CF35A-L, CF07-L, CF2A-L and

CF8E-L). In Figure 6B, the distribution of the MIC for gentamicin for these strains in absence (0 µg/ml) or presence of tomatidine (8 µg/ml) are compared. Median values (bars) of both distributions are indicated. Distributions were compared with a Mann Whitney test (***, $P < 0.001$). MIC results are presented as the means from at least two independent experiments.

[00119] Figure 7A displays the MIC of the aminoglycoside tobramycin in absence (0 µg/ml) or presence of tomatidine (8 µg/ml) for the same normal *S. aureus* strains as in Figures 6A and B (i.e. 8325-4, SHY-3906, Newman, ATCC 29213, CF07-L, Newbould, MRSA COL, CF4B-L, CF35A-L, Sa220c, CF1A-L, CF2A-L and CF8E-L). In Figure 7B, the distribution of the MIC for tobramycin among these same strains in absence (0 µg/ml) or presence of tomatidine (8 µg/ml) were compared. Median values (bars) of both distributions are indicated. Distributions were compared with a Mann Whitney test (***, $P < 0.001$). MIC results are presented as the means from at least two independent experiments.

[00120] Figure 8 shows the effect of tomatidine (at 8 µg/ml), erythromycin (at 2 to 4xMIC; 0.5 µg/ml), ciprofloxacin (at 2xMIC; 1.0 µg/ml), gentamicin (at 1/8 to 1/16xMIC; 0.06 µg/ml), and of the combination of gentamicin and tomatidine (TO) (at 0.06 and 8 µg/ml, respectively) on the growth and viability of the normal (i.e. non electron transport-deficient strain *S. aureus* ATCC 29213). The no drug control culture is also shown.

[00121] Figure 9 shows the effect of (A) gentamicin (GEN) or (B) tobramycin (TOB) (at ~1xMIC; 1 µg/ml) alone or in combination with tomatidine (TO) (at 8 µg/ml) on the growth and viability of the strain *S. aureus* ATCC 29213. The no drug control culture (Ctrl) is also shown.

[00122] Figure 10 shows the effect of gentamicin (GEN) at concentrations ranging from 0.25 to 4 µg/ml combined or not with 8 µg/ml of TO on the viability of 24 h cultures of the strain *S. aureus* ATCC 29213. Significant differences between the control (CTRL) and tomatidine (TO) conditions are shown (**P<0.01 and *P<0.05; unpaired *t*-test). Data are presented as means with standard deviations from at least two independent experiments.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Antimicrobial activity of compounds of the present invention

[00123] In certain embodiments, the present invention relates to the unexpected discovery that compounds of the present invention may have a very potent growth inhibitory activity against electron transport-deficient microbes whereas the growth of normal non electron transport-deficient bacterial strains is not significantly altered by compounds of the present invention. The action of compounds of the present invention on electron transport-deficient microbes is bacteriostatic and without being bound

by this theory, seems to results from the inhibition of the biosynthesis of macromolecules and more specifically protein biosynthesis. Furthermore, the action of compounds of the present invention have the ability to target intracellular bacteria, *i.e.*, to reach and act on bacteria even if they are present into a host cell. Thus, the antimicrobial activity of compounds of the present invention against electron transport-deficient microbes is clinically relevant (*i.e.*, requires minimal amounts of compound for potency) and is also effective against intracellular pathogenic bacteria. The clinical use of the compounds of the present invention may thus help to selectively defeat difficult-to-treat and relapsing bacterial infections caused by extracellular or intracellular electron transport-deficient microbes.

[00124] The present invention also encompasses using a compound of the present invention with another active ingredient (*e.g.*,another anibiotic agent).

Potentiating activity of compounds of the present invention

[00125] In other embodiments, the present invention hence also relates to the surprising discovery that compounds of the present invention may selectively potentiate the inhibitory activity of aminoglycoside antimicrobial agents against normal (i.e. non electron transport-deficient (*e.g.*, non-SCVs)) bacteria) such as *Staphylococcus* spp. This potentiating action may be efficient against clinical isolates that are not antibiotic resistant, but also against antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), aminoglycoside-resistant *S. aureus* and multi-resistant *S. aureus*. As aminoglycoside antimicrobial agents are currently used in clinic to treat, among others, staphylococcal infections, the use of compounds of the present invention in order to increase the potency of aminoglycoside-based antimicrobial therapies may be useful in human and veterinary medicine. In addition to increasing the potency of aminoglycoside-based therapies, compounds of the present invention used in combination with aminoglycosides may also reduce the development of resistance to aminoglycosides in bacteria. The present invention thus also relates to the use of at least one compound of the present invention in combination with an aminoglycoside antimicrobial agent to improve the antibiotic efficacy of the aminoglycoside (*i.e.*, to create a synergy and to reduce the development of resistance) in a therapeutic approach that selectively treat or prevent bacterial infections in subjects in need thereof.

Antimicrobial activity of compounds of the present invention in polymicrobic infections

[00126] In accordance with yet a further embodiment, the present invention relates to the surprising discovery that compounds of the present invention may have a very potent growth inhibitory activity against normal (i.e. non electron transport-defective bacteria (*e.g.*, non SCV *Staphylococcus* spp.)) when such bacteria are present in a polymicrobic community comprising at least one organism producing at least one inhibitor of the electron transport chain (*e.g.*, *Pseudomonas aeruginosa*). The

clinical use of compounds of the present invention, used alone or in combination with other active ingredients, may thus help to selectively defeat difficult-to-treat and relapsing polymicrobic bacterial infections.

DEFINITIONS

[00127] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one" but it is also consistent with the meaning of "one or more", "at least one", and "one or more than one".

[00128] As used herein the term "microbe" includes without being limited to a bacterium.

[00129] As used here in the term "infection" refers to a monomicrobic or a polymicrobic infection. It refers to infections involving at least one microbial target of the present invention (*e.g.*, an electron transport-deficient bacteria (SCVs, anaerobes, etc.), a bacterial pathogen targeted by aminoglycoside). In a particular embodiment, such bacteria are of the Firmicutes phylum. Without being so limited, infections targeted by the compounds of the present invention includes food-borne infections, an infection of the airways of cystic fibrosis patients, hospital-acquired pneumonia, or an infection associated with burns, implantation of catheter, or endotracheal tube, etc.

[00130] As used herein the terms "polymicrobic infection" are interchangable with the terms "mixed infection", "co-infection" or "polymicrobial infection". As used herein, they refer to a co-culture, an infection, a colonization, a community or a population of microbes of different species or strains found together either as planktonic organisms or embedded in a biofilm structure. More particularly, polymicrobic infections targeted by compounds of the present invention include at least one microorganism (*e.g.*, bacteria) producing at least one electron transport inhibitor (*e.g.*, *Pseudomonas aeruginosa* (Lightbown and Jackson, 1956; Machan *et al.*, 1992; Mitchell *et al.*, 2010b; Voggu *et al.*, 2006)) and/or at least one molecule related to 4-hydroxy-2-alkylquinolines produced by certain microorganisms (*e.g.*, bacteria) (*e.g.*, *Burkholderia* species (Vial *et al.*, 2008)). Without being so limited, such polymicrobic infections may be found in any pathologic situation where staphylococci and *P. aeruginosa* co-infect a same host (*e.g.*, cystic fibrosis and hospital-acquired infections (*e.g.*, pneumonia and infections associated with burns, catheters, and endotracheal tubes)) (Chastre and Fagon, 2002; Harlid *et al.*, 1996; Harrison, 2007; Hoffman *et al.*, 2006).

[00131] The use of the word "bacterium" in this specification and claim(s) may be interchanged with the words "bacteria", "bacterial pathogen", "infectious agent", "strain" or "bacterial strain" (*e.g.,* living either as planktonic microorganism, embedded in a biofilm structure or intracellular).

[00132] As used herein the terms "reducing the development of resistance" toward an

antimicrobial agent (*e.g.*, aminoglycoside) refers to a reduction in the number of bacteria that become resistant to the antimicrobial agent when treated with the antimicrobial agent in combination with a compound of the present invention as compared to when treated with the antimicrobial agent alone . As used herein the term "reduce", "reduction" or "decrease" or "prevention" of development of resistance toward an antimicrobial agent refers to a reduction in development of resistance toward an antimicrobial agent of at least 10% as compared to reference (*e.g.*, treatment with antimicrobial agent alone) development of resistance, in an embodiment of at least 20% lower, in a further embodiment of at least 30%, in a further embodiment of at least 40%, in a further embodiment of at least 50%, in a further embodiment of at least 60%, in a further embodiment of at least 70%, in a further embodiment of at least 80%, in a further embodiment of at least 90%, in a further embodiment of 100% (complete prevention).

[00133] As used herein, the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, un-recited elements or method steps.

Compound

[00134] As used herein, the terms "molecule", "compound" and "agent" are used interchangeably and broadly to refer to natural, synthetic or semi-synthetic molecules or compounds. The term "compound" therefore denotes, for example, chemicals, macromolecules, cell or tissue extracts (from plants or animals) and the like. Non-limiting examples of compounds include peptides, antibodies, carbohydrates, nucleic acid molecules and pharmaceutical agents. The compound can be selected and screened by a variety of means including random screening, rational selection and by rational design using, for example, ligand modeling methods such as computer modeling. As will be understood by the person of ordinary skill, molecules having non-naturally occurring modifications are also within the scope of the term "compound". For example, the compounds of the present invention can be modified to enhance their activity, stability, and/or bioavailability, and also to lower its toxicity. The compounds or molecules identified in accordance with the teachings of the present invention have a therapeutic value in diseases or conditions related to microbial infections.

[00135] As used herein the term "aryl" refers to substituted or unsubstituted aryl (*e.g.*, C5-C6), wherein the substituent, if any, is an halide, OH, OMe, NO_2, NH_2 or CO_2H, including heterocycles. Het cycles 1 (het1), 2 (het2) and 3 (het 3) defined herein are also examples of aryls.

[00136] As used herein the term "alkyl" refers to saturated or unsaturated (*e.g.*, allyle),

substituted or unsubstituted, linear or branched alkyl (C1 to C10), wherein the substituent is an halide, OH, OMe, NO_2, NH_2 or CO_2H. Without being so limited, it includes $-CH_2-CH=CH_2$, and $-(CH_2)_3-CH(CH_3)CH_2$.

[00137] As used herein the term "aralkyl" refers to a radical derived from an alkyl radical by replacing one or more hydrogen atoms by aryl groups. It includes saturated or unsaturated, substituted or unsubstituted, linear or branched aralkyl (C1 to C10), comprising wherein the substituent is an halide, OH, OMe, NO_2, NH_2 or CO_2H.

[00138] As used herein the term « CO » refers to a carbonyl.

[00139] As used herein the term "aminoglycoside" refers to an aminoglycoside antimicrobial agent and include without being so limited to amikacin, arbekacin, gentamicin, kanamycin, dideoxykanamycin, neomycin, neamine, lividomycin, butirosin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, framycetin, ribostamycin, bekanamycin, dibekacin, hygromycin B, sisomicin, isepamicin, verdamicin, astromicin, apramycin, fortimycin, sorbistin, kasugamycin, istamycin, sagamicin, spectinomycin and other known aminoglycosides. The term aminoglycoside also includes herein the 4,5-disubstituted deoxystreptamines, 4,6-disubstituted deoxystreptamines, aminocyclitols, streptidines, actinanimes, deoxystreptamines, destomycins. It also includes neoglycosides or "next-generation aminoglycosides" (*e.g.*, plazomycin, ACHN-490) namely aminoglycosides able to circumvent bacterial resistance mechanisms used against previous aminoglycosides.

[00140] As used herein the term "combination" when used in reference to the use of the compound of the invention in combination with at least one other antibiotic (*e.g.*, aminoglycoside) means i) simultaneously (*e.g.*, in separate compositions or a single composition); ii) simultaneously as a single dual action compound (*e.g.*, a conjugate of the two or more, the compound of the invention chemically linked with at least another antibiotic) in a single composition; or iii) subsequently (*e.g.*, in separate compositions wherein the compound of the present invention is administered before (*e.g.*, immediately before) or after (*e.g.*, immediately after) the at least other antibiotic).

[00141] The present invention encompasses therefore the use of a combination of two, three or more active ingredients including at least one compound of the present invention. A combination of three compounds in accordance of the present invention can include a compound of the present invention, an aminoglycoside and a beta-lactam (*e.g.*, Ubrolexin™ (*i.e.* cephalexin and kanamycin)).

Microbial Targets

[00142] Compounds of the present invention may be used as antimicrobial agents. In this

respect, the compounds of the present invention are used against "electron transport-deficient microbes". As used herein the term "electron transport-deficient microbes" refers for example to SCVs that have a defect in the electron transport chain, to bacteria that are facultative anaerobes but that are grown in anaerobic environments, to bacteria that naturally have a low redox-potential electron transport (*e.g.*, anaerobes) and to bacteria of a polymicrobic infection that have been affected by at least one electron transport inhibitor and/or at least one molecule related to 4-hydroxy-2-alkylquinolines produced by at least one microorganism (*e.g.*, bacteria) (*e.g.*, *Pseudomonas aeruginosa, Burkholderia* species) or also present in the infection. In a specific embodiment, the electron transport-deficient microbe is a gram positive bacteria.

[00143] SCVs may have a defect in the electron transport chain caused by mutation, sub-optimal expression, sub-optimal biosynthesis or alteration of electron transport proteins, necessary coenzymes, cofactors or precursors, a defect in the bacterial F_0F_1-ATPase or proton pumps or an overall reduction of certain metabolic pathways such as the tricarboxilic cycle that ultimately affects and reduces electron transport. SCVs of a variety of bacterial species of human or animal origins are thus microbial targets of the compounds of the present invention. The microbial species include but are not limited to coagulase-positive and -negative staphylococci such as *S. aureus*, *S. intermedius*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus*, *S. chromogenes*, *S. stimulans, S. saprophyticus*, *S. hominis, S. lugdunensis, S. capitis* as well as *Micrococcus luteus*. Also targeted are the enterococci (such as *E. faecium*, *E. faecalis*, *E. hirae*, *E. gallinarum*), the streptococci of group A, of group B, of the viridans group, of the mitis group, such as *Streptococcus pneumoniae*, *S. pyogenes*, *S. mitis*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. suis*, *S. bovis* and *S. intermedius*. Other SCV targets are from *Bacillus spp.*, and *Listeria spp.* that include *Bacillus subtilis*, *Bacillus anthracis, Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* and *Listeria ivanovii*, with also the inclusion of other bacterial genus like *Corynebacterium, Lactobacillus* and *Gardnerella*. The compounds of the present invention may be used against bacteria of the Firmicutes phylum. While there are currently more than 274 genera within the Firmicutes phylum, notable genera of Firmicutes include **Bacilli, order Bacillales**, *Bacillus*, *Listeria*, *Staphylococcus*, **Bacilli, order Lactobacillales**, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, **Clostridia**, *Acetobacterium*, *Clostridium*, *Eubacterium*, *Heliobacterium*, *Heliospirillum*, *Megasphaera*, *Pectinatus*, *Selenomonas*, *Zymophilus*, *Sporohalobacter*, *Sporomusa*, and **Erysipelotrichi**, *Erysipelothrix*.

[00144] Bacteria that can grow either in the presence or in absence of oxygen such as the facultative anaerobes (Ginnes and Stewart, 1996) are also microbial targets of the present invention. Such facultative anaerobe bacteria growing in an anaerobic environment are considered "electron transport-deficient microbes" since their electron transport chain is not functioning to the full potential in the absence of oxygen. For example, it has been shown that the membrane potential of the facultative

anaerobe *S. aureus* grown anaerobically causes a substantial decrease of the electrical potential across the cytoplasmic membrane (Mates *et al.*, 1983).

[00145] The terms "electron transport-deficient microbes" also refer to bacteria that naturally have a low redox-potential electron transport such as anaerobes. Such electron transport systems contain electron transport proteins with a low redox potential (ferridoxin-like and flavodoxin-like proteins) that allow energy production in the absence of oxygen. Anaerobes use fermentation or only parts of the Krebs' cycle and the electron transport system, which is leading to an energetic deficit in comparison to aerobic organism using their more complexed metabolic pathways (Black, 2008). Disease causing anaerobic bacteria such as of those of the *Clostridium* (*e.g.*, *C. difficile*, *C. perfringens*, *C. botulinum*, *C. tetani*), *Peptococcus*, *Peptostreptococcus* and *Propionibacterium* genus can thus be considered to have a defective electron transport system generating a different membrane potential and are also microbial targets of the compounds of the present invention.

[00146] The term "electron transport-deficient microbes" also refers to bacteria of a polymicrobic infection that are affected by at least one electron transport inhibitor and/or at least one molecule related to 4-hydroxy-2-alkylquinolines produced by at least one microorganism (*e.g.*, bacteria (*e.g.*, *Pseudomonas aeruginosa*, *Burkholderia* species) also present in the infection.

[00147] In a specific embodiment, electron transport-deficient microbes according to the invention are SCVs. In another embodiment, electron transport-deficient microbes according to the invention are intracellular SCVs. In another more specific embodiment, electron transport-deficient microbes according to the invention are staphylococcal SCVs. In another embodiment, the electron transport-deficient microbe according to the invention is *Staphylococcus aureus* SCV, *Staphylococcus epidermidis* SCV, another coagulase-negative staphylococci SCV, *Bacillus subtilis* SCV, *Bacillus anthracis* SCV, *Bacillus cereus* SCV, *Bacillus coagulans* SCV, *Listeria monocytogenes* SCV or *Listeria ivanovii* SCV. In another specific embodiment electron transport-deficient microbes are anaerobic bacteria (*e.g., Clostridium spp.*). In another specific embodiment electron transport-deficient microbes are facultative anaerobic bacteria grown in anaerobic environments (*e.g.*, *S. aureus*). In another specific embodiment, the electron transport-deficient microbe is a bacterium that is affected by another organism producing at list one inhibitor of the electron transport chain and/or at least one molecule related to a 4-hydroxy-2-alkylquinoline. In another specific embodiment, the organism producing at least one inhibitor of the electron transport chain is *Pseudomonas aeruginosa* or any other microorganism found in the polymicrobic infection and producing at least one electron transport inhibitor. In another specific embodiment, the polymicrobic infection is an infection of the airways of cystic fibrosis patients, hospital-acquired pneumonia, or an infection associated with burns, implantation of catheter, or endotracheal tube.

[00148] Compounds of the present invention may also be used as potentiators of antimicrobial agents. As used herein, the term "potentiator" in the context of an "antimicrobial agent potentiator" refers to an agent which increases the antimicrobial activity of another antimicrobial agent on a bacterium and thus creates a synergy, *i.e.*, the activity of the combination of agents is superior to that observed for either agent individually.

[00149] In this respect, the compounds of the present invention may be used in combination with aminoglycosides against "normal" (i.e. non electron transport-deficient) bacterial targets of human or animal origins that include but are not limited to coagulase-positive and -negative staphylococci such as *S. aureus*, *S. intermedius*, *S. epidermidis*, *S. haemolyticus*, *S. hyicus*, *S. chromogenes*, *S. stimulans*, *S. saprophyticus*, *S. hominis*, *S. lugdunensis*, *S. capitis* as well as against *Micrococcus luteus*. Also targeted are the streptococci of group A, of group B, of the viridans group, of the mitis group, such as *S. pneumoniae*, *S. pyogenes*, *S. mitis*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. suis*, *S. bovis* and *S. intermedius*. Other bacterial targets of the compounds in combination with aminoglycosides are *Bacillus spp.*, and *Listeria spp.* that include *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* and *Listeria ivanovii*, with also the inclusion of other bacterial genus like *Corynebacterium*, *Lactobacillus* and *Gardnerella*. In a specific embodiment, the non electron transport-deficient target of the compounds of the invention as potentiators of aminoglycosides is a gram positive bacteria.

[00150] In a particular embodiment, the compounds of the present invention are used as potentiators of aminoglycosides against normal staphylococcal strains (*e.g.*, *Staphyloccocus aureus*, *Staphyloccocus epidermidis*) and other coagulase-negative staphylococci strains.

Subjects and objects

[00151] As used herein the term "object" refers to an animal or to an animal tissue (*e.g.*, skin, hands), an animal cells (*e.g.*, in cell cultures for laboratory purpose or for use for administration to subjects), food (*e.g.*, packaged food preparation, meat, milk, milk products, etc.), a synthetic material or a natural material. Synthetic materials include, without being so limited, working surfaces (*e.g.*, table, counter), instruments, prosthetic devices and biomaterials. The term "Natural material" includes, without being so limited, skin grafts, tissue cultures and organs.

[00152] As used herein the term "subject" or "patient" refers to an animal, preferably a mammal such as but not limited to a human, cow, goat, ewe, ass, horse, pig, chicken, cat, dog, etc. who is the object of treatment, observation or experiment.

Excipients/carriers

[00153] As used herein, the terms "pharmaceutically acceptable" refer to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to animals (*e.g.*, cows, humans). Preferably, as used herein, the term "pharmaceutically acceptable" means approved by regulatory agency of the federal or state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[00154] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compounds of the present invention may be administered. Sterile water or aqueous saline solutions and aqueous dextrose and glycerol solutions may be employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin.

Routes of administration

[00155] Compounds of the invention may be administered in a pharmaceutical composition. Pharmaceutical compositions may be administered in unit dosage form. Any appropriate route of administration may be employed, for example, transdermal (topical), parenteral, subcutaneous, intramuscular, intramammary, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraarticular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, or oral administration. Examples of specific routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, intramammary; oral (*e.g.*, inhalation); transdermal (topical); transmucosal, and rectal administration.

[00156] Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer such compositions to patients. Methods well known in the art for making pharmaceutical compositions and formulations are found in, for example, Remington: The Science and Practice of Pharmacy, (20th ed.) ed. A. R. Gennaro A R., 2000, Lippincott: Philadelphia.

Formulations

[00157] Therapeutic formulations for oral administration, may be in the form of tablets or capsules; for transmucosal (*e.g.*, rectal, intranasal) or transdermal/percutaneous administration may be in the form of ointments, powders, nasal drops, sprays/aerosols or suppositories; for topical administration, may be in the form of ointments, creams, gels or solutions; for parenteral administration (*e.g.*, intravenously, intramuscularly, intradermal, intramammary, subcutaneously, intrathecally or transdermally), using for example injectable solutions. Furthermore, administration can be carried out sublingually or as ophthalmological preparations or as an aerosol, for example in the form of a spray.

Intravenous, intramuscular or oral administration is a preferred form of use.

[00158] The pharmaceutical compositions of the present invention may also contain excipients/carriers such as preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, odorants, salts for the variation of osmotic pressure, buffers, coating agents or antioxidants. As mentioned earlier, they may also contain other therapeutically valuable agents.

Oral

[00159] For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used for example in the form of tablets, troches, dragees, hard or soft gelatin capsules, solutions (*e.g.*, syrups), aerosols, emulsions or suspensions, or capsules. For the preparation of formulations for oral administration, the compounds of the present invention may be admixed with pharmaceutically inert, inorganic or organic excipients (*e.g.*, pharmaceutically compatible binding agents, and/or adjuvant). The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring. Examples of suitable excipients for tablets, dragees or hard gelatin capsules for example include lactose, maize starch or derivatives thereof, talc or stearic acid or salts thereof. Suitable excipients for use with soft gelatin capsules include for example vegetable oils, waxes, fats, semi-solid or liquid polyols etc.; according to the nature of the active ingredients it may however be the case that no excipient is needed at all for soft gelatin capsules.

[00160] For the preparation of solutions and syrups, excipients which may be used include for example water, polyols, saccharose, invert sugar and glucose.

Nasal

[00161] For administration by inhalation, the compounds may be delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Formulations for inhalation may contain excipients, for example, lactose, or may be aqueous solutions containing, for example, polyoxyethylene-9-lauryl ether, glycocholate and deoxycholate, or may be oily solutions for administration in the form of nasal drops, or as a gel.

Transmucosal or transdermal (topical)

[00162] For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. For suppositories, and local or percutaneous application, excipients which may be used include for example natural or hardened oils, waxes, fats and semi-solid or liquid polyols.

Parenteral

[00163] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Solutions or suspensions used for parenteral application can include the following components: a sterile diluent such as water for injection (where water soluble), saline solution, fixed oils (*e.g.*, paraffin oil), polyalkylene glycols such as polyethylene glycols, glycerine, propylene glycol or other synthetic solvents, oils of vegetable origin, or hydrogenated napthalenes; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; reducing agents such as dithiothreitol, buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be used to control the release of the compounds. Other potentially useful parenteral delivery systems for compounds of the invention include ethylenevinyl acetate copolymer particles, osmotic pumps, implantable infusion systems, and liposomes. The parenteral preparation can also be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00164] For intravenous or intramammary administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS).

[00165] Liposomal suspensions (including liposomes targeted to specific cell types) can also be used as pharmaceutically acceptable carriers. A variety of liposomal formulations suitable for delivering a compound to an animal have been described and demonstrated to be effective in delivering a variety of compound, including, *e.g.*, small molecules, nucleic acids, and polypeptides.

[00166] As mentioned earlier, medicaments containing the compounds of the present invention

are also an object of the present invention, as is a process for the manufacture of such medicaments, which process comprises bringing one or more of the compounds of the present invention to, if desired, one or more other therapeutically valuable substances into a galenical administration form.

Compounds

Protective group

[00167] The compounds of the present invention may include protective groups. As used herein, and without being so limited, the term "protective group" is meant to refer to a substituent on a heteroatom that may be cleaved in specified reaction conditions to unmask the heteroatom and includes without being so limited tert-butoxycarbonyle (BOC), *t*-butyldimethylsilyl (TBDMS), methoxymethyl (MOM), etc. Further examples of protecting groups may be found in Protective groups in organic synthesis, 4th edition, Peter G. M. Wuts & Theodora W. Greene editors, Wiley 2007.

Salts, esters, hydrates and solvates

[00168] The compounds of the present invention include pharmacologically acceptable salts and ester derivatives thereof as well as hydrates or solvates thereof and all stereoisomeric forms of the referenced compounds. The compounds and pharmacologically acceptable esters thereof of the present invention can form pharmacologically acceptable salts if necessary.

Salts

[00169] The terms "pharmacologically acceptable salt thereof" refer to a salt to which the compounds of the present invention can be converted. Preferred examples of such a salt include alkali metal salts such as a sodium salt, a potassium salt, a lithium salt, magnesium or calcium salts; alkaline earth metal salts such as a calcium salt and a magnesium salt; metal salts such as an aluminium salt, an iron salt, a zinc salt, a copper salt, a nickel salt and a cobalt salt; amine salts such as inorganic salts including an ammonium salt; organic salts or ammonium salts such as a t-octylamine salt, a dibenzylamine salt, a morpholine salt, a glucosamine salt, a phenylglycine alkyl ester salt, an ethylenediamine salt, an N-methylglucamine salt, a guanidine salt, a diethylamine salt, a triethylamine salt, a dicyclohexylamine salt, an N,N'-dibenzylethylenediamine salt, a chloroprocaine salt, a procaine salt, a diethanolamine salt, an N-benzyl-phenethylamine salt, a piperazine salt, a tetramethylammonium salt and a tris(hydroxymethyl)aminomethane salt; inorganic acid salts such as hydrohalic acid salts such as a hydrofluoride, a hydrochloride, a hydrobromide or a hydroiodide, a nitrate, a perchlorate, a sulfate or a phosphate; lower alkanesulfonates such as a methanesulfonate (mesylate), trifluoromethanesulfonate or an ethanesulfonate; arylsulfonates such as a benzenesulfonate or a p-toluenesulfonate and the like, which are non toxic to living organisms; organic acid salts such as an

acetate, a malate, adipate, a fumarate, a succinate, a citrate, alginate, ascorbate, benzoate, benzenesulfonate, bisulfate, butyrate, camphorate, camphorsulfonate, cinnamate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, itaconate, lactate, maleate, mandelate, sulfonate, methanesulfonate, trifluoromethanesulfonates, ethanesulfonates 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, tartrate, thiocyanate, tosylate, and undecanoate, a tartrate, an oxalate or a maleate; and amino acid salts such as a glycine salt, a lysine salt, an arginine salt, an ornithine salt, histidine, a glutamate or an aspartate salt. Additionally, basic nitrogen containing groups may be quaternized with such agents as lower alkyl halides such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates including dimethyl, diethyl, and dibutyl sulfate; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and strearyl chlorides, bromides and iodides, aralkyl halides including benzyl and phenethyl bromides, and others. For further example, see S. M. Berge, et al. "Pharmaceutical Salts," J. Pharm. Sci. 1977, 66, 1-19. Such salts can be formed quite readily by those skilled in the art using standard techniques.

[00170] More specific examples of the salts formed with an acidic group present in the compounds of the present invention include metal salts such as alkali metal salts (*e.g.*, sodium salts, potassium salts and lithium salts), alkali earth metal salts (*e.g.*, calcium salts and magnesium salts), aluminum salts and iron salts; amine salts such as inorganic amine salts (*e.g.*, ammonium salts) and organic amine salts (*e.g.*, t-octylamine salts, dibenzylamine salts, morpholine salts, glucosamine salts, phenylglycinealkyl ester salts, ethylenediamine salts, N-methylglucamine salts, guanidine salts, diethylamine salts, triethylamine salts, dicyclohexylamine salts, N,N'-dibenzylethylenediamine salts, chloroprocaine salts, procaine salts, diethanolamine salts. N-benzylphenethylamine salts, piperazine salts, tetramethylammonium salts and tris(hydroxymethyl)aminomethane salts; and amino acid salts such as glycine salts, lysine salts, arginine salts, ornithine salts, glutamates and aspartates.

[00171] All salts are intended to be pharmaceutically acceptable salts within the scope of the invention and all salts are considered equivalent to the free forms of the corresponding compounds for purposes of the invention.

Esters

[00172] Physiologically/pharmaceutically acceptable esters are also useful as active medicaments. The term "pharmaceutically acceptable esters" embraces esters of the compounds of the present invention, in which hydroxy groups (*e.g.*, in carboxylic acid) have been converted to the

corresponding esters and may act as a prodrug which, when absorbed into the bloodstream of a warm-blooded animal, may cleave in such a manner as to release the drug form and permit the drug to afford improved therapeutic efficacy. Such esters can be formed with inorganic or organic acids such as nitric acid, sulphuric acid, phosphoric acid, citric acid, formic acid, maleic acid, acetic acid, succinic acid, tartaric acid, methanesulphonic acid, p-toluenesulphonic acid and the like, which are non toxic to living organisms. Further examples are the esters with aliphatic or aromatic acids such as acetic acid or with aliphatic alcohol (*e.g.*, alkyl esters, including methyl, ethyl, propyl, isopropyl, butyl, isobutyl or pentyl esters, and the like) or aromatic alcohols (*e.g.*, benzyl ester).

[00173] Esters can be prepared from their corresponding acids or salts by a variety of methods known to those skilled in the art, such as, for example, by first transforming the acid to the acid chloride and then reacting the acid chloride with a suitable alcohol. Other suitable methods for making esters are described in Kemp and Vellaccio, 1980.

[00174] Where esters of the invention have a basic group, such as an amino group, the compound can be converted to a salt by reacting it with an acid, and in the case where the esters have an acidic group, such as a sulfonamide group, the compound can be converted to a salt by reacting it with a base. The compounds of the present invention encompass such salts.

[00175] Salts and esters of the compounds of the present invention may be prepared by known method by employing appropriate starting materials or intermediate compounds that are readily available and/or are described herein.

[00176] Generally, a desired salt of a compound of this invention can be prepared *in situ* during the final isolation and purification of a compound by means well known in the art. For example, a desired salt can be prepared by separately reacting the purified compound in its free base or free acid form with a suitable organic or inorganic acid, or suitable organic or inorganic base, respectively, and isolating the salt thus formed. In the case of basic compounds, for example, the free base is treated with anhydrous HCl in a suitable solvent such as THF, and the salt isolated as a hydrochloride salt. In the case of acidic compounds, the salts may be obtained, for example, by treatment of the free acid with anhydrous ammonia in a suitable solvent such as ether and subsequent isolation of the ammonium salt. These methods are conventional and would be readily apparent to one skilled in the art.

[00177] The compounds of this invention may be esterified by a variety of conventional procedures including reacting the appropriate anhydride, carboxylic acid or acid chloride with the alcohol group of a compound of this invention. The appropriate anhydride is reacted with the alcohol in the presence of a base to facilitate acylation such as 1,8-bis[dimethylamino]naphthalene or N,N-dimethylaminopyridine. Or, an appropriate carboxylic acid can be reacted with the alcohol in the

presence of a dehydrating agent such as dicyclohexylcarbodiimide, 1-[3-dimethylaminopropyl]-3-ethylcarbodiimide or other water soluble dehydrating agents which are used to drive the reaction by the removal of water, and, optionally, an acylation catalyst. Esterification can also be effected using the appropriate carboxylic acid in the presence of trifluoroacetic anhydride and, optionally, pyridine, or in the presence of N,N-carbonyldiimidazole with pyridine. Reaction of an acid chloride with the alcohol can be carried out with an acylation catalyst such as 4-DMAP or pyridine.

[00178] One skilled in the art would readily know how to successfully carry out these as well as other known methods of etherification of alcohols.

Hydrates

[00179] As used herein the terms, "pharmaceutically acceptable hydrate" refer to the compounds of the instant invention crystallized with one or more molecules of water to form a hydrated form.

Prodrugs and solvates

[00180] Prodrugs and solvates of the compounds of the invention are also contemplated herein. A discussion of prodrugs is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems (1987) 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, (1987) Edward B. Roche, ed., American Pharmaceutical Association and Pergamon Press. The term "prodrug" means a compound (*e.g.*, a drug precursor) that is transformed *in vivo* to yield a compound of the present invention or a pharmaceutically acceptable salt, hydrate or solvate of the compound. The transformation may occur by various mechanisms (*e.g.*, by metabolic or chemical processes), such as, for example, through hydrolysis in blood. A discussion of the use of prodrugs is provided by T. Higuchi and W. Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in Bioreversible Carriers in Drug Design, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987.

[00181] For example, if a compound of the present invention or a pharmaceutically acceptable salt, hydrate or solvate of the compound contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a group such as, for example, (C1–C8)alkyl, (C2–C12)alkanoyloxymethyl, 1-(alkanoyloxy)ethyl having from 4 to 9 carbon atoms, 1-methyl-1-(alkanoyloxy)-ethyl having from 5 to 10 carbon atoms, alkoxycarbonyloxymethyl having from 3 to 6 carbon atoms, 1-(alkoxycarbonyloxy)ethyl having from 4 to 7 carbon atoms, 1-methyl-1-(alkoxycarbonyloxy)ethyl having from 5 to 8 carbon atoms, N-(alkoxycarbonyl)aminomethyl having from 3 to 9 carbon atoms, 1-(N-(alkoxycarbonyl)amino)ethyl having

from 4 to 10 carbon atoms, 3-phthalidyl, 4-crotonolactonyl, gamma-butyrolacton-4-yl, di-N,N-(C1–C2)alkylamino(C2–C3)alkyl (such as β-dimethylaminoethyl), carbamoyl-(C1–C2)alkyl, N,N-di(C1–C2)alkylcarbamoyl-(C1–C2)alkyl and piperidino-, pyrrolidino- or morpholino(C2–C3)alkyl, and the like.

[00182] Similarly, if a compound of the present invention contains an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a group such as, for example, (C1–C6)alkanoyloxymethyl, 1-((C1–C6)alkanoyloxy)ethyl, 1-methyl-1-((C1–C6)alkanoyloxy)ethyl, (C1–C6)alkoxycarbonyloxymethyl, N-(C1–C6)alkoxycarbonylaminomethyl, succinoyl, (C1–C6)alkanoyl, α-amino(C1–C4)alkanyl, arylacyl and α-aminoacyl, or α-aminoacyl-α-aminoacyl, where each α-aminoacyl group is independently selected from the naturally occurring L-amino acids, P(O)(OH)2, —P(O)(O(C1–C6)alkyl)2 or glycosyl (the radical resulting from the removal of a hydroxyl group of the hemiacetal form of a carbohydrate), and the like.

[00183] If a compound of the present invention incorporates an amine functional group, a prodrug can be formed by the replacement of a hydrogen atom in the amine group with a group such as, for example, R-carbonyl, RO-carbonyl, NRR'-carbonyl where R and R' are each independently (C1–C10)alkyl, (C3–C7)cycloalkyl, benzyl, or R-carbonyl is a natural α-aminoacyl or natural β-aminoacyl, —C(OH)COOY1 wherein Y1 is H, (C1–C6)alkyl or benzyl, —C(OY2)Y3 wherein Y2 is (C1–C4) alkyl and Y3 is (C1–C6)alkyl, carboxy (C1–C6)alkyl, amino(C1–C4)alkyl or mono-N— or di-N,N-(C1–C6)alkylaminoalkyl, —C(Y4)Y5 wherein Y4 is H or methyl and Y5 is mono-N— or di-N,N-(C1–C6)alkylamino morpholino, piperidin-1-yl or pyrrolidin-1-yl, and the like.

[00184] One or more compounds of the invention may exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, and it is intended that the invention embrace both solvated and unsolvated forms. "Solvate" means a physical association of a compound of this invention with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like. "Hydrate" is a solvate wherein the solvent molecule is H_2O.

[00185] One or more compounds of the invention may optionally be converted to a solvate. Preparation of solvates is generally known. Thus, for example, M. Caira et al, J. Pharmaceutical Sci., 93(3), 601–611 (2004) describe the preparation of the solvates of the antifungal fluconazole in ethyl acetate as well as from water. Similar preparations of solvates, hemisolvate, hydrates and the like are described by E. C. van Tonder et al, AAPS Pharm Sci Tech., 5(1), article 12 (2004); and A. L. Bingham

et al, Chem. Commun., 603–604 (2001). A typical, non-limiting, process involves dissolving the inventive compound in desired amounts of the desired solvent (organic or water or mixtures thereof) at a higher than ambient temperature, and cooling the solution at a rate sufficient to form crystals which are then isolated by standard methods. Analytical techniques such as, for example I. R. spectroscopy, show the presence of the solvent (or water) in the crystals as a solvate (or hydrate).

Stereoisomers, diastereomers, enantiomers, racemates, tautomers

[00186] The compounds of the present invention have asymmetric carbon atoms and can exist in the form of stereoisomers (*e.g.*, diastereomers, optically pure enantiomers) or as racemates or mixtures of two or more stereoisomers of each compound.. The term "compound" as used herein embraces all of these forms.

[00187] Diastereomers (sometimes called diastereoisomers) are stereoisomers that are not enantiomers. Diastereomerism occurs when two or more stereoisomers of a compound have different configurations at one or more (but not all) of the equivalent (related) stereocenters and are not mirror images of each other. When two diastereoisomers differ from each other at only one stereocenter they are epimers. Each stereocenter gives rise to two different configurations and thus to two different stereoisomers.

[00188] Diastereomers differ from enantiomers in that the latter are pairs of stereoisomers which differ in all stereocenters and are therefore mirror images of one another. Enantiomers of a compound with more than one stereocenter are also diastereomers of the other stereoisomers of that compound that are not their mirror image. Diastereomers have different physical properties and different reactivity, unlike enantiomers. Diastereomers of the present invention include tomatidine and 3-alpha-hydroxy-tomatidine for example.

[00189] For purposes of this Specification, "pharmaceutically acceptable tautomer" means any tautomeric form of any compound of the present invention.

[00190] The purification of enantiomers and the separation of isomeric mixtures of a compound of the present invention may be accomplished by standard techniques known in the art.

Dosages

[00191] The dosages in which the compounds of the present invention are administered in effective amounts depend on the nature of the specific active ingredient, the body weight, the age and the requirements of the patient and the mode of application. In general, daily dosages of about 1 mg - 5000 mg, preferably 5 mg - 500 mg, per day come into consideration.

[00192] The skilled artisan will appreciate that certain factors may influence the dosage required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a compound of the present invention can include a series of treatments.

Toxicity and therapeutic efficacy

[00193] Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00194] Data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

Kits

[00195] The present invention also encompasses kits comprising the compounds of the present invention. For example, the kit can comprise one or more compounds inhibiting the growth of electron transport-deficient microbes (*e.g.*, SCVs) or potentiating the antimicrobial activity of aminoglycoside antibiotics against normal bacterial strains (*e.g.*, staphylococci). The kit may optionally include one or more control sample(s). The compounds or agents can be packaged in a suitable container. The kit can further comprise instructions for using the kit.

[00196] The present invention also relates to methods for preparing the above-mentioned

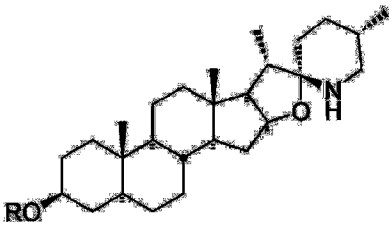
compounds.

[00197] The present invention is illustrated in further details by the following non-limiting examples.

EXAMPLE 1

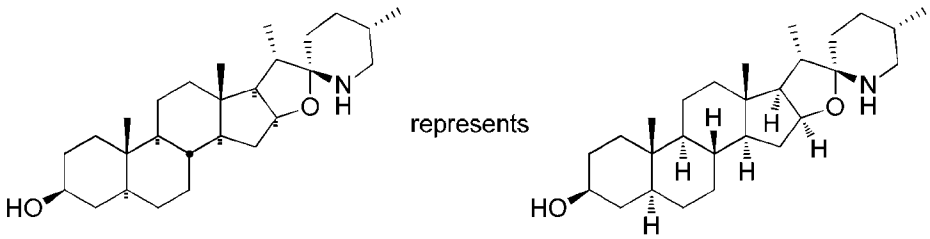
Antibacterial activity of tomatidine against electron transport-deficient *Staphylococcus aureus* small-colony variants (SCVs) measured with MIC

[00198] Tomatidine (formula 1.1 wherein R is H) specifically and selectively inhibits the growth of *S. aureus* SCVs whereas it has no significant impact on the growth of normal *S. aureus* strains.



(1.1)

[00199] The symbols used herein to denote the orientation of the hydrogen atoms are those used in the tomatidine formula presented below at the left, wherein "=" denotes □-H and "•" □-H. They are used to identify the stereochemistry of tertiary carbons (having three direct neighbors other than hydrogens). The classical representation of the hydrogens is shown in the right for comparison purposes. Such convention is used to simplify the formulas.

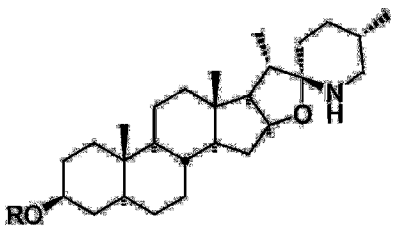


Tomatidine

[00200] **Method:** The minimal inhibitory concentrations (MICs) (i.e. lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation), of tomatidine (formula 1.1 above, wherein R is H), tomatine (formula 1.2 below) and control antibiotics (gentamicin, vancomycin, erythromycin, ciprofloxacin and oxacillin) were determined against "normal" (i.e. non electron transport-deficient) (ATCC 29213, Newbould, CF07-L and CF1A-L) and electron transport-deficient SCV (NewbouldΔ*hemB*, CF07-S and CF1D-S) *S. aureus* strains. Of note, CF07-L and CF07-S

and CF1A-L and CF1D-S are genetically-related pairs of strains co-isolated from CF patients (Mitchell *et al.*, 2010b) whereas Newbould is a laboratory strain of bovine origin (ATCC 29740). Results are reported in Table 1 below.

[00201] **Results:** Table 1 below shows that tomatidine's MIC against all SCVs was remarkably low (0.12 µg/ml) whereas no clinically significant MIC was measurable for normal strains. Also, no MIC was observed for tomatine, the lycotetraose-substituted derivative of tomatidine, against SCVs, which confirmed the specificity of the growth inhibitory activity of tomatidine against SCVs. MICs of gentamicin for the different strains were in accordance with the known decreased susceptibility of SCVs to aminoglycosides (Proctor *et al.*, 2006). The MIC of erythromycin against the laboratory-derived SCV strain NewbouldΔ*hemB* (>16 µg/ml) is explained by the insertion of the macrolide resistance gene *ermA* in the *hemB* gene of this strain to create the SCV phenotype (defective electron transport chain and respiratory deficiency) through inactivation of hemin biosynthesis (Brouillette *et al.*, 2004). MICs obtained for the other control antibiotics were in the expected Clinical and Laboratory Standards Institute (CLSI) (2006) ranges and did not seem to vary significantly among strains. Briefly, MICs were determined using the microdilution method in 96-well microplates. Bacteria were inoculated at ~10^5-10^6 CFU/ml and incubated at 35ºC for 48h in brain heart infusion (BHI) broth (BD, Mississauga, ON, Canada) in order to allow SCVs to reach maximal growth as previously described (Atalla *et al.*, 2008; Mitchell *et al.*, 2010b). Then OD_{595nm} was read on a microplate reader. The MICs obtained against the quality control strain ATCC 29213 for all antibiotics tested were similar in BHI and in cation-adjusted Mueller-Hinton broth (CAMHB) (BD) showing that the type of cultivation medium did not influence results.



(1.2)

R is lycotetraose

[00202] TABLE 1: Susceptibility (MIC in µg/ml) of normal and SCV *S. aureus* strains to tomatidine, tomatine and control antibiotics.

Strain[a]	TO	TN	GEN	VAN	ERY	CIP	OXA
ATCC 29213 in BHI	>16	>16	1	2	0.12-0.25	0.5	0.12-0.25
ATCC 29213 in MHBCA	>16	>16	0.5-1	1-2	0.12-0.5	0.5-1	0.12-0.5
Newbould	>16	>16	0.5-1	1	0.25	0.25-0.5	0.06-0.12
NewbouldΔ*hemB*	0.12	>16	4-8	2	>16	0.12-0.25	0.03-0.06
CF07-L	>16	>16	1-2	2	0.25	0.5	0.06-0.12
CF07-S	0.12	>16	8	2	0.12	0.12	0.06-0.12
CF1A-L	>16	>16	1-2	1-2	0.25	0.5	0.25
CF1D-S	0.12	>16	8	2	0.12	0.12	0.06-0.12

[a]ATCC 29213, Newbould, CF07-L and CF1A-L are normal strains whereas NewbouldΔ*hemB*, CF07-S and CF1D-S are SCVs.

TO: tomatidine, TN: tomatine, GEN: gentamicin, VAN: vancomycin, ERY: erythromycin, CIP: ciprofloxacin, OXA: oxacillin.

EXAMPLE 2

Antibacterial activity of tomatidine against electron transport-deficient *Staphylococcus aureus* small-colony variants (SCVs) and against the anaerobic bacterium *Clostridium perfringens* measured with an agar diffusion method

[00203] Tomatidine (formula 1.1, wherein R is H) specifically and selectively inhibits the growth of all types of *S. aureus* SCVs whereas it has no significant impact on the growth of normal *S. aureus* strains. The growth of the anaerobic strain *C. perfringens* (also considered herein to be electron transport-deficient) is also inhibited by tomatidine.

[00204] **Method:** The susceptibility of various *S. aureus* SCVs as well as of the anaerobe strain *Clostridium perfringens* ATCC 13124 to tomatidine was tested by an agar diffusion method. *S. aureus* strains SCV NewbouldΔ*hemB* (hemin auxotroph), SCV CF07-S (menadione auxotroph), SCV CF6A-S (thymidine auxotroph), SCV CF41A-S (unknown auxotrophy), and strain *C. perfringens* ATCC 13124 were spread on the surface of Tryptic Soy agar plates and 50 µg of tomatidine diluted in DMSO was added to wells for diffusion. After incubation in aerobic conditions for *S. aureus* and anaerobic conditions for *C. perfringens* (using the Anaero pack system no.10-01, Mitsubishi gas chemical co., Tokyo), the diameters of the zones of inhibition around the wells (for the DMSO control and for the tomatidine well) were measured and reported in mm in Table 2.

[00205] **Results:** Table 2 shows the diameters of the zones of inhibition caused by tomatidine against various *S. aureus* SCVs as well as against an anaerobe, *C. perfringens*. Results show that all types of *S. aureus* SCVs, whether they are hemin (NewbouldΔ*hemB*), menadione (CF07-S), thymidine (CF6A-S) or unknown auxotroph (CF41A-S), are all susceptible to the inhibitory action of tomatidine. This is also true for the *S. aureus* SCV strain CF6A-S which is multi-resistant to several antibiotics such

as tobramycin (MIC >32 µg/ml), gentamicin (MIC >32 µg/ml) as well as trimetoprim (MIC >32 µg/ml). Also, as it did against the electron transport-deficient *S. aureus* SCVs, tomatidine caused a growth inhibition against the anaerobic strain *C. perfringens*, which naturally possess a low redox-potential electron transport.

[00206] TABLE 2: Diameters of the zone of inhibition (in mm) caused by tomatidine on a variety of *S. aureus* SCVs and against the anaerobic strain *C. perfringens*.

Antibiotic effect of tomatidine on *Staphylococcus aureus* SCVs and *Clostridium perfringens*				
Organism	Strain	Auxotrophy	Diameter of inhibition zone (mm)	
			Control (DMSO)	Tomatidine (50 µg)
Staphylococcus aureus				
	SCV Newbould Δ*hemB*	hemin	0	23.5
	SCV CF07S	menadione	0	22.5
	SCV CF6A-S	thymidine	0	23.0
	SCV CF41A-S	unknown	0	21.5
Clostridium perfringens				
	ATCC 13124		6.5	11
S. aureus strains were incubated for 24 hours at 37°C with O_2.				
C. perfringens was incubated for 48 hours at 37°C without O_2.				

EXAMPLE 3

Effect of inducing an electron transport chain defect in normal *Staphylococcus aureus* strains on their susceptibility to tomatidine

[00207] The inhibition of electron transport by 4-hydroxy-2-heptylquinoline-N-oxide (HQNO), a known electron transport inhibitor (Hoffman *et al.*, 2006; Mitchell *et al.*, 2010b), sensitizes normal strains to tomatidine. This shows that tomatidine possesses a specific antibacterial activity against strains that have a defective electron transport system like SCVs.

[00208] **Method:** The MICs of tomatidine, tomatine and control antibiotics (gentamicin, vancomycin, erythromycin, ciprofloxacin and oxacillin) were determined against the normal strains ATCC 29213 and CF07-L as well as against the SCV strain CF07-S in the presence of 20 µg HQNO/ml. Results are reported in Table 3 below. Also, the normal *S. aureus* strain CF07-L was inoculated at ~10^5-10^6 CFU/ml in BHI in absence or presence of HQNO and/or tomatidine at 20 µg/ml and 8 µg/ml, respectively. Cultures were incubated 48 h at 35°C/225 RPM and the growth was visually evaluated. Results are reported in Figure 2.

[00209] **Results:** As shown in Table 3 below, HQNO allowed tomatidine to inhibit the growth of

normal strains as it does of SCVs. HQNO did not however alter the susceptibility of SCVs, which already have an altered electron transport, to tomatidine or any other antibiotic. HQNO also increased resistance of normal strains to the aminoglycoside gentamicin (see also (Hoffman *et al.*, 2006)), further supporting that the effect of HQNO on normal strains generates the SCV phenotype. Figure 2 confirms that the combination of HQNO (20 µg/ml) and tomatidine (8 µg/ml) has an inhibitory activity on normal *S. aureus* strains and that this inhibitory activity is not observed with either of these molecules alone.

[00210] Accordingly, addition of 1 µg/ml sub inhibitory concentration of the proton motive force uncoupler carbonyl cyanide *m*-chlorophenylhydrazone, CCCP (*i.e.*, another electron transport inhibitor), also caused ATCC 29213 to become susceptible to the growth inhibitory activity of tomatidine (tomatidine MIC of 0.12 µg/ml in presence of CCCP) and increased resistance to gentamicin (MIC of gentamicin of 4-8 µg/ml in presence of CCCP). MICs were determined as described in Example 1 above.

[00211] TABLE 3: Susceptibility (MIC in µg/ml) of normal and SCV *S. aureus* strains to tomatidine, tomatine and control antibiotics with or without the presence of HQNO.

Strain[a]	TO	TN	GEN	VAN	ERY	CIP	OXA
ATCC 29213	>16	>16	1	2	0.12-0.25	0.5	0.12-0.25
ATCC 29213 + HQNO	0.12-0.25	>16	4	2	0.25	0.25	0.12
CF07-L	>16	>16	1-2	2	0.25	0.5	0.06-0.12
CF07-L + HQNO	0.5	>16	4	2	0.25	0.25	0.06-0.12
CF07-S	0.12	>16	8	2	0.12	0.12	0.06-0.12
CF07-S + HQNO	0.12	>16	4-8	2	0.06-0.12	0.12	0.06-0.12

[a]ATCC 29213 and CF07-L are normal strains whereas CF07-S is a SCV.

4-hydroxy-2-heptylquinoline-N-oxide (HQNO) was used at 20 µg/ml.

TO: tomatidine, TN: tomatine, GEN: gentamicin, VAN: vancomycin, ERY: erythromycin, CIP: ciprofloxacin, OXA: oxacillin.

EXAMPLE 4

Effect of counteracting the electron transport chain defect of *Staphylococcus aureus* SCV strains on their susceptibility to tomatidine

[00212] The susceptibility of electron transport-deficient strains to tomatidine is abolished when the strain defect is compensated.

[00213] **Method:** Normal (Newbould, CF07-L and CF1A-L), and SCV (NewbouldΔ*hemB* (in the presence and absence of hemin), CF07-S (in the presence and absence of menadione) and CF1D-S)

S. aureus strains were treated with various concentrations of tomatidine (4, 2, 1, 0.5, 0.25, 0.12 and 0.06 µg/ml) for 48h in the Brain Hearth Infusion (BHI) medium at 35°C and 10 µl samples were thereafter spotted on agar plated which were further incubated for 48h before a picture was taken. Results are reported in Figure 1A.

[00214] **Results:** As shown in Figure 1A, the susceptibility of the hemin-dependent electron transport-deficient SCV NewbouldΔ*hemB* and of the menadione-dependent electron transport-deficient SCV CF07-S to tomatidine was abolished in the presence of supplemental hemin and menadione, respectively, which further confirmed that a defective electron transport is required for the antibacterial activity of tomatidine to occur.

EXAMPLE 5

Bacteriostatic activities of tomatidine against normal *Staphylococcus aureus* strains and small-colony variants (SCVs)

[00215] Time-kill experiments were performed in order to determine whether the effect of tomatidine on SCVs is bacteriostatic (prevents growth) or bactericidal (kills cells).

[00216] **Method:** Bacteria were inoculated at ~10^5-10^6 CFU/ml in BHI in the absence or presence of antibiotics at the specified concentrations (concentrations of 16 µg/ml of tomatidine (TO) (n=3), 0.5 µg/ml of erythromycin (ERY) (n=3) and 1.0 µg/ml of ciprofloxacin (CIP) (n=3) were used against CF07-L (Figure 1B), whereas concentrations of 0.25 µg/ml of TO (n=4), 16 µg/ml of TN (n=3), 0.25 µg/ml of ERY (n=3) and 0.5 µg/ml of CIP (n=2) were used against the SCV strain CF07-S). At several time points during growth at 35°C (225 RPM), bacteria were sampled, serially diluted and plated on tryptic soy agar (TSA) for colony-forming unit (CFU) determinations (*i.e.*, viable bacterial counts). Plates were incubated for 24 or 48 h at 35°C for normal and SCV strains, respectively. The antibacterial activities of tomatidine and control antibiotics (erythromycin (a bacteriostatic macrolide) and ciprofloxacin (a bactericidal fluoroquinolone) against the normal CF07-L strain and the SCV CF07-S as a function of time are presented in Figures 1B and 1C, respectively. The antibacterial activity of tomatine against the SCV strain was also evaluated (TN in Figure 1C).

[00217] **Results:** Figure 1C clearly demonstrates that the presence of tomatidine at 0.25 µg/ml (2XMIC) induced bacteriostasis in SCVs whereas it does not affect the growth of normal strains (Figure 1B). Tomatidine is thus bacteriostatic like the widely used macrolide class of antibiotics.

EXAMPLE 6

Effect of tomatidine on the biosynthesis of macromolecules in untreated and HQNO-treated normal *Staphylococcus aureus* strains

[00218] Tomatidine causes inhibition of the biosynthesis of macromolecules and more specifically protein biosynthesis in electron transport-deficient *S. aureus*.

[00219] In order to get insight into the mechanism of action of tomatidine on SCVs, macromolecular biosynthesis assays were performed with the normal strain ATCC 29313 in the absence or presence of 20 µg HQNO/ml. HQNO-treated bacteria were used to create the SCV phenotype because it allowed to achieve an elevated cell densities before the addition of HQNO.

[00220] **Method:** The complete defined medium (CDM) was used for macromolecular biosynthesis assays. CDM was constituted of the following chemicals per liter: 5 g glucose, 50 mg $MgSO_4$, 7 g K_2HPO_4, 2 g KH_2PO_4, 0.5 g of Na-Citrate dihydrate, 1 g $(NH_4)_2SO_4$, 1 mg thiamine, 1.2 mg niacin, 0.25 mg calcium pantothenate, 0.005 mg of biotin, 10 mg of L-tryptophan, 5 mg adenine, 5 mg guanine, 5 mg cytosine, 5 mg uracil, 100 mg L-glutamic acid, 90 mg L-aspartic acid, 80 mg L-proline, 50 mg L-arginine, 50 g glycine, 50 mg L-lysine, 60 mg L-alanine, 30 mg L-serine, 20 mg L-cysteine, 10 mg L-methionine, 50 mg L-tyrosine, 40 mg L-phenylalanine, 20 mg L-histidine, 30 mg L-threonine, 30 mg L-isoleucine, 80 mg L-valine, 90 mg L-leucine and 20 mg thymine. The medium CDM-LEU had 22.5 mg/l of L-Leucine instead of 90 mg/l whereas the medium CDM-ALA had 15 mg/l of L-alanine instead of 60 mg/l. Protein, DNA, RNA and cell wall peptidoglycan biosynthesis were evaluated by measuring the incorporation of the appropriate radiolabeled precursors into bacteria prior to treatment with trichloroacetic acid (TCA). Inocula were prepared by incubating bacteria overnight at 35°C (225 RPM) in the CDM medium. Cultures were then adjusted to an optical density at 600nm (A_{600nm}) of 0.1 and grown until an A_{600nm} of 0.3 in CDM, CDM-LEU (protein) or CDM-ALA (cell wall) was achieved. An amount of 3µCi/ml of [^{3}H]leucine, 1µCi/ml of [^{3}H]thymine, 1µCi/ml of [^{3}H]uridine or 2µCi/ml [^{3}H]D-alanine was added to aliquots of cultures in presence of the different antimicrobial compounds at approximately 4XMIC in order to evaluate protein, DNA, RNA or cell wall peptidoglycan synthesis, respectively. The incorporation of [^{3}H]-molecules into macromolecules were allowed for 45 min for the protein and cell wall assays, and for 35 min for the DNA and RNA assays. Cold 10% TCA was then added to all samples to stop the incorporation and precipitate macromolecules for 1 h on ice. All samples were filtered through a glass microfiber filter (Piscataway, NJ, USA) by using a dot-blot filtration system. Each filter was washed with 100 µl of 10% TCA containing 1.5 M NaCl and 100 µl of 10% TCA. Filters were dried overnight and their radioactivity was measured in a liquid scintillation counter. MICs of the control antibiotics chloramphenicol, norfloxacin, rifampicin and vancomycin against *S. aureus* ATCC 29213 were 8-16, 1, 0.008-0.015 and 0.5-1 µg/ml, respectively (Data not shown).

[00221] **Results:** The effect of control antibiotics chloramphenicol (CHL), norfloxacin (NOR), rifampicin (RIF) and vancomycin (VAN) that are well-known to inhibit protein synthesis, DNA replication, RNA transcription and cell wall peptidoglycan synthesis, respectively, were tested on the normal strain ATCC 29213 at approximately 4XMIC (four times their minimal inhibitory concentration) (Figure 3A). As expected, each of these antibiotics preferentially inhibited the incorporation of radiolabeled precursors into the targeted macromolecules i.e. (chloramphenicol (CHL); 64 µg/ml), DNA (Norfloxacin (NOR); 4

µg/ml), RNA (Rifampicin (RIF); 0.06 µg/ml) and cell wall peptidoglycan synthesis (Vancomycin (VAN); 4 µg/ml). Tomatidine diluted in dimethyl sulphoxide (DMSO) at a concentration of up to 125 µg/ml did not alter the synthesis of any macromolecule in ATCC 29213 in comparison to the DMSO-treated control (Figure 3B). However, in the presence of 20 µg HQNO/ml, tomatidine decreased the biosynthesis of all macromolecules at all tested concentrations above 0.12 µg/ml when compared to the HQNO-treated control (Figure 3C). In presence of HQNO, the inhibition of protein synthesis was significantly more affected by tomatidine than was the biosynthesis of all other macromolecules (Figure 3C). This indicates that the primary cellular target of tomatidine is the bacterial protein biosynthesis machinery.

EXAMPLE 7

Effect of tomatidine on the replication of a clinical SCV of *Staphylococcus aureus* in polarized airway epithelial cells

[00222] Results herein show that tomatidine has an antimicrobial activity against intracellular SCVs. This is particularly relevant because the ability of SCVs to persist within host cells is thought to be involved in the development of chronic and difficult-to-treat infections (Sendi and Proctor, 2009). More precisely, the following results demonstrate that tomatidine can significantly decrease the infection of polarized airway epithelial cells by SCVs by inhibiting their ability to replicate inside cells.

[00223] **Method:** The human airway epithelial cells, shCFTR, which mimic the CFTR defect, were derived from the Calu-3 cell line ATCC HTB 55 (Palmer *et al.*, 2006). The shCFTR cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with 0.1mM MEM nonessential amino acids, 1 mM of sodium pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 2.5 µg/ml of Fungizone and 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. For routine culture, 4 µg/ml of puromycin was added to culture media. All cell culture reagents were purchased from Wisent (St-Bruno, QC, Canada). Cell infection assays were performed as previously described with few adaptations for the Transwell™ system (Mitchell *et al.*, 2010c). Cells were seeded at 2.5 x 10^5 cells/inserts on 12-well Transwell™ plates and cultured for 9 to 10 days in an air:liquid system. The complete medium in the basal compartment was replaced by the invasion medium (1% FBS and no antibiotics) 18 h before assays. Inocula were prepared by suspending bacteria grown 20 h on BHIA plates in ice-cold PBS. Bacteria (CF07-L or CF07-S) were then washed three times in ice-cold PBS and suspended in the invasion medium supplemented with 0.5% BSA at a density of approximately 4 x 10^8 CFU/ml. Cells were washed twice with PBS and 250 µl of bacterial suspension were apically added to each insert. Invasion was allowed for 3 h, inserts were emptied and washed three times with PBS. Invasion medium supplemented with 20 µg/ml of lysostaphin (Sigma) was then added to kill extracellular bacteria and the cells were further incubated 24 or 48 h in presence of lysostaphin. DMSO or the different concentrations of tomatidine were added after invasion. Cells were washed once with PBS and the invasion medium supplemented with lysostaphin, DMSO and/or tomatidine was replaced at 24 h post-internalization.

Fresh invasion medium supplemented with lysostaphin was also added 1 h before cell lysis to ensure that only intracellular bacteria were counted. Following three washes with PBS, cells were detached with 100 µl of trypsin 0.25% and lyzed for 10 min by the addition of 400 µl of water containing 0.05% of Triton X-100. Lysates were serially diluted 10-fold and plated on agar for CFU determination. Plates were incubated for 24 or 48 h at 35°C for normal and SCV strains, respectively. Results are reported in Figure 4A and B.

[00224] **Results:** Figure 4A shows that, although both normal and SCV strains caused similar level of infection at 24 h post-internalization, the intracellular load of the SCV strain CF07-S 48 h post-internalization was clearly larger than that resulting from the normal strain CF07-L. A significant difference between cells infected with CF07-L and CF07-S 48 h post-internalization is shown. These differences in cellular infection levels are explained by the ability of SCVs to persist and replicate within epithelial cells (Moisan *et al.*, 2006; Sendi and Proctor, 2009). The impact of tomatidine on the infection of epithelial cells by SCVs was evaluated. Figure 4B demonstrated that cells treated with 1.25 and 12.5 µg/ml of tomatidine (diluted in DMSO) contained significantly less SCVs CF07-S than the DMSO-treated control cells 48 h post-internalization.

EXAMPLE 8

Effect of tomatidine on normal *S. aureus* bacteria in co-culture with *Pseudomonas aeruginosa*

[00225] Given that *S. aureus* and *P. aeruginosa* are often co-isolated from the airways of CF patients and that *P. aeruginosa* is known to produce respiratory inhibitors targeting *S. aureus* such as HQNO and pyocyanin (Mitchell et al., 2010b; Voggu *et al.*, 2006) as well as other antisapthylococcal compounds (Kessler *et al.*, 1993; Qazi *et al.*, 2006, the effect of tomatidine on the viability of *S. aureus* in co-culture with *P. aeruginosa* was tested. Results herein demonstrate that tomatidine kills normal *S. aureus* bacteria when grown in presence of *P. aeruginosa*.

[00226] **Methods**: *S. aureus* bacteria were inoculated at ~10^5-10^6 CFU/ml in Cation-adjusted Mueller-Hinton broth (CAMHB) and grown at 35°C with shaking in the absence or presence of 8 µg/ml tomatidine. Bacteria were sampled, serially diluted and plated on tryptic soy agar for CFU determinations. For experiments in co-culture, both *S. aureus* ATCC 29213 and *P. aeruginosa* PA14 were inoculated at ~10^5-10^6 CFU/ml. Mannitol-salt agar plates were used to selectively evaluate *S. aureus* CFU when in co-culture with *P. aeruginosa*.

[00227] **Results:** Table 4 shows that while tomatidine does not significantly alter the growth of the normal *S. aureus* strain ATCC 29213 when in mono-culture, the viability of this bacterium is decreased by the presence of tomatidine when in co-culture with the *P. aeruginosa* strain PA14. More precisely, exposure of ATCC 29213 to tomatidine significantly decreases its viability when in co-culture with PA14

in comparison to all other conditions ($P < 0.01$; one-way ANOVA with Tuckey's post test). In contrast to the bacteriostatic effect of tomatidine on SCVs, tomatidine is bactericidal against *S. aureus* bacteria in co-culture with *P. aeruginosa.*

[00228] TABLE 4: Effect of tomatidine (TO) at 8 µg/ml on the viability (in Log_{10} CFU/ml) of the normal *S. aureus* (SA) ATCC 29213 alone or in co-culture with *P. aeruginosa* (PA).

Conditions	Viability of *S. aureus* (in Log_{10} CFU/ml) at two time points [a]	
	0 h	24 h
SA alone	5.3 ± 0.1	10.0 ± 0.1
SA alone + TO	5.1 ± 0.4	9.64 ± 0.08
SA + PA	5.36 ± 0.03	5.4 ± 0.7
SA + PA + TO	5.4 ± 0.1	2 ± 1 [b]

[a] Results are presented as means ± standard deviations from 2 to 3 independent experiments.

[b] $P < 0.01$; one-way ANOVA with Tuckey's post test.

EXAMPLE 9

Combined effect of tomatidine and gentamicin against heterogeneous *Staphylococcus aureus* populations composed of both normal and SCV strains

[00229] Tomatidine can be used in combination with classical antibiotics during therapies, especially in patients simultaneously infected by SCVs and *S. aureus* having the normal phenotype. Tomatidine can complement the antibacterial effect of the aminoglycoside antibiotics (*e.g.*, gentamicin) against a bacterial population composed of both normal and SCV strains of *S. aureus*.

[00230] **Method:** Bacteria were inoculated at ~10^5-10^6 CFU/ml in BHI in absence or presence of gentamicin and/or tomatidine at 4 and 0.12 µg/ml, respectively. Cultures were incubated 48 h at 35°C/225 RPM and the growth was visually evaluated. Results are reported in Figures 5A and B.

[00231] **Results**: Figure 5A shows that gentamicin at 4 µg/ml inhibits the growth of the normal strain CF07-L whereas tomatidine at 0.12 µg/ml does not. Figure 5B shows that gentamicin at 4 µg/ml does not inhibit the growth of the SCV CF07-S while tomatidine at 0.12 µg /ml does. Finally, in Figure 5C, a combination of gentamicin at 4 µg /ml and tomatidine at 0.12 µg/ml inhibits the growth of a heterogeneous population composed of both the normal strain CF07-L and the SCV CF07-S whereas neither antibiotic molecule alone can.

EXAMPLE 10

Potentiating effect of tomatidine on aminoglycoside antibiotics against normal staphylococcal strains

[00232] Results of the assays used in this example report the unexpected discovery that tomatidine specifically and selectively increases the antibacterial activity of aminoglycoside antibiotics against *Staphylococcus* Spp. that are not electron transport-deficient.

[00233] **Method**: The MICs of gentamicin (aminoglycoside), tobramycin (aminoglycoside), amikacin (aminoglycoside), streptomycin (aminoglycoside), kanamycin (aminoglycoside), oxacillin (beta-lactam), erythromycin (macrolide), norfloxacin (fluoroquinolone), ciprofloxacin (fluoroquinolone), tetracycline and vancomycin (glycopeptide) with or without tomatidine (TO) against normal *S. aureus* strain ATCC 29213 were determined using the microdilution method in 96-well microplates. Bacteria were inoculated at ~10^5-10^6 CFU/ml and incubated at 35ºC for 24 h in CAMHB. Then OD_{595nm} was read on a microplate reader. Results are reported in Table 5 below.

[00234] **Results:** Table 5 below shows that tomatidine decreases the MICs (*i.e.* increases the susceptibility) of the aminoglycoside antibiotics gentamicin, tobramycin, amikacin, streptomycin and kanamycin against the non electron transport-deficient *S. aureus* ATCC 29213. As an example, tomatidine at 8 µg/ml increases the antibacterial activity of gentamicin and tobramycin against ATCC 29213 between 8-32 and 4-8 fold, respectively.

[00235] TABLE 5: Susceptibility (MIC in µg/ml) of *S. aureus* ATCC 29213 to several antibiotics in absence or presence of tomatidine at 8 µg/ml.

Antibiotic	-TO	+TO	Fold (-TO/+TO)[a]
Gentamicin	0.5-1	0.03-0.06	8-32
Tobramycin	0.25-0.5	0.06	4-8
Amikacin	2	0.5	4
Streptomycin	4-8	1	4-8
Kanamycin	2-4	0.5	4-8
Oxacillin	0.25	0.25	1
Erythromycin	0.5	0.5	1
Norfloxacin	1-2	1-2	1
Ciprofloxacin	0.5	0.5	1
Tetracycline	0.25-0.5	0.25-0.5	1
Vancomycin	1	1	1

[a] Increased susceptibility measured in fold differences. Differences between unexposed (-TO) and exposed (+TO) results were determined for each independent experiments and are presented as intervals.

[00236] **Results:** Table 6 below shows that the potentiating effect of tomatidine on the antibacterial activity of aminoglycoside antibiotics is also efficient against other clinically important *Staphylococcus spp.* (*e.g., S. epidermidis, S. haemolyticus, S. saprophyticus and S. hominis*). Results are from MIC experiments.

[00237] TABLE 6: Susceptibility (MIC in µg/ml) of several *Staphylococcus spp.* strains to the aminoglycoside antibiotics gentamicin and tobramycin in absence or presence of 8 µg/ml of tomatidine.

Species	Strain	Antibiotic	-TO	+TO	Fold (-TO/+TO)[a]
S. epidermidis					
	ATCC 12228				
		Gentamicin	0.12	0.06	2
		Tobramycin	0.12	0.06	2
	ATCC 35984				
		Gentamicin	32	8	4
		Tobramycin	16	2	8
S. haemolyticus					
	sh022				
		Gentamicin	16	4	4
		Tobramycin	32	4	8
	sh032				
		Gentamicin	64	8	8
		Tobramycin	32	2	16
S. saprophyticus					
	ATCC 15305				
		Gentamicin	ND	ND	ND
		Tobramycin	0.12	0.016	8
S. hominis					
	ssp008c				
		Gentamicin	8	2	4
		Tobramycin	32	8	4
	sho23				
		Gentamicin	0.12	0.06	2
		Tobramycin	4	1	4

[a] Increased susceptibility measured in fold differences. Differences between unexposed (-TO) and exposed (+TO) results were determined for each independent experiments and are

presented as intervals. ND not determined.

[00238] **Method:** A checkerboard protocol was used in order to determine the effect of aminoglycoside antibiotics on ATCC 29213 as a function of tomatidine concentration. This checkerboard protocol (Eliopoulos and Moellering, 1996) was conducted by a microdilution method similar to that use for standard MICs determination. In 96 wells plates, antibiotics were loaded at a 4X concentration (where X is the maximal tested concentration) in well A1 and at a 2X concentration in the others wells of the column 1. Antibiotics were serially diluted 1:1 from the column 2 to column 10. Tomatidine was then loaded in wells A1 to A11 at a 4X concentration and serially diluted 1:1 from row B to row G. Row H was without tomatidine whereas column 11 was without antibiotic. Wells A12, B12, C12 and D12 are positive untreated controls whereas wells E12, F12, G12 and H12 are negative non-inoculated controls. Bacteria were inoculated at ~10^5-10^6 CFU/ml and incubated at 35ºC for 24 h in CAMHB. Then OD_{595nm} was read on microplate reader. Results shown in Table 7 below are the MICs determined for the aminoglycosides by this checkerboard method in presence of the indicated amounts of tomatidine. The FIC index was calculated as follow (Eliopoulos and Moellering, 1996): FIC index = FICA + FICB = A/MICA+ B/MICB, where A and B are MICs of compounds A and B in combination and where MICA and MICB are the MICs of compound A alone and of compound B alone, respectively, and FICA and FICB are the FICs of compound A and of compound B, respectively. The analysis of the FIC index demonstrates a total synergy if the FIC index is ≤0.5, a partial synergy if the FIC index is >0.5 and ≤0.75, an additive effect of both compound if the FIC index is >0.75 and ≤1, and an antagonistic effect if the FIC index is >2.

[00239] **Results:** Table 7 below shows that in a checkerboard assay, tomatidine creates a synergy with all tested aminoglycoside antibiotics (i.e. tobramycin (TOB), gentamicin (GEN), amikacin (AMI), streptomycin (STR) and kanamycin (KAN)) with a calculated Fractional Inhibitory Concentration (FIC) index below 0.5.

[00240] TABLE 7: Susceptibility (MIC in µg/ml) of *S. aureus* ATCC 29213 to several aminoglycoside antibiotics as a function of tomatidine concentration.

Tomatidine (µg/ml)	Aminoglycoside MIC in µg/ml				
	Gentamicin	Tobramycin	Amikacin	Streptomycin	Kanamycin
0	0.5-1	0.25-0.5	2	4-8	2-4
0.06	0.25	0.25	2	4	2
0.12	0.06-0.25	0.03-0.12	0.5	2	0.5-1

0.25	0.06	0.03-0.06	0.25	1-2	0.5
0.5	0.06	0.03	0.25-0.5	1	0.5
1	0.03-0.06	0.03-0.06	0.25-1	1	0.5-1
2	0.06-0.12	0.03-0.06	0.25-1	1	0.25-0.5
4	0.03-0.12	0.03-0.06	0.25	1	0.5
8	0.03-0.06	0.06	0.5	1	0.5
FIC index[a]	≤ 0.116	≤ 0.133	≤ 0.133	≤ 0.199	≤ 0.193

[a] Although tomatidine alone did not inhibit the growth of normal *S. aureus* strains, a MIC value of 32 µg/ml was considered for tomatidine in order to approximate FIC indexes. The symbol "≤" in of the FIC index values indicates that these values are overestimated. A FIC index below 0.5 indicates a strong synergy.

EXAMPLE 11

Potentiating effect of tomatidine on aminoglycoside antibiotics against staphylococcal strains of multiple clinical origins and against multi-resistant staphylococcal strains

[00241] The potentiating effect of tomatidine on the antibacterial activity of aminoglycoside antibiotics against *S. aureus* is efficient against several strains isolated from human and veterinary infections, including antibiotic-resistant *S. aureus* strains.

[00242] **Results**: Table 8 below shows the antibiotic susceptibility profile of several normal *S. aureus* strains isolated from both human and veterinary infections. While several strains are susceptible to all of the antibiotics tested (*e.g.*, CF1A-L), others are resistant to one or several antibiotics (*e.g.*, Sa228c) and may include methicillin-resistant (*e.g.*, MRSA COL) or vancomycin-intermediate (*e.g.*, Mu50) *S. aureus* and the like (MRSA, Vancomycin-intermediate *Staphylococcus aureus* (VISA), glycopeptide-intermediate *Staphylococcus aureus* (GISA), Vancomycin-resistant *Staphylococcus aureus* (VRSA)). Table 9 below shows that tomatidine decreases the MICs (*i.e.* increases the susceptibility) of *S. aureus* isolates, including antibiotic-resistant *S. aureus*, to gentamicin, tobramycin and kanamycin. This potentiating effect of tomatidine on the gentamicin and tobramycin activity is also illustrated in Figures 6A and 7A, respectively. Figure 6B and 7B show that this potentiating effect of tomatidine is highly significant ($P < 0.001$). For example, tomatidine at 8 µg/ml increases in average the antibacterial activity of gentamicin and tobramycin against all tested *S. aureus* strains by 8±3 fold. The determination of MICs was performed as described in Example 10 above.

[00243] TABLE 8: Antibiotic susceptibility profile (MIC in µg/ml) of several *S. aureus* strains isolated from both human and veterinary infections

Strain	Origin (infection)	GEN	TOB	KAN	OXA	ERY	NOR	TET	VAN	CIP
8325-4	Laboratory strain	0.12-0.25	0.12-0.25	2-8	0.12	0.25-0.5	1-2	0.25	1	0.25-0.5
Newbould	Cow (mastitis)	0.5-1	0.5-1	4	0.12	0.5	0.5	0.25-0.5	0.5-1	0.25
SHY97-3906	Cow (mastitis)	0.25	0.25	2	0.12-0.25	0.12-0.25	0.5-1	0.25-0.5	0.5-1	0.25
ATCC 43300	Human (HA)	64-128 (R)	512-1024 (R)	512-1024 (R)	16-32 (R)	>64 (R)	ND	0.5	0.5-1.0	0.5
ATCC BAA-41	Human (HA)	0.5	512 (R)	256-512 (R)	>64 (R)	>64 (R)	ND	0.5	1	>64 (R)
N315	Human (HA)	1	512 (R)	256 (R)	8 (R)	>64 (R)	ND	0.5	0.5	0.25
MA078038	Human (CA)	0.25-0.5	0.5	>1024 (R)	64 (R)	64 (R)	ND	0.5	0.5-1.0	16 (R)
Newman	Human (Osteo)	0.5-1	0.25-0.5	4	0.5-1	0.5	1	0.25-0.5	1	0.25
ATCC 29213	Human (SSTI)	0.5-1	0.25-0.5	2-4	0.25	0.5	1-2	0.25-0.5	1	0.5
MRSA COL	Human (SSTI)	0.5-1	0.5-1	4	>64 (R)	0.5	2-4	1-2	2	0.5
Mu50	Human (SSTI)	128 (R)	1024 (R)	>1024 (R)	>64 (R)	>64 (R)	>64 (R)	>16 (R)	4 (I)	32-64 (R)
Sa220c	Human (SSTI)	0.5	0.5-1	4	16-32 (R)	0.5-1	>64 (R)	0.5	1	32 (R)
Sa228c	Human (SSTI)	64-128 (R)	1024 (R)	512-1024 (R)	>64 (R)	>64 (R)	>64 (R)	>16 (R)	1	>64 (R)
CF1A-L	Human (CF lungs)	0.5-1	1	4	0.25-0.5	0.25-0.5	1	0.25	1	0.5-1
CF2A-L	Human (CF lungs)	1	1	4	0.25	0.5	0.5-1	2	1	0.25
CF4B-L	Human	0.5	0.5-1	4	0.5	0.5	1	0.25	1	0.25-0.5

CF6B-L	(CF lungs) Human (CF lungs)	128-256 (R)	256 (R)	1024 (R)	0.25-1	0.5-2	>64 (R)	0.25	0.5-1	>64 (R)
CF7A-L	(CF lungs) Human (CF lungs)	0.5-1	1024 (R)	256-512 (R)	>64 (R)	>64 (R)	>64 (R)	0.25-0.5	1	>64 (R)
CF8E-L	(CF lungs) Human (CF lungs)	1	1	16	0.125	0.5	4	0.25-0.5	1-2	1
CF9A-L	(CF lungs) Human (CF lungs)	0.5-1	512-1024 (R)	256 (R)	>64 (R)	>64 (R)	>64 (R)	0.25-0.5	1	>64 (R)
CF35A-L	(CF lungs) Human (CF lungs)	0.5-1	0.5-1	4	>64 (R)	>64 (R)	>64 (R)	0.5	1	>64 (R)
CF07-L	(CF lungs) Human (CF lungs)	0.5-1	0.5-1	4	0.12-0.25	0.5	1-2	0.5	1-2	0.5

ATCC 29213, Newman and 8325-4 are control strains.

Newbould (ATCC 29740) and SHY-3906 are strains isolated from bovine mastitis.

MRSA COL, ATCC 43300, ATCC BAA-41, N315, MA078038, Mu50, CF7A-L, CF9A-L, CF35A-L, Sa220c and Sa228c are methicillin-resistant strains (MRSA).
Mu50 is also a vancomycin-intermediate resistant *S. aureus* (VISA).

CF1A-L, CF2A-L, CF4B-L, CF6B-L, CF7A-L, CF8E-L, CF9A-L, CF35A-L and CF07-L are pulmonary isolates (MRSA or not) from human patients with cystic fibrosis.
GEN: gentamicin, TOB: tobramycin, KAN: kanamycin, OXA: oxacillin, ERY: erythromycin, NOR: norfloxacin, TET: tetracycline, VAN: vancomycin, CIP: ciprofloxacin.
Intermediate resistance (I) and resistance (R) to antibiotics.

HA: Hospital-associated isolate; CA: Community-associated isolate; SSTI: Skin and soft tissue infection/wound; CF: Cystic fibrosis; Osteo: Osteomyelitis; ND: Not determined.

[00244] TABLE 9: Susceptibility (MIC in µg/ml) of several *S. aureus* strains to the aminoglycoside antibiotics gentamicin, tobramycin and kanamycin in absence or presence of 8 µg/ml of tomatidine.

Strain	Antibiotic	-TO	+TO	Fold (-TO/+TO)[a]
ATCC 29123				
	Gentamicin	0.5-1	0.03-0.06	8-32
	Tobramycin	0.25-0.5	0.03-0.06	4-16
	Kanamycin	2-4	0.5	4-8
Newman				
	Gentamicin	0.5-1	0.06	8-16
	Tobramycin	0.25-0.5	0.06-0.12	2-8
	Kanamycin	4	0.5	8
8325-4				
	Gentamicin	0.12-0.25	0.03-0.06	4
	Tobramycin	0.12-0.25	0.03-0.06	4
	Kanamycin	2-8	0.5-4	2-4
Newbould				
	Gentamicin	0.5-1	0.06	8-16
	Tobramycin	0.5-1	0.06-0.12	8
	Kanamycin	4	0.5	8
SHY-3906				
	Gentamicin	0.25	0.03-0.06	4-8
	Tobramycin	0.25	0.03	8
	Kanamycin	2	0.5	4
MRSA COL				
	Gentamicin	0.5-1	0.06	8-16
	Tobramycin	0.5-1	0.06	8-16
	Kanamycin	4	0.5	8
CF1A-L				
	Gentamicin	0.5-1	0.12	4-8
	Tobramycin	1	0.06-0.25	4-16
	Kanamycin	4	0.5	8
CF2A-L				
	Gentamicin	1	0.12-0.25	4-8
	Tobramycin	1	0.06-0.12	8-16
	Kanamycin	4	0.5	8
CF4B-L				
	Gentamicin	0.5	0.06-0.12	4-8
	Tobramycin	0.5-1	0.06-0.12	4-16

	Kanamycin	4	0.5	8
CF8E-L				
	Gentamicin	1	0.25-0.5	2-4
	Tobramycin	1	0.25	4
	Kanamycin	16	2-8	2-4
CF35A-L				
	Gentamicin	0.5-1	0.06-0.12	8
	Tobramycin	0.5-1	0.06	8-16
	Kanamycin	4	1	4
CF07-L				
	Gentamicin	0.5-1	0.06-0.25	4-8
	Tobramycin	0.5-1	0.06-0.12	4-8
	Kanamycin	4	0.5-1	4-8
Sa220c				
	Gentamicin	0.5	0.06-0.12	4-8
	Tobramycin	0.5-1	0.06-0.12	4-16
	Kanamycin	4	0.5	8

[a] Increased susceptibility measured in fold differences. Differences between unexposed (-TO) and exposed (+TO) results were determined for each independent experiments and are presented as intervals.

EXAMPLE 12

Potentiating effect of tomatidine on aminoglycoside antibiotics against staphylococcal strains that are specifically resistant to aminoglycosides

[00245] The potentiating effect of tomatidine on the antibacterial activity of aminoglycoside antibiotics against staphylococci is also efficient against aminoglycoside-resistant strains.

[00246] **Results:** Table 10 below shows that tomatidine increased the susceptibility of gentamicin-resistant, tobramycin-resistant and kanamycin-resistant strains to gentamicin, tobramycin and kanamycin despite their resistance against one or several of these antibiotics. More particularly, the nine strains included in Table 10 below are resistant to several antibiotics (see Table 8 above) and are thus multi-resistant strains likely to cause difficult-to-treat infections. The determination of MICs was conducted as described in Example 10 above. The strains used in Table 10 below were also characterized for their content in some resistance genes responsible for aminoglycoside resistance and coding for aminoglycoside-modifying enzymes, following the PCR detection procedure of Schmitz *et al* (1999). The aminoglycoside resistance determinants that were detected are reported in Table 10 below.

[00247] TABLE 10: Susceptibility (MIC in µg/ml) of several aminoglycoside-resistant *S. aureus*

strains to the aminoglycoside antibiotics gentamicin, tobramycin and kanamycin in absence or presence of 8 µg/ml of tomatidine.

Strain	Resistance determinant	Antibiotic	-TO	+TO	Fold (-TO/+TO)[a]
ATCC 43300	ND				
		Gentamicin	64-128	16-32	4
		Tobramycin	512-1024	128	4-8
		Kanamycin	512-1024	256	2-4
ATCC BAA-41	*ant(4')-Ia*	Gentamicin	0.5	0.12-0.25	2-4
		Tobramycin	512	128	4
		Kanamycin	256-512	64	4-8
N315	*ant(4')-Ia*	Gentamicin	1	0.12	8
		Tobramycin	512	128-256	2-4
		Kanamycin	256	64	4
MA078038	*aph(3')-IIIa*	Gentamicin	0.25-0.5	0.06	4-8
		Tobramycin	0.5	0.06-0.12	4-8
		Kanamycin	>1024	1024	>1
Mu50	*aac(6')-aph(2'')*, *ant(4')-Ia*	Gentamicin	128	16	8
		Tobramycin	1024	128	8
		Kanamycin	>1024	256-512	>2-4
CF6B-L	*aac(6')-aph(2'')*				
		Gentamicin	128-256	16-32	4-8
		Tobramycin	256	16-64	4-16
		Kanamycin	1024	128-256	4-8
CF7A-L	*ant(4')-Ia*				
		Gentamicin	0.5-1	0.12-0.25	2-8
		Tobramycin	1024	128-512	2-8
		Kanamycin	256-512	64-256	2-4
CF9A-L	*ant(4')-Ia*				
		Gentamicin	0.5-1	0.06-0.12	4-8
		Tobramycin	512-1024	64-256	4-8
		Kanamycin	256	128-256	1-2
Sa228c	*aac(6')-aph(2'')*, *ant(4')-Ia*				
		Gentamicin	64-128	8-16	8
		Tobramycin	1024	128-256	4-8

	Kanamycin	512-1024	128-256	2-8

[a] Differences between unexposed (-TO) and exposed (+TO) results were determined for each independent experiments and are presented as intervals.
ND: Not determined.

EXAMPLE 13

Bacteriostatic and bactericidal activities of steroid alkaloid compounds alone or in combination with aminoglycosides

[00248] The antibacterial activity of steroid alkaloids were determined in time-kill experiments using the method described in Example 5 above, alone or in combination with aminoglycosides against the electron transport-deficient variants or the normal (i.e. non electron transport-deficient) strains, respectively, of a variety of bacterial species. Bacteria were inoculated at ~10^5-10^6 CFU/ml in BHI or MHBCA in the absence or presence of antibiotics at the specified concentrations. At several time points during growth at 35°C, cultures were sampled, serially diluted and plated on TSA for CFU determinations.

[00249] **Results:** Figure 8 shows that tomatidine greatly potentiates the bactericidal action of aminoglycosides such as gentamicin against "normal", non electron transport-deficient *S. aureus* ATCC 29213. Results show that while neither gentamicin nor tomatidine used alone at 0.06 or 8 μg/ml, respectively, had antibacterial activity on *S. aureus*, the combination of both provided a strong bactericidal activity; the combination killed *S. aureus* better and faster than the well-known bactericidal drug ciprofloxacin used at 2xMIC (1.0 μg/ml). The concentration of gentamicin used in the assay was 0.06 μg/ml, which represented only 1/8 to 1/16 of the MIC of the drug alone against *S. aureus* ATCC 29213.

EXAMPLE 14

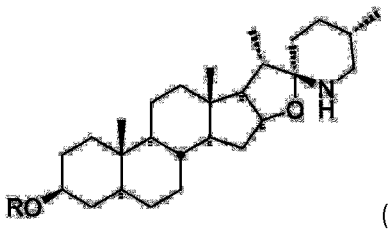
Prevention of the emergence of bacteria with decreased susceptibility to aminoglycoside antibiotics with steroid alkaloid compounds

[00250] Regrowth of bacteria with reduced susceptibility to aminoglycosides is often observed within 24 hours following antibiotic exposure (Miller *et al.*, 1978; Wilson and Sanders, 1976). The effect of compounds of the present invention on the emergence of bacteria with reduced susceptibility to aminoglycosides was determined. Bacteria were inoculated at ~10^5-10^6 CFU/ml in BHI or MHBCA in the absence or presence of antibiotics at the specified concentrations. At several time points during growth at 35°C, cultures were sampled, serially diluted and plated on TSA for CFU determinations.

[00251] **Results**: Figures 9A and B show that gentamicin (GEN) and tobramycin (TOB) alone at ≥ 1×MIC (1 µg/ml) are bactericidal against *S. aureus* ATCC 29213 although, as anticipated for aminoglycosides, regrowth is observed within 24 h. Accordingly, colonies isolated from 24h-cultures exposed to gentamicin were often normal-growing bacteria with a decreased susceptibility to gentamicin (MIC ranging from 1 to 4 µg/ml) or SCVs (MIC for gentamicin ranging from 4 to 8 µg/ml). Tomatidine (TO) at 8 µg/ml markedly reduced the regrowth of bacteria exposed to gentamicin (9A) or tobramycin (9B). Figure 10 further demonstrated that the presence of tomatidine can significantly decrease the number of CFU recovered from cultures exposed to concentrations of gentamicin ranging from 0.5 to 4 µg/ml for 24 h. From these time-kill experiments, isolated colonies obtained from cultures exposed to gentamicin combined or not with 8 µg/ml tomatidine were analyzed for their susceptibility to gentamicin. When ATCC 29213 was exposed to gentamicin alone, the emergence of numerous normal-growing isolates showing decreased susceptibility to gentamicin (MIC ranging from 1 to 4 mg/L) was easily detected. The combination of tomatidine and gentamicin significantly reduced the emergence of such resistant CFU (Figure 10).

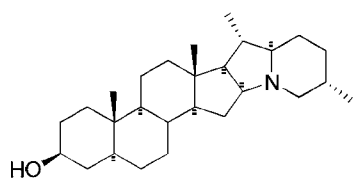
EXAMPLE 15

Natural steroid alkaloids

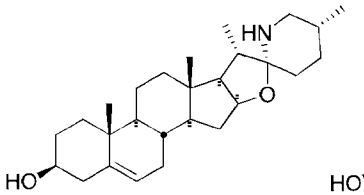


(1.1)

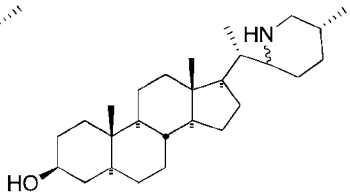
R=H, Tomatidine



demissidine

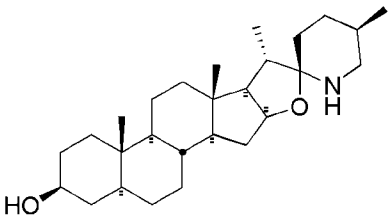


solasodine



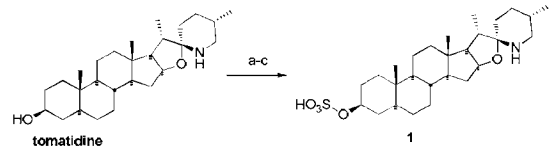
dihydrosolacongestidine

[00252] The compounds above (including tomatidine formula 1.1) are commercially available through Sigma-Aldrich, Acros or Molekula for example. Solasodan (see structure below) was purchased from Sigma.



EXAMPLE 16

Synthesis of tomatidine 3-sulfate of formula 1.0



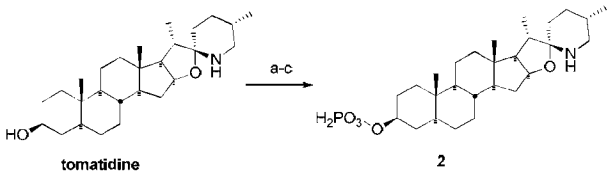
tomatidine 1

a. Cbz-OSu, $NaHCO_3$, $THF:H_2$); b. NaH, $BnOSO_2Cl$, THF; c. H_2, Pd/C

[00253] Tomatidine 3-sulfate **1** is synthesized in 3 steps by initially protecting the aminal via a carbobenzyloxy (Cbz) group in standard conditions. Subsequently, the free hydroxyl is sulphated using benzyloxy sulfuryl chloride and sodium hydride in THF. Finally, simultaneous hydrogenolysis of both benzyl groups will give the desired compound.

EXAMPLE 17

Synthesis of tomatidine 3-phosphate of formula 1.0



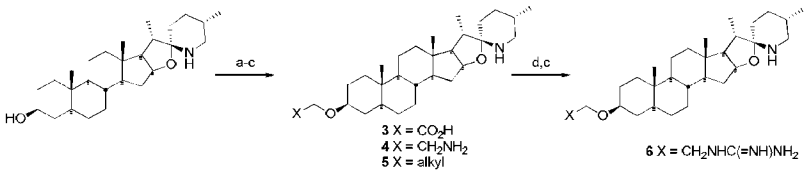
tomatidine 2

a. Cbz-OSu, $NaHCO_3$, $THF:H_2$); b. NaH, $(BnO)_2POCl$, THF; c. H_2, Pd/C

[00254] Tomatidine 3-phosphate **2** is synthesized in 3 steps by initially protecting the aminal via a Cbz group in standard conditions. Subsequently, the free hydroxyl is phosphorylated using bis(benzyloxy)phosphoryl chloride and sodium hydride in THF. Finally, simultaneous hydrogenolysis of both benzyl groups gives the desired compound.

EXAMPLE 18

Synthesis of 3-substituted tomatidine analogues of formula 1.0

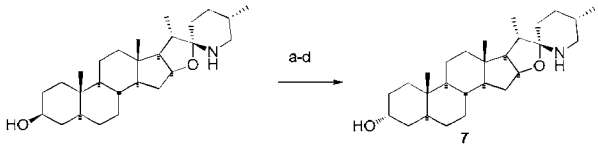


a. Cbz-OSu, $NaHCO_3$, THF; b. (i. **3**): NaH, THF, $BrCH_2CO_2Bn$; (ii. **4**) $BrCH_2CH_2NHCO_2Bn$, THF; (iii. **5**) ICH_2alkyl, NaH, THF; c. H_2, Pd/C; d. CbzNH(C=NTf)NHCbz, DiPEA, DCM

[00255] 3-substituted tomatidine analogs 3-5 are synthesized starting from tomatidine by initial Cbz protection of the hemiaminal portion of the molecule, followed by alkylation using sodium hydride and the required electrophile (benzyl chloroacetate for **3**, Cbz-bromoethylamine for **4**, iodoalkanes for **5**). Subsequent hydrogenolysis of both protective groups delivers the desired compounds **3**-**5**. Synthesis of 6 starts **4**, which is reacted with Goodman's triflimide reagent. Subsequent hydrogenolysis delivers **6**. Analogues bearing additional substitution on the amine moiety of **4** are synthesized by further functionalization of the primary amine using standard methods such as amide bond formation, sulfonylation or reductive amination.

EXAMPLE 19

Synthesis of 3α-hydroxytomatidine of formula 1.0

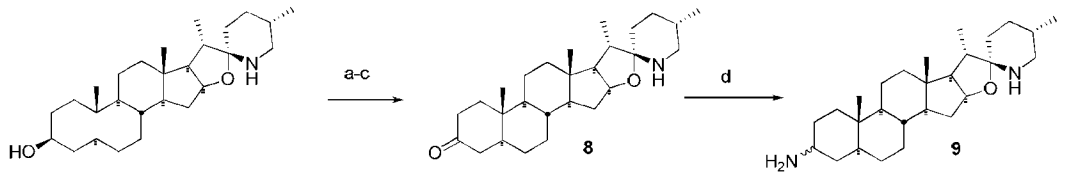


a. Cbz-OSu, $NaHCO_3$, THF; b. BzOH, DIAD, PPh_3; c. LiOH, H_2O:THF; d. H_2, Pd/C

[00256] 3α-hydroxytomatidine **7** is synthesized by initial protection of the amine group as described above in Example 16 followed by Mitsubobu reaction with benzoic acid, benzoate cleavage with lithium hydroxyde then hydrogenolysis of the Cbz group.

EXAMPLE 20

Synthesis of 3-oxo-tomatidine and 3-aminotomatidine of formula 1.0

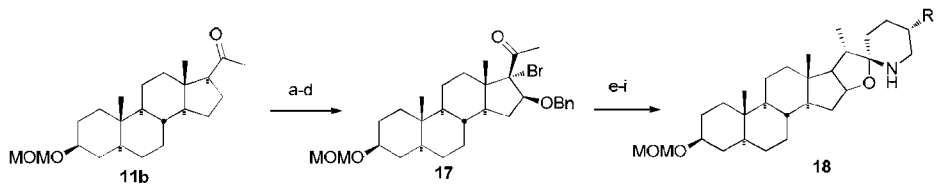


a. Cbz-OSu, $NaHCO_3$, THF; b. Swern oxydation; c. H_2, Pd/C; d. $(NH_4)_2CO_3$, $NaBH_3CN$, MeOH

[00257] 3-oxo-tomatidine **8** is synthesized by protection of the amine group with a Cbz, followed by Swern oxidation of the alcohol and subsequent hydrogenolysis. 3-aminotomatidine **9** is obtained from **8** by reductive amination using ammonium carbonate and sodium cyanoborohydride.

EXAMPLE 21

Synthesis of analogues 18 of formula 1.0

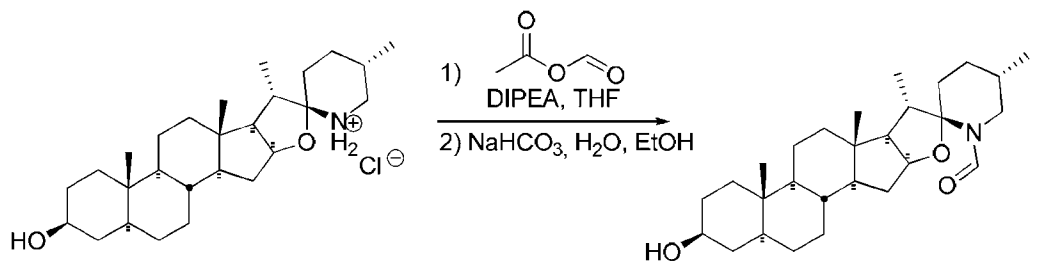


a. NBS, benzoyl peroxide, CCl_4; b. DBU; c. N-bromoacetamide, THF, H_2O; d. $BnOC(=NH)CCl_3$, H^+;
e. LDA, Comins' reagent; f. R-$C_5H_4NB(OH)_2$, base, $Pd(PPh_3)_4$; g. Et_3B, Bu_3SnH; h. H_2, Pd/C; i. HCl, EtOAc

[00258] Common intermediate **11b** is first treated with NBS and benzoyl peroxide followed by base treatment to give the unsaturated ketone (Bolger *et al.*, 1996). The latter undergoes bromination with N-bromoacetamide followed by opening of the bromonium ion with water (Li *et al.*, 2009). Subsequent benzyl protection gives intermediate **17**. Subsequently, the enol triflate is formed using Comins' reagent, then undergoes a Suzuki cross coupling with variously substituted pyridines. The bromide is then cleaved in reducing conditions, and the spirohemiaminal closed to give the desired analogues satisfying formula **18** having the alcohol in position 3 protected by a protective group methoxymethyl (MOM).

EXAMPLE 22

Synthesis of N-formyl tomatidine (21) of formula 1.0



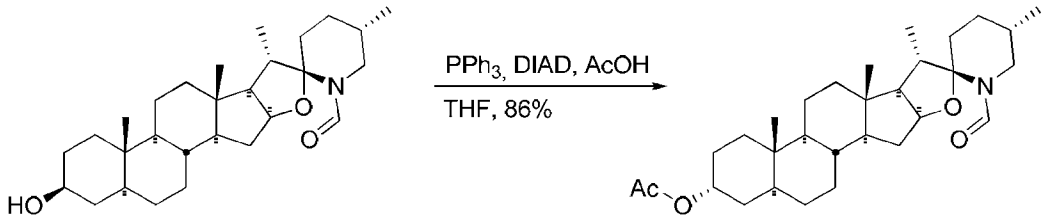
[00259] In a 250 mL round flask, tomatidine hydrochloride (200 mg, 0.442 mmol, 1.0 eq) was suspended in dry THF (20 mL) and acetic formic anhydride (380 mg, 4.420 mmol, 10.0 eq) and DIPEA (390 µL, 2.210 eq, 5.0 eq) were added. The reaction was stirred for 15 minutes, then monitored by TLC (thin layer chromatography) (50% AcoEt/Hexanes). Solvents were removed by evaporation under reduced pressure. The compound was then diluted in 125 mL EtOH and 50 mL of aquous $NaHCO_3$ buffer (pH = 9.5) and stirred for one week, monitored by TLC until complete disappearance of diformylated compound. EtOH was then evaporated, and the resulting aqueous phase was extracted with 3X 25 mL AcOEt. The combined organic phases were dried on anhydrous $MgSO_4$ and evaporated under reduced pressure.

[00260] Crude product was purified by flash chromatography (25% AcOEt/Hexanes) to give 155 mg (79%) of the desired compound 21.

[00261] **^{1}H NMR**(300 MHz, $CDCl_3$) δ (ppm) 8.41 (s, 1H), 4.29 (d, 1H, J = 11.5 Hz), 4.13 (dd., 1H, J_1 = 7.3 Hz, J_2 = 15.5 Hz), 3.58 (quint, 1H, J = 4.7 Hz), 2.65 (t, 1H, J = 11.5), 7.1 (quint, 1H, J = 7.1 Hz), 1.9 (quint, 1H, J = 5.28 Hz) 1.87 (d, 1H, J = 13.7 Hz), 1.82-1.72 (m, 3H), 1.72-1.63 (m, 3H), 1.61-1.45 (m, 7H), 1.40 (d, 1H, J = 13.0 Hz), 1.38-1.22 (m, 8H), 1.15 (dt, 1H, J_1 = 12.3 Hz, J_2 = 3.9 Hz), 1.12-1.06 (m, 2H), 1.05 (d, 3H, J = 6.8 Hz), 0.95 (dt, 1H, J_1 = 13.7 Hz, J_2 = 3.6 Hz), 0.91 (d, 3H, J = 5.9 Hz), 0.89-0.84 (m, 1H), 0.82 (s, 6H), 0.64 (dt, 1H, J_1 = 11.39, J_2 = 3.6 Hz). **HRMS** calculated for $C_{28}H_{45}O_3N$: 443.6618, calculated for MNa^+: 466.6510 found: 466.3308 (MNa^+).

EXAMPLE 23

Synthesis of N-formyl-3α-acetyltomatidine (22) of formula 1.0



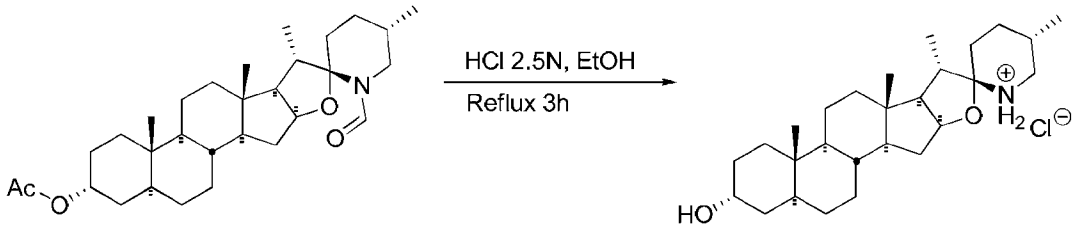
[00262] In a 10 mL round bottom flask, N-formyl tomatidine (**1**) (60 mg, 0.135 mmol, 1.0 eq) was dissolved in 3 mL anhydrous THF, along with triphenylphosphine (71 mg, 0.270 mmol, 2.0 eq) and acetic acid (22 µL, 378 eq, 2.8 eq). Diisopropylazodicarboxylate (40 µL, 202 mmol, 1.5 eq) was added, and the reaction was stirred at room temperature for 4h, monitored by TLC (25% AcOEt/hexanes, rf: 0.10 (uv). An

additional 20 µL DIAD, 30 mg PPh_3 and 20 µL of acetic acid were added to drive the reaction to completion, and the reaction was stirred overnight. The reaction was then concentrated under reduced pressure, suspended in water and extracted 3X with AcOEt. The combined organic fractions were washed with brine, dried on anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography (10% AcOEt/hexanes) and 56 mg (86%) of compound **22** were obtained.

[00263] **^{1}H NMR**(300 MHz, $CDCl_3$) δ (ppm) 8.43 (s, 1H), 5.00 (m, 3H, DIAD) 4.31 (d, 1H, *J* = 11.8 Hz), 4.99 (m, 1H), 4.16 (quad, 1H, *J* = 7.1 Hz), 2.68 (t, 1H, *J* = 12.1 Hz), 2.56 (s, 4H), 2.08-1.98 (m, 5H), 1.90 (d, 1H, *J* = 12.4 Hz), 1.84-1.74 (m, 3H), 1.74-1.51 (m, 5H), 1.48 (s, 4H), 1.42-1.20 (m, 25H, DIAD), 1.14 (d, 2H, *J* = 4.7 Hz), 1.08 (d, 3H, *J* = 7.1 Hz), 0.93 (d, 4H, *J* = 6.0 Hz), 0.85 (s, 3H), 0.83 (s, 3H), 0.81-0.73 (dt, 1H, J_1 = 11.5 Hz, J_2 = 3.3 Hz).

EXAMPLE 24

Synthesis of 3α-hydroxytomatidine hydrochloride salt (23) of formula 1.0



[00264] In a 25 mL round flask, 28 mg **22** (0.058 mmol) was refluxed for 3 hours in 6 mL EtOH and 3 mL aquous HCl 2.5 N. Upon completion, the ethanol and HCL were removed under reduced pressure and the remaining water was removed by lyophilization. Compound **23** was obtained as the hydrochloride salt of compound **7** (free base).

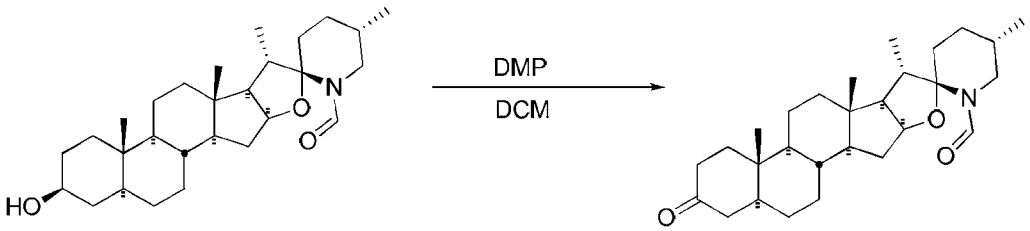
[00265] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 4.40 (quad, 1H, *J* = 8.8 Hz), 3.98 (s large, 1H), 3.56 (m, 1H), 3.14 (t, 1H, *J* = 12.6 Hz), 2.94 (t, 1H, *J* = 11.0 Hz), 2.23 (t, 1H, *J* = 6.0 Hz), 2.13-1.99 (m, 2H), 1.91 (t, 1H, *J* = 7.1 Hz), 1.85-1.54 (m, 8H), 1.54-1.37 (m, 6H), 1.36-1.18 (s large, 21H), 1.12 (d, 3H, *J* = 6.6 Hz), 1.01 (d, 3H, *J* = 6.0 Hz), 0.93 (s, 3H), 0.86 (s large, 5H).

[00266] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 96.2 (s), 81.1 (s), 69.1 (s), 66.9 (s), 65.7 (s), 65.4 (s), 61.7 (s), 55.6 (s), 54.2 (s), 48-46 (m, CD_3OD) 40.8 (s), 38.8 (s), 35.8 (s), 35.3 (s), 34.9 (s), 32.0 (s), 28.1

(s), 25.8 (s), 25.3 (s), 20.1 (s), 20.0 (s), 17.3 (s).

EXAMPLE 25

Synthesis of N-formyl-3-oxotomatidine (24) of formula 1.0

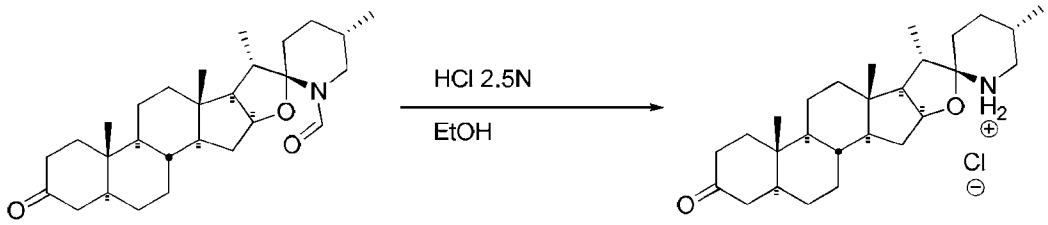


[00267] In a 10 mL round bottom flask, N-formyltomatidine **21** (50 mg, 0.113 mmol, 1.0 eq) and Dess-Martin periodinane (95 mg, 0.225 mmol, 2.0 eq) were stirred in 6.5 mL DCM. The reaction was monitored by TLC (50% AcOEt/Hexanes). Upon completion, the reaction was quenched for 30 minutes with $Na_2S_2O_3$ 0.2M, then extracted 3X with AcOEt. The combined organic phases were washed with brine, dried on anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography (50% AcOEt/Hexanes) to yield 34 mg (68%) of desired compound **24**.

[00268] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 8.46 ppm (s, 1H), 4.32 (d, 1H, *J* = 12.8 Hz), 4.17 (quad, 1H, *J* = 8.9 Hz), 2.69 (t, 1H, *J* = 12.6 Hz), 2.58 (quint, 1H, *J* = 6.6 Hz), 2.52-2.24 (m, 3H), 2.16-2.11 (m, 1H), 2.11-1.98 (m, 2H), 1.91 (d, 1H, *J* = 14.0 Hz), 1.86-1.68 (m, 3H), 1.69-1.47 (m, 6H), 1.47-1.29 (m, 6H), 1.29-1.23 (m, 1H), 1.21 (s, 3H), 1.19-1.12 (m, 2H), 1.09 (d, 3H. *J* = 7.1 Hz), 1.05 (s, 2H), 0.94 (d, 4H, *J* = 5.5 Hz), 0.88 (s, 3H), 0.77 (dd, 1H, J_1 = 10.4 Hz, J_2 = 4.4 Hz).

EXAMPLE 26

Synthesis of 3-oxotomatidine hydrochloride salt (25) of formula 1.0



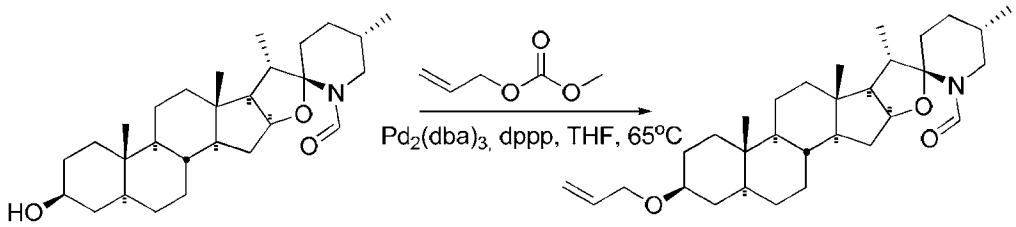
[00269] In a 25 mL round flask, 34 mg **22** (0.077 mmol) was refluxed for 2 hours in 10 mL EtOH and 5 mL aquous HCl 2.5 N. Upon completion, the ethanol and HCL were removed under reduced pressure and the remaining water was removed by lyophilization. Compound **25** was obtained as the hydrochloride salt of compound **8** (free base).

[00270] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 4.37 (quad, 1H, *J* = 9.0 Hz), 3.11 (d, 2H, *J* = 15.0 Hz), 2.89 (t, 1H, *J* = 12.0 Hz), 2.53-2.30 (m, 1H), 2.24-2.14 (m, 1H), 2.08-1.94 (m, 3H), 1.91-1.63 (m, 7H), 1.63-1.50 (m, 5H), 1.49-1.15 (m, 12H), 1.10 (s, 2H), 1.06 (d, 3H, *J* = 7.1 Hz), 0.96 (d, 3H, *J* = 5.5 Hz), 0.89 (s, 3H), 0.82 (s, 3H), 0.76-0.66 (m, 1H).

[00271] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 100.2 (s), 96.1 (s), 81.1 (s), 61.7 (s), 55.5 (s). 54.1 (s), 42.2 (s), 40.8 (s), 40.7 (s), 39.6 (s), 35.5 (s), 34.9 (s), 34.8 (s), 32.0 (s), 31.4 (s), 28.2 (s), 28.1 (s), 27.9 (s), 25.7 (s), 25.3 (s), 20.7 (s), 17.2 (s), 15.9 (s), 13.2 (s), 10.6 (s), 10.5 (s).

EXAMPLE 27

Synthesis of O-allyl-N-formyltomatidine (55) of formula 1.0



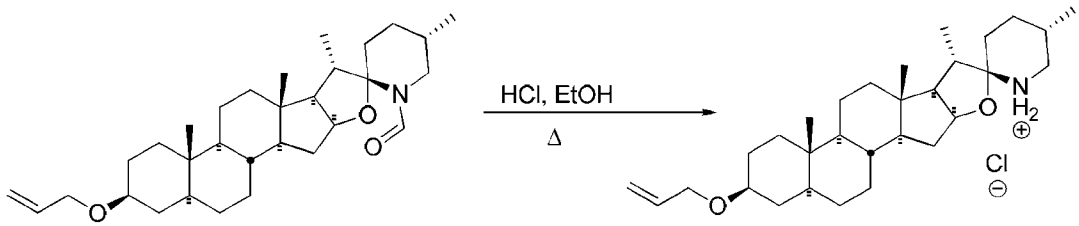
[00272] In a 3 mL round botton flask equipped with a condenser tube and placed under argon atmosphere, compound **21** (30 mg, 0.068 mmol) was dissolved in 1 mL THF. $Pd_2(dba)_3$ (3 mg, 0.003 mmol, 0.005 eq), 1,3-bis(diphenylphosphino)propane (5 mg, 0.012 mmol, 0.18 eq) and allyl methyl carbonate (0.2 mL, 1.76 eq, 26 eq) were successively added. The reaction was brought to 65°C for 6H, monitored by TLC. (50% AcOEt/hexanes). Upon completion, the reaction was allowed to cool to room temperature, then the solvent was removed *in vacuo*. The crude compound was purified by flash chromatography (20%

AcOEt/Hexanes) to yield 25 mg (76%) of desired compound **55**.

[00273] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 8.41 (s, 1H), 5.93 (ddt, 1H, J_1 = 17.3 Hz, J_2 = 10.5 Hz, J_3 = 5.7 Hz), 5.34 (d quad, 1H, J_1 = 17.1 Hz, J_2 = 1.4 Hz), 1.25 (d quad, 1H, J_1 = 10.3 Hz, J_2 = 1.3 Hz), 4.60 (dt, 2H, J_1 = 5.8 Hz, J_2 = 1.4 Hz), 4.53 (quint, 1H, J = 5.5 Hz), 4.29 (d, 1H, J = 11.9 Hz), 4.16-4.08 (m, 2H), 2.65 (t, 1H, J = 11.3 Hz), 2.54 (t, 1H, J = 7.0 Hz), 2.01-1.94 (m, 1H), 1.94-1.84 (m, 2H), 1.81-1.68 (m, 4H), 1.65 (s, 2H), 1.62-1.47 (m, 6H), 1.46-1.35 (m, 2H) 1.35-1.28 (m, 41H) 1.25 (t, 3H, J = 7.3 Hz), 1.22-1.08 (m, 2H), 1.05 (d, 3H, J = 7.0 Hz), 1.02-0.92 (m, 1H), 0.90 (d, 4H, J = 5.8 Hz), 0.83 (s, 3H), 0.82 (s, 1H), 0.65 (dt, 1H, J_1 = 10.7 Hz, J_2 = 4.3 Hz).

EXAMPLE 28

Synthesis of O-allyltomatidine hydrochloride salt (56) of formula 1.0

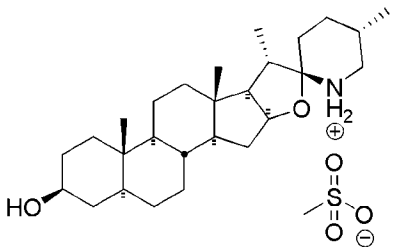


[00274] In a 20 mL vial, **55** (8.7 mg, 0.018 mmol), was dissolved in 10 mL EtOH and 3 mL conc. HCl. The mixture was brought to 65°C for 1H, then solvant was removed *in vacuo*. The remaining water was lyophilized to yield 7.9 mg (90%) of crude compound **56**.

[00275] **^{1}H NMR**(300 MHz, CD_3OD) δ (ppm) 5.96-5.82 (m, 1H), 5.28 (d, 1H, J = 17.1 Hz), 5.18 (d, 1H, J = 10.4 Hz), 4.53 (d, 2H, J = 5.9 Hz), 4.47 (m, 1H), 4.36 (m, 1H), 3.58-3.42 (m, 1H), 3.15-3.04 (m, 1H), 2.87 (t, 1H, J = 11.7 Hz), 2.76-2.58 (m, 1H), 2.38-2.26 (m, 1H), 2.21-1.90 (m, 4H), 1.84 (d, 2H, J = 11.1 Hz), 1.78-1.44 (m, 12H), 1.40-1.10 (m, 15H), 1.09-1.92 (m, 8H), 0.87 (s, 2H), 0.86-0.79 (m, 5H), 0.75-0.57 (m, 4H).

EXAMPLE 29

Synthesis of tomatidine methanesulfonate (57) of formula 1.0



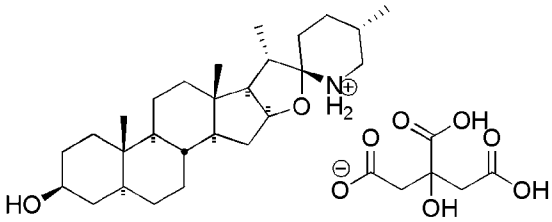
[00276] In a 25 mL round bottom flask, tomatidine (60 mg, 0.132 mmol) was suspended in 15 mL EtOH along with silver oxide (60 mg). The mixtured was mixed in a sonic bath for 1H, then filtered on diatomaceous earth pad to yield 50 mg (91%) of tomatidine free base (formula 1.1, R=H).

[00277] In a 20 mL vial, 21.5 mg (0.052 mmol) of tomatidine free base was solubilised in THF. 63 µL of a solution of methanesulfonic acid 1M in THF was added (1.2 eq), and the mixture was stirred for 5 minutes. Solvant was removed *in vacuo* to yield 20 mg (75%) of desired compound **57**.

[00278] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 4.37 ppm (quad, 1H, *J* = 7.2 Hz), 3.73-3.67 (m, 1H), 4.52-3.42 (m, 1H), 3.12 (d, 1H, *J* = 12.5 Hz), 2.89 (t, 1H, *J* = 12.3 Hz), 2.69 (s, 3H), 2.37 (t, 1H, *J* = 7.2 Hz), 2.24-2.13 (m, 1H), 2.08-1.96 (m, 2H), 1.90-1.81 (m, 2H), 1.78-1.64 (m, 5H), 1.63-1.42 (m, 4H), 1.40-1.32 (m, 7H), 1.31-1.13 (m, 7H), 1.08 (d, 3H, *J* = 7.2 Hz), 0.96 (d, 3H, *J* = 6.5 Hz), 0.89 (s, 3H), 0.84 (s, 3H), 0.74-0.62 (m, 1H).

EXAMPLE 30

Synthesis of tomatidine citrate (58) of formula 1.0



[00279] In a 25 mL round bottom flask, tomatidine (60 mg, 0.132 mmol) was suspended in 15 mL EtOH along with silver oxide (60 mg). The mixture was mixed in a sonic bath for 1H, then filtered on diatomaceous earth pad to yield 50 mg (91%) of tomatidine free base (formula 1.1, R=H).

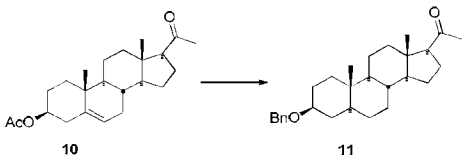
[00280] In a 20 mL vial, 27 mg (0.065 mmol) of tomatidine free base was solubilised in THF. 235 µL of a solution of citric acid, 0.33M in THF was added (1.2 eq), and the mixture was stirred for 5 minutes.

Solvent was removed *in vacuo* to yield 30 mg (76%) of desired compound **58**.

[00281] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 4.40-4.29 (m, 1H), 3.54-3.39 (m, 2H), 3.22-3.10 (s large, 1H), 1.93-2.83 (m, 1H), 2.78 (quad, 10H, *J* = 14.5 Hz), 2.33 (t, 1H, *J* = 8.1 Hz), 2.07-1.95 (m, 3H), 1.88-1.80 (m, 12H), 1.79-1.63 (m, 8H), 1.62-1.45 (m, 5H), 1.38 (s, 15H), 1.30-1.24 (m, 4H), 1.24-1.05 (m, 9H), 1.00-0.90 (m, 6H), 0.89 (s, 4H), 0.84 (s, 4H), 0.68 (dt, 1H, J_1 = 11.8 Hz, J_2 = 4.5 Hz).

EXAMPLE 31

Synthesis of common intermediate 11



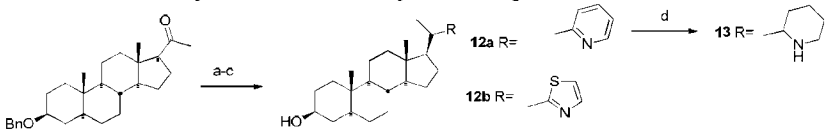
10 11

a. H_2, Pd/C, EtOH; b. NaOH, MeOH:H_2O; c. BnoC(=NH)CCl_3, TfOH, DCM

[00282] Intermediate **11** is synthesized from pregnenolone acetate, starting with hydrogenation that delivered the reduced product. Subsequent methanolysis of the acetyl group with methanolic sodium hydroxide was followed by benzylation using benzyl trichloroacetimidate and triflic acid, delivering intermediate **11**.

EXAMPLE 32

Synthesis of heterocyclic analogues 12a, 12b and 13 of formula 2.0



11 a. NaH, Comins' reagent; b. heteroarylboronic acid, $Pd(PPh_3)_4$, base; c. H_2, Pd/C; d. H_2, PtO_2, 1000 psi

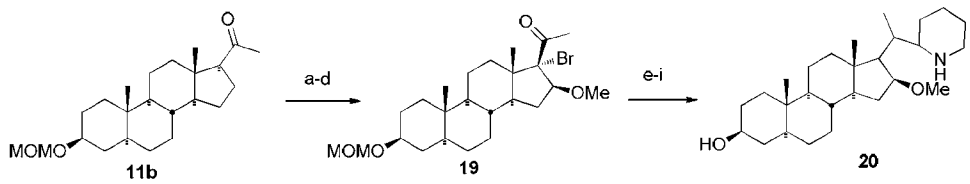
[00283] Synthesis of heterocyclic analogues starts from common intermediate **11**. Initial formation of the triflyl enol using Comins' reagent followed by Suzuki cross-coupling with pyridineboronic acid using a palladium catalyst and subsequent hydrogenation of the double bond gives pyridine analogue **12a**. Analogous derivatives with either alternative branching on the pyridine ring or additional substituents on pyridine are synthesized by the same method. Thiazole-substituted analogues **12b** are synthesized using

the same sequence with a cross-coupling with 2-thiazolylboronic acid. Piperidine derivatives **13** are obtained by high pressure hydrogenation of the pyridine derivative using platinum oxide.

EXAMPLE 33

Synthesis of analogue 20 of formula 2.0

[00284] Intermediate **11b** is first treated with NBS and benzoyl peroxide followed by base treatment to give the unsaturated ketone (Bolger *et al.*, 1996). The latter undergoes bromination with N-bromoacetamide followed by opening of the bromonium ion with water (Li *et al.*, 2009). Subsequent methyl protection leads to intermediate **19**. Subsequent transformations yields analogue **20** in which ring the E of tomatidine is open.

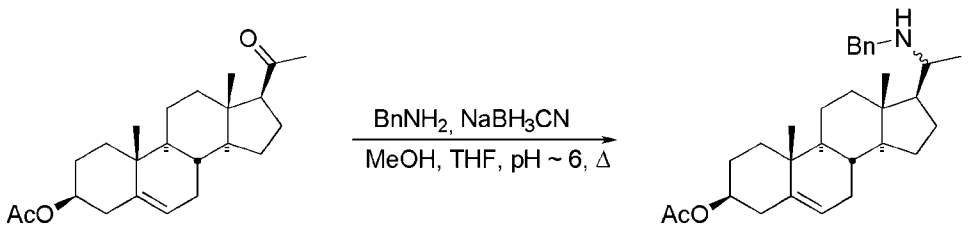


a. NBS, benzoyl peroxide, CCl_4; b. DBU; c. N-bromoacetamide, THF, H_2O; d. $BnOC(=NH)CCl_3$, H^+;
e. LDA, Comins' reagent; f. R-$C_5H_4NB(OH)_2$, base, $Pd(PPh_3)_4$; g. Et_3B, Bu_3SnH; h. H_2, Pd/C; i. HCl, EtOAc

Pregnenolone Acetate derivatives

EXAMPLE 34

Synthesis of O-acetyl-N-benzylpregn-5,6-en-3β-ol-20-amine (29a, 29b) of formula 2.0



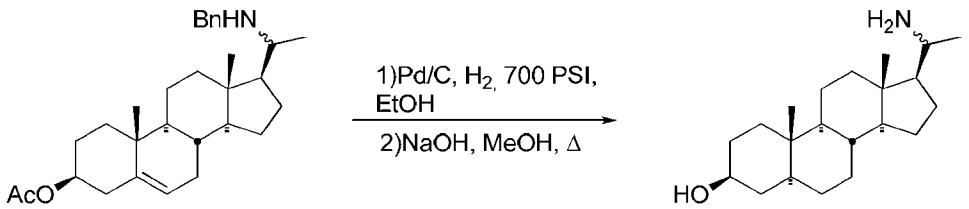
[00285] In a 25 mL round bottom flask equipped with a condenser tube, pregnenolone acetate (200 mg, 0.558 mmol) and benzylamine (366 µL, 3.347 mmol, 6.0 eq) were dissolved in 10 mL anhydrous MeOH. pH was set to ≈6 with conc. acetic acid, and 10 mL anhydrous THF was added. $NaBH_3CN$ (39 mg, 0.614 mmol, 1.1 eq) was added before the reaction was heated to reflux and stirred overnight. The next day, the reaction was monitored by TLC (25% AcOEt/Hexanes, UV/CAM, rf: 0.46 (starting material), 0.07 and 0.04

(desired compound)). The solvents were removed under reduced pressure, and the material was suspended in water. The pH was ajusted to 8 with saturated aquous $NaHCO_3$, then the mixture was extracted 3X with DCM. The combined organic fractions were washed with brine, dried on anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The crude compound was purified by flash chromatography (50% AcOEt/Hexanes) to yield 122 mg (48%) and 79 mg (32%) of each diastereisomer of the desired compound **29**. The absolute stereochemistry of each compound was not identified.

[00286] **^{1}H NMR** (300 MHz, CD_3OH) δ (ppm) 7.34-7.21 (m, 5H), 5.36 (d, 1H, J = 4.8 Hz), 3.76 (dd, 2H, J_1 = 80.6 Hz, J_2 = 12.9 Hz), 2.62 (dt, 1H, J_1 = 15.6 Hz, J_2 = 9.5 Hz), 2.31 (d, 2H, J = 7.1 Hz), 2.08 (dt, 1H, J_1 = 12.0 Hz, J_2 = 3.1 Hz), 2.02 (s, 3H) 2.01-1.74 (m, 4H), 1.65-1.07 (m, 12H), 1.05 (d, 4H, J = 6.1 Hz), 1.01 (s, 4H), 0.65 (s, 3H).

EXAMPLE 35

Synthesis of pregnan-3β-ol-20-amine (30a, 30b) of formula 2.0



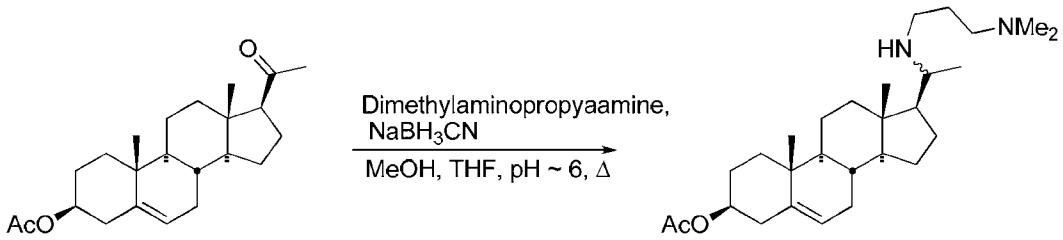
[00287] In two separate 20 mL vials, **29a** and **29b** (20 mg each, 0.080 mmol) were dissolved in 3 mL EtOH. Pd/C 10% w/w was added and the vials were placed under 700 PSI of hydrogen in a hydrogenation bomb overnight. The following morning, the compounds were filtered on diatomaceous earth pad and the solvent was evaporated under reduced pressure.

[00288] The compounds were then refluxed in 5 mL MeOH and 2 mL NaOH 1M for 1h. MeOH was evaporated under reduced pressure, then wather was added to give a white solid wich was isolated by filtration to yield 2 mg of **30a** and 1.5 mg of **30b**.

[00289] **^{1}H NMR** (300 MHz, CD_3OH) δ (ppm) 3.59 (sept, 1H, J = 5 Hz), 2.89-2.79 (m, 1H), 1.99-1.90 (m, 1H), 1.80 (d, 1H, J = 13.3 Hz), 1.75-1.62 (m, 3H), 1.60-1.48 (s large, 18H), 1.43-1.20 (m, 8H), 1.19-1.02 (m, 5H), 0.99 (d, 4H, J = 5.7 Hz), 0.95-0.83 (m, 2H), 0.81 (s, 3H), 0.72 (s) 0.69-0.63 (m, 1H).

EXAMPLE 36

Synthesis of O-acetylpregn-5,6-en-3β-ol-20-((N,N-dimethylamino)propyl)amine (31) of formula 2.0

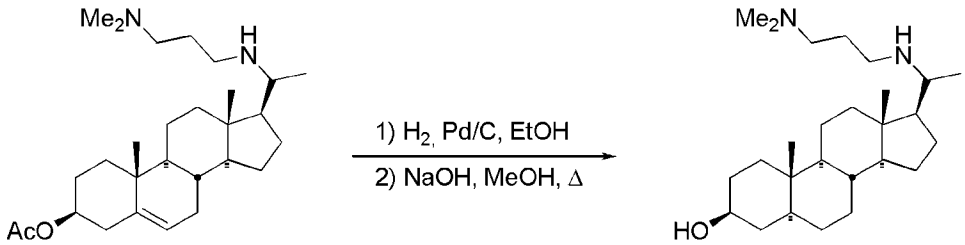


[00290] In a 25 mL round bottom flask equipped with a condenser tube, pregnenolone acetate (200 mg, 0.558 mmol) and N,N-dimethylaminopropylamine (421 µL, 3.347 mmol, 6.0 eq) were dissolved in 10 mL anhydrous MeOH. pH was set to ≈6 with conc. acetic acid, and 10 mL anhydrous THF was added. $NaBH_3CN$ (39 mg, 0.614 mmol, 1.1 eq) was added then the reaction was heated to reflux and stirred overnight. The next day, the reaction was monitored by TLC (75% AcOEt/Hexanes). The solvents were removed under reduced pressure, and the material was suspended in water. pH was ajusted to 8 with saturated aquous $NaHCO_3$, then the mixture was extracted 3X with DCM. The combined organic fractions were washed with brine, dried on anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The crude compound **31** (141 mg, 57%) was used without further purification.

[00291] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.90-5.60 (s large, 3H), 5.27 (d, 1H, *J* = 4.9 Hz), 5.22 (t, 1H, *J* = 3.1 Hz), 4.55-4.43 (m, 1H), 3.31-3.12 (m, 1H), 2.97-2.32 (m, 5H), 2.22 (s, 3H), 2.17 (m, 4H), 1.93 (s, 3H), 1.87-1.69 (m, 6H), 1.68-1.54 (m, 4H), 1.52-1.29 (m, 8H), 1.27 (d, 3H, *J* = 6.3 Hz), 1.24-1.17 (m, 2H), 1.14 (d, 3H, *J* = 5.5 Hz), 1.11 (s, 1H), 1.09-0.98 (m, 3H), 0.94-0.88 (m, 4H) 0.78-0.74 (m, 1H), 0.65 (s, 2H), 0.62 (s, 1H).

EXAMPLE 37

Synthesis of pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine (32) of formula 2.0



[00292] In a 50 mL round bottom flask, **31** (40 mg 0.090 mmol) was dissolved in 20 mL EtOH. Pd/C 10% w/w was added and the solution was placed under 700 PSI of hydrogen in a hydrogenation bomb for

6h. The mixture was filtered on diatomaceous earth pad and the solvent was evaporated under reduced pressure.

[00293] The compound was then refluxed in 15 mL MeOH and 6 mL NaOH 1M for 1h. MeOH was evaporated under reduced pressure, then water was added to give a white solid wich was isolated by filtration to yield 18 mg (50%) of the desired compound **32**.

[00294] **^{1}H NMR** (300 MHz, $CDCl_3$) □ (ppm) 3.58 (sept, 1H, *J* = 7.6 Hz), 2.80-2.68 (m, 1H), 2.59-2.37 (m, 2H), 2.34-2.28 (m, 2H), 2.20 (s, 6H), 1.99-1.43 (m, 14H), 1.43-1.14 (m, 10H), 1.14-0.98 (m, 5H), 0.95 (d, 3H, *J* = 5.8 Hz), 0.92-0.81 (m, 1H), 0.80 (s, 3H), 0.68 (s, 2H), 0.65 (s, 2H).

[00295] **^{13}C NMR** (75.5 MHz, $CDCl_3$) □ (ppm) 71.3 (s), 58.4 (s), 56.4 (s), 56.2 (s), 54.2 (s), 45.8 (s), 45.6 (s), 44.8 (s), 42.3 (s), 40.3 (s), 39.5 (s), 38.2 (s), 37.0 (s), 35.4 (s), 32.0 (s), 31.5 (s), 28.7 (s), 28.3 (s), 27.1 (s), 26.9 (s), 24.1 (s), 21.3 (s) , 21.1 (s), 19.2 (s), 12.5 (s), 12.3.

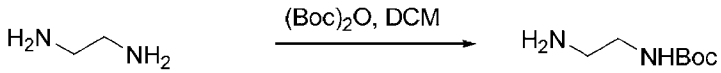
EXAMPLE 38

General procedure for synthesis of Boc- diaminoalkanes (Boc = *tert*-butoxycarbonyle)

[00296] In a 5 mL round bottom flask, 11.5 mmol (10 eq) of desired diaminoalkane was solubilised in 2.5 mL DCM. A solution of $(Boc)_2O$ (265 □L, 1.15 mmol, 1.0 eq) in 1 mL DCM was added dropwise. The resulting mixture was stirred for 24h at room temerature, then washed with water, brine, then dried on anhydrous $MgSO_4$. The solvant was removed under reduced pressure to yield the desired compound (see also Mingyu Hu, 2011).

EXAMPLE 39

Synthesis of N-Boc-1,2-diaminoethane (33)

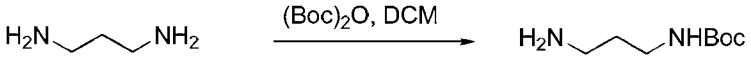


[00297] Following the procedure described in Example 38 above, 76 mg (41%) of desired compound **33** were obtained.

[00298] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.28-5.11 (s large, 1H), 3.13 (quad, 2H, *J* = 5.7 Hz), 2.76 (t, 2H, *J* = 5.7 Hz), 2.47-2.20 (s large, 2H), 1.38 (s, 9H).

EXAMPLE 40

Synthesis of N-Boc-1,3-diaminopropane (34)

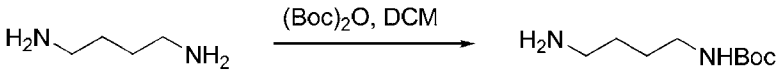


[00299] Following the procedure described in Example 38 above, 100 mg (50%) of desired compound **34** were obtained.

[00300] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.16 (s large, 1H), 3.60 (s large, 2H), 3.20 (quad, 2H, *J* = 6.1 Hz), 2.83 (t, 2H, *J* = 6.1 Hz), 1.69 (quint, 2H, *J* = 6.1 Hz), 1.42 (s, 9H).

EXAMPLE 41

Synthesis of N-Boc-1,4-diaminobutane (35)



[00301] Following the procedure described in Example 38 above, 171 mg (79%) of desired product were obtained.

[00302] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 4.94 (s large, 1H), 3.04 (s large, 4H), 2.69 (s large, 2H), 1.45 (s large, 4H), 1.36 (s, 9H).

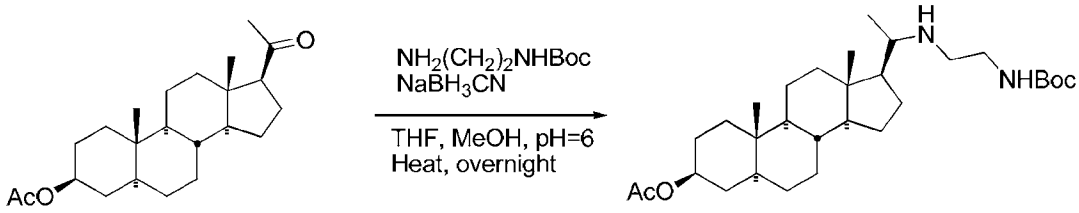
EXAMPLE 42

General procedure for reductive amination with pregnanolone acetate

[00303] In a 25 mL round bottom flask equipped with a condenser tube, pregnenolone acetate (75 mg, 0.208 mmol) and corresponding amine (2-6 eq) were solubilised in 5 mL MeOH and pH was ajusted to 6 with conc. acetic acid. 5 mL THF were then added, followed by $NaBH_3CN$ (15 mg, 0.230 mmol, 1.1 eq). The reaction was brought to reflux overnight and monitored by TLC. Solvents were removed under reduced pressure, and the solid was suspended in water and pH was adjusted to 8 with saturated aquous $NaHCO_3$. The mixture was extracted with 3X DCM, and the combined organic fractions were washed with brine, dried on anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude compound was purified by flash chromatography (50% AcOEt/Hexanes then 10% MeOH/ 89%AcOEt / 1% NEt_3).

EXAMPLE 43

Synthesis of O-acetylpregnan-3β-ol-20-(boc-aminoethyl)amine (36) of formula 2.0

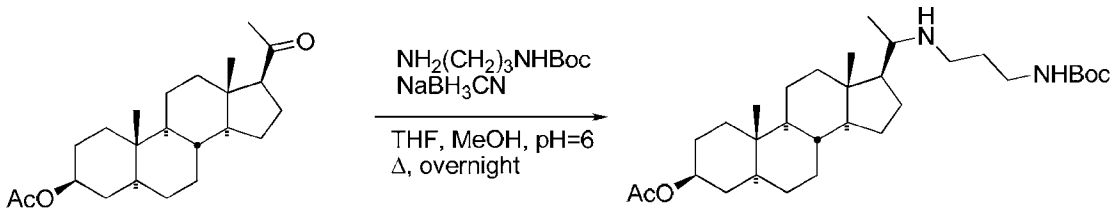


[00304] Following the procedure described in Example 42 above, 76 mg of compound **23** (0.474 mmol, 2.3 eq) was used to yield 84 mg (81%) of desired compound **36**.

[00305] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.02 (d large, 1H), 4.66 (m, 1H, *J* = 4.9 Hz), 3.27-3.05 (m, 2H), 2.85-2.68 (m, 1H), 2.65-2.45 (m, 3H), 2.03-1.92 (m, 4H), 1.88-1.51 (m, 7H), 1.47-1.35 (s large, 13H), 1.33-1.10 (m, 11H), 1.09-1.82 (m, 9H), 0.80 (s large, 4H), 0.69-0.55 d large, 4H).

EXAMPLE 44

Synthesis of O-acetylpregnan-3β-ol-20-(boc-aminopropyl)amine (37) of formula 2.0

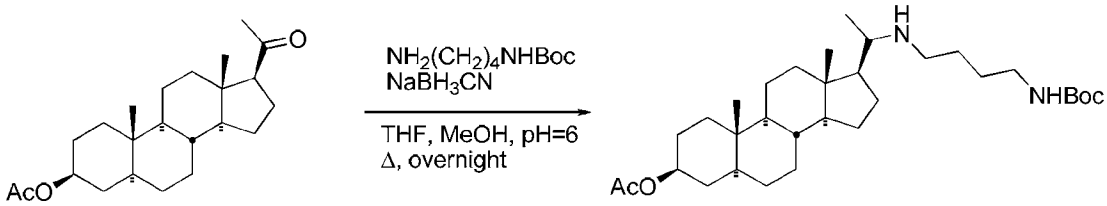


[00306] Following the procedure described in Example 42, 100 mg of compound **34** (0.574mmol, 2.8 eq) was used to yield 51 mg (47%) of desired compound **37**.

[00307] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.60 (s large, 0.2 H) 5.07 (s large, 0.6 H), 4.65 (sept, 1H, *J* = 5.1 Hz), 3.16 (sept, 1H, *J* = 3.6 Hz), 1.99 (s, 3H), 1.98-1.84 (m, 2H), 1.84-1.51 (m, 9H), 1.41 (s, 13H), 1.36-1.08 (m, 11H), 0.95 (d, 3H, *J* = 6.1 Hz), 0.92-0.81 (m, 1H), 0.79 (s, 3H), 0.66 (s, 2H), 0.63 (s, 1H), 0.62-0.57 (m, 1H).

EXAMPLE 45

Synthesis of O-acetylpregnan-3β-ol-20-(boc-aminobutyl)amine (38) of formula 2.0



[00308] Following the procedure described in Example 42 above, 171 mg of compound **35** (0.908 mmol, 4.4 eq) was used to yield 70 mg (63%) of desired compound **38**.

[00309] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 4.92 (d large), 4.65 (sept, 1H, *J* = 6.2 Hz), 3.09 (s large, 2H), 2.78-2.52 (m, 2H), 2.52-2.38 (m, 1H), 1.99 (s, 3H), 1.95-1.84 (m, 1H), 1.82-1.45 (m, 10H, 1.41 (s, 10H), 1.35-1.10 (m 10H), 1.05-1.10 (m, 1H) 0.97 (d, 3H, *J* - 5.7 Hz), 0.91-0.81 (m, 1H), 0.66 (s, 2H), 0.63 (s, 1H), 0.61-0.56 (m, 1H).

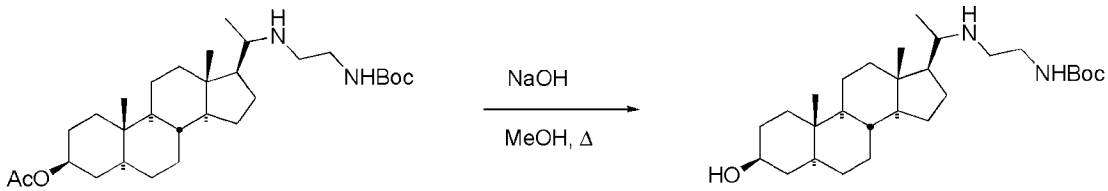
EXAMPLE 46

General procedure for saponification of acetate protective group

[00310] In a 25 mL round bottom flask, 0.150 mmol of starting material were dissolved in 10 mL MeOH and 4 mL NaOH 1M, then refluxed overnight. The following morning, methanol was removed *in vacuo* and the remaining aqueous layer was extracted 3X with AcOEt. The combined organic layers were treated with brine, dried on anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude product was used without further purification.

EXAMPLE 47

Synthesis of pregnan-3β-ol-20-(boc-aminoethyl)amine (39) of formula 2.0



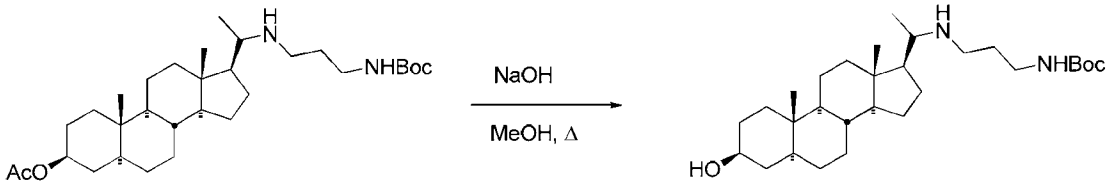
[00311] Following the procedure described in Example 46 above, 84 mg compound **36** (0.166 mmol) were used to yield 77 mg (100%) of desired compound **39**.

[00312] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.08 ppm (d large, 1H), 3.57 (sept, 1H, *J* = 5.0 Hz), 3.20 (sept, 2H, *J* = 5.8 Hz), 2.79 (sext, 1H, *J* = 5.8 Hz), 2.67-2.52 (m, 2H), 2.26-2.10 (m, 3H), 2.00-1.47 (m, 10H), 2.43 (s, 10H), 2.38-1.19 (m, 11H), 1.08 (quad, 3H, *J* = 6.7 Hz), 1.05-1.00 (m, 2H), 0.98 (d, 3H, *J* = 6.1

Hz), 0.94-0.81 (m, 2H), 0.79 (s, 3H), 0.67 (s, 2H), 0.64 (s, 1H0, 0.63-0.57 (m, 1H).

EXAMPLE 48

Synthesis of pregnan-3β-ol-20-(boc-aminopropyl)amine (40) of formula 2.0

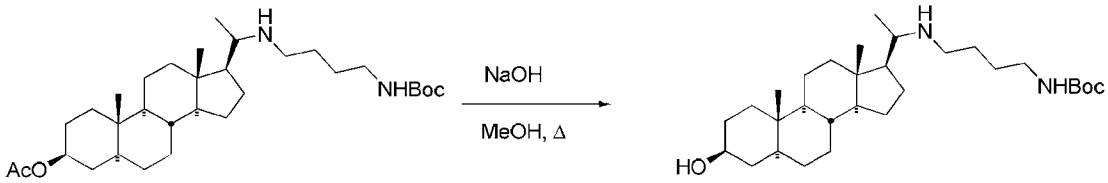


[00313] Following the procedure described in Example 46 above, 51 mg of compound **37** (0.099 mmol) were used to yield 40 mg (85%) of desired compound **40**.

[00314] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 5.07 (s large, 1H), 3.57 (sept, 1H, 5.0 Hz), 3.17 (quad, 2H, *J* = 5.9 Hz), 2.86-2.69 (m, 1H), 2.61-2.43 (m, 2H), 2.03-1.86 (m, 5H), 1.83-1.47 (m, 11H), 1.43 (s, 11H), 1.38-1.19 (m, 11H), 1.13-1.01 (m, 4H), 0.96 (d, 4H, *J* = 6.3 Hz), 0.91-0.82 (m, 2H), 0.79 (s, 3H), 0.67 (s, 2H), 0.64 (s, 1H) 0.62 (dt, 1H, J_1 = 11.7 Hz, J_2 = 3.3 Hz).

EXAMPLE 49

Synthesis of pregnan-3β-ol-20-(boc-aminobutyl)amine (41) of formula 2.0



[00315] Following the procedure described in Example 46 above, 70 mg of compound **38** (0.131 mmol) were used to yield 64 mg (98%) of desired compound **41**.

[00316] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 4.98-4.82 (m, 1H), 3.57 (sept, 1H, *J* = 4.9 Hz), 3.11 (quad, 2H, *J* = 6.1 Hz), 2.75-2.65 (m, 1H0, 2.61-2.39 (m, 2H), 1.97-1.46 (m, 14H), 1.42 (s, 9H), 1.38-1.20 (M, 9H), 1.08 (d, 3H, *J* = 6.1 Hz), 0.96 (d, 3H, *J* = 6.1 Hz), 0.93-0.81 (m, 2H), 0.79 (s, 3H), 0.67 (s, 2H), 0.65 (s, 1H), 0.63-0.56 (m, 1H).

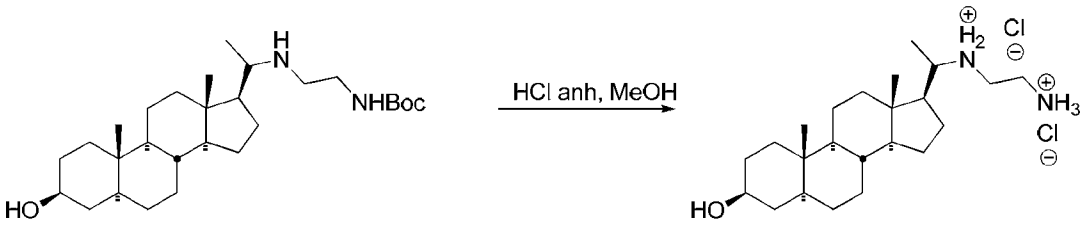
EXAMPLE 50

General procedure for Boc removal

[00317] In a 25 mL round bottom flask, the starting material was dissolved in 5 mL MeOH. A solution of anhydrous HCl (5 mL MeOH + 75 µL AcCl) was added, and the reaction was allowed to stir for 1h. The solvent was then removed *in vacuo*. The product was purified by trituration with ether or used as such.

EXAMPLE 51

Synthesis of pregnan-3β-ol-20-(aminoethyl)amine hydrochloride salt (42) of formula 2.0

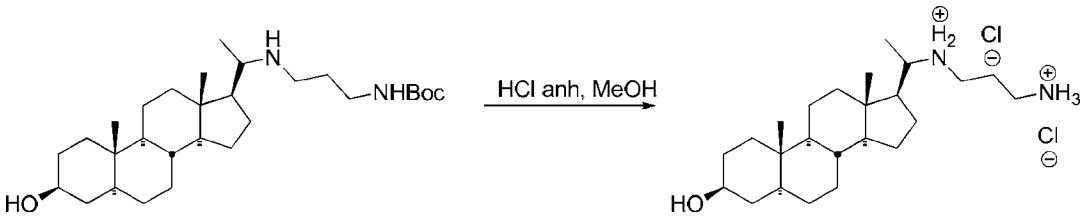


[00318] Following the procedure described in Example 50 above, 77 mg of compound **39** (0.166 mmol) were used to yield 64 mg (77%) of desired compound **42**.

[00319] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 3.49 (sept, 1H, *J* = 4.8 Hz), 3.41-3.31 (m, 3H), 3.30-3.24 (m, 1H), 3.11-3.01 (m, 1H), 2.05-1.83 (m, 2H), 1.82-1.61 (m, 6H), 1.58 (s, 3H), 1.43 (s, 3H), 1.40-1.29 (m, 4H), 1.30-1.21 (m, 6H), 1.17-1.06 (m, 3H), 1.03-0.86 (m, 2H), 0.82 (s, 3H), 0.76 (s, 1H), 0.73 (s, 2H), 0.69-0.61 (m, 1H).

EXAMPLE 52

Synthesis of pregnan-3β-ol-20-(aminopropyl)amine hydrochloride salt (43) of formula 2.0



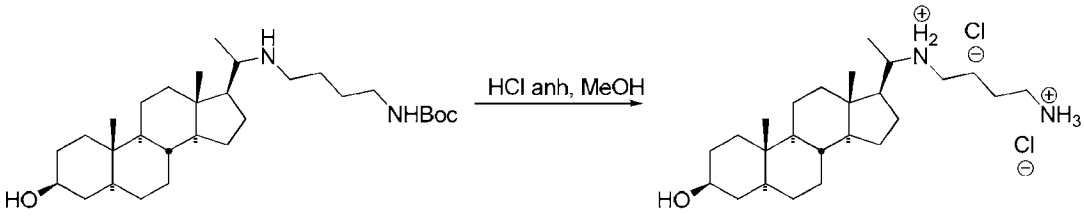
[00320] Following the procedure described in Example 50 above, 40 mg of compound **40** (0.084 mmol) were used to yield 23 mg (61%) of desired compound **43**.

[00321] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 3.45-3.34 (m, 2H) 3.28 (quint, 2H, *J* = 1.7 Hz), 3.13 (t, 2H, *J* = 6.9 Hz), 3.05 (t, 2H, *J* = 7.9 Hz) 2.09 (quint, 1H, *J* = 7.2 Hz), 1.97-1.79 (m, 2H), 1.79-1.62 (m, 5H),

1.58 (s, 1H), 1.56-1.45 (m, 2H), 1.43 (s, 4H), 1.40-1.33 (m, 3H), 1.33-1.21 (m, 7H), 1.17-1.04 (m, 3H), 1.03-1.86 (m, 2H), 0.82 (s, 3H), 0.73 (s, 3H), 0.71-0.60 (m, 1H).

EXAMPLE 53

Synthesis of pregnan-3β-ol-20-(aminobutyl)amine hydrochloride salt (44) of formula 2.0

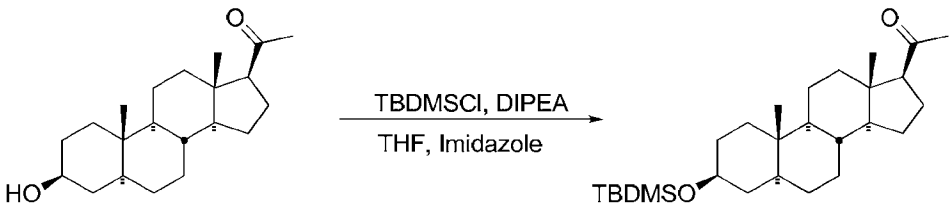


[00322] Following the procedure described in Example 50 above, 63 mg of compound **41** (0.128 mmol) were used to yield 50 mg (85%) of desired compound **44**.

[00323] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 3.55-3.37 (m, 2H), 3.28 (m, 2H, *J* = 1.5 Hz), 3.09-3.01 (s large, 2H), 3.01-2.92 (s large, 2H), 2.00-1.82 (m, 2H), 1.81-1.62 (m, 8H,)1.62-1.45 (m, 1H), 1.45-1.33 (m, 4H), 1.33-1.21 (m, 7H), 1.19-1.07 (m, 3H), 1.03-0.86 (m, 2H), 0.82 (s, 3H), 0.73 (s, 4H).

EXAMPLE 54

Synthesis of O-*t*-butyldimethylsilylpregnanolone (45) of formula 2.0

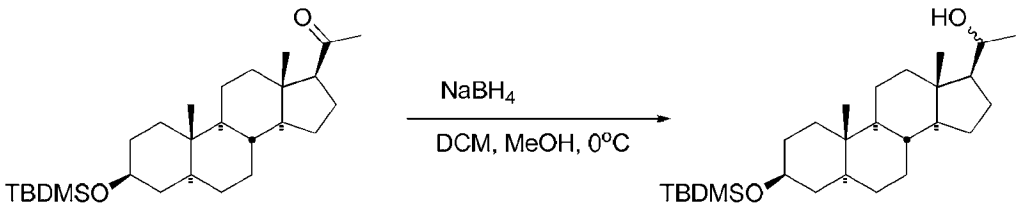


[00324] In a 250 mL round bottom flask, pregnanolone **28** (4.34g, 13.6 mmol) was dissolved in 120 mL THF. Imidazole (2.3g, 34 mmol, 2.5 eq), *t*-butyldimethylsilyl chloride (2.56g, 17 mmol, 1.25 eq) and DIPEA (4.7 mL, 27.2 mmol, 2.0 eq) were successively added and the reaction was allowed to stir overnight at room temperature. The mixture was then concentrated under reduced pressure and diluted in AcOEt. The mixture was washed with water, 2X $NaHCO_3$ sat., 2X brine, dried over $MgSO_4$, and the solvents were removed *in vacuo*. The crude product was purified by flash chromatography (25% AcOEt/Hexanes) to yield 5.26g (89%) of the desired compound **45**.

[00325] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.54 (sept, 1H, *J* = 5.8 Hz), 2.51 (t, 1H, *J* = 9.4 Hz), 2.21-2.11 (m, 1H), 2.10 (s, 3H), 2.05-1.96 (m, 1H), 2.72-1.54 (m, 8H), 1.49-1.01 (m, 13H), 1.00-0.90 (m, 3H), 0.88 (s, 9H), 0.79 (s, 3H), 0.66 (dt, 3H, J_1 = *11.6 Hz*, J_2 = 4.8 Hz), 0.59 (s, 3H), 0.05 (s, 6H).

EXAMPLE 55

Synthesis of *t*-Butyldimethysilylpregnane-3,20-diol (53) of formula 2.0

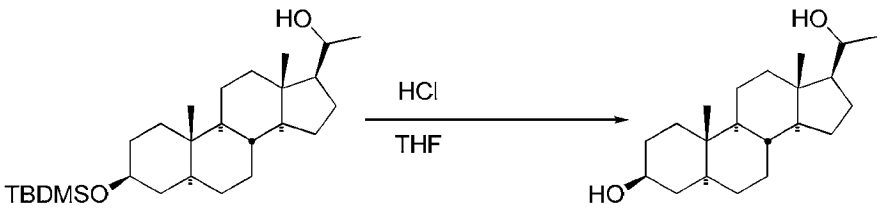


[00326] In a 25 mL round bottom flask, compound **45** (100 mg, 0.231 mmol) was dissolved in 5 mL DCM and 5 mL MeOH, then cooled at 0°C. $NaBH_4$ (9.6 mg, 0.254 mmol, 1.1 eq) was added and the reaction was stirred for 1h at 0°C, monitored by TLC (50% AcOEt/Hexanes). Upon completion, the reaction was quenched with acetone for 30 minutes, then concentrated *in vacuo*. The resulting compound was suspended in water, and extracted 3X with AcOEt. The combined organic layers were washed with brine, dried on anhydrous $MgSO_4$ and concentrated *in vacuo*. The crude compound was purified by flash chromatography (10% AcOEt/Hexanes) to yield 66 mg (66%) of the desired compound **53**.

[00327] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.78-3.66 (m, 1H), 3.54 (sept, 1H, *J* = 5.8 Hz), 2.17 (s, 3H), 2.04-1.96 (m, 1H), 1.72-1.59 (m, 5H), 1.58-1.43 (m, 6H, 1.43-1.18 (m, 8H), 1.12 (d, 3H, *J* = 5.8 Hz), 1.08-0.91 (m, 3H), 0.88 (s, 9H), 0.80 (s, 3H), 0.73 (s, 3H), 0.67-0.57 (m, 1H), 0.04 (s, 6H).

EXAMPLE 56

Synthesis of pregnane -3,20-diol (54) of formula 2.0



[00328] In a 5 mL round bottom flask, compound **53** (10 mg, 0.023 mmol) was dissolved in 2 mL

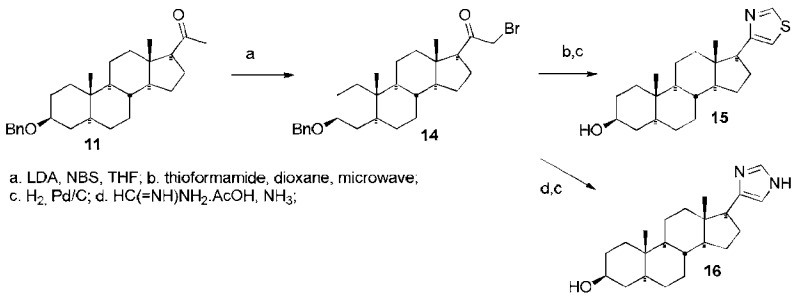
THF and 0.5 mL HCl 1M. The reaction was stirred for 2h, monitored by TLC. Upon completion, the reaction was concentrated *in vacuo* and AcOEt was added. The organic layer was washed successively with saturated aquous $NaHCO_3$, water and brine, then dried on anh. $MgSO_4$ and concentrated *in vacuo*. The crude material was purified by flash chromatography (50% AcOEt/Hexanes) to produce the desired compound **54** in a quantitative yield.

[00329] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.77-3.67 (m, 1H), 3.59 (sept, 1H, *J* = 5.4 Hz), 1.85-1.45 (m, 14H), 1.44-1.18 (m, 14H), 1.13 (d, 5H, *J* = 6.1 Hz), 1.06-0.83 (m, 5H), 0.81 (s, 3H) 0.74 (s, 3H), 0.70-0.59 (m, 1H).

[00330] **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ (ppm) 71.4 (s), 70.6 (s), 58.6 (s), 55.9 (s), 54.3 (s), 44.9 (s), 42.5 (s), 40.1 (s), 38.2 (s), 37.0 (s), 35.5 (s), 35.4 (s), 32.1 (s), 31.5 (s), 28.7 (s), 25.6 (s), 24.5 (s), 23.6 (s), 21.1 (s), 12.6 (s), 12.3 (s). **HRMS** calculated for $C_{21}H_{36}O_2$: 320.2715, found: 320.2708.

EXAMPLE 57

Synthesis of heterocyclic analogues 15,16 of formula 3.0

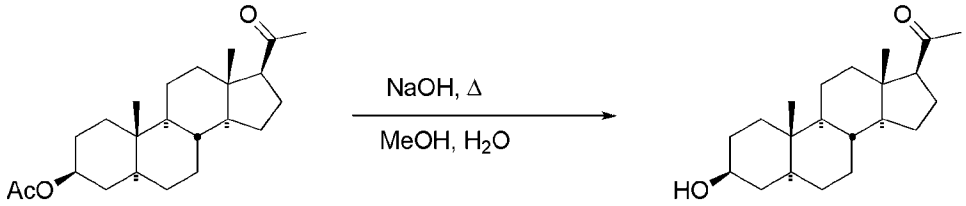


a. LDA, NBS, THF; b. thioformamide, dioxane, microwave;
c. H_2, Pd/C; d. $HC(=NH)NH_2$.AcOH, NH_3;

[00331] Thiazole analogue **15** is synthesized by initial bromination of exocyclic ketone **11** to generate intermediate bromoketone **14**, treatment of bromoketone **14** with thioformamide followed by hydrogenolysis delivers analogue **15** (Ayesa *et al.*, 2009). Treatment of bromoketone **14** with formamidine acetate followed by ammonia gives imidazole derivative **16** (Wong, 1995). Additional heterocyclic derivatives are obtained by the same approach (pyridines, substituted pyrimidines, thiazoles, imidazoles and pyridines).

EXAMPLE 58

Synthesis of pregnanolone (28) of formula 3.0



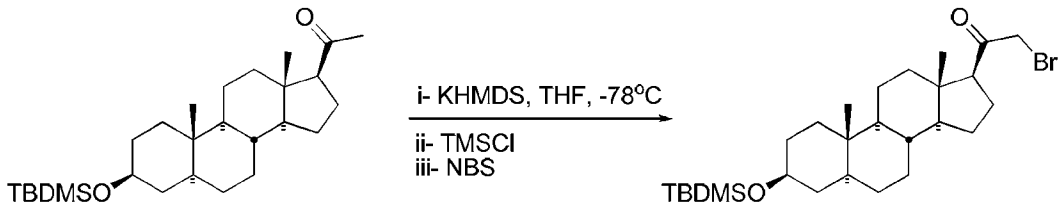
[00332] In a 500 mL round bottom flask, pregnanolone acetate (1.5g, 4.18 mmol) was dissolved in 200 mL of MeOH, 70 mL EtOH, 80 mL H_2O and 10 mL NaOH 1M. The mixture was refluxed for 4 hours. The organic solvents were then removed under reduced pressure as a solid suspension could be observed in the remaining aquous phase. The solid was isolated by filtration, rinced with cold water and dried overnight at room temperature to yield 1.1g (81%) of desired compound.

[00333] **^{1}H NMR** (300 MHz, CD_3OH) δ (ppm) 3.60 (m, 1H, *J* = 4.5 Hz), 2.52 (t, 1H, *J* = 8.8 Hz), 2.22-2.12 (m, 1H), 2.11 (s, 1H), 2.04-1.96 (m, 1H), 1.86-1.76 (m, 1H), 1.77-1.57 (m, 6H), 1.49-1.20 (m, 9H), 1.20-1.06 (m, 3H), 1.04-0.84 (m, 3H), 0.81 (s, 3H), 0.68 (dt, 1H, J_1 = 10.4 Hz, J_2 = 3.8 Hz), 0.60 (s, 3H).

[00334] **^{13}C NMR** (75.5 MHz, $CDCl_3$) δ (ppm) 209.7 (s), 71.2 (s), 63.8 (s), 56.7 (s). 54.2 (s), 44.8 (s), 44.3 (s), 39.1 (s), 38.2 (s), 37.0 (s), 35.5 (s), 32.0 (s), 31.4 (s), 31.0 (s), 28.6 (s), 24.4 (s), 22.8 (s), 21.3 (s), 13.5 (s), 12.3 (s). **HRMS** calculated for $C_{21}H_{34}O_2$: 318.2559, found: 318.2552.

EXAMPLE 59

Synthesis of O-*t*-butyldimehylsilyl-21-bromopregnanolone (46) of formula 3.0



[00335] In a 50 mL round bottom flask under argon atmosphere, compound **45** (500 mg, 1.15 mmol) was cooled to -78°C in anhydrous THF. KHMDS 1M in THF (1.27 ml, 1.27 mmol, 1.1 eq) was added and the mixture was stirred for 15 minutes. TMSCl (150 µL, 1.15 mmol, 1.0 eq) was added and the mixture was stirred for 1h at room temperature, and monitored by TLC. (50% AcOEt/Hexanes,). The reaction was cooled down to -78°C before addition of N-Bromosuccinimide (204 mg, 1.15 mmol, 1.0 eq). After 1 hour of stirring at -78°C, the reaction was quenched with saturated aquous $NaHCO_3$ and THF was evaporated under reduced pressure. Water was added, and the solution was extracted with 3X AcOEt. The combined organic layers were washed with brine, dried on anhydrous $MgSO_4$ and concentrated *in vacuo*. The

compound was purified by flash chromatography (2% AcOEt/Hexanes to 6% AcOEt/Hexanes) to yield 510 mg (87%) of desired compound **46**.

[00336] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.87 (d, 2H, J = 2.7 Hz), 3.51 (sept, 1H, J = 5.3 Hz), 2.78 (t, 1H, J = 8.9 Hz), 2.14 (quad, 1H, J = 9.2 Hz), 1.87 (dt, 2H, J_1 = 11.7 Hz, J_2 = 2.7 Hz), 1.73-1.52 (m, 8H), 1.46-1.29 (m, 5H), 1.29-1.11 (m,6H), 1.08-0.88 (m, 2H), 0.85 (s, 10H), 0.76 (s, 3H), 0.63 (dt, 1H, J_1 = 12.1 Hz, J_2 = 2.9 Hz), 0.59 (s, 3H), 0.01 (s, 6H) .

EXAMPLE 60

General procedure for substitution of bromine by amino compounds

[00337] In a 20 mL vial, compound **46** was solubilised in THF (≈ 0.1M). 2.0 eq of corresponding amine was added, and the reaction was stirred for 1h at room temperature. THF was removed *in vacuo*. The obtained solid was suspended into water, and extracted 3X with AcOEt. The organic combined layers were washed with brine, dired on anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. Crude compound was purified by flash chromatography.

[00338] Compound was then solubilised in THF:HCl (4:1 solution) and stirred for 2h. Upon completion (TLC), saturated aq. $NaHCO_3$ was added until the solution is alkaline and THF was removed *in vacuo*. The remaining aquous layer was extracted 3X with AcOEt, and the combined organic layers were washed with brine, dried on anhydrous $MgSO_4$ and concentrated under reduced pressure. The crude compound was purified by flash chromatography.

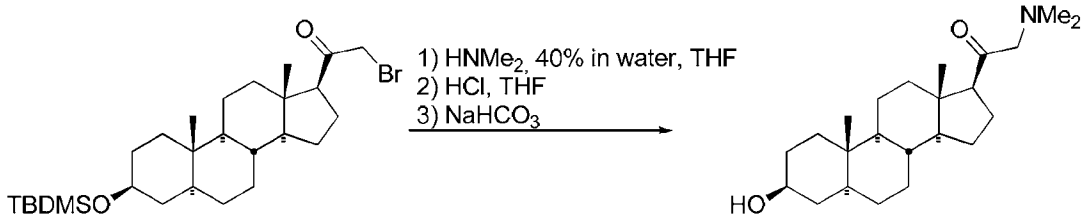
EXAMPLE 61

General procedure for substitution of bromine by amino compounds

[00339] In a 5 mL vial, compound **46** was solubilised in THF (0.09M) and 2-5 eq of corresponding amine was added. The reaction was stirred overnight, monitored by TLC for completion. The reaction was then acidified with HCl 1M, and allowed to stir upon completion, monitored by TLC. THF was removed *in vacuo* and the remaining water was removed via lyophilization. The crude product was purified via reverse-phase preparative chromatography.

EXAMPLE 62

Synthesis of N,N-dimethyl-21-aminopregnanolone (47) of formula 3.0



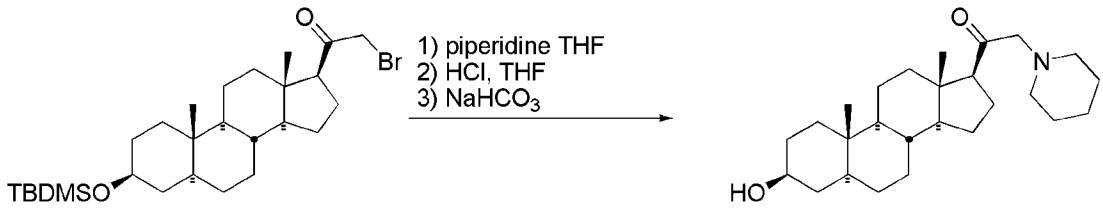
[00340] Following the procedure described in Example 60 above, 125 mg of compound **46** (0.244 mmol) were used to obtain 68 mg of silylated intermediate. Upon deprotection, 20 mg (38% overall yield) of compound **47** were obtained.

[00341] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.58 (sept, 1H, J = 5.2 Hz), 3.13 (dd, 2H, J_1 = 21.4 Hz, J_2 = 21.7 Hz), 2.55 (t, 1H, J = 8.8 Hz), 2.28 (s, 6H), 2.16 (d, 1H, J = 9.3 Hz), 1.91-175 (m, 4H), 1.74-1.52 (m, 6H), 1.43-1.20 (m, 8H), 1.20-1.04 (m, 3H), 1.03-0.83 (m, 3H), 0.79 (s, 1H), 0.72-0.61 (m, 1H), 0.60 (s, 3H).

[00342] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 206.5 (s), 70.3 (s), 60.3 (s), 56.6 (s), 55.9 (s), 55.7 (s), 54.4 (s), 54.1 (s), 44.7 (s), 44.4 (s), 43.8 (s), 43.5 (s), 38.7 (s), 38.2 (s), 37.5 (s), 36.8 (s), 35.6 (s), 35.4 (s), 35.2 (s), 31.8 (s), 30.6 (s), 29.5 (s), 24.1 (s), 23.4 (s), 22.4 (s), 20.9 (s), 20.8 (s), 11.3 (s). **HRMS** calculated for $C_{23}H_{39}O_2N$: 362.3059, found: 362.3059.

EXAMPLE 63

Synthesis of 21-piperidinopregnanolone (48) of formula 3.0



[00343] Following the procedure described in Example 60 above, 100 mg of compound **46** (0.195 mmol) were used to obtain 80 mg of silylated intermediate. Upon deprotection, 46 mg (74% overall yield) of compound **48** were obtained.

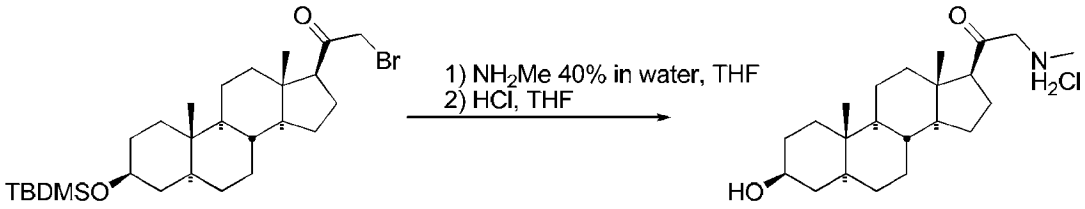
[00344] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.56 (sept, 1H, J = 4.8 Hz), 3.08 (s, 2H), 2.58 (t, 1H, J = 9.8 Hz), 2.37 (s large, 4H), 2.13 (d, 1H, J = 10.3 Hz), 1.86 (dt, 2H, J_1 = 11.1 Hz, J_2 = 3.9 Hz), 1.78 (d, 1H, J = 11.1 Hz), 1.72-1.48 (m, 10H), 1.45-1.19 (m, 10H), 1.18-1.03 (m, 3H), 1.01-0.83 (m, 3H), 0.77 (s, 3H), 0.70-0.62 (m, 1H), 0.58 (s, 3H).

[00345] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 213.5 (s), 71.3 (s), 60.2 (s), 58.8 (s), 56.8 (s), 54.7

(s), 54.2 (s), 44.8 (s), 39.0 (s), 38.1 (s), 37.0 (s), 35.5 (s), 34.5 (s), 32.0 (s), 31.5 (s), 28.6 (s), 25.6 (s), 24.5 (s), 23.9 (s), 23.0 (s), 21.3 (s), 13.6 (s), 12.4 (s). **HRMS** calculated for $C_{26}H_{43}O_2N$: 402.3372, found: 402.3380.

EXAMPLE 64

Synthesis of N-methyl-21-aminopregnanolone hydrochloride salt (49) of formula 3.0



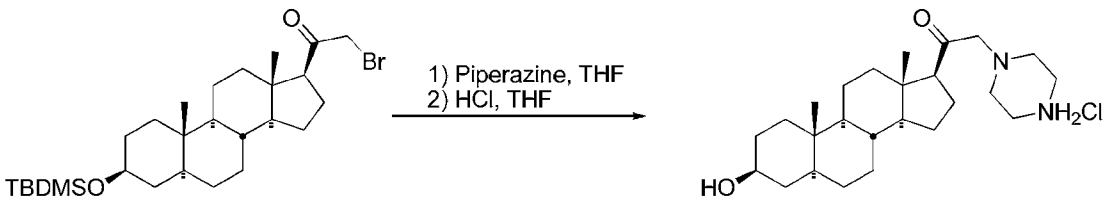
[00346] Following the procedure described in Example 61 above, 150 mg of compound **46** (0.293 mmol) were used to obtain 108 mg (96%) of crude compound. 50 mg were purified by reverse-phase preparative chromatography to yield 32 mg of pure compound **49**.

[00347] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.49 (sept, 1H, *J* = 5.1 Hz), 2.68 (s, 2H), 2.67 (t, 1H, *J* = 9.2 Hz), 2.52 (s, 3H), 2.18-2.07 (m, 1H), 2.00-1.92 (m, 1H), 1.80-1.58 (m, 6H), 1.54-1.46 (m, 1H), 1.45-1.32 (m, 5H), 1.17-1.07 (m, 2H), 1.05-1.90 (m, 3H), 0.82 (s, 3H), 0.76-0.57 (m, 1H), 0.65 (s, 3H).

[00348] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 203.1 (s), 70.4 (s), 60.2 (s), 57.2 (s), 56.4 (s), 54.1 (s), 44.7 (s), 38.2 (s), 37.4 (s), 36.8 (s), 35.4 (s), 35.2 (s), 31.9 (s), 30.6 (s), 28.4 (s), 24.0 (s), 22.3 (s), 20.9 (s), 12.5 (s), 11.2 (s).δ

EXAMPLE 65

Synthesis of 21-piperazinopregnanolone hydrochloride salt (50) of formula 3.0



[00349] Following the procedure described in Example 61 above, 200 mg of compound **46** (0.391 mmol) were used to obtain 172 mg (100%) of the desired compound **50**.

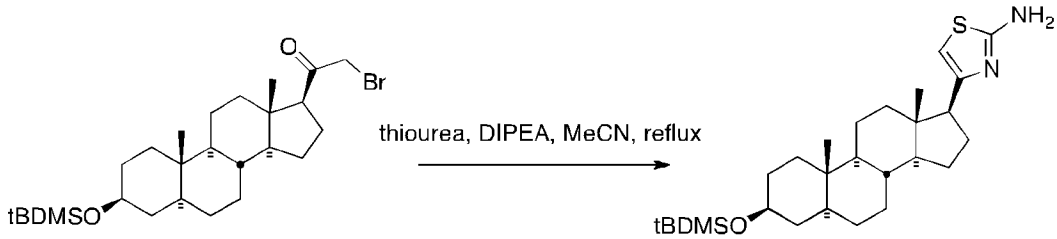
[00350] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 3.36 (m, 1H), 2.63 (t, 1H, *J* = 6.9 Hz), 2.21-2.09 (m, 1H), 2.06-1.98 (m, 1H), 1.81-1.58 (m, 6H), 1.55-1.46 (m, 2H), 1.45-1.33 (m, 3H), 1.33-1.18 (m, 6H), 1.17-

1.06 (m, 2H), 1.05-0.89 (m, 3H), 0.82 (s, 3H), 0.72 (dt, 1H, J_1 = 12.0 Hz, J_2 = 2.8 Hz), 0.67 (s, 3H).

[00351] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 202.6 (s), 70.4 (s), 64.2 (s), 60.5 (s), 56.5 (s), 54.1 (s), 44.9 (s), 44.7 (s), 40.5 (s), 40.2 (s), 38.2 (s), 37.4 (s), 36.8 (s), 35.4 (s), 35.2 (s), 31.8 (s), 30.7 (s), 28.4 (s), 24.0 (s), 22.4 (s), 20.9 (s), 12.5 (s), 11.3 (s).

EXAMPLE 66

Synthesis of aminothiazole (51) of formula 3.0

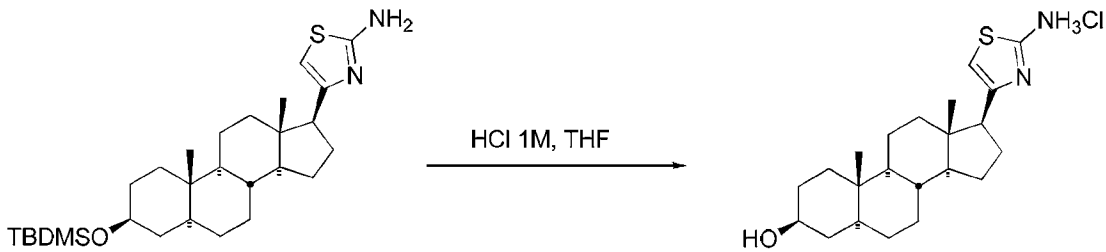


[00352] In a 50 mL round bottom flask, compound **46** (47 mg, 0.091 mmol) was dissolved in 7 mL acetonitrile. Thiourea (8 mg, 0.105 mmol, 1.15 eq) and DIPEA (31 µL, 0.178 mmol, 1.95 eq) were added and the reaction was brought to reflux for 5h, monitored by TLC (50% AcOEt/Hexanes). Upon cooling to room temperature, solvents were removed *in vacuo*. The crude product was purified by flash chromatography (25% AcOEt/Hexanes) to yield 21 mg (44%) of the desired compound **51**.

[00353] **^{1}H NMR** (300 MHz, $CDCl_3$) δ (ppm) 6.09 (s, 1H), 5.03 (s large, 2H), 3.55 (sept, 1H, J = 5.3 Hz), 2.58 (t, 1H, J = 9.7 Hz), 1.98-1.86 (m, 3H), 1.75-1.62 (m, 4H), 1.58-1.33 (m, 4H), 1.32-1.18 (m, 7H), 1.17-1.04 (m, 2H), 1.00-0.91 (m, 1H), 0.88 (s, 9H), 0.79 (s, 3H), 0.66 (dt, 1H, J_1 = 11.0 Hz, J_2 = 3.5 Hz), 0.49 (s, 3H), 0.10 (s, 1H), 0.04 (s, 5H).

EXAMPLE 68

Deprotection of 51 to form compound (52) of formula 3.0



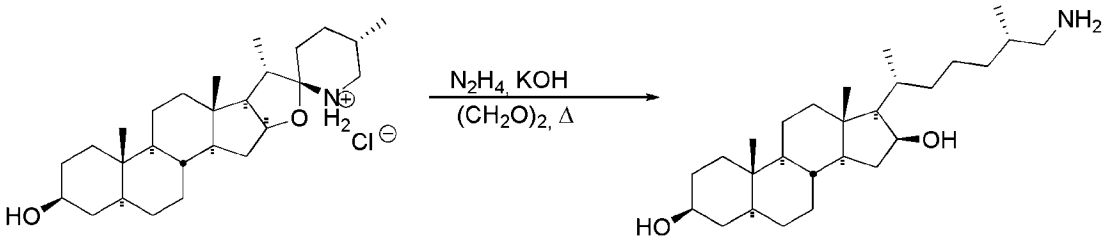
[00354] In a 10 mL round bottom flask, 5 mg compound **51** (10 mmol) was dissolved in 4 mL THF and 1 mL HCl 1M. The reaction was stirred at room temperature until completion (2h, monitored by TLC). Solvent was removed *in vacuo*, and the remaining aquous phase was removed by 2 co-evaporations with THF. The crude compound was triturated with diethyl ether to yield 4 mg of desired compound **52** (hydrochloric salt) (100% yield).

[00355] **^{1}H NMR** (300 MHz, CD_3OD) δ (ppm) 6.52 (s, 1H), 3.49 (sext, 1H, J = 5.8 Hz), 2.61 (t, 1H, J = 2.64 Hz), 2.07-1.86 (m, 2H), 1.81-1.79 (m, 5H), 1.56-1.08 (m, 11H), 1.05-0.91 (m, 2H),0.82 (s, 3H), 0.72 (dt, 1H, J_1 = 11.0 Hz, J_2 = 4.2 Hz), 0.59 (s, 3H).

[00356] **^{13}C NMR** (75.5 MHz, CD_3OD) δ (ppm) 167.5 (s), 142-6 (s), 101.7 (s), 70.3 (s), 55.5 (s), 54.4 (s), 50.0 (s), 44.8 (s), 44.0 (s), 37.5 (s), 37.4 (s), 36.8 (s), 35.9 (s), 35.3 (s), 31.8 (s), 30.7 (s), 28.4 (s), 25.4 (s), 23.7 (s), 20.7 (s), 12.3 (s), 11.3 (s). **HRMS** calculated for $C_{22}H_{35}ON_2S$: 374.2392, found: 374.2388.

EXAMPLE 69

Synthesis of compound 26 of formula 5.0



[00357] In a 5 mL round bottom flask, tomatidine hydrochloride salt (10 mg, 0.022 mmol), hydrazine (10.5 µL, 0.332 mmol, 15 eq.) and KOH (18 mg, 0.320 mmol, 14.5 eq) in 2.5 mL ethylene glycol were heated to 100°C for 1h, then to 200°C for 4h. The reaction was monitored by TLC until completion, then allowed to cool to room temperature. The resulting mixture was diluted with water, then extracted 5X with diethyl ether. The organic fractions were combined, washed with brine, dried on anhydrous $MgSO_4$ and solvent was removed under reduced pressure. The crude product was purified by flash chromatography (25% AcOEt/Hexanes) to yield 5 mg (54%) of the desired compound **26**.

EXAMPLE 70

Potentiating effect of steroid alkaloids on aminoglycoside antibiotics against normal *S. aureus strains* and antimicrobial effect of steroid alkaloids against SCV *S. aureus* bacteria

[00358] Compounds of the present invention potentiate aminoglycosides' effect on normal *S. aureus* strains and are antibacterial against against *S. aureus* SCVs.

[00359] Compounds of the invention were tested for their ability to potentiate the aminoglycoside antibiotic gentamicin against *S. aureus* ATCC 29213 and for their antibacterial activity against ATCC 29213 and the SCV strain NewbouldΔ*hemB*. MICs were determined as described in the Example 10 above.

[00360] **Results:** Table 11 below shows that compounds of the present invention have the ability to potentiate gentamicin against normal *S. aureus* and to inhibit the growth of SCV strain NewbouldΔ*hemB*. Compounds were divided in categories according to their potentiating activity level with gentamicin (no or mild potentiation (1-2 fold increase in gentamicin activity) vs. moderate or strong potentiation (4-16 fold increase in gentamicin activity)) and antibacterial activity level against SCVs (low, MIC > about 8 µg/ml; moderate, MIC = about 4 to about 8 µg/ml; and strong activity, MIC ≤about 0.5 µg/ml).

[00361] TABLE 11: Antibacterial efficacy (MIC, Minimal Inhibitory Concentration) of compounds (Cpd) of the invention as determined by 1) the susceptibility (MIC in µg/ml) of *S. aureus* ATCC 29213 to the aminoglycoside antibiotic gentamicin (GEN) in the presence of 8 µg/ml of the Cpd and/or 2) their antibacterial activity against *S. aureus* SCVs.

Compound (Cpd)	MIC of Cpd against ATCC29213 (µg/ml)	Fold[a] (MIC of GEN alone/MIC of GEN with Cpd)	MIC of Cpd against SCV *hemB*[b] (µg/ml)
Tomatidine hydrochloride salt	>16	4-16	≤ 0.5
Tomatidine mesylate (57)	>16	4-16	≤ 0.5
Tomatidine citrate (58)	>16	4-16	≤ 0.5
Solasodan	>16	1-2	>8
N-formyl tomatidine (21)	>16	1-2	>8
3-alpha-hydroxytomatidine hydrochloride salt (23)	>16	4-16	4-8
3-oxotomatidine hydrochloride salt (25)	>16	4-16	≤ 0.5

Compound 26	>16	4-16	≤ 0.5
Pregnanolone (28)	>16	1-2	>8
pregnan-3β-ol-20-amine Diasterioisomer (30a)	>16	1-2	>8
pregnan-3β-ol-20-amine Diasterioisomer (30b)	>16	1-2	>8
Pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine (32)	>16	4-16	>8
Pregnan-3β-ol-20-(aminoethyl)amine hydrochloride salt (42)	>16	1-2	4-8
Pregnan-3β-ol-20-(aminopropyl)amine hydrochloride salt (43)	>16	1-2	4-8
Pregnan-3β-ol-20-(aminobutyl)amine hydrochloride salt (44)	>16	1-2	>8
N,N-dimehyl-21-aminopregnanolone (47)	>16	1-2	>8
21-piperidinopregnanolone (48)	>16	1-2	>8
N-methyl-21-aminopregnanolone hydrochloride salt (49)	>16	1-2	>8
21-piperazinopregnanolone hydrochloride salt (50)	>16	1-2	>8
Compound 52	>16	1-2	>8
pregnane -3,20-diol (54)	>16	1-2	>8
O-allyltomatidine hydrochloride salt (56)	8-16	4-16	≤ 0.5

[a]The fold is the ratio of the MIC of gentamicin (GEN) alone against *S. aureus* ATCC 29213 (MIC of 0.5-1 µg/ml) over the MIC of gentamicin obtained in the presence of 8 µg/ml of compound (Cpd). The exception was Compound **56** that was used at 4 µg/ml. Results are provided in categories of synergy (no or mild synergy, 1-2 synergy fold; moderate or strong synergy, 4-16 synergy fold).

[b]Results are provided in categories of inhibitory activities against *S. aureus* SCV (strong activity, MIC ≤ 0.5 µg/ml; moderate activity, MIC = 4-8 µg/ml; low activity, MIC >8 µg/ml).

EXAMPLE 71

Antibacterial activity of compounds of the present invention on *Bacillus spp.*, and on *Listeria spp.*

[00362] Susceptibility of the *Bacillus spp.*, and of the *Listeria spp.* to steroid alkaloids was determined as follows.

[00363] **Method:** The effect of tomatidine on the growth of *Bacillus subtilis* strains ATCC 6633 and ATCC 9372, *Bacillus cereus* strain ATCC 11778 and *Listeria monocytogenes* strain ATCC 13932 was tested by an agar diffusion method. *Bacillus* spp. strains and *Listeria monocytogenes* were spread on the surface of Mueller-Hinton agar and Mueller-Hinton supplemented with 5% sheep blood, respectively. 35 µg of tomatidine diluted in DMSO or DMSO alone were added to wells for diffusion and plates were incubated for 24 hours at 35°C. The diameters of the zones of inhibition around the wells (for the DMSO control and for the tomatidine well) were measured and reported in mm in TABLE 12 below.

TABLE 12. Tomatidine biological activity against *Bacillus* and *Listeria* spp.

Species	Strains	Diameter of inhibition zone (mm)	
		DMSO	Tomatidine (35 µg)
Bacillus subtilis	ATCC 9372	0	25.5
Bacillus subtilis	ATCC 6633	5	25.5
Bacillus cereus	ATCC 11778	0	21.5
Listeria monocytogenes	ATCC 13932	0	12.5

[00364] Together with results presented in Examples 1, 2, 8 and 10, results from TABLE 12 show that compounds of the present invention have biological activities against bacteria within the Firmicutes phylum.

EXAMPLE 72

[00365] The biological activity of compounds of the present invention can be determined using techniques as described in Examples 1 (i.e., antibacterial activity against *S. aureus* SCVs), 2 (i.e., antibacterial activity against anaerobic bacterium (*e.g.*, *C. perfringens*)), 8 (i.e., antibacterial activity against normal *S. aureus* in co-culture with *P. aeruginosa*), 10 (i.e., potentiating effect on aminoglycoside antibiotics against normal *S. aureus*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. hominis*) and 70

(i.e.,antibacterial activity against *Bacillus* spp.and *Listeria* spp.) above.

[00366] Also determined is the antibacterial activity against the streptococci of group A, of group B, of the viridans group, of the mitis group, whereas the strains and species are of human or animal origins, such as *S. pneumoniae*, *S. pyogenes*, *S. mitis*, *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, *S. suis*, *S. bovis* and *S. intermedius*. Additional coagulase-positive and -negative staphylococci are tested including *S. intermedius*, *S. hyicus*, *S. chromogenes*, *S. stimulans*, *S. lugdenensis* *S. capitis*.

[00367] Additional anaerobes are tested including the *C. difficile*, the *Peptostreptococcus*, *Peptococcus* following the method described in Example 2 above. Cultivation techniques for aerobes, anaerobes and fastidious bacteria are as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2006).

[00368] Susceptibility of other bacterial genus such as *Corynebacterium* and *Gardnerella* is also tested.

EXAMPLE 73

Inhibitory effect of compounds of the present invention measured in cell cultures

[00369] The compounds of the present invention are tested for their ability to inhibit the growth of microbial pathogens with electron transport deficiencies (or with normal electron transport when used in combination with aminoglycosides) during infection of cell cultures such as those used in Example 7.

EXAMPLE 74

Inhibitory effect of compounds of the present invention measured during infection in animals (*in vivo*)

[00370] The compounds of the present invention are able to inhibit the growth of microbial pathogens with electron transport deficiencies (or with normal electron transport when used in combination with aminoglycosides) during infection of an animal (*in vivo*). The antibacterial activity *in vivo* is demonstrated through the use of various infection models using, for example mice models of septicemia, soft tissue infections, pneumonia and mastitis.

Septicemia model

[00371] The septicemia model (Deslouches et al, 2005) allows testing the efficacy of compounds to clear or diminish an infection. Bacteria are injected iv or ip with an inoculum that leads to 50-70% mortality in untreated mice (3-5 mice per test group). Following inoculation, compounds are administered either iv, ip, sc

or im and treatment efficacy is measured by the reduction of bacterial CFU in various organs (*e.g.*, liver, kidneys), in the peritoneal liquid or in blood or is evaluated based on the animals' survival rate.

Neutropenic mouse thigh model

[00372] Compound efficacy in a neutropenic mouse thigh model is evaluated as follows (Malouin et al, 2005): Mice (immune suppressed with cyclophosphamide treatments prior to infection) are challenged with bacteria (10^4 CFU per thigh im). To determine efficacy, compounds are delivered iv, sc, ip or im 2 h post-infection. Mice (3-5 mice per treatment) are euthanized 8 h post-infection. The thigh tissues (two samples per animal) are recovered, homogenized, and bacterial CFU per g of tissue are determined by plating appropriate dilutions.

Lung infection (pneumonia) model

[00373] Compound efficacy in a lung infection (pneumonia) model is evaluated as follow (Ragle et al, 2010): Mice are challenged with intra-tracheal injection of bacteria (10^8 CFU). To determine efficacy, compounds are delivered iv, sc, ip, im or by aerosol, 2 h post-infection. Mice (3-5 mice per treatment) are euthanized 24 h post-infection. The lungs are recovered, homogenized, and bacterial CFU per g of tissue are determined by plating appropriate dilutions.

Mouse mastitis model

[00374] Compound efficacy in a mouse mastitis model is evaluated as follow (Brouillette et al, 2004b): Lactating CD-1 mice are challenged with bacteria injected through the teat canal. A Hamilton syringe with a blunt needle is used to inoculate with 10^2 CFU per gland in both L4 and R4 mammary glands. Compounds are delivered by an intra-mammary injection 4 h following challenge. Each experimental group is composed of 3-6 mice (i.e., 6-12 glands). Mammary glands are harvested, weighed and homogenized in PBS at 18 h. Homogenates are serially diluted and plated on agar for bacterial CFU determination.

[00375] Although the present invention has been described hereinabove by way of specific embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claims.

REFERENCES

Alexander, E.H., and Hudson, M.C. (2001) Factors influencing the internalization of *Staphylococcus aureus* and impacts on the course of infections in humans. *Appl Microbiol Biotechnol* **56**: 361-366.

Allegrucci, M., and Sauer, K. (2008) Formation of *Streptococcus pneumoniae* non-phase-variable colony variants is due to increased mutation frequency present under biofilm growth conditions. *J Bacteriol* **190**: 6330-6339.

Archer, G.L. (1998) *Staphylococcus aureus*: a well-armed pathogen. *Clin Infect Dis* **26**: 1179-1181.

Atalla, H., Gyles, C., Jacob, C.L., Moisan, H., Malouin, F., and Mallard, B. (2008) Characterization of a *Staphylococcus aureus* small colony variant (SCV) associated with persistent bovine mastitis. *Foodborne Pathog Dis* **5**: 785-799.

Ayesa, S.; Lindquist, C.; Agback, T.; Benkestock, K.; Classon, B.; Henderson, I.; Hewitt, E.; Jansson, K.; Kallin, A.; Sheppard, D.; Samuelsson, B. Solid-phase parallel synthesis and SAR of 4-amidofuran-3-one inhibitors of cathepsin S: effect of sulfonamides P3 substituents on potency and selectivity. *Bioorg Med Chem* **2009,** 17, 1307-24.

Bad Bug Book. *Bacillus cereus* and other *Bacillus* spp. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. Food and Drug Administration (www.fda.gov)

Bednarek, P., and Osbourn, A. (2009) Plant-microbe interactions: chemical diversity in plant defense. *Science* **324**: 746-748.

Beierlein JM, Anderson AC. 2011. New developments in vaccines, inhibitors of anthrax toxins, and antibiotic therapeutics for *Bacillus anthracis*. Curr Med Chem. 18(33):5083-94.

Black, J.G. (2008) *Microbiology: Principles and Explorations* 7th ed. John Wiley & Sons.

Bolger, M. B.; Wieland, S.; Hawkinson, J. E.; Xia, H.; Upasani, R.; Lan, N. C. *In vitro* and *in vivo* activity of 16,17-dehydro-epipregnanolones: 17,20-bond torsional energy analysis and D-ring conformation. *Pharm Res* **1996,** 13, 1488-94.Bouarab, K., El ordi, M., Gattuso, M., Moisan, H. and Malouin, F. (2007) Plant stress response agents affect *Staphylococcus aureus* virulence genes. Abstr. 47th Intersci. Conf. *Antimicrob. Agents Chemother.*, abstr. C1-1483.

Brouillette, E., Martinez, A., Boyll, B.J., Allen, N.E., and Malouin, F. (2004) Persistence of a *Staphylococcus aureus* small-colony variant under antibiotic pressure *in vivo*. *FEMS Immunol Med Microbiol* **41**: 35-41.

Brouillette, E., G. Grondin, C. Lefebvre, B.G. Talbot, and F. Malouin. 2004b. Mouse mastitis model of infection for antimicrobial compound efficacy studies against intracellular and extracellular forms of *Staphylococcus aureus*. Vet. Microbiol. 101:253-262.

Bryan, L.E., and Kwan, S. (1981) Mechanisms of aminoglycoside resistance of anaerobic bacteria and facultative bacteria grown anaerobically. *J Antimicrob Chemother* **8 Suppl D**: 1-8.

Canadian Cystic Fibrosis Foundation (2007) Patient data registry report. Toronto, ON, Canada.

Casey, A.L., Lambert, P.A., and Elliott, T.S. (2007) Staphylococci. *Int J Antimicrob Agents* **29 Suppl 3**: S23-32.

Chambers, H.F., and Deleo, F.R. (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol* **7**: 629-641.

Chastre, J., and Fagon, J.Y. (2002) Ventilator-associated pneumonia. *Am J Respir Crit Care Med* **165**: 867-903.

Chatterjee, I., Herrmann, M., Proctor, R.A., Peters, G., and Kahl, B.C. (2007) Enhanced post-stationary-phase survival of a clinical thymidine-dependent small-colony variant of *Staphylococcus aureus* results from lack of a functional tricarboxylic acid cycle. *J Bacteriol* **189**: 2936-2940.

Clinical and Laboratory Standards Institute (CLSI) (2006) Methods for dilution antimicrobial susceptibility tests for bacteria: Approved Standard.

Cystic Fibrosis Foundation (2008) Patient registry annual report. Washington, D.C.

Dasenbrook, E.C., Checkley, W., Merlo, C.A., Konstan, M.W., Lechtzin, N., and Boyle, M.P. (2010) Association between respiratory tract methicillin-resistant *Staphylococcus aureus* and survival in cystic fibrosis. *Jama* **303**: 2386-2392.

Deslouches, B., K. Islam, J. K. Craigo, S. M. Paranjape, R. C. Montelaro, and T. A. Mietzner. 2005. Activity of the De Novo Engineered Antimicrobial Peptide WLBU2 against *Pseudomonas aeruginosa* in Human Serum and Whole Blood: Implications for Systemic Applications. *Antimicrob. Agents chemother.* 49: 3208–3216

Eliopoulos, G.M., and R.C. Moellering. 1996. Antimicrobial combinations. In Antibiotics in Laboratory Medicine, 4th ed. (V. Lorian, Ed.), pp330-396. Williams and Wilkins, Baltimore, MD.

Friedman, M. (2002) Tomato glycoalkaloids: role in the plant and in the diet. *J Agric Food Chem* **50**: 5751-5780.

Galli, J., Ardito, F., Calo, L., Mancinelli, L., Imperiali, M., Parrilla, C., Picciotti, P.M., and Fadda, G. (2007) Recurrent upper airway infections and bacterial biofilms. *J Laryngol Otol* **121**: 341-344.

Gibson, R.L., Burns, J.L., and Ramsey, B.W. (2003) Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* **168**: 918-951.

Ginnes, R. B., and V. Stewart. 1996. Respiration. In *Escherichia coli* and *Salmonella*, cellular and molecular biology, p. 217-261. Ed. F. C. Neidhardt. American Society for Microbiology, Washington.

Goerke, C., and Wolz, C. (2004) Regulatory and genomic plasticity of *Staphylococcus aureus* during persistent colonization and infection. *Int J Med Microbiol* **294**: 195-202.

Gonzalez-Lamothe, R., Mitchell, G., Gattuso, M., Diarra, M.S., Malouin, F., and Bouarab, K. (2009) Plant antimicrobial agents and their effects on plant and human pathogens. *Int J Mol Sci* **10**: 3400-3419.

Guillet, C., O. Join-Lambert, A. Le Monnier, A. Leclercq, F. Mechaï, M.-F. Mamzer-Bruneel, M. K. Bielecka, M. Scortti, O. Disson, P. Berche, J. Vazquez-Boland, O. Lortholary and M. Lecuit. 2010. Human Listeriosis Caused by *Listeria ivanovii*. Emerging Infectious Diseases, 16:136-138.

Harlid, R., Andersson, G., Frostell, C.G., Jorbeck, H.J., and Ortqvist, A.B. (1996) Respiratory tract colonization and infection in patients with chronic tracheostomy. A one-year study in patients living at home. *Am J Respir Crit Care Med* **154**: 124-129.

Hecht, D.W. (2006) Anaerobes: antibiotic resistance, clinical significance, and the role of susceptibility testing. *Anaerobe* **12**: 115-121.

Harrison, F. (2007) Microbial ecology of the cystic fibrosis lung. *Microbiology* 153: 917-923.

Hoffman, L.R., Deziel, E., D'Argenio, D.A., Lepine, F., Emerson, J., McNamara, S., Gibson, R.L., Ramsey, B.W., and Miller, S.I. (2006) Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* **103**: 19890-19895.

Jacques, M., Aragon, V., and Tremblay, Y.D. (2011) Biofilm formation in bacterial pathogens of veterinary importance. *Anim Health Res Rev* **11**: 97-121.

Kessler, E., Safrin, M., Olson, J.C., and Ohman, D.E. (1993) Secreted LasA of *Pseudomonas aeruginosa* is a staphylolytic protease. *J Biol Chem* **268**: 7503-7508.

Kloos, W.E., and Bannerman, T.L. (1994) Update on clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* **7**: 117-140.

Li, W.; Dang, Y.; Liu, J. O.; Yu, B. Expeditious synthesis of hippuristanol and congeners with potent antiproliferative activities. *Chemistry* **2009,** 15, 10356-9.

Lightbown, J.W., and Jackson, F.L. (1956) Inhibition of cytochrome systems of heart muscle and certain bacteria by the antagonists of dihydrostreptomycin: 2-alkyl-4-hydroxyquinoline N-oxides. *Biochem J* **63**: 130-137.

Lyczak, J.B., Cannon, C.L., and Pier, G.B. (2002) Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* **15**: 194-222.

Machan, Z.A., Taylor, G.W., Pitt, T.L., Cole, P.J., and Wilson, R. (1992) 2-Heptyl-4-hydroxyquinoline N-oxide, an antistaphylococcal agent produced by *Pseudomonas aeruginosa*. *J Antimicrob Chemother* **30**: 615-623.

Malouin, F., E. Brouillette, A. Martinez, B. J. Boyll, J. L. Toth, J. L. Gage and N. E. Allen. 2005. Identification of antimicrobial compounds active against intracellular *Staphylococcus aureus*. *FEMS Immunol. Med. Microbiol.* **45**:245-252.

Martin-Hernandez, A.M., Dufresne, M., Hugouvieux, V., Melton, R., and Osbourn, A. (2000) Effects of targeted replacement of the tomatinase gene on the interaction of *Septoria lycopersici* with tomato plants. *Mol Plant Microbe Interact* **13**: 1301-1311.

Mates, S. M., L. Patel, H. R. Kaback, and M. H. Miller. 1983. Membrane potential in anaerobically growing *Staphylococcus aureus* and its relationship to gentamicin uptake. *Antimicrob. Agents Chemother.* 23:526-530.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V. (1999) Food-related illness and death in the United States. *Emerg Infect Dis* **5**: 607-625.

Melter, O., and Radojevic, B. (2010) Small colony variants of *Staphylococcus aureus* - review. *Folia Microbiol (Praha)* **55**: 548-558.

Miller, M.H., Wexler, M.A., and Steigbigel, N.H. (1978) Single and combination antibiotic therapy of *Staphylococcus aureus* experimental endocarditis: emergence of gentamicin-resistant mutants. *Antimicrob Agents Chemother* **14**: 336-343.

Mingyu, H., Lin, L., Hao, W., Ying, S., Peng-Yu. Y., Mahesh. U., Qing-Hua, X., and Shao, Q. Y. (2011) Multicolor, One- and Two-Photon Imaging of Enzymatic Activities in Live Cells with Fluorescently Quenched Activity-Based Probes (qABPs), *JACS* **133**(31): 12009-12020.

Mitchell, G., Brouillette, E., Seguin, D.L., Asselin, A.E., Jacob, C.L., and Malouin, F. (2010a) A role for sigma factor B in the emergence of *Staphylococcus aureus* small-colony variants and elevated biofilm production resulting from an exposure to aminoglycosides. *Microb Pathog* **48**: 18-27.

Mitchell, G., Seguin, D.L., Asselin, A.E., Deziel, E., Cantin, A.M., Frost, E.H., Michaud, S., and Malouin, F. (2010b) *Staphylococcus aureus* sigma B-dependent emergence of small-colony variants and biofilm production following exposure to *Pseudomonas aeruginosa* 4-hydroxy-2-heptylquinoline-N-oxide. *BMC Microbiol* **10**: 33.

Mitchell, G., Bilodeau, G., Grondin, G., Cantin, A. and Malouin, F (2010c) Defects in the cystic fibrosis transmembrane conductance regulator (CFTR) increase *Staphylococcus aureus* intracellular infection of human pulmonary cells. Abstr. 100th *Gen. Meet. Am. Soc. Microbiol.*, abstr. D-1179.

Mitchell, G., Gattuso, M., Bouarab, K. and Malouin, F (2009) Tomatidine (TO) affects virulence regulators of prototypical *Staphylococcus aureus* (SA) and small-colony variants (SCV) of cystic fibrosis patients. Abstr. 49th Intersci. Conf. *Antimicrob. Agents Chemother.*, abstr. C1-1341.

Moisan, H., Brouillette, E., Jacob, C.L., Langlois-Begin, P., Michaud, S., and Malouin, F. (2006) Transcription of virulence factors in *Staphylococcus aureus* small-colony variants isolated from cystic fibrosis patients is influenced by SigB. *J Bacteriol* **188**: 64-76.

Nagy, E. (2010) Anaerobic infections: update on treatment considerations. *Drugs* **70**: 841-858.
Osbourn, A.E. (1996) Preformed Antimicrobial Compounds and Plant Defense against Fungal Attack. *Plant Cell* **8**: 1821-1831.

Palmer, M.L., Lee, S.Y., Maniak, P.J., Carlson, D., Fahrenkrug, S.C., and O'Grady, S.M. (2006) Protease-activated receptor regulation of Cl- secretion in Calu-3 cells requires prostaglandin release and CFTR activation. *Am J Physiol Cell Physiol* **290**: C1189-1198.

Parkins, M.D., and Elborn, J.S. (2010) Newer antibacterial agents and their potential role in cystic fibrosis pulmonary exacerbation management. *J Antimicrob Chemother* **65**: 1853-1861.

Peter G. M. Wuts & Theodora W. Greene editors, Protective groups in organic synthesis, 4th edition, Wiley 2007.

Proctor, R.A., von Eiff, C., Kahl, B.C., Becker, K., McNamara, P., Herrmann, M., and Peters, G. (2006) Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* **4**: 295-305.

Pyorala, S., and Taponen, S. (2009) Coagulase-negative staphylococci-emerging mastitis pathogens. *Vet Microbiol* **134**: 3-8.

Qazi, S., Middleton, B., Muharram, S.H., Cockayne, A., Hill, P., O'Shea, P., Chhabra, S.R., Camara, M., and Williams, P. (2006) N-acylhomoserine lactones antagonize virulence gene expression and quorum sensing in *Staphylococcus aureus*. *Infect Immun* **74**: 910-919.

Ragle, B.E., Karginov, V.A., and Bubeck Wardenburg, J. (2010) Prevention and treatment of *Staphylococcus aureus* pneumonia with a beta-cyclodextrin derivative. *Antimicrob Agents Chemother* 54, 298-304

Roddick, J.G. (1974) Steroidal glycoalkaloid alpha-tomatine. *Phytochemistry* **13**: 9-25.

Ruiz-Rubio, M., Perez-Espinosa, A., Lairini, K., Roldan-Arjona, T., Dipietro, A., and Anaya, N. (2001) Metabolism of the tomato saponin alpha-tomatine by phytopathogenic fungi. In *Studies in Natural Products Chemistry*. Vol. 25. Rahman, A. (ed). Oxford: Elsevier, pp. 293-326.

Rupnik, M., Wilcox, M.H., and Gerding, D.N. (2009) *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat Rev Microbiol* **7**: 526-536.

Sears, P.M., and McCarthy, K.K. (2003) Management and treatment of staphylococcal mastitis. *Vet Clin North Am Food Anim Pract* **19**: 171-185, vii.

Sears, P. M. and K. K. McCarthy (2003) Management and treatment of staphylococcal mastitis. Vet. Clin. Food Anim. Pract. 19:171-185.

Sendi, P., and Proctor, R.A. (2009) Staphylococcus aureus as an intracellular pathogen: the role of small colony variants. *Trends Microbiol* **17**: 54-58.

Shah, P.M. (2005) The need for new therapeutic agents: what is the pipeline? *Clin Microbiol Infect* **11 Suppl 3**: 36-42.

Sibley, C.D., Parkins, M.D., Rabin, H.R., and Surette, M.G. (2009) The relevance of the polymicrobial nature of airway infection in the acute and chronic management of patients with cystic fibrosis. *Curr Opin Investig Drugs* 10: 787-794.

Sibley, C.D., and Surette, M.G. (2011) The polymicrobial nature of airway infections in cystic fibrosis: Cangene Gold Medal Lecture. *Can J Microbiol* 57: 69-77.

Simons, V., Morrissey, J.P., Latijnhouwers, M., Csukai, M., Cleaver, A., Yarrow, C., and Osbourn, A. (2006) Dual effects of plant steroidal alkaloids on *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* **50**: 2732-2740.

Schmitz, F.-J., A. C. Fluit, M. Gondolf, R. Beyrau, E. Lindenlauf, J. Verhoef, H.-P. Heinz and M. E. Jones. 1999. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. J. Antimicrob. Chemother. 43 : 253–259

Songer, J.G. (2010) Clostridia as agents of zoonotic disease. *Vet Microbiol* **140**: 399-404.

Songer, J. G., and F. A. Uzal. 2005. Clostridial enteric infections in pigs. J. Vet. Diagn. Invest. 17:528-536.

Stepan, J., Pantucek, R., and Doskar, J. (2004) Molecular diagnostics of clinically important staphylococci. *Folia Microbiol (Praha)* **49**: 353-386.

Stewart, P.S. (2002) Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol* **292**: 107-113.

Talbot, G.H., Bradley, J., Edwards, J.E., Jr., Gilbert, D., Scheld, M., and Bartlett, J.G. (2006) Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. *Clin Infect Dis* **42**: 657-668.

Tenhagen, B. A., G. Koster, et al. (2006). "Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany." J Dairy Sci 89(7): 2542-51.

Tuchscherr, L., Medina, E., Hussain, M., Volker, W., Heitmann, V., Niemann, S., Holzinger, D., Roth, J., Proctor, R.A., Becker, K., Peters, G., and Loffler, B. (2011) *Staphylococcus aureus* phenotype switching: an effective bacterial strategy to escape host immune response and establish a chronic infection. *EMBO Mol Med* (in press).

Van Immerseel, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. 2004. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. *Avian Pathol.* 33:537-549.

Vergison, A., O. Denis, A. Deplano, G. Casimir, G. Claeys, F. DeBaets, K. DeBoeck, N. Douat, H. Franckx, J. Gigi, M. Ieven, C. Knoop, P. Lebeque, F. Lebrun, A. Malfroot, F. Paucquay, D. Pierard, J. Van Eldere and M. J. Struelens. 2007. National survey of molecular epidemiology of *Staphylococcus aureus* colonization in Belgian cystic fibrosis patients. *J. of Antimicrob Chemother.* 59:893–899.

Vial, L., Lepine, F., Milot, S., Groleau, M.C., Dekimpe, V., Woods, D.E., and Deziel, E. (2008) *Burkholderia pseudomallei*, *B. thailandensis*, and *B. ambifaria* produce 4-hydroxy-2-alkylquinoline analogues with a methyl group at the 3 position that is required for quorum-sensing regulation. J Bacteriol 190: 5339-5352.

Voggu, L., Schlag, S., Biswas, R., Rosenstein, R., Rausch, C., and Gotz, F. (2006) Microevolution of cytochrome bd oxidase in Staphylococci and its implication in resistance to respiratory toxins released by Pseudomonas. *J Bacteriol* **188**: 8079-8086.

Vuong, C., and Otto, M. (2002) *Staphylococcus epidermidis* infections. *Microbes Infect* **4**: 481-489.

Wellinghausen, N., Chatterjee, I., Berger, A., Niederfuehr, A., Proctor, R.A., and Kahl, B.C. (2009) Characterization of clinical *Enterococcus faecalis* small-colony variants. *J Clin Microbiol* **47**: 2802-2811.

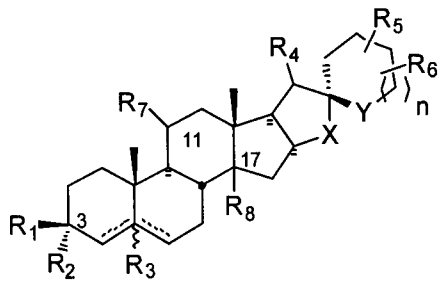
Wilson, S.G., and Sanders, C.C. (1976) Selection and characterization of strains of *Staphylococcus aureus* displaying unusual resistance to aminoglycosides. *Antimicrob Agents Chemother* **10**: 519-525.

Witte, W., Cuny, C., Klare, I., Nubel, U., Strommenger, B., and Werner, G. (2008) Emergence and spread of antibiotic-resistant Gram-positive bacterial pathogens. *Int J Med Microbiol* **298**: 365-377.

Wong, W. C. G., C. *Synthesis* **1995**, 139.

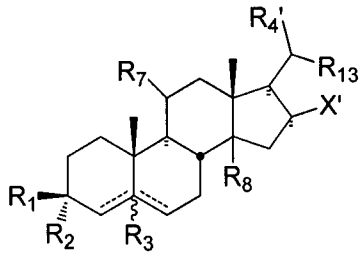
CLAIMS

1. A compound of formula:



(1.0)

wherein,
(1) R1 is H, OH, NH_2, NHR12, N(R12)(R12'), OR12 or SR12; and R2=H; or
(2) R2 is H, OH, NH_2, NHR12, N(R12)(R12'), OR12 or SR12; and R1=H; or
(3) R1 and R2 together form =O or =NR12;
R3 is α-H, β-H, α-alkyl, β-alkyl, α-OH or β-OH, or is absent when the double bond is present either in C4=C5, or in C5=C6;
- - - - - is an optional double bond;
R4-R6 are identical or different and are H, alkyl, OH, OR18, NHR18 or N(R18)(R18');
R7 is H, α-OH or β-OH;
R8 is α-H, β-H, α-OH or β-OH;
X and Y are identical or different and are O, NR19, or CH_2;
R12 and R12' are identical or different and are H, alkyl, aryl, COalkyl, COaryl, CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R14)_p$, PO_3H_2, CO-CH(R20)NH_2, $(CH_2)_{n'}$-NH-R14, C(=NH)NHR21, CH_3OCH_2 ,Silylalkyl, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$, $(CH_2)_mNHC(=NH)NH_2$, $(CH_2)_m$-C(=NH)NH_2, NHalkyl or NHaryl;
R14, R22 and R22' are identical or different and are H, alkyl, aryl, COalkyl, CO_2alkyl, COaryl, CO_2aryl, SO_2alkyl, SO_2aryl, $SO_2N(alkyl)p'$ or CO-CH(R20)NH_2;
R18 and R18' are identical or different and are H, alkyl, aryl, COalkyl, COaryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(alkyl)_{p''}$, PO_3H_2, CO-CH(R20')NH_2, $(CH_2)_{n''}$-NH-R22, C(=NH)NHR21', $(CH_2)_{m'}CO_2H$, $(CH_2)_{m'}SO_3H$, $(CH_2)_{m'}NH_2$, $(CH_2)_{m'}NHC(=NH)NH_2$, $(CH_2)_{m'}$-C(=NH)NH_2, NHalkyl or NHaryl;
R19 is H, alkyl, aryl, COH, COalkyl, COaryl, CO_2alkyl, CO_2aryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(Ralkyl)_{p'''}$, PO_3H_2, CO-CH(R20'')NH_2, $(CH_2)_{n'''}$-NH-R22', C(=NH)NHR21'', $(CH_2)_{m''}CO_2H$, $(CH_2)_{m''}SO_3H$, $(CH_2)_{m''}NH_2$, $(CH_2)_{m''}NHC(=NH)NH_2$, $(CH_2)_{m''}$-C(=NH)NH_2, NHalkyl or NHaryl;
R20, R20' and R20'' are identical or different and correspond to the side chain of any L- and D-amino acid;
R21, R21' and R21'' are identical or different are are H, alkyl, OH, Oalkyl, Oaryl, NHalkyl, NHaryl, $N(alkyl)_2$, $N(aryl)_2$, or N(alkyl)(aryl);
n, n', n'' and n''' are identical or different and are 0-5;
m, m' and m'' are identical or different and are 1-5; and
p, p', p'' and p''' are identical or different and are 1-2;
wherein the compound of formula 1.0 is not tomatidine, solasodine, 3α-hydroxytomatidine or 3-oxo-tomatidine;
or



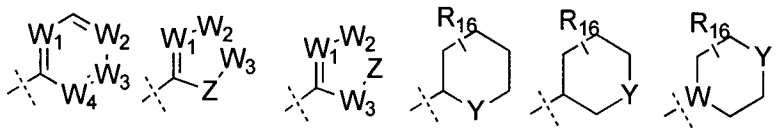
(2.0)

wherein,
R1, R2, R3, R7 and R8 are as defined above;
- - - - - is an optional double bond;
X' is H, OR15 or NHR15,

wherein R15 is H, alkyl, aryl, COalkyl, COaryl, CONHalkyl, CONHaryl, SO_3H, SO_2alkyl, SO_2aryl, $SO_2N(R14)_p$, PO_3H_2, $COCH(R20)NH_2$, $(CH_2)_{n'}$-NH-R14, C(=NH)NHR21, $(CH_2)_mCO_2H$, $(CH_2)_mSO_3H$, $(CH_2)_mNH_2$, $(CH_2)_mNHC(=NH)NH_2$, $(CH_2)_m$-$C(=NH)NH_2$, alkylNHalkyl, alkylNalkyl, alkylN(alkyl)$_2$, alkylNH$_2$, alkylNHCO$_2$alkyl or Silylalkyl; wherein p, n', R14, R21 and m are as defined above;

R4' is H, alkyl or aryl;

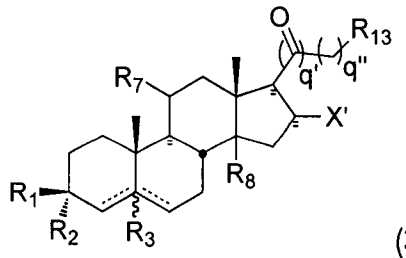
R13 is halogen, $N(CH_3)_2$, OR15', NHR15' or COR15', wherein R15' is defined as is R15 and is identical or different from R15; or



Het1 Het2 Het3 Het4 Het5 Het6

wherein W, W1, W2, W3, W4 are identical or different and are N or CH or CR16;
R16 is H, alkyl, aryl, NHR15' or OR15', wherein R15' is as defined above;
Y is CH_2, NH, N-alkyl, N-COalkyl, N-COaryl, N-SO_2alkyl, N-SO_2aryl, NH-$C(=NH)NH_2$, N-CO_2alkyl or N-CO_2aryl; and
Z is NH, NR15', S or O, wherein R15' is as defined above;

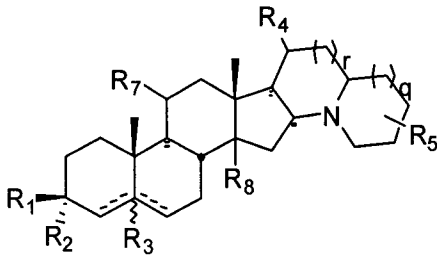
wherein the compound of formula 2.0 is not dihydrosolacongestidine, pregnan-3β-ol-20-amine, pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine or pregnane -3,20-diol;
or



(3.0)

wherein R1, R2, R3, R7, R8, R13 and X' are as defined above;
- - - - is an optional double bond; and
q' and q'' are identical or different and are 0-1;
wherein the compound of formula 3.0 is not pregnanolone, pregnan-3β-ol-20-(aminopropyl)amine, pregnan-3β-ol-20-(aminobutyl)amine or O-*t*-butyldimethylsilylpregnanolone;

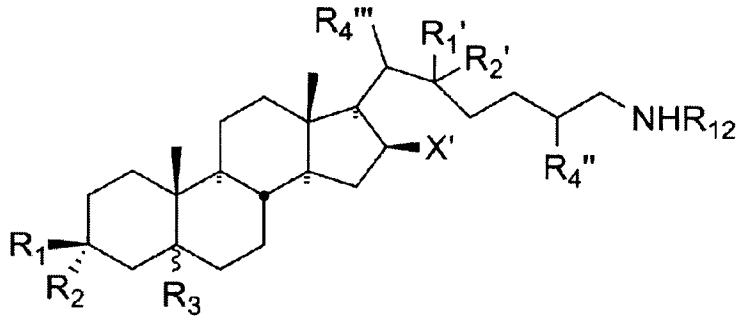
or



(4.0)

wherein R1, R2, R3, R4, R5, R7, R8 are as defined above;
- - - - - is an optional double bond;
r is 0-5; and
q is 0-5,
wherein the compound of formula 4.0 is not demissidine;

or



(5.0)

wherein
R1, R2, R3 and R12 are as defined above;
R4" and R4"' are identifical or different and are H or CH_3;
R1' and R2' are identical or different and are H, OH, Oalkyl or NHalkyl; and
X' is as defined above;
or a salt, stereoisomer or any mixture of stereoisomers of the compound of formula 1.0. 2.0, 3.0, 4.0 or 5.0.

2. The compound of claim 1, wherein the compound is of formula 1.0 and wherein

(i) R1 is OR12 or H;

(ii) R2 is OR12 or H;

(iii) R3 is H;

(iv) R4 is an alkyl;

(v) R5 is H;

(vi) R6 is an alkyl;

(vii) R7 is H;

(ix) R8 is H;

(x) n is 1;

(xi) X is O;

(xii) Y is NR19;

(xiii) there is no double bond; or

(xiv) any combination of (i) to (xiii).

3. The compound of claim 1 or 2, wherein the compound is of formula 1.0 and wherein:

(i) R1 is OR12 and R2 is H;

(ii) R3 is H;

(iii) R4 is CH3;

(iv) R5 is H;

(v) R6 is CH3;

(vi) R7 is H;

(vii) R8 is H;

(viii) n is 1;

(ix) X is O;

(x) Y is NR19 ;

(xi) there is no double bond; or

(xii) any combination of (i) to (xi).

4. The compound of claim 1, wherein the compound is of formula 1.0 and wherein R3 is H, R4 is alkyl, R5 is H, R6 is alkyl, R7 is H, R8 is H, n is 1, X is O, Y is NR19 or $N^{+}R(19)(R19')$ and there is no double bond.

5. The compound of claim 4, wherein Y is NR19.

6. The compound of claim 5, wherein R1 is H, R2 is OR12, R4 is CH_3 and R6 is CH_3.

7. The compound of claim 5, wherein R1 is OR12, R2 is H, R4 is CH_3 and R6 is CH_3.

8. The compound of claim 7, wherein R12 is SO_3H and R19 is H.

9. The compound of claim 7, wherein R12 is PO_3H_2 and R19 is H.

10. The compound of claim 7, wherein R12 is $(CH_2)_m$-CO_2H, m is 1 and R19 is H.

11. The compound of claim 7, wherein R12 is $(CH_2)_mNH_2$, m is 2 and R19 is H.

12. The compound of claim 7, wherein R12 is alkyl, and R19 is H.

13. The compound of claim 7, wherein R12 is $(CH_2)_mNHC(=NH)NH_2$, m is 2 and R19 is H.

14. The compound of claim 5, wherein R1 is NH2 and R2 is H or R1 is H and R2 is NH2 R4 is CH_3,R6 is CH_3, and R19 is H.

15. The compound of claim 6, wherein R12 is a CH_3OCH_2 and R19 is H.

16. The compound of claim 7, wherein wherein R12 is H and R19 is COH.

17. The compound of claim 6, wherein R12 is COalkyl, and R19 is COH.

18. The compound of claim 17, wherein COalkyl is $COCH_3$.

19. A methanesulfonate salt of a compound as defined in claim 6, wherein R12 is H and R19 is H.

20. A citrate salt of a compound as defined in claim 6, wherein R12 is H and R19 is H.

21. The compound of claim 5, wherein R1 and R2 together form =O, R4 is CH_3 and R6 is CH_3 and R19 is (C=O)H.

22. The compound of claim 5, wherein R1 and R2 together form =O, R4 is CH_3, R6 is CH_3 and R19 is H.

23. A hydrochloride salt of the compound of claim 22.

24. The compound of claim 7, wherein R12 is an alkyl and R19 is COH.

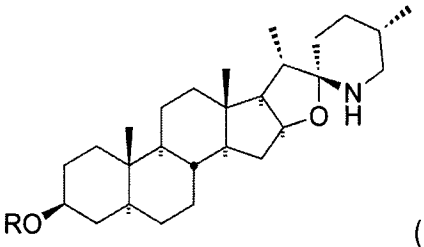
25. The compound of claim 24, wherein the alkyl is $-CH_2-CH=CH_2$.

26. The compound of claim 7, wherein R12 is an alkyl and R19 is H.

27. The compound of claim 26, wherein the alkyl is $-CH_2-CH=CH_2$.

28. A hydrochloride salt of the compound of claim 27.

29. The compound of claim 1, wherein the compound is of formula 1.1:



(1.1),

wherein R is defined as R12 in claim 1.

30. The compound of claim 1, wherein the compound is of formula 2.0 and wherein:
(i) R1 is OR12;
(ii) R2 is H;
(iii) R3 is H or absent;
(iv) R7 is H;
(v) R8 is H;
(vi) X' is H or OR15;
(vii) there is no double bond; or
(viii) any combination of (i) to (vii).

31. The compound of claim 1 or 30, wherein the compound is of formula 2.0 and wherein:
(i) R1 is OR12;
(ii) R2 is H;
(iii) R3 is H or absent;
(iv) R4' is alkyl or aralkyl
(v) R7 is H;
(vi) R8 is H;
(vii) X' is H or OR15;
(viii) there is no double bond; or
(ix) any combination of (i) to (viii).

32. The compound of claim 30, wherein the compound is of formula 2.0 and wherein R1 is OR12, R2 is H, R3 is H or absent, R7 is H, R8 is H, X' is H or OR15.

33. The compound of claim 32, wherein R3 is H.

34. The compound of claim 33, wherein R4' is alkyl.

35. The compound of claim 33, wherein R4' is CH_3.

36. The compound of claim 35, wherein R12 is H.

37. The compound of claim 36, wherein X' is H, there is no double bond and R13 is of formula Het1.

38. The compound of claim 37, wherein W1, W2 and W3 are CH, W4 is N and R16 is H.

39. The compound of claim 36, wherein X' is H, there is no double bond and R13 is of formula Het2.

40. The compound of claim 39, wherein W1 is N, W2 and W3 are CH, Z is S and R16 is H.

41. The compound of claim 36, wherein X' is H, there is no double bond and R13 is of formula Het4.

42. The compound of claim 41, wherein Y is NH and R16 is H.

43. The compound of claim 36, wherein X' is OR15, there is no double bond and R13 is of formula Het4.

44. The compound of claim 43, wherein R15 is CH_3, Y is NH and R16 is H.

45. The compound of claim 32, wherein R3 is absent, R4' is alkyl, X' is H, R12 is COalkyl, R13 is NHR15, and there is a double bond.

46. The compound of claim 32, wherein R3 is H, R4' is alkyl, X' is H, R12 is H, R13 is NHR15, and there is no double bond.

47. The compound of claim 45, wherein R4' is CH_3, R12 is $COCH_3$ and R15 is aryl.

48. The compound of claim 47, wherein the aryl is benzyl.

49. The compound of claim 45, wherein R4' is CH_3, R12 is $COCH_3$ and R15 is alkylN(alkyl)$_2$.

50. The compound of claim 49, wherein R15 is $(CH_2)_3$-N$(CH_3)_2$.

51. The compound of claim 46, wherein R4' is CH_3.

52. The compound of claim 32, wherein R3 is H, R4' is alkyl, X' is H, R12 is COalkyl, R13 is NHR15, and there is no double bond.

53. The compound of claim 32, wherein R3 is H, R4' is alkyl, X' is H, R12 is H, R13 is NHR15, and there is no double bond.

54. The compound of claim 53, wherein R4' is CH_3, R12 is $COCH_3$.

55. The compound of claim 54, wherein R15 is alkylNHCO_2alkyl.

56. The compound of claim 55, wherein R15 is $(CH_2)_2$-NH$CO_2C(CH_3)_3$.

57. The compound of claim 55, wherein R15 is $(CH_2)_3$-NH$CO_2C(CH_3)_3$.

58. The compound of claim 55, wherein R15 is $(CH_2)_4$-NH$CO_2C(CH_3)_3$.

59. The compound of claim 51, wherein R15 is alkylNHCO_2alkyl.

60. The compound of claim 59, wherein R15 is$(CH_2)_2$-NH$CO_2C(CH_3)_3$.

61. The compound of claim 59, wherein R15 is $(CH_2)_3$-NH$CO_2C(CH_3)_3$.

62. The compound of claim 59, wherein R15 is $(CH_2)_4$-$NHCO_2C(CH_3)_3$.

63. The compound of claim 51, wherein R15 is $(CH_2)_mNH_2$.

64. The compound of claim 63, wherein R15 is $(CH_2)_2NH_2$.

65. A hydrochloride salt of the compound of claim 64.

66. The compound of claim 36, wherein X' is H, R13 is OR15 and there is no double bond.

67. The compound of claim 38, wherein R15 is H.

68. The compound of claim 1, wherein the compound is of formula 3.0 and wherein:
(i) R1 is OR12;
(ii) R2 is H;
(iii) R3 is H;
(iv) R7 is H;
(v) R8 is H;
(vi) X' is H; or
(vii) any combination of (i) to (vi).

69. The compound of claim 68, wherein R1 is OR12, R2 is H, R3 is H, R7 is H, R8 is H, X' is H and there is no double bond.

70. The compound of claim 69, wherein R12 is H.

71. The compound of claim 70, wherein q' and q" are 0.

72. The compound of claim 69, wherein R12 is $Si(CH_3)_2C(CH_3)_3$.

73. The compound of claim 72, wherein q' and q" are 0.

74. The compound of claim 71, wherein R13 is of formula Het3.

75. The compound of claim 73, wherein R13 is of formula Het3.

76. The compound of claim 74, wherein W1 is N, W2 and W3 are CH and Z is S.

77. The compound of claim 74, wherein W1 is N, W2 and W3 are CH and Z is NH.

78. The compound of claim 74, wherein W1 is N, W2 is CR16, W3 is CH and Z is S.

79. The compound of claim 78, wherein R16 is NH_2.

80. A hydrochloride salt of the compound of claim 79.

81. The compound of claim 75, wherein R12 is $Si(CH_3)_2C(CH_3)_3$.

82. The compound of claim 81, wherein W1 is N, W2 is CR16, W3 is CH and Z is S.

83. The compound of claim 82, wherein R16 is NH_2.

84. The compound of claim 70, wherein q' and q" are 1.

85. The compound of claim 72, wherein q' and q" are 1.

86. The compound of claim 84, wherein R13 is $N(CH_3)_2$.

87. The compound of claim 84, wherein R13 is Het6.

88. The compound of claim 87, wherein W is N, Y is CH and R16 is H.

89. The compound of claim 87, wherein W is N, Y is NH and R16 is H.

90. A hydrochloride salt of the compound of claim 89.

91. The compound of claim 84, wherein R13 is $NHCH_3$.

92. A hydrochloride salt of the compound of claim 91.

93. The compound of claim 85, wherein R13 is halogen.

94. The compound of claim 93, wherein the halogen is bromium.

95. The compound of claim 1, wherein the compound is of formula 4.0 and wherein:

(i) R1 is OR12;

(ii) R2 is H;

(iii) R3 is H;

(iv) R7 is H;

(v) R8 is H;

(vi) R12 is H;

(vii) there is no double bond; or

(viii) any combination of (i) to (vii).

96. The compound of claim 1, wherein the compound is of formula 5.0 and wherein:

(i) R1 is OR12;

(ii) R2 is H;

(iii) R3 is H;

(iv) R4" is H or CH_3;

(v) R4"' is H or CH_3;

(vi) X' is H or OR15;

(vii) any combination of (i) to (vi).

97. The compound of claim 96, wherein the compound is of formula 5.0 and wherein R1 is OR12, R2 is H, R3 is H, R4" is H or CH_3, R4"' is H or CH_3, and X' is H or OR15.

98. The compound of claim 97, wherein R4" and R4"' are CH_3.

99. The compound of claim 98, wherein R12 is H.

100. The compound of claim 99, wherein X' is OH and R1' and R2' are H.

101. A composition comprising

(A) (i) the compound of formula 1.0, 2.0, 3.0, 4.0 or 5.0 as defined in any one of claims 1 to 100;

(ii) tomatidine;

(iii) demissidine;

(iv) solasodine;

(v) 3α-hydroxytomatidine;

(vi) 3-oxo-tomatidine;

(vii) pregnanolone;

(viii) pregnan-3β-ol-20-amine;

(ix) pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine;

(x) pregnan-3β-ol-20-(aminopropyl)amine

(xi) pregnan-3β-ol-20-(aminobutyl)amine;

(xii) O-*t*-butyldimethylsilylpregnanolone;

(xiii) pregnane -3,20-diol;

(xiv) dihydrosolacongestidine; or

(xv) a salt, stereoisomer or any mixture of stereoisomers of any one of (ii) to (xiv); and

(B) (a) an antibiotic;

(b) an antiseptic;

(c) a disinfectant; or

(d) any combination of (a)-(c).

102. A composition comprising the compound as defined in any one of claims 1 to 100, and

(a) an antibiotic;

(b) an antiseptic;

(c) a disinfectant;

(d) a diluent;

(e) an excipient;

(f) a pharmaceutically acceptable carrier; or

(g) any combination of (a)-(f).

103. The composition of claim 102, wherein said composition is a pharmaceutical composition.

104. A method of preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe, said method comprising administering to said subject a therapeutically effective amount of a compound or a composition comprising the compound and a pharmaceutically acceptable carrier, the compound being:

(i) of formula 1.0, 2.0, 3.0, 4.0 or 5.0 as defined in any one of claims 1 to 100;

(ii) tomatidine;

(iii) demissidine;

(iv) solasodine;

(v) 3α-hydroxytomatidine;

(vi) 3-oxo-tomatidine;

(vii) pregnanolone;

(viii) pregnan-3β -ol-20-amine;

(ix) pregnan-3β-ol-20-((N,N-dimethylamino)propyl)amine;

(x) pregnan-3β-ol-20-(aminopropyl)amine

(xi) pregnan-3β-ol-20-(aminobutyl)amine;

(xii) O-*t*-butyldimethylsilylpregnanolone;

(xiii) pregnane -3,20-diol;

(xiv) dihydrosolacongestidine; or

(xv) a salt, stereoisomer or any mixture of stereoisomers of any one of (ii) to (xiv),

whereby said bacterial infection is prevented or treated.

105. A method of disinfecting and/or sterilizing an object of an electron transport-deficient microbe, said method comprising applying an effective amount of the compound as defined in claim 104 or of a composition comprising said compound to said object, whereby said object is disinfected and/or sterilized.

106. The method of claim 105, wherein said object is an animal, an animal tissue, animal cells, food, a synthetic material or a natural material.

107. The method of any one of claims 104 to 106, wherein the electron transport-deficient microbe is an electron transport-deficient bacteria.

108. The method of any one of claims 104 to 107, wherein the electron transport-deficient microbe is an intracellular bacteria.

109. The method of claim 107 or 108, wherein the electron transport-deficient microbe is a bacterial small-colony variant (SCV).

110. The method of claim 109, wherein the SCV is a coagulase-positive or -negative staphylococci, an enterococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella.*

111. The method of claim 109, wherein the SCV is of the Firmicutes phylum.

112. The method of claim 111, wherein the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

113. The method of claim 112, wherein the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.*

114. The method of claim 110, wherein the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii.*

115. The method of any one of claims 104 to 111, wherein the electron transport-deficient microbe is a staphylococci.

116. The method of claim 115, wherein the staphylococci is an antibiotic-resistant *Staphylococcus*.

117. The method of claim 115 or 116, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*.

118. The method of claim 117, wherein the staphylococci is a *Staphylococcus aureus*.

119. The method of claim 118, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

120. The method of any one of claims 104 to 108, wherein the electron transport-deficient microbe is an anaerobe bacteria.

121. The method of claim 120, wherein the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium*.

122. The method of claim 121, wherein the electron transport-deficient microbe is a *Clostridium*.

123. The method of claim 122, wherein the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*.

124. The method of any one of claims 104 to 108, wherein the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen.

125. The method of any one of claims 104 to 108, wherein the electron transport-deficient microbe is a bacterium that is affected by another microorganism producing at least one electron transport inhibitor.

126. A method of preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or

composition as defined in claim 104, whereby said polymicrobial infection is prevented or treated.

127. The method of claim 126, wherein the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*.

128. The method of claim 126 or 127, wherein the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof.

129. The method of any one of claims 126 to 128, wherein the subject has cystic fibrosis.

130. The method of any one of any one of claims 126 to 128, wherein the subject has a polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

131. A method of disinfecting and/or sterilizing an object of a polymicrobial infection as defined in claim 126, said method comprising applying an effective amount of the compound as defined in claim 104 or of a composition comprising the compound, to said object, whereby said object is disinfected and/or sterilized.

132. The method of claim 131, wherein said object is an animal, an animal tissue, animal cells, a food, a synthetic material or a natural material.

133. A method of preventing or treating a microbial infection caused by a bacterial pathogen in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or composition as defined in claim 104, in combination with an aminoglycoside antibiotic, whereby said microbial infection is prevented or treated.

134. A method of disinfecting and/or sterilizing an object of a bacterial pathogen, said method comprising applying an effective amount of the compound as defined in claim 104 or of a composition comprising the compound, in combination with an aminoglycoside antibiotic to said object, whereby said object is disinfected and/or sterilized.

135. (removed)

136. The method of any one of claim 133 to 134, wherein the bacterial pathogen is an intracellular bacteria.

137. The method of any one of claims 133, 134 and 136, wherein the bacterial pathogen is a coagulase-positive or -negative staphylococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella.*

138. The method of claim 136, wherein the bacterial pathogen is of the Firmicutes phylum.

139. The method of claim 138, wherein the bacterial pathogen of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

140. The method of claim 139, wherein the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.*

141. The method of any one of claims 133, 134 and 136, wherein the bacterial pathogen is a *Staphylococcus aureus, Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis, Micrococcus luteus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus mitis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus suis, Streptococcus bovis, Streptococcus intermedius, Bacillus subtilis, Bacillus anthracis, Bacillus cereus, Bacillus coagulans, Listeria monocytogenes* or *Listeria ivanovii.*

142. The method of any one of claims 133, 134 and 136, wherein the bacterial pathogen is a staphylococci.

143. The method of claim 142, wherein the staphylococci is a chronic and/or an antibiotic-resistant *Staphylococcus*.

144. The method of claim 142 or 143, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*.

145. The method of claim 144, wherein the staphylococci is a *Staphylococcus aureus*.

146. The method of claim 145, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

147. The method of any one of claims 133, 134 and 136 to 146, wherein the aminoglycoside antibiotic is amikacin, gentamicin, kanamycin, streptomycin or tobramycin.

148. The method of any one of claims 133, 134 and 136 to 147, further comprising a beta-lactam antibiotic.

149. A method for reducing the development of resistance toward aminoglycosides in a bacteria, or treating a bacteria resistant to aminoglycoside in a subject, said method comprising administering to said subject a therapeutically effective amount of the compound or composition as defined in claim 104, whereby said development of resistance toward aminoglycosides in a bacteria is prevented or said bacteria resistant to aminoglycoside is treated.

150. The method of any one of claims 104 to 134 and 136 to 149, wherein said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

151. The method of any one of claims 104 to 134 and 136 to 150, wherein said subject or object is food, a cow or a human.

152. The method of any one of claims 104 to 134 and 136 to 150, wherein said subject is a human.

153. Use of the compound as defined in claim 104 or of a composition comprising the compound, for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from a an electron transport-deficient microbe.

154. Use of the compound as defined in claim 104 or of a composition comprising the compound, in the manufacture of a medicament for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from a an electron transport-deficient microbe.

155. The use of claim 153 or 154, wherein said object is an animal, an animal tissue, animal cells, a food, a synthetic material or a natural material.

156. The use of any one of claims 153 to 155, wherein the electron transport-deficient microbe is an electron transport-deficient bacteria.

157. The use of any one of claims 153 to 156, wherein the electron transport-deficient microbe is an intracellular bacteria.

158. The use of claim 156 or 157, wherein the electron transport-deficient microbe is a bacterial small-colony variant (SCV).

159. The use of claim 158, wherein the SCV is a coagulase-positive or -negative staphylococci, an enterococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella.*

160. The use of claim 158, wherein the SCV is of the Firmicutes phylum.

161. The use of claim 160, wherein the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

162. The use of claim 161, wherein the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.*

163. The use of claim 159, wherein the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii.*

164. The use of any one of claims 153 to 160, wherein the electron transport-deficient microbe is a staphylococci.

165. The use of claim 164, wherein the staphylococci is an antibiotic-resistant *Staphylococcus.*

166. The use of claim 164 or 165, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis,* a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis.*

167. The use of claim 166, wherein the staphylococcus is a *Staphylococcus aureus.*

168. The use of claim 167, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

169. The use of any one of claims 153 to 157, wherein the electron transport-deficient microbe is an anaerobe bacteria.

170. The use of claim 169, wherein the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium.*

171. The use of claim 169, wherein the electron transport-deficient microbe is a *Clostridium*.

172. The use of claim 171, wherein the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*.

173. The use of any one of claims 153 to 157, wherein the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen.

174. (removed)

175. Use of the compound as defined in claim 104 or of a composition comprising the compound, for: (a) preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor; or (b) the disinfection, sterilization and/or antisepsis of an object from a the polymicrobial infection.

176. (removed)

177. The use of claim 175, wherein the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*.

178. The use of claim 175 or 177, wherein the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof.

179. The use of any one of claims 175, 177 and 178, wherein the polymicrobial infection is an infection of the airways of a cystic fibrosis subject, a polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

180. Use of the compound as defined in claim 104 or of a composition comprising the compound, in combination with an aminoglycoside antibiotic, for: (a) preventing or treating a bacterial pathogen infection in a subject; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

181. Use of the compound as defined in claim 104 or of a composition comprising the compound, in combination with an aminoglycoside antibiotic, in the manufacture of a medicament for: (a) preventing or treating a bacterial pathogen infection in a subject; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

182. The use of any one of claims 175, 180 and 181, wherein said object is an animal, an animal tissue, animal cells, a food, a synthetic material or a natural material.

183. The use of any one of claims 180 to 182, wherein the bacterial pathogen is an intracellular bacteria.

184. The use of any one of claim 180 to 183, wherein the bacterial pathogen is a coagulase-positive or -negative staphylococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella.*

185. The use of claim 183, wherein the bacterial pathogen is of the Firmicutes phylum.

186. The use of claim 185, wherein the bacterial pathogen of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

187. The use of claim 186, wherein the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.*

188. The use of any one of claim 180 to 183, wherein the bacterial pathogen is a *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hyicus, Staphylococcus chromogenes, Staphylococcus stimulans, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus mitis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus suis, Streptococcus bovis, Streptococcus intermedius, Bacillus subtilis, Bacillus anthracis, Bacillus cereus, Bacillus coagulans, Listeria monocytogenes* or *Listeria ivanovii.*

189. The use of any one of claims 180 to 185, wherein the bacterial pathogen is a staphylococci.

190. The use of claim 189, wherein the staphylococci is an antibiotic-resistant *Staphylococcus*.

191. The use of claim 189 or 190, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*.

192. The use of claim 191, wherein the staphylococci is a *Staphylococcus aureus*.

193. The use of claim 192, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

194. The use of any one of claims 180 to 193, wherein the aminoglycoside antibiotic is amikacin, gentamicin, kanamycin, streptomycin or tobramycin.

195. The use of any one of claims 180 to 194, further comprising a beta-lactam antibiotic.

196. (removed)

197. (removed)

198. The use of any one of claims 153 to 195, wherein said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

199. The use of any one of claims 153 to 195 and 198, wherein said subject or object is food, a cow or a human.

200. The use of any one of claims 153 to 195 and198, wherein said subject is a human.

201. Compound as defined in claim 104 or of a composition comprising the compound, for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by an electron transport-deficient microbe; or (b) the disinfection, sterilization and/or antisepsis of an object from an electron transport-deficient microbe.

202. The compound of claim 201, wherein said object is an animal, an animal tissue, animal cells, food, a synthetic material or a natural material.

203. The compound of claim 201 or 202, wherein the electron transport-deficient microbe is an electron transport-deficient bacteria.

204. The compound of any one of claims 201 to 203, wherein the electron transport-deficient microbe is an intracellular bacteria.

205. The compound of any one of claims 201 to 204, wherein the electron transport-deficient microbe is a bacterial small-colony variant (SCV).

206. The compound of claim 205, wherein the SCV is a coagulase-positive or -negative staphylococci, an enterococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium,* a *Lactobacillus* or a *Gardnerella*.

207. The compound of claim 205, wherein the SCV is of the Firmicutes phylum.

208. The compound of claim 207, wherein the SCV of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

209. The compound of claim 208, wherein the SCV is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes.*

210. The compound of claim 206, wherein the SCV is a *Staphylococcus aureus*, *Staphylococcus intermedius*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus hyicus*, *Staphylococcus chromogenes*, *Staphylococcus stimulans*, *Staphylococcus saprophyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus capitis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Enterococcus hirae*, *Enterococcus gallinarum*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mitis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus suis*, *Streptococcus bovis*, *Streptococcus intermedius*, *Bacillus subtilis*, *Bacillus anthracis*, *Bacillus cereus*, *Bacillus coagulans*, *Listeria monocytogenes* or *Listeria ivanovii*.

211. The compound of any one of claims 201 to 207, wherein the electron transport-deficient microbe is a staphylococci.

212. The compound of claim 211, wherein the staphylococci is an antibiotic-resistant *Staphylococcus*.

213. The compound of claim 211 or 212, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*.

214. The compound of claim 213, wherein the staphylococci is a *Staphylococcus aureus*.

215. The compound of claim 214, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

216. The compound of any one of claims 201 to 204, wherein the electron transport-deficient microbe is an anaerobe bacteria.

217. The compound of claim 216, wherein the anaerobe is a *Clostridium*, a *Peptostreptococcus*, a *Peptococcus*, or a *Propionibacterium*.

218. The compound of claim 217, wherein the electron transport-deficient microbe is a *Clostridium*.

219. The compound of claim 218, wherein the *Clostridium* is *Clostridium perfringens* or *Clostridium difficile*.

220. The compound of any one of claims 201 to 204, wherein the electron transport-deficient microbe is a facultative anaerobic bacterium grown in the absence of oxygen.

221. (removed)

222. Compound as defined in claim 104 or of a composition comprising the compound, for: (a) preventing or treating a polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor; or (b) the disinfection, sterilization and/or antisepsis of an object from a the polymicrobial infection.

223. The compound of claim 222, wherein the polymicrobial infection involving at least one microorganism that produces at least one electron transport inhibitor comprises *Pseudomonas aeruginosa*.

224. The compound of claim 222 or 223, wherein the electron transport inhibitor is a 4-hydroxy-2-alkylquinoline or an analogue thereof.

225. The compound of any one of claims 222 to 224, wherein the polymicrobial infection is an infection of the airways of a cystic fibrosis subject, a polymicrobic hospital-acquired pneumonia or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

226. Compound as defined in claim 104 or of a composition comprising the compound, in combination with an aminoglycoside antibiotic for: (a) preventing or treating a microbial infection in a subject, wherein said microbial infection is caused by a bacterial pathogen; or (b) the disinfection, sterilization and/or antisepsis of an object from a bacterial pathogen.

227. The compound of claim 226, wherein said object is an animal, an animal tissue, animal cells, a food, a synthetic material or a natural material.

228. The compound of claim 226 or 227, wherein the bacterial pathogen is an intracellular bacteria.

229. The compound of any one of claims 226 to 228, wherein the bacterial pathogen is a coagulase-positive or -negative staphylococci, a streptococci of group A, a streptococci of group B, a streptococci of the viridans group, a streptococci of the mitis group, a *Bacillus spp.*, a *Listeria spp.*, a *Corynebacterium*, a *Lactobacillus* or a *Gardnerella*.

230. The compound of claim 228, wherein the bacterial pathogen is of the Firmicutes phylum.

231. The compound of claim 230, wherein the bacterial pathogen is of the Firmicutes phylum is a *Bacillus spp.* or a *Listeria spp.*

232. The compound of claim 231, wherein the bacterial pathogen is a *Bacillus subtilis*, a *Bacillus cereus* or a *Listeria monocytogenes*.

233. The compound of any one of claim 226 to 229, wherein the bacterial pathogen is a *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hyicus, Staphylococcus chromogenes, Staphylococcus stimulans, Staphylococcus saprophyticus, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus capitis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus mitis, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus suis, Streptococcus bovis, Streptococcus intermedius, Bacillus subtilis, Bacillus anthracis, Bacillus cereus, Bacillus coagulans, Listeria monocytogenes* or *Listeria ivanovii*.

234. The compound of any one of claims 226 to 229, wherein the bacterial pathogen is a staphylococci.

235. The compound of claim 234, wherein the staphylococci is an antibiotic-resistant *Staphylococcus*.

236. The compound of claim 234 or 235, wherein the staphylococci is a *Staphylococcus aureus*, a *Staphylococcus epidermidis*, a *Staphylococcus haemolyticus*, a *Staphylococcus saprophyticus*, or a *Staphylococcus hominis*.

237. The compound of claim 236, wherein the staphylococci is a *Staphylococcus aureus*.

238. The compound of claim 237, wherein said staphylococci is a methicillin-resistant *Staphylococcus aureus* (MRSA), community acquired MRSA, a vancomycin-intermediate *Staphylococcus aureus* (VISA), a vancomycin-resistant *Staphylococcus aureus* (VRSA) or a glycopeptide-resistant *Staphylococcus aureus* (GISA).

239. The compound of any one of claims 226 to 238, wherein the aminoglycoside antibiotic is amikacin, gentamicin, kanamycin, streptomycin or tobramycin.

240. The compound of any one of claims 226 to 239, further comprising a beta-lactam antibiotic.

241. (removed)

242. The compound of any one of claims 201 to 220 and 222 to 240, wherein said infection is a pulmonary infection, a mammary gland infection, a skin and soft tissue infection, a septicemia, a polymicrobic hospital-acquired pneumonia, or a polymicrobic infection associated with a burn, a catheter, or an endotracheal tube.

243. The compound of any one of claims 201 to 220 and 222 to 240, wherein said subject or object is food, a cow or a human.

244. The compound of any one of claims 201 to 220 and 222 to 240, wherein said subject is a human.

245. A method of identifying a pathogen, the microbial infection of which is treatable by the compound as defined in claim 104 or a composition comprising the compound, said method comprising contacting said bacterial pathogen with said compound or composition and determining the effect of said compound or composition on the growth or survival of said pathogen, wherein a decrease in the growth or survival of said pathogen in the presence as compared to in the absence of said compound or composition is an indication that said bacterial pathogen is treatable by said compound or composition.

246. A kit comprising the compound as defined in claim 104, and instructions to use same in (a) the prevention or treatment of a microbial infection; or (b) the disinfection, sterilization and/or antisepsis of an object.

247. The kit of claim 246, further comprising an aminoglycoside antimicrobial agent.

248. The kit of claim 246 or 247, wherein the aminoglycoside antibiotic is amikacin, gentamicin, kanamycin, streptomycin or tobramycin.

249. The kit of any one of claims 246 to 248, further comprising a beta-lactam antibiotic.

250. The kit of any one of claims 246 to 249, further comprising

(iii) an antiseptic;

(iv) a disinfectant;

(v) a diluent;

(vi) an excipient;

(vii) a pharmaceutically acceptable carrier; or

(viii) any combination of (iii)-(vii).

251. The kit of any one of claims 246 to 249, further comprising

(a) an antibiotic;

(b) an antiseptic;

(c) a disinfectant; or

(d) any combination of (a)-(c).

252. A composition comprising a combination of:

(i) the compound as defined in claim 104; and

(ii) an aminoglycoside antimicrobial agent.

253. The composition of claim 252, wherein the aminoglycoside antibiotic is amikacin, gentamicin, kanamycin, streptomycin or tobramycin.

254. The composition of claim 252 or 253, further comprising a beta-lactam antibiotic.

255. The composition of any one of claims 252 to 254, further comprising:

(iii) an antiseptic;

(iv) a disinfectant;

(v) a diluent;

(vi) an excipient;

(vii) a pharmaceutically acceptable carrier; or

(viii) any combination of (iii)-(viii).

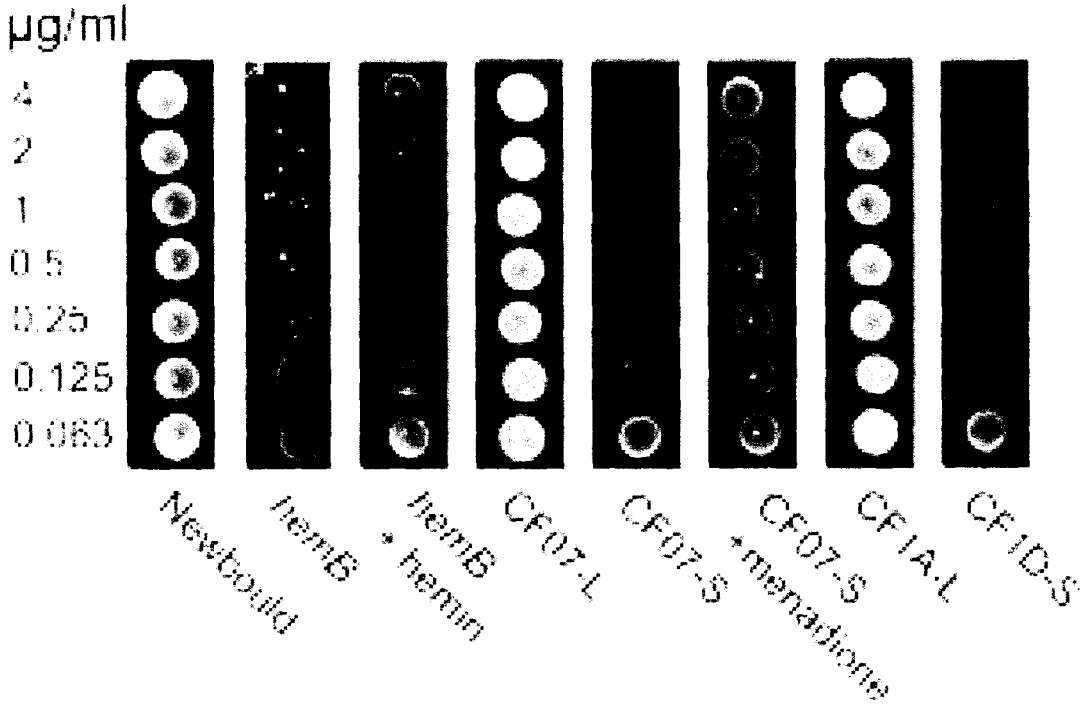
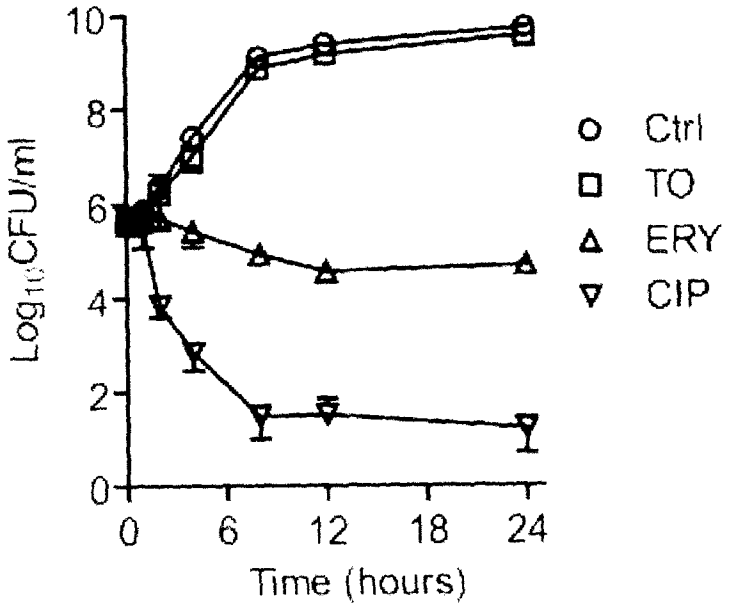

µg/ml
4
2
1
0.5
0.25
0.125
0.063
Newbould
hemB
hemB + hemin
CF07-L
CF07-S
CF07-S + menadione
CF1A-L
CF1D-S

FIG 1A


10
8
6
4
2
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Log10CFU/ml
0
6
12
18
24
Time (hours)
Ctrl
TO
ERY
CIP

FIG 1B

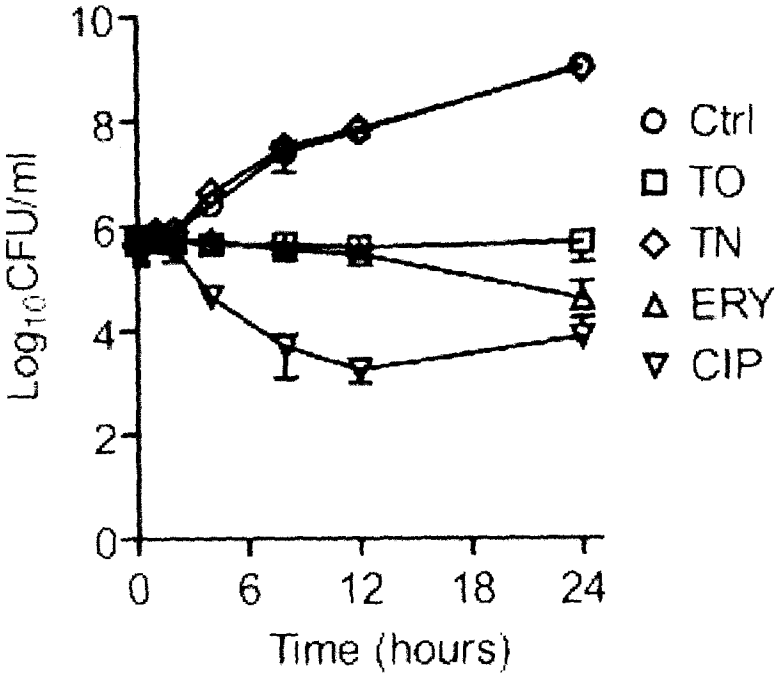
10
8
6
4
2
0
Log10CFU/ml
0
6
12
18
24
Time (hours)
Ctrl
TO
TN
ERY
CIP

FIG 1C

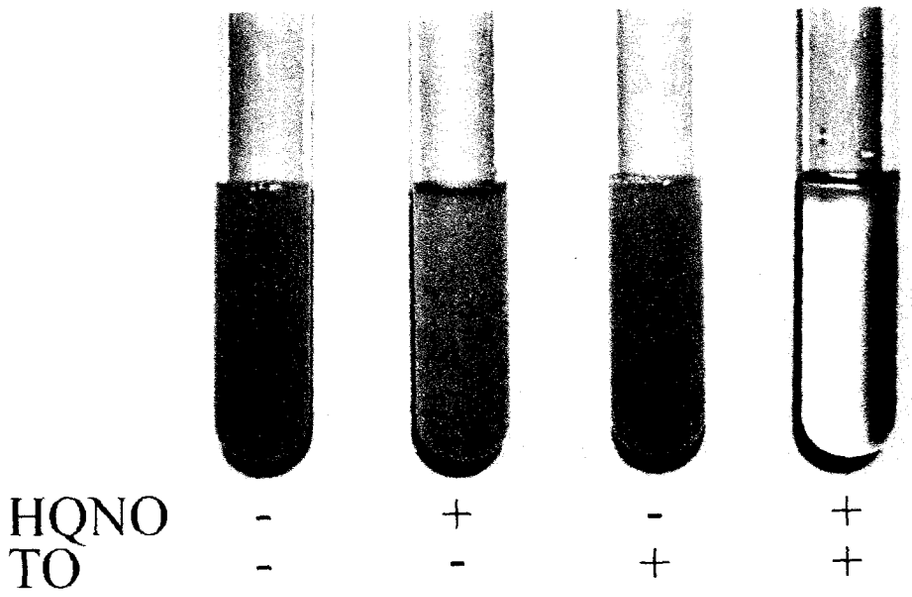

FIG 2

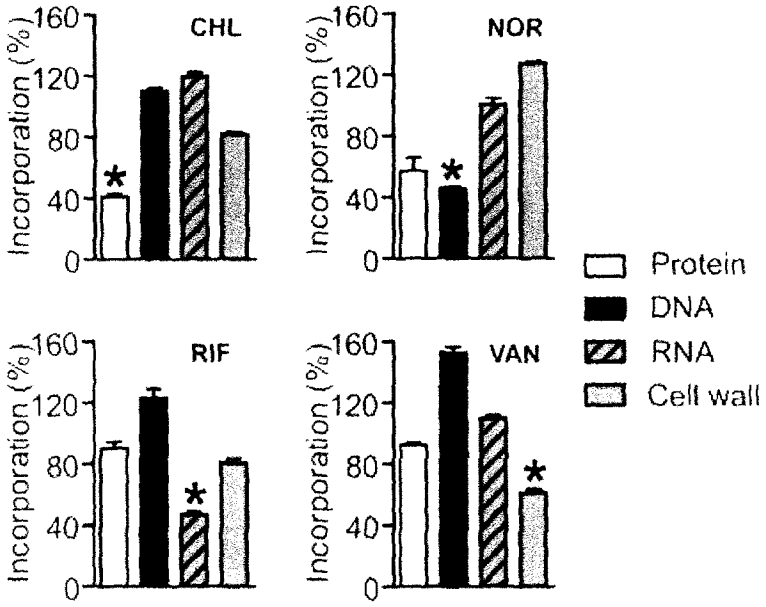

FIG 3A

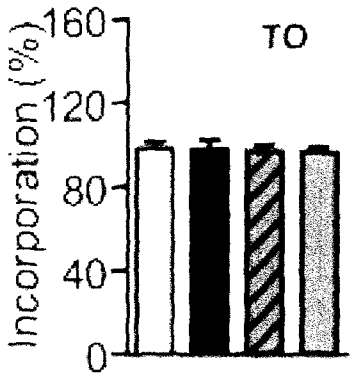

FIG 3B

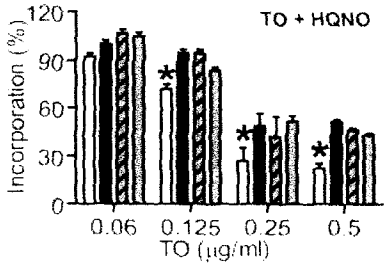

FIG 3C

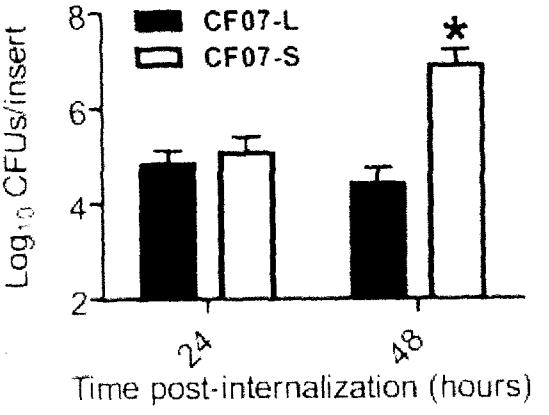
CF07-L
CF07-S
*
8
6
4
2
Log10 CFUs/insert
24
48
Time post-internalization (hours)

FIG 4A

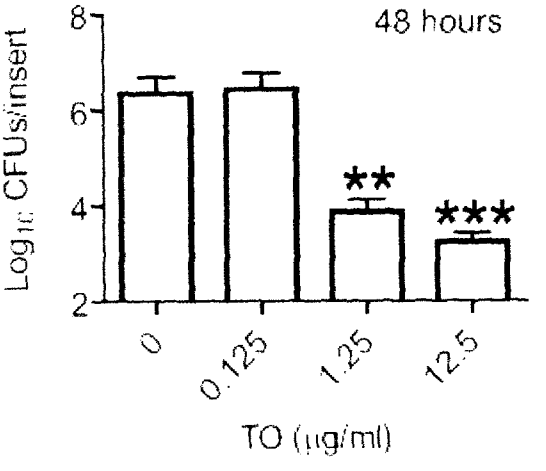
48 hours
**
***
8
6
4
2
Log10 CFUs/insert
0
0.125
1.25
12.5
TO (µg/ml)

FIG 4B

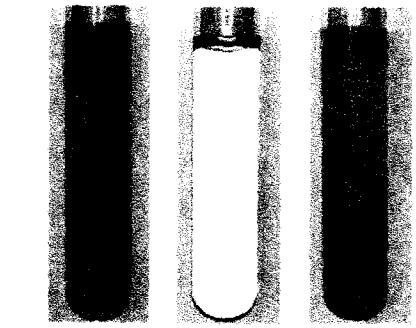

FIG 5A

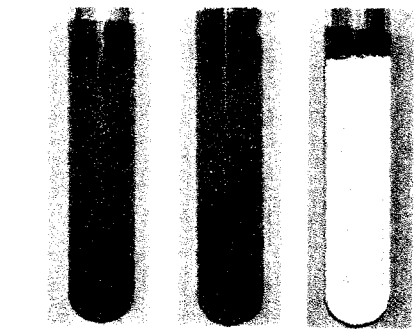

FIG 5B

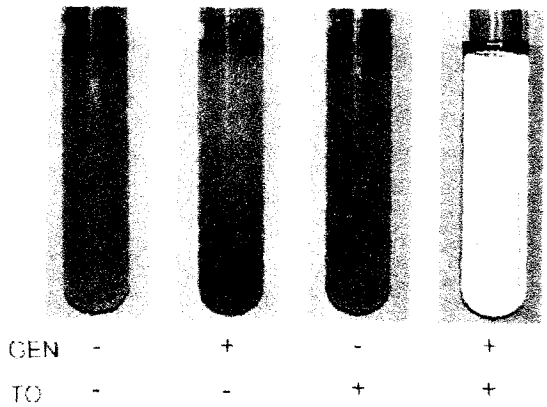

FIG 5C

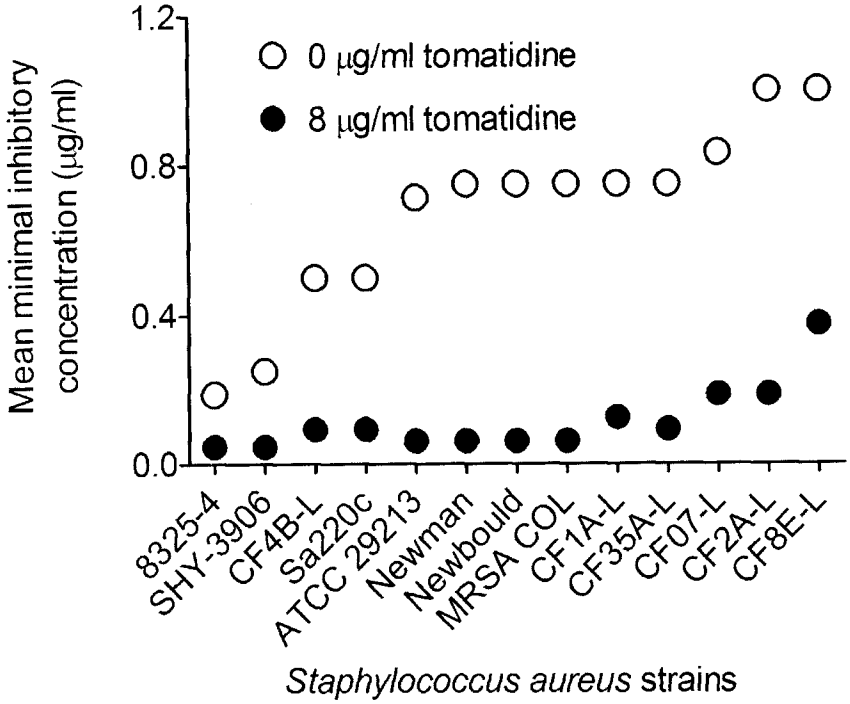

FIG 6A

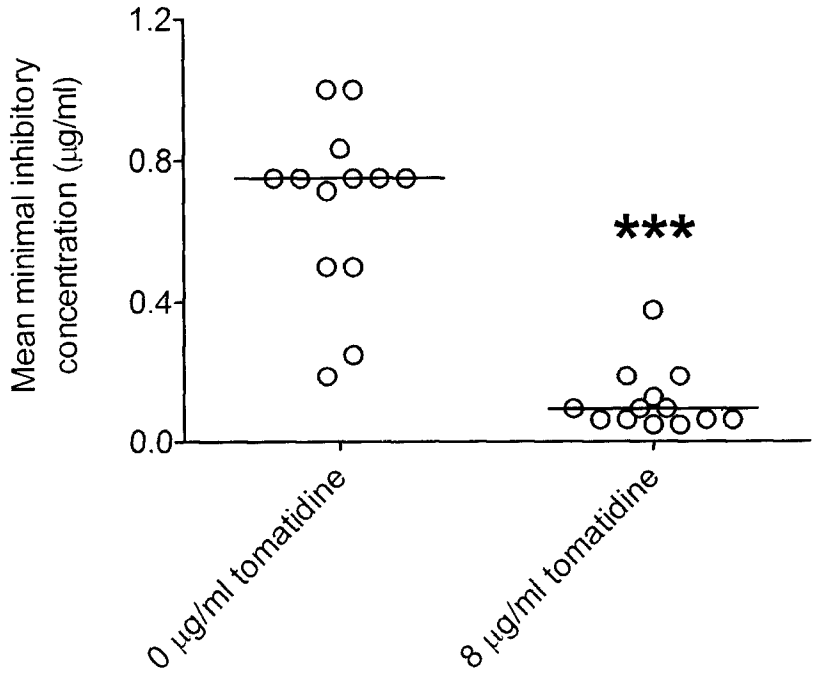

Figure 6B

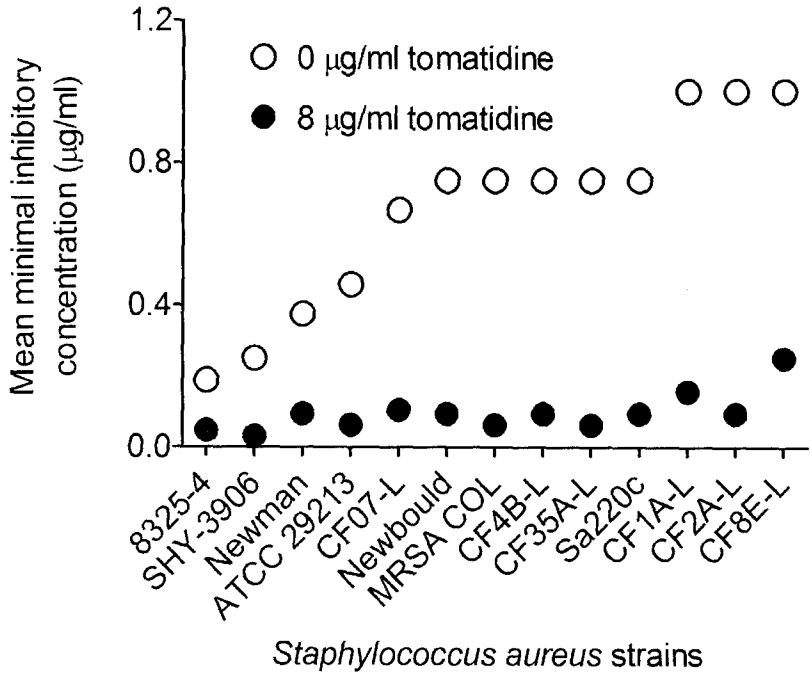

FIG 7A

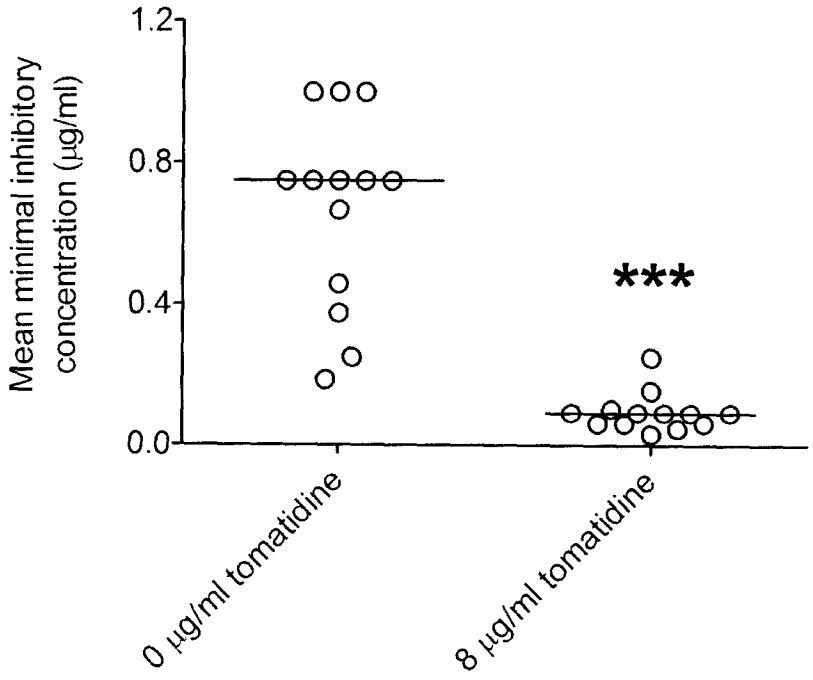

Figure 7B

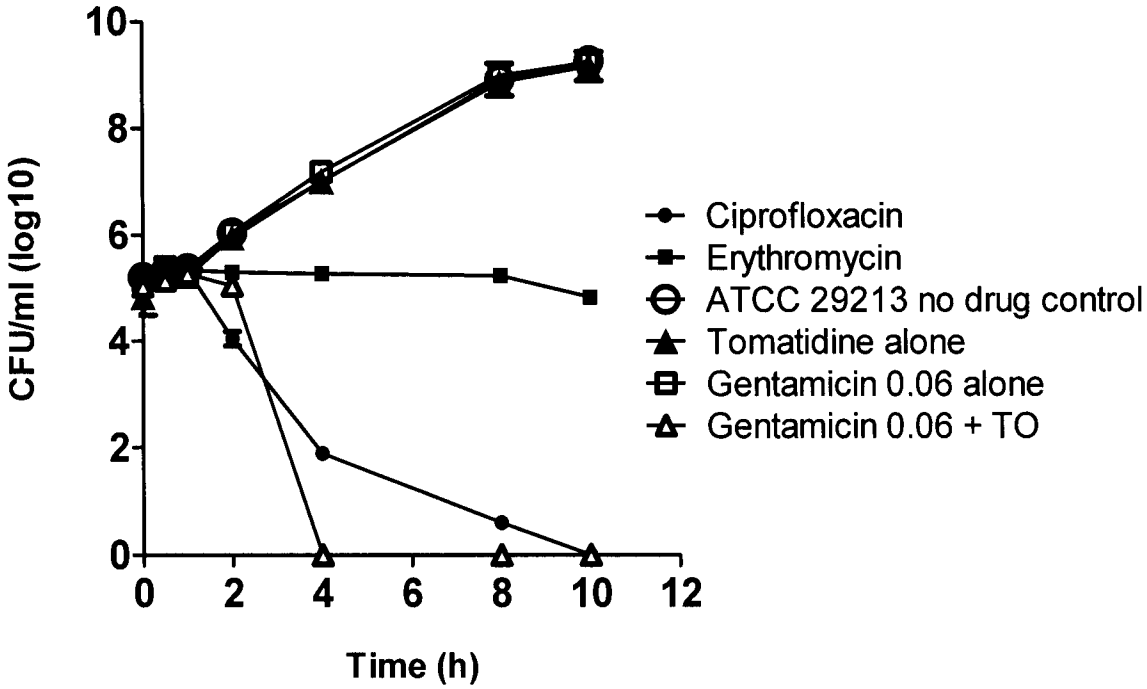
10
8
6
4
2
0
CFU/ml (log10)
0
2
4
6
8
10
12
Time (h)
Ciprofloxacin
Erythromycin
ATCC 29213 no drug control
Tomatidine alone
Gentamicin 0.06 alone
Gentamicin 0.06 + TO

FIG 8

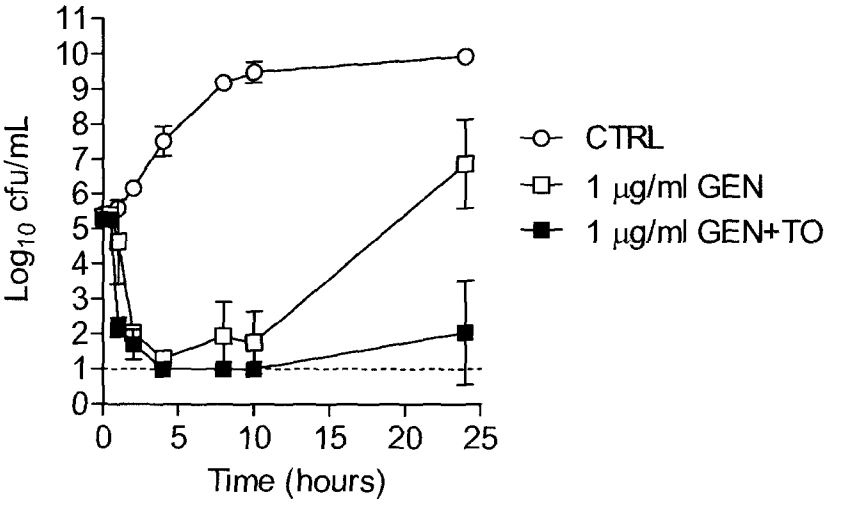
Log10 cfu/mL
Time (hours)
CTRL
1 µg/ml GEN
1 µg/ml GEN+TO

FIG 9A

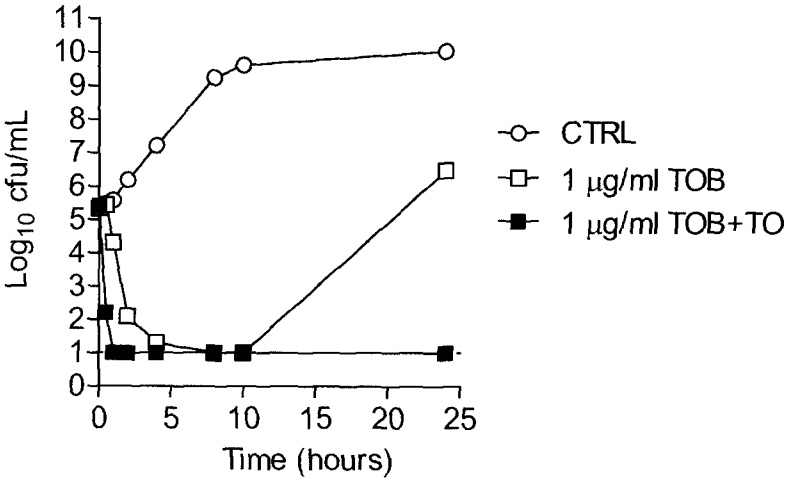
Log10 cfu/mL
Time (hours)
CTRL
1 µg/ml TOB
1 µg/ml TOB+TO

FIG 9B

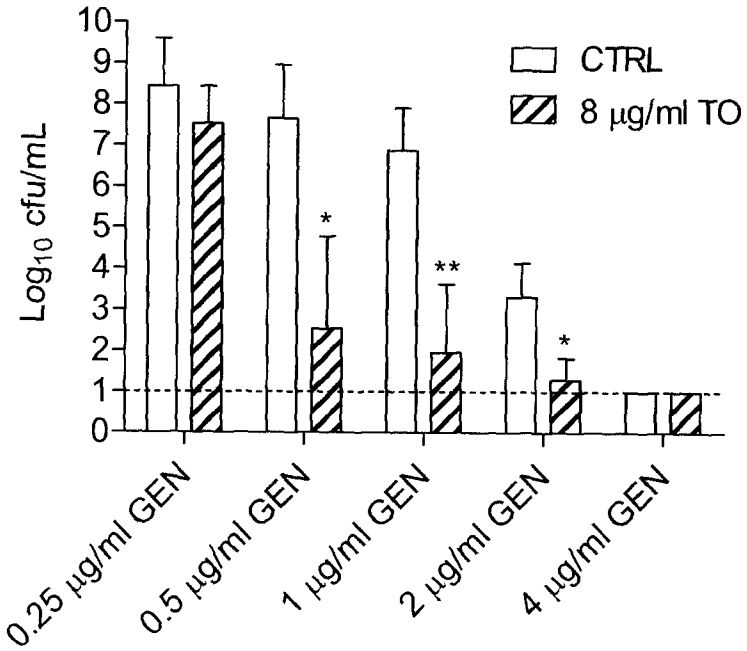
10
9
8
7
6
5
4
3
2
1
0
Log10 cfu/mL
CTRL
8 µg/ml TO
*
**
*
0.25 µg/ml GEN
0.5 µg/ml GEN
1 µg/ml GEN
2 µg/ml GEN
4 µg/ml GEN

FIG 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050087

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07J 71/00*** (2006.01) , ***A61K 31/58*** (2006.01) , ***A61L 2/16*** (2006.01) , ***A61P 31/00*** (2006.01) , ***A61P 31/04*** (2006.01) , ***C07J 53/00*** (2006.01) ***C12Q 1/18*** (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)

C07J 71/00 (2006.01) , ***A61K 31/58*** (2006.01) , ***A61L 2/16*** (2006.01) , ***A61P 31/00*** (2006.01) , ***A61P 31/04*** (2006.01) , ***C07J 53/00*** (2006.01) ***C12Q 1/18*** (2006.01)

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

C07J, A61K

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

EPOQUE, Canadian Patent Database, Pubmed, STN, Google Scholar: tomatidine, antimicrobial, antibiotic, steroidal alkaloids, tomatidenol, spirosolane, solanum, solanidine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	MALOUIN, *et al.*, "Tomatidine Inhibits Replication of *Staphylococcus aureus* Small Colony Variants in Cystic Fibrosis Airway Epithelial Cells", *Antimicrob. Agents Chemother.*, **2011**, *55*, p1937-1945. applicant disclosure	all
P, X	MALOUIN, *et al.*, "Tomatidine acts in synergy with aminoglycoside antibiotics against multiresistant *Staphylococcus aureus* and prevents virulence gene expression", *J. Antimicrob. Chemother.*, **2012**, *67*, p559-568. applicant disclosure	all
P, X	MILNER, *et al.*, "Bioactivities of Glycoalkaloids and Their Aglycones from *Solanum* Species", *J. Agric. Food Chem.*, **2011**, *59*, p3454-3484. see whole article, especially page 3468 under heading "Antimicrobial activity"	all
X	CA2395642 (LAVIE, ET AL) 12 July 2001, 12.07.2001 see page 4, lines 13-15, page 6, lines 1-13, 20-25, see chart pages 10, 11 and claims 5, 15-21, 27	101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	LAVIE, *et al.*, "Inhibitory Effect of Steroidal Alkaloids on Drug Transport and Multidrug Resistance in Human Cancer Cells", *Anticancer Res.*, **2001**, *21*(2A), p1189-1194. See whole document, especially figures	101-103

[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
26 April 2012 (26-04-2012)	3 May 2012 (03-05-2012)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Karol Gajewski (819) 934-6734

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHA, *et al.*, "Synthesis and *in vitro* antitumor activities of novel soladulcidine derivatives", *Zhongguo Yaoke Daxue Xuebao (Journal of China Pharmaceutical University)*, **2010**, *41*(6), p493-498. See scheme 1, especially compounds 10-17.	1-36, 41, 43, 101-103
X	US2770618 (KUHN, ET AL) 13 Nov 1956, 13.11.1956. see example	1-7, 12, 17, 18, 29
X	QUYEN, *et al.*, "Synthesis of the Steroid Alkaloid Soladunalinidine and Other 5α-Spirosolan-3-amines", *Liebigs Ann. Chem.*, **1990**, *6*, p519-524 See compounds 1-5	1-7, 14, 22, 29
X	BIRD, *et al.*, "Soladunalinidine, a new steroidal alkaloid from *Solanum dunalianum*", *Tett. Lett.*, **1978**, *2*, p159-160. See structures 1-4.	1-7, 14, 29
X	SCHREIBER, *et al.*, "Über Tomatid-5-en-3β-ol aus *Solanum dulcamara* L. und dessen abbau zu 3β-acetoxy-pregna-5,16-dien-20-on", *Justus Liebigs Annalen der Chemie*, **1965**, *681*, p187-195.	1-7, 14, 29
X	PAULSEN, *et al.*, "Monosaccharide mit stickstoffhaltigem Ring, XIV Untersuchungen über die magnetische Anisotropie der Amidgruppe", *Chem. Ber.*, **1967**, *100*(10), p3385-3396. See N-formyl compound under γ-Methyl on page 3390.	1-7, 16
X	ROWAN, *et al.*, "Antifungal stress metabolites from *Solanum aviculare*", *Phytochem.*, **1983**, *22*(9), p2102-2104. See structures 1-3.	1-7, 22
X	NAGAOKA, *et al.*, "Steroidal alkaloids from roots of tomato stock", *Phytochem.* **1993**, *34*(4), p1153-1157. See compounds 1-3	1-7, 22
X	SATO, *et al.*, "Alkaloids from *Solanum congestiflorum*", *J. Org. Chem.*, **1969**, *34*(6), p1577-1582. See scheme 1	1-7, 29, 32-38, 41, 42
X	FRIEDMAN, "Tomato Glycoalkaloids: Role in the Plant and in the Diet", *J. Agric. Food Chem.*, **2002**, *50*(21), p5751–5780 see whole document	1-7, 29, 32, 101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	UHLE, "The Synthesis pf Azaoxaspirane Steroid Alkaloids", *J. Am. Chem. Soc.*, **1961**, *83*(6) , p1460–1472. See structure XXIV	1-7, 29, 34-38, 41, 42
X	SATO, *et al.*, "Chemistry of the Spiroaminoketal Side Chain of Solasodine and Tomatidine. IV. Chemistry of the Tomatidine Side Chain", *J. Org. Chem.*, **1960**, *25*, p1962-1965. See compounds I, Ia, IV	1-7, 29, 95-98
X	SIMONS, *et al.*, "Dual Effects of Plant Steroidal Alkaloids on *Saccharomyces cerevisaiae*", *Antimicrob. Agents Chemother.*, **2006**, *50*, p2732-2740. See whole document, especially Fig 1(C)	1-7, 29, 101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	SATO, *et al.*, "New Dihydro Derivatives of Tomatidine and Solasodine", *J. Am. Chem. Soc.*, **1956**, *78*(13), p3150–3153. See structures II, IIa	1-7, 29
X	CN101054399A (TIAN) 17 October 2007, 17-10-2007 see structures 5, 6 in abstract; page 1 of description; structures 5a, 6a on page 8; 5b, 6b on page 9	1-7, 29

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	TSCHESCHE, *et al.*, "Zur Biogenese Des Aza-Oxa-Spiran-Systems Der Steroidalkoide Vom Spirosolan-typ in Solanaceen", *Phytochem.*, **1978**, *17*(2), p251-255. See pages 252.	1-7, 30-36, 41
X	MCKENNA, "Steroidal Alkaloids", *Quarterly Reviews, Chemical Society*, **1953**, *7*(3), p231-254. See pages 238, 240	1-7, 30-36, 41
X	MECCIA, *et al.*, "On the Configuration of Solaquidine", *J. Nat. Prod.*, **1987**, *50*(4), p642-645. See page 643.	1-7, 30-36, 41
X	NIÑO, *et al.*, "Biological activities of steroidal alkaloids isolated from *Solanum leucocarpum*", *Pharmaceutical Biology*, **2009**, *47*(3), p255-259. See Figure 1 on page 257 and Table 1 on page 258.	1-7, 30-36, 41, 101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	MAXWELL, *et al.*, "3-Aminospirosolane alkaloids from *Solanum arboreum*", *Phytochemistry*, **1996**, *43*(4), p913-915. See compounds 1-7	1-5, 14
X	MAXWELL, *et al.*, "3β-Aminospirosolane alkaloids from *Solanum triste*", *J. Nat. Prod.*, **1995**, *58*(4), p625-628. See compounds 1-3	1-5, 14
X	COLEMAN, *et al.*, "Characterization of Plant-Derived Saponin Natural Products against *Candida albicans*", *ACS Chem. Biol.*, **2010**, *5*(3), p321-332. See compounds A11 and A20.	1-3, 101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	MAZUR, *et al.*, "The Synthesis of the Steroidal Sapogenins", *J. Am. Chem. Soc.*, **1960**, *82*(22), p5889–5908. see whole document, especially compounds LXIII, LXIV, LXVII, etc.	1-3
X	SATO, *et al.*, "Structure of Tomatillidine", *J. Org. Chem.*, **1965**, *30*(3), p754-760. See compounds III, VII, IX, X, XI, XII and second column on page 755	1, 30-38, 41, 42
X	ADAM, *et al.*, "*Solanum*-Alkaloide XXVI. Präparative Trennung stereoisomerer Imino-choelstane und weiterer Steroide durch Dünnschicht-Chromatographie unter Verwendung von Jod als indifferentes Nachweisreagens", *Z. Chem.*, **1963**, *3*(3), p100-102. See structures 1 and 2	1, 30-38, 41, 42
X	ADAM, *et al.*, "*Solanum*-Alkaloide XXXIX. Synthese von 22,26-imino-5α-cholestan-3β-olen aus 3β-acetoxy-pregn-5-en-20-on und deren sterische zuordnung", *Tetrahedron*, **1964**, *20*(7), p1707-1718. see page 1708.	1, 30-38, 41, 42
X	XIE, *et al.*, "Structure-Activity Relationship of Aza-Steroids as PI-PLC Inhibitors", *Bioorg. Med. Chem.*, **2001**, *9*(5), p1073-1085. See compounds in scheme 1: 2a,b through 8a,b	1, 30-38, 45-65
X	KUSANO, *et al.*, "Antifungal Properties of Solanum Alkaloids", *Chem. Pharm. Bull.*, **1987**, *35*(12), p4862-4867. See tables 1 and 2.	1, 30-36, 41, 101-134, 136-173, 175, 177-195, 198-220, 222-240, 242-244
X	TSCHESCHE, *et al.*, "Zur Syntheese von 22,26-Epiminocholestanolen", *Chem. Ber.*, **1978**, *111*(2), p801-802.	1, 30-36, 41
X	BIRD, *et al.*, "Structures of the Steroidal Alkaloids 25-Isosolaforidine and Solacallinidine Isolated from *Solanum callium*", *Aust. J. Chem.*, **1979**, *32*(3), p597-609. See page 600, scheme 1.	1, 30-36, 43
X		1, 30-36, 45, 49, 50

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UBUKA, "Experimental isovalthinuria. III. Induction by bile acids, and hypocholesterolemic agents", *Acta Med. Okayama*, **1963**, *17*(6), p273-278. see compounds a and d in table 2.	1, 30-36, 45, 49, 50
X	KRAML, "Agents Affecting Lipid Metabolism. XXVI. Specificity of Some Inhibitors of the Late Stages of Cholesterol Biosynthesis", *Lipids*, **1967**, *2*(1), p5-7. See compound 22,25-DAC in table 1.	1, 30-36, 45, 49, 50
X	US3419661, (ELDER), 31 December 1968, 31-12-1968, see column 2.	1, 30-36, 46, 53
X	US3558608, (KLIMSTRA), 26 January 1971, 26-01-1971. See bottom of col. 1	1, 30-36, 46, 53
X	ARMAS, *et al.*, "Steroidal *N*-Nitrosoamines. Part 4. Intramolecular Functionalization of *N*-Nitroamine Radicals: Synthesis of 1,4-Nitroimine Compounds", *J. Chem. Soc. Perkin Trans. I*, **1988**, *12*, p3255-3265. See compounds on page 3257.	1, 30-32, 45
X	KIRCHER, *et al.*, "Preparation of Some Unsaturated Side-Chain Derivatives of Cholesterol", *J. Org. Chem.*, **1982**, *47*(9), p1722-1724. see scheme 1, compounds 4a, 5a	1, 95-99

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2012/050087

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [X] Claim Nos. : 104-134, 136-152

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 104-134, 136-152 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claim 153.

2. [X] Claim Nos. : 1-7, 37-42

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

certain claims encompassed too many possibilities to produce reasonable results. However, every effort has been made to cite as many representative pieces of prior art as time would allow.

3. [ ] Claim Nos. :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. (claims 1, 101-255 in part, 2-29 in full) is aimed at compounds of formula 1.0, compositions thereof and uses thereof.
2. (claims 1, 101-255 in part, 30-67 in full) is aimed at compounds of formula 2.0, compositions thereof and uses thereof.
3. (claims 1, 101-255 in part, 68-94 in full) is aimed at compounds of formula 3.0, compositions thereof and uses thereof.
4. (claims 1, 101-255 in part, 95 in full) is aimed at compounds of formula 4.0, compositions thereof and uses thereof.
5. (claims 1, 101-255 in part, 96-100 in full) is aimed at compounds of formula 5.0, compositions thereof and uses thereof.

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. : 1, 101-255 (in part), 2-67, 96-100 (in full)

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[X] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2012/050087

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
CA2395642A1	12 July 2001 (12-07-2001)	AT304360T	15 September 2005 (15-09-2005)
		AU2022901A	16 July 2001 (16-07-2001)
		DE60022687D1	20 October 2005 (20-10-2005)
		DE60022687T2	06 July 2006 (06-07-2006)
		EP1274445A2	15 January 2003 (15-01-2003)
		EP1274445B1	14 September 2005 (14-09-2005)
		IL133809D0	30 April 2001 (30-04-2001)
		IL149913A	01 August 2006 (01-08-2006)
		IL149913D0	10 November 2002 (10-11-2002)
		JP2003519178A	17 June 2003 (17-06-2003)
		US2003114393A1	19 June 2003 (19-06-2003)
		WO0149279A2	12 July 2001 (12-07-2001)
		WO0149279A3	17 October 2002 (17-10-2002)
US2770618A	13 November 1956 (13-11-1956)	None	
CN101054399A	17 October 2007 (17-10-2007)	CN101054399A	17 October 2007 (17-10-2007)
		CN100569792C	16 December 2009 (16-12-2009)
US3013008A	12 December 1961 (12-12-1961)	GB983660A	17 February 1965 (17-02-1965)
US3419661A	31 December 1968 (31-12-1968)	None	
US3558608A	26 January 1971 (26-01-1971)	None	