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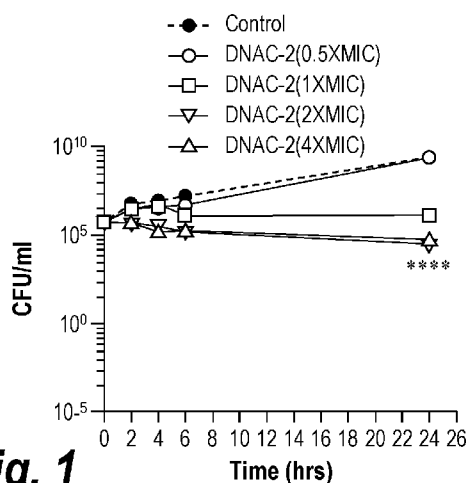


Fig. 1

(57) Abstract: The present disclosure relates to compounds having antimicrobial activity, compositions comprising said compounds in combination with β -lactam antibiotics, and methods of using the compounds and compositions.

ANTIBIOTIC COMPOSITIONS

U.S. GOVERNMENT RIGHTS

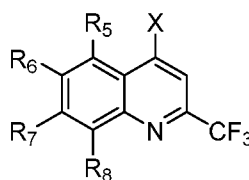
[0001] This invention was made with government support under grant number U54AI057159 awarded by the NERCE-BEID and grant number P30GM106394 awarded
 5 by the COBRE (NIH). The government has certain rights in the invention.

BACKGROUND

[0002] Antibiotic resistance is recognized as a global public health threat. Methicillin-resistant *Staphylococcus aureus* (MRSA) is on the CDC's report of deadly pathogens. The CDC estimates that there were 80,461 invasive MRSA infections
 10 and 11,285 related deaths in the United States alone in 2011. An unknown but much higher number of less severe infections occurred in both the community and in healthcare settings. Concurrently, rates of antibiotic resistance in MRSA are surpassing 50% in 5 of the 6 World Health Organization world regions. Despite this, the success rate of discovery of new anti-MRSA therapeutics has been low. The rapid evolution of multidrug
 15 resistant bacteria necessitates the development of new, efficient antibiotics.

SUMMARY

[0003] Accordingly, the present disclosure provides compounds having antimicrobial activity both in monotherapy and in combination therapy with β -lactam antibiotics. In one aspect, provided herein is a composition comprising an antibiotic and
 20 a compound of formula (I), or a pharmaceutically acceptable salt thereof:

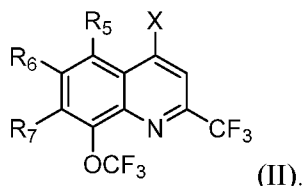


(I)

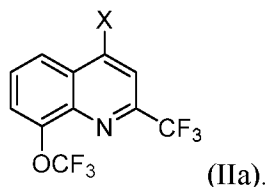
wherein X is OR or NHR; R is hydrogen or R_1 - R_2 -(R_3)_n; R_1 is selected from C_{1-6} alkyl, C_{2-6} alkynyl, aryl and heteroaryl; R_2 is selected from aryl and heteroaryl, or is absent;
 25 each R_3 is independently selected from C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} fluoroalkyl, C_{1-6} fluoroalkoxy, halogen, cyano and $-CO_2R_4$; R_4 is hydrogen or C_{1-6} alkyl; R_5 , R_6 , R_7 and R_8 are independently selected from hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} fluoroalkyl and C_{1-6} fluoroalkoxy; and n is 0-3; provided that at least one of R_5 , R_6 , R_7 and R_8 is C_{1-6} fluoroalkyl or C_{1-6} fluoroalkoxy.

[0004] In certain embodiments of formula (I), R_8 is C_{1-6} fluoroalkoxy. In one embodiment, R_5 , R_6 and R_7 are hydrogen, and R_8 is C_{1-6} fluoroalkoxy. In a particular embodiment, R_8 is trifluoromethoxy.

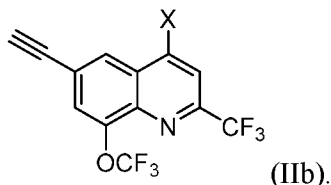
[0005] In another embodiment, the compound of formula (I) has the structure of formula (II), or a pharmaceutically acceptable salt thereof:



[0006] In a particular embodiment, the compound of formula (II) has the structure of formula (IIa), or a pharmaceutically acceptable salt thereof:

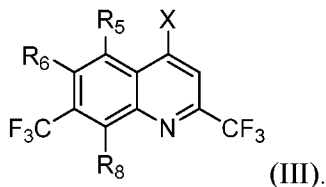


[0007] In another particular embodiment, the compound of formula (II) has the structure of formula (IIb), or a pharmaceutically acceptable salt thereof:

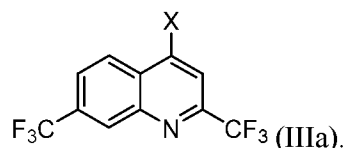


[0008] In certain embodiments of formula (I), R_7 is C_{1-6} fluoroalkyl. In one embodiment, R_5 , R_6 and R_8 are independently selected from hydrogen and C_{1-6} fluoroalkyl. In another embodiment, R_5 , R_6 and R_8 are hydrogen. In another embodiment, R_5 is C_{1-6} fluoroalkyl and R_6 and R_8 are hydrogen. In a particular embodiment, said C_{1-6} fluoroalkyl is trifluoromethyl. In another particular embodiment, R_7 is trifluoromethyl.

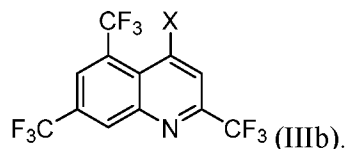
[0009] In one embodiment, the compound of formula (I) has the structure of formula (III), or a pharmaceutically acceptable salt thereof:



[0010] In a particular embodiment, the compound of formula (III) has the structure of formula (IIIa), or a pharmaceutically acceptable salt thereof:

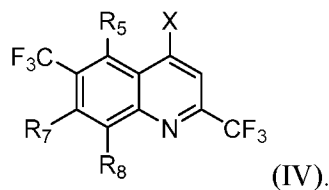


[0011] In another particular embodiment, the compound of formula (III) has the structure of formula (IIIb), or a pharmaceutically acceptable salt thereof:

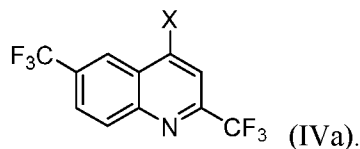


[0012] In certain embodiments of formula (I), R₆ is C₁₋₆fluoroalkyl. In one embodiment, R₅, R₇ and R₈ are independently selected from hydrogen and C₁₋₆fluoroalkyl. In another embodiment, R₅, R₇ and R₈ are hydrogen. In a particular embodiment, R₆ is trifluoromethyl.

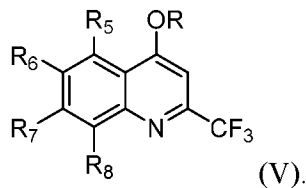
[0013] In one embodiment, the compound of formula (I) has the structure of formula (IV), or a pharmaceutically acceptable salt thereof:



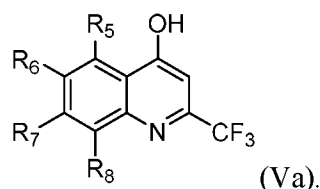
[0014] In a particular embodiment, the compound of formula (IV) has the structure of formula (IVa), or a pharmaceutically acceptable salt thereof:



[0015] In certain embodiments, X is OR and the compound has the structure of formula (V), or a pharmaceutically acceptable salt thereof:

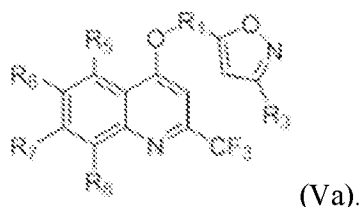


[0016] In one embodiment, R is hydrogen and the compound of formula (V) has the structure of formula (Va), or a pharmaceutically acceptable salt thereof:

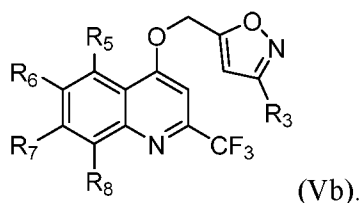


[0017] In a particular embodiment, the compound of formula (Va) is selected from compounds (1), (1b), (1c), and (1f) of Table 1(a), and pharmaceutically acceptable salts thereof.

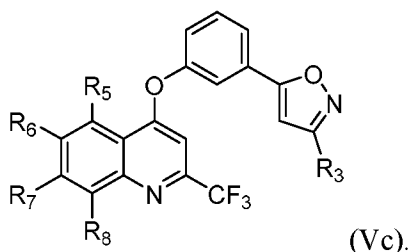
5 [0018] In another embodiment of the compound of formula (V), R is R₁-R₂-R₃; wherein R₁ is selected from C₁₋₆ alkyl, C₂₋₆ alkynyl, aryl and heteroaryl; R₂ is heteroaryl; R₃ is -CO₂H or -CO₂C₁₋₆; and n is 1. In one embodiment, R₂ is selected from furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, oxazolyl, isoxazolyl, oxadiazolyl, and thiadiazolyl. In one embodiment, R₂ is isoxazol-3-yl. In another embodiment, the
10 compound of formula (V) has the structure of formula (Va):



[0019] In a particular embodiment, the compound of formula (Va) has the structure of formula (Vb), or a pharmaceutically acceptable salt thereof:

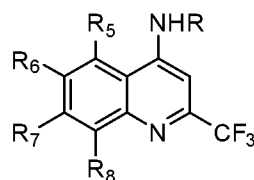


15 [0020] In another particular embodiment, the compound of formula (Va) has the structure of formula (Vc), or a pharmaceutically acceptable salt thereof:



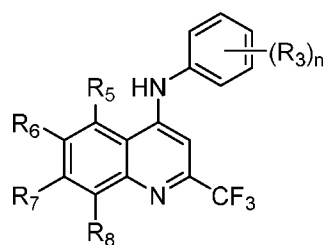
[0021] In one embodiment, R₃ is -CO₂H or -CO₂CH₂CH₃.

[0022] In certain embodiments X is NHR and the compound of formula (I)
20 has the structure of formula (VI):



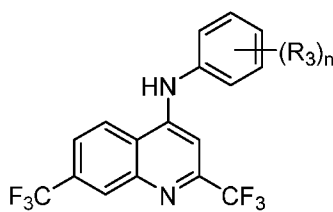
(VI).

[0023] In one embodiment, R is R₁-R₂-R₃; R₁ is aryl or heteroaryl; R₂ is absent; and each R₃ is independently selected from C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆fluoroalkyl, C₁₋₆fluoroalkoxy, halogen and cyano. In another embodiment, R₁ is phenyl. In a particular embodiment, the compound has the structure of formula (VII):



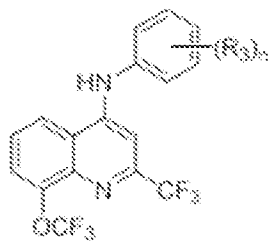
(VII).

[0024] In one embodiment, R₆ is hydrogen and R₅, R₇ and R₈ are independently selected from hydrogen, C₁₋₆fluoroalkyl and C₁₋₆fluoroalkoxy. In a particular embodiment, the compound of formula (VII) has the structure of formula (VIIa):



(VIIa).

[0025] In another particular embodiment, the compound of formula (VII) has the structure of formula (VIIb):



(VIIb).

[0026] In certain embodiments, R₃ is trifluoromethyl and n is 0 or 1. In a particular embodiment, the compound of formula (VII) is selected from compounds (3a) and (3b) of Table 1(b), and pharmaceutically acceptable salts thereof.

[0027] In certain embodiments of the above compositions, the antibiotic is selected from cefoxitin, nafcillin and oxacillin.

[0028] In one embodiment, antibiotic is cefoxitin. In a particular embodiment, the antibiotic is cefoxitin and the compound is compound (1), or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is cefoxitin and the compound is compound (1b), or a pharmaceutically acceptable salt thereof.

[0029] In one embodiment, the antibiotic is nafcillin. In a particular embodiment, the antibiotic is nafcillin and the compound is compound (1b), or a pharmaceutically acceptable salt thereof.

[0030] In one embodiment, the antibiotic is oxacillin. In a particular embodiment, the antibiotic is oxacillin and the compound is compound (1), or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is oxacillin and the compound is compound (1b),

[0031] or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is oxacillin and the compound is compound (1c), or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is oxacillin and the compound is compound (1f), or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is oxacillin and the compound is compound (3a), or a pharmaceutically acceptable salt thereof. In another particular embodiment, the antibiotic is oxacillin and the compound is compound (3b), or a pharmaceutically acceptable salt thereof.

Methods and Uses

[0032] In another aspect, provided herein is a method of treating or preventing a bacterial infection a subject in need thereof, comprising administering to the subject a therapeutically-effective amount of the composition comprising a compound of formula (I) (for example, compounds of formulas (II)-(VII)) and an antibiotic.

[0033] In one embodiment, the method comprises the steps of: (a) administering to the subject a compound of formula (I) (including, for example, compounds of formulas (II)-(VII)); and (b) administering to the subject a therapeutically-effective amount of an antibiotic.

[0034] In another embodiment, the method comprises the steps of: (a) administering to the subject a therapeutically-effective amount of an antibiotic; and (b) administering to the subject a compound of formula (I) (for example, compounds of formulas (II)-(VII)).

[0035] In another aspect, provided herein is a method of treating or preventing a bacterial infection a subject in need thereof, comprising administering to the subject a therapeutically-effective amount of the composition described herein.

[0036] In one embodiment, the method comprises the steps of: (a)
5 administering to the subject a compound of formula (I) (for example, compounds of formulas (II)-(VII)); and (b) administering to the subject a therapeutically-effective amount of an antibiotic selected from cefoxitin, nafcillin and oxacillin.

[0037] In another embodiment, the method comprises the steps of: (a)
administering to the subject a therapeutically-effective amount of an antibiotic selected
10 from cefoxitin, nafcillin and oxacillin; and (b) administering to the subject a compound of formula (I) (for example, compounds of formulas (II)-(VII)).

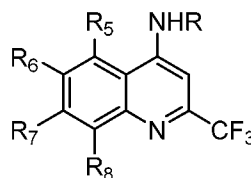
[0038] In a particular embodiment of the above methods, the antibiotic is oxacillin.

[0039] In a particular embodiment of the above methods, the compound is
15 selected from the group of compounds (1), (1b), (1c), (1f), (3a), (3b), and pharmaceutically acceptable salts thereof.

[0040] In one embodiment of the above methods, the bacterial infection is caused by a gram positive bacterium. In certain embodiments, the gram positive bacterium is selected from *Staphylococcus aureus*, *Staphylococcus epidermidis*,
20 *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, Group B *Streptococci*, Group C *Streptococci*, and Group G *Streptococci*. In a particular embodiment, the bacterial infection is caused by *Staphylococcus aureus*. In another particular embodiment, the bacterial infection is caused by methicillin-resistant *Staphylococcus aureus*. In another particular embodiment,

25 [0041] the methicillin-resistant *Staphylococcus aureus* is a community-acquired strain. In another particular embodiment, the methicillin-resistant *Staphylococcus aureus* is a hospital-acquired strain. In another particular embodiment, the bacterial infection is caused by the USA300 strain of methicillin-resistant *Staphylococcus aureus*.

30 [0042] In another aspect, provided herein is a compound of formula (VI), or a pharmaceutically acceptable salt thereof:

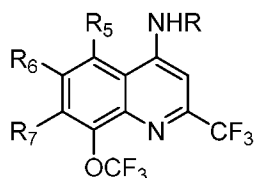


(VI)

wherein R is R₁-R₂-(R₃)_n; R₁ is aryl or heteroaryl; R₂ is absent; each R₃ is independently selected from C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆fluoroalkyl, C₁₋₆fluoroalkoxy, halogen and cyano; R₅, R₆, R₇ and R₈ are independently selected from hydrogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₁₋₆fluoroalkyl and C₁₋₆fluoroalkoxy; and n is 0-3.

[0043] In one embodiment, at least one of R₅, R₆, R₇ and R₈ is C₁₋₆fluoroalkyl or C₁₋₆fluoroalkoxy. In another embodiment, R₈ is C₁₋₆fluoroalkoxy. In still another embodiment, R₅, R₆ and R₇ are hydrogen, and R₈ is C₁₋₆fluoroalkoxy. In a particular embodiment, R₈ is trifluoromethoxy.

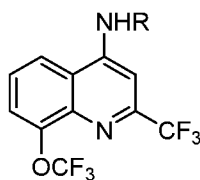
[0044] In one embodiment, the compound of formula (VI) has the structure of formula (VIa):



(VIa)

or a pharmaceutically acceptable salt thereof:

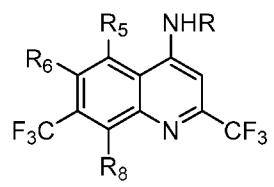
[0045] In one embodiment, the compound of formula (VIa) has the structure of formula (VIb), or a pharmaceutically acceptable salt thereof:



(VIb).

[0046] In another embodiment of the compound of formula (VI), R₇ is C₁₋₆fluoroalkyl. In another embodiment, R₅, R₆ and R₈ are independently selected from hydrogen and C₁₋₆fluoroalkyl. In a particular embodiment, R₅, R₆ and R₈ are hydrogen. In another particular embodiment, R₅ is C₁₋₆fluoroalkyl and R₆ and R₈ are hydrogen. In another particular embodiment, said C₁₋₆fluoroalkyl is trifluoromethyl.

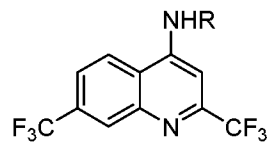
[0047] In another embodiment of the compound of formula (VI), R₇ is trifluoromethyl and the compound of formula (VI) has the structure of formula (VIc):



(VIc)

or a pharmaceutically acceptable salt thereof.

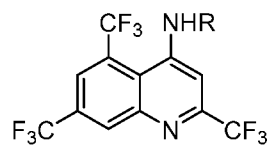
[0048] In one embodiment, the compound of formula (VIc) has the structure
5 of formula (VIId):



(VIId)

or a pharmaceutically acceptable salt thereof.

[0049] In another embodiment, the compound of formula (VIc) has the
10 structure of formula (VId):

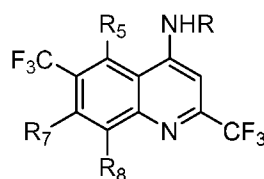


(VId)

or a pharmaceutically acceptable salt thereof

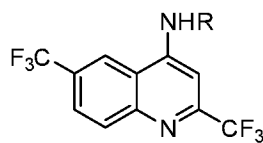
[0050] In another embodiment of the compound of formula (VI), R₆ is C₁-
15 6fluoroalkyl. In one embodiment, R₅, R₇ and R₈ are independently selected from
hydrogen and C₁₋₆fluoroalkyl. In another embodiment, R₅, R₇ and R₈ are hydrogen. In a
particular embodiment, R₆ is trifluoromethyl.

[0051] In another embodiment, the compound of formula (VI) has the
structure of formula (VIf), or a pharmaceutically acceptable salt thereof:



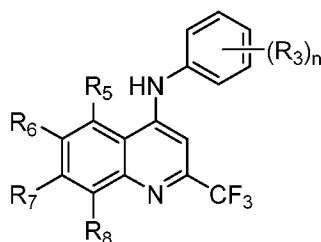
(VIf).

[0052] In a particular embodiment, the compound of formula (VIf) has the
structure of formula (VIg), or a pharmaceutically acceptable salt thereof:



(VIg).

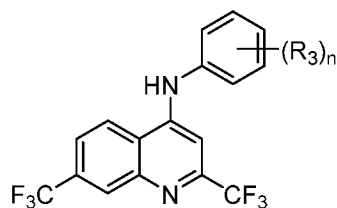
[0053] In another embodiment, the compound of formula (VI) has the structure of formula (VII):



(VII).

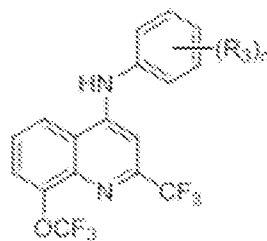
[0054] In one embodiment of the compound of formula (VII), R₆ is hydrogen and R₅, R₇ and R₈ are independently selected from hydrogen, C₁₋₆fluoroalkyl and C₁₋₆fluoroalkoxy.

10 [0055] In one embodiment, the compound of formula (VII) has the structure of formula (VIIa):



(VIIa).

15 [0056] In another embodiment, the compound of formula (VII) has the structure of formula (VIIb):



(VIIb).

[0057] In a particular embodiment of the above compounds, R₃ is trifluoromethyl and n is 0 or 1.

20 [0058] In another particular embodiment, the compound is selected from compounds (3a) and (3b), and pharmaceutically acceptable salts thereof. In yet particular

embodiment, the compound is compound (3a), or a pharmaceutically acceptable salt thereof. In still another particular embodiment, the compound is compound (3b), or a pharmaceutically acceptable salt thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0059] **FIG. 1** depicts time kill curves of MRSA USA300 with different concentrations of compound 1. USA300 cells were grown to mid-log phase, back diluted 100-fold and treated with (a) 0.5X compound 1 (open circle), 1X compound 1 (closed square), 2X compound 1 (open square) and 4X MIC of compound 1 (closed triangle) or left untreated (closed circle). Samples were taken at 0, 2, 4, 6 and 24 hrs and a viable count carried out. Asterisk denotes *P* values of <0.05 as determined by Tukey's multiple comparison test of two way analysis of variance. The lines show the mean and SE.

10 [0060] **FIG. 2** depicts time kill curves of MRSA USA300 with different concentrations of compound 1b. USA300 cells were grown to mid-log phase, back diluted 100-fold and treated with 0.125X MIC (open circle), 0.25X MIC (open square), 15 0.5X MIC (closed square) and 1X MIC of compound 1b (closed triangle) or left untreated (closed circle). Samples were taken at 0, 2, 4, 6 and 24 hrs and a viable count carried out. Asterisk denotes *P* values of <0.05 as determined by Tukey's multiple comparison test of two way analysis of variance. The lines show the mean and SE.

20 [0061] **FIG. 3** depicts percent inhibition of macromolecular biosynthesis of *Staphylococcus simulans* 22 by compound 1 as measured by inhibition of radiolabeled precursor incorporation into nascent DNA (open square), RNA (open circle), peptidoglycan (closed circle) and protein (closed square). Bacteria were treated with compound 1 for 30 minutes in the presence of radioactive precursors. The data are presented as percent inhibition compared to control with no antibiotic.

25 [0062] **FIG. 4** depicts percent inhibition of DNA biosynthesis of *S. simulans* 22 by compound 1: 0.5X MIC (open square), 1X MIC (closed square), 2X MIC (open triangle) of compound 1 normalized to control with no antibiotic, over a period of time. Control with no antibiotic is denoted by open circle and ciprofloxacin (10X MIC) (closed circle) was used as a positive control.

30 [0063] **FIG. 5** depicts percent inhibition of cell wall biosynthesis of *S. simulans* 22 by compound 1: 0.5X MIC (open square), 1X MIC (closed square), 2X MIC (open triangle) of compound 1 normalized to control with no antibiotic, over a period of time. Control with no antibiotic is denoted by (open circle) and vancomycin (10X MIC) (closed circle) was used as a positive control.

[0064] FIG. 6 depicts control cells and compound 1 treated cells, shown at 1200X magnification, showing the effect of compound 1 on cell wall by TEM. USA300 cells were grown to mid-log phase and treated with compound 1 for 90 minutes, or left untreated (control). Cells were then collected and processed for EM as described in

5 Material and Methods.

[0065] FIG. 7 depicts the effect of compound 1 on cell wall by TEM. USA300 cells were grown to mid-log phase and treated with compound 1 for 90 minutes, or left untreated (control). Cells were then collected and processed for EM as described in Material and Methods. Compound 1 treated cells have significantly more

10 septate cells.

[0066] FIG. 8 depicts the effect of compound 1 on cell wall by TEM. USA300 cells were grown to mid-log phase and treated with compound 1 for 90 minutes, or left untreated (control). Cells were then collected and processed for EM as described in Material and Methods. Compound 1 treated cells have a significantly thicker

15 cell wall as demonstrated in the box and whiskers plot showing STD. The symbol (*) represents statistically significant difference, with $P < 0.05$ comparing control to compound 1 treated cells.

[0067] FIG. 9 depicts the effect of compound 1 on cell wall by TEM. USA300 cells were grown to mid-log phase and treated with compound 1 for 90

20 minutes, or left untreated (control). Cells were then collected and processed for EM as described in Material and Methods. Mesosome-like invaginations are seen both along the cell wall and along the septum in cells at 15000X magnification (arrows, right panel).

[0068] FIG. 10 depicts microscopy showing activation of the *vraRS* cell wall stress stimulon using strains containing a *vraRS* promoter driving sfGFP expression. In

25 the presence of 1X MIC compound 1, the mean cell fluorescence was $8246 \pm 1178^*$ a.u. (arbitrary units), $n=406$, while in the presence of 1x MIC vancomycin (positive control), the mean cell fluorescence was $29072 \pm 7573^*$ a.u., $n=379$. In the absence of antibiotics, the average cell fluorescence was $4521 \pm 781^*$ a.u. (arbitrary units), $n=377$. *p value < 0.0001 between DMSO control and vancomycin-treated cells and < 0.05 between control

30 and compound 1 treated cells.

[0069] FIG. 11 depicts microscopy showing localization of PBP2 and FtsZ by compound 1. Localization of the PBP2 and FtsZ was tracked using strains expressing GFP-PBP2 or FtsZ-CFP after treatment with 1x MIC compound 1 for 30 min.

[0070] FIG. 12 depicts microscopy showing the disruption of cell membrane and depolarization by compound 1. Cells were either untreated (control) or treated with 1X MIC compound 1 for 30 min and stained with FM 4-64 to visualize the membrane, Van-FL to visualize the cell wall (middle panels) or Hoechst 33342 to stain the DNA.

5 [0071] FIG. 13 depicts a graph showing the disruption of cell membrane and depolarization by compound 1. Actively growing USA300 cells were back-diluted, resuspended in PBS with glucose and treated with DiOC₂ in the dark for 30 minutes at 25°C. Loss in fluorescence was monitored over time upon adding increasing concentrations of DNAC-1 at 2X MIC (open square), 4X MIC (closed square) or 8X MIC
10 (open triangle). The untreated cells are shown as open circles and CCCP was used as a positive control (closed circle). Each line represents the means accompanied by standard errors of 3 independent readings.

[0072] FIG. 14 depicts the effects of compound 1, alone and in combination with oxacillin, against strains of community-acquired MRSA (CA-MRSA) and hospital-
15 acquired MRSA (HA-MRSA).

DETAILED DESCRIPTION

[0073] The present disclosure is related to compounds having antimicrobial activity both in monotherapy and in combination therapy with β -lactams. The compounds described herein, e.g., compounds of formulas (I)-(VII), and embodiments
20 and pharmaceutically acceptable salts thereof, as well as the compounds of Tables 1(a) and 1(b), and pharmaceutically acceptable salts thereof, are collectively referred to as “compounds of the invention.” “Compositions of the invention” refers to one or more compounds of the invention in combination with one or more antibiotics, as described herein.

25 [0074] Exposure of the community-acquired strain of methicillin-resistant staphylococcus aureus (MRSA) strain USA300 to compounds of the invention led to reduced daughter cell separation after cross wall synthesis and an increase in cell wall thickness with mesosome formation mostly along the septum. Additionally, penicillin binding protein 2 (PBP-2) was mislocalized in an FtsZ-independent manner. Compounds
30 of the invention (e.g., compound 1), similar to novobiocin (a DNA gyrase inhibitor), affected both DNA synthesis and transcription, as demonstrated by the macromolecular synthesis assay and the antagonism of both rifampicin and levofloxacin against compound 1 in the checkerboard assays. Compound 1b, in combination with oxacillin, was also active against USA300. Compounds of the invention have a novel mechanism

of action which causes growth arrest by inhibiting daughter cell separation due to disruption in cell wall and DNA synthesis.

[0075] Unlike other well-known agents that affect DNA replication, compounds of the invention affect the cell wall. For example, compound 1 is effective on
5 its own against a variety MRSA strains, and is synergistic with β -lactams such as oxacillin and cefoxitin. Evaluation of the pharmacokinetic and toxicity profiles of compound 1 show that it is stable in human microsomes, is cell permeable and does not exhibit any major cytotoxicity.

[0076] An SAR study was undertaken to synthesize analogues of compound 1.
10 The 4-hydroxyquinoline analogues showed various levels of antibiotic activity against MRSA, depending on the identity and the position of the substitution, with substitution on both rings of the 4-hydroxyquinoline being required for greatest efficacy against MRSA. Table 1(a) and 1(b) summarize the MIC data for these analogues. Aromatic substitution resulting in compounds 1c and 1f (Table 1a) showed a four-fold improvement in
15 MIC. Compound 1b exhibited a ten-fold improvement in activity when used in combination with sub-MIC (16 mg/L) of oxacillin vs. the compound alone. Comparison of compounds 1a and 1d vs. the parental compound compound 1 suggests that an electron-withdrawing group such as $-\text{CF}_3$ or $-\text{OCF}_3$ is a requisite for efficacy. Moreover, the position of substitution appears to have a strong effect on the activity. For example,
20 compound 1c, with a CF_3 - group on the 7- position, has similar inhibitory properties as compound 1, while its regioisomer compound 1e, though not completely inactive, is considerably less effective. Intriguingly, compound 1b is only as active as compound 1 on its own, but its activity in combination with oxacillin is 10 fold better than compound 1. Analogues of compound 1 in which the hydroxyl group was modified were also
25 examined. Alkylation of the 4-hydroxy group with either a propargyl group or isoxazole completely neutralized the antimicrobial activity of compound 2a-i as shown in Table 1(b). This observation suggests that a hydrogen bond donor moiety in that region of the molecule may be required for antimicrobial activity. Interestingly, hydrolysis of compound 2i to yield a carboxylic acid moiety in compound 2j (a hydrogen bond donor)
30 recovered the activity to the level of compound 1c from which compound 2j is derived. Notably, compounds 3a and 3b, which are both hydrogen bond donors, exhibit good activity against MRSA with oxacillin. These observations may also indicate a correlation between hydrogen bond donors and antimicrobial activity.

[0077] As the screen for compound 1 is a mechanistically unbiased screen against MRSA, a direct target was not immediately apparent. In an attempt to separate secondary effects from the primary target, the effect of compound 1 was monitored over time in macromolecular synthesis assays. In these assays, compound 1 was most efficient in inhibiting DNA and cell wall synthesis in a concentration dependent manner and transcription to a lesser degree. Similar macromolecular assays have shown that other inhibitors of DNA replication behave differently from each other. Specifically, ciprofloxacin exhibits a rapid concentration dependent inhibition of DNA synthesis in 30 min, however, novobiocin exerts effects on both DNA and RNA synthesis in the same time frame. Neither of these drugs had any effect on cell wall synthesis either directly or indirectly. In contrast, the inhibitory effect of compound 1 on cell wall synthesis, occurring within 30 minutes of exposure, is concentration dependent. These target profiles differentiate compound 1 from other drugs that inhibit DNA replication.

[0078] Activation of the *vraRS* system is a reliable way to assess cell wall stress. The effect of compound 1 on the cell wall was confirmed using a *vraRS* promoter fusion to GFP. Treatment with compound 1 showed a significant activation of the cell-wall stress response operon, albeit to lower level compared to vancomycin. Kuroda *et al.* have previously shown that treatment of cells with levofloxacin, unlike cell-wall active compounds, does not induce transcription of *vraRS*. This induction is thus a specific cell-wall stress response, as general stresses like heat, high osmolarity and pH shift do not appear to affect *vraRS* transcription thus indicating that this is not likely to be a secondary affect due to growth inhibition.

[0079] Using EM, as seen in Figure 8, a significant thickening of the cell wall was observed in compound 1 treated cells without any major differences in cell size. Notably however, ~55 % of compound 1 treated cells showed cross wall/septum formation and a reduced or undetectable splitting system compared to just 20% of untreated cells. That *S. aureus* cells seemed to be “arrested” in the growth cycle without loss of cell size may suggest impairment in cell division or a change in cell wall as opposed to major changes in metabolic activity. These data were reminiscent of the lack of daughter cell separation seen upon treatment of vancomycin sensitive cells with sub-MIC vancomycin. In that case of vancomycin sensitive cells, the effect was traced back to an inability of the murein hydrolases to access the cell wall due to association of the cell wall subunits to vancomycin as shown by an inhibition of autolytic activity. Compound 1, however, did not have a significant effect on autolysis as monitored by a Triton X-100

induced autolysis assay (data not shown). Mislocalization of the cell wall synthesis enzyme PBP2 upon compound 1 treatment confirmed the cell wall as a plausible target for compound 1. The appearance of the normal looking FtsZ rings ruled out mislocalization of FtsZ as a cause of the PBP2 defect in the cell wall. As substrate
5 binding directs PBP2 localization to the septum, the mislocalization of PBP2 points to impairment in peptidoglycan synthesis at the cross wall. Bactericidal activity by way of inhibiting cell wall synthesis is generally a slow process, and the time kill assay mirrors this temporal requirement.

[0080] The data suggest a secondary effect of compound 1 on the
10 membrane. First, membrane blebbing in USA300 cells treated with compound 1 at the crosswalls was not a concentration dependent event nor did we see significant membrane damage upon membrane staining within 30 minutes. Secondly, compound 1 did not induce pore-formation in the membrane as reflected by a lack of potassium and ATP release from treated cells. Third, antibiotics that depolarize membrane as a primary
15 mechanism of action, like otrivancin, act very rapidly and in a concentration dependent manner, unlike compound 1.

[0081] Zhou *et al.* recently showed that cell-wall stress together with mechanical failure can result in ultrafast cell separation. Turgor pressure on the cytoplasmic membrane is the primary source of cell wall stress and membrane disruption
20 leads to a decrease in turgor pressure in the cell. It is conceivable that the non-specific nature of compound 1 mediated membrane depolarization results in a reduction in turgor pressure, thus disrupting the mechanics for the cell separation, resulting in a large number of cells being arrested at that stage.

[0082] It has been previously demonstrated that in *E.coli mazEF*-mediated cell
25 death is triggered under the specific physiological condition where RNA and/or protein synthesis was inhibited. Upon treating a previously characterized strain containing a *mazEF* deletion with compound 1, reduced killing of the *mazEF* mutant was observed compared to the wild type (data not shown). This may suggest a role for *mazEF*-mediated cell death upon treatment with compound 1.

30 [0083] The precise mode of action of compound 1 has not been determined and no compound 1 resistant mutants have been observed so far even after several passages at sub-MIC concentrations of compound 1. In Gram positive bacteria, resistance to 4-quinolones occurs mainly due to mutations in topoisomerase IV and gyrase; but this effect is reversible. Arjes *et al.*, showed that cells subjected to prolonged inhibition of

DNA synthesis do not show the FtsZ ring, and conversely, inhibiting cell division triggers an arrest in DNA replication. Without being bound by a particular mechanistic theory, it appears that compound 1 inhibits cell separation after the cross wall is laid down, thus preventing cell division and ushering in growth arrest of MRSA. Cells with growth arrest
5 do not replicate their DNA, thereby reducing the chance of mutations arising in the population. Nevertheless, it is not clear if all of these effects exert simultaneously to account for cell death or is a sequential process. It is also possible that the multiple effects of compound 1 reduce the emergence of resistant mutants in the population.

[0084] The term “composition” as used herein may also refer to a
10 pharmaceutical composition.

[0085] The term “pharmaceutical composition” is defined herein to refer to a mixture or solution containing at least one therapeutic agent to be administered to a subject, e.g., a mammal or human, in order to prevent or treat a particular disease or condition affecting the mammal or human.

15 [0086] The term “pharmaceutically acceptable” is defined herein to refer to those compounds, materials, compositions and/or dosage forms, which are, within the scope of sound medical judgment, suitable for contact with the tissues a subject, e.g., a mammal or human, without excessive toxicity, irritation allergic response and other problem complications commensurate with a reasonable benefit / risk ratio.

20 [0087] The term “treating” or “treatment” as used herein comprises a treatment relieving, reducing or alleviating at least one symptom in a subject or effecting a delay of progression of a disease. For example, treatment can be the diminishment of one or several symptoms of a disorder or complete eradication of a disorder, such as cancer. Within the meaning of the present invention, the term “treat” also denotes to
25 arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease) and/or reduce the risk of developing or worsening a disease. The term "prevent", "preventing" or "prevention" as used herein comprises the prevention of at least one symptom associated with or caused by the state, disease or disorder being prevented.

[0088] The term “therapeutically effective amount” of a compound or
30 combination of the invention is an amount of the compound, combination, or a constituent of a combination sufficient to provide an observable improvement over the baseline clinically observable signs and symptoms of the disorder treated with the combination.

[0089] The term “administering” or “administration” and the like, refers to providing the compound or combination of the invention to the subject in need of treatment.

[0090] As used herein, the term “alkyl” refers to a fully saturated branched or unbranched hydrocarbon moiety. The alkyl can comprise 1 to 20 carbon atoms, 1 to 16 carbon atoms, 1 to 10 carbon atoms, 1 to 7 carbon atoms, 1 to 6 carbons, 1 to 4 carbons, or 1 to 3 carbon atoms. In a particular embodiment, the alkyl comprises 1-6 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2- dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, n-decyl and the like. Furthermore, the expression “C_{x-y}-alkyl”, wherein x is 1-5 and y is 2-10 indicates a particular alkyl group (straight- or branched-chain) of a particular range of carbons. For example, the expression C₁₋₄alkyl includes, but is not limited to, methyl, ethyl, propyl, butyl, isopropyl, tert-butyl and isobutyl. In certain contexts, the term “alkyl” may be understood to represent a monovalent radical (e.g., methyl), a divalent radical (e.g., methylene), or a trivalent radical (e.g., methane).

[0091] As used herein, the term “alkenyl” refers to a straight-chain, cyclic or branched hydrocarbon residue comprising at least one carbon-carbon double bond (i.e., an olefinic bond) and the indicated number of carbon atoms. Alkenyl groups may have up to 8, up to 6, particularly up to 4, and more particularly up to 2 carbon atoms. Examples of alkenyl groups are ethenyl, 1-propenyl, 2-propenyl, isopropenyl, 1-butenyl, 2-butenyl, 3-butenyl, isobutenyl, 1-cyclohexenyl, 1-cyclopentenyl. In certain contexts, the term “alkenyl” may be understood to represent a monovalent radical, a divalent radical, or a trivalent radical.

[0092] As used herein, the term “alkynyl” refers to a straight-chain or branched hydrocarbon residue comprising at least one triple carbon-carbon bond and the indicated number of carbon atoms. Alkynyl groups may comprise 2 to 20 carbon atoms, 2 to 16 carbon atoms, 2 to 10 carbon atoms, 2 to 7 carbon atoms, 2 to 6 carbons, 2 to 4 carbons, or 2 to 3 carbon atoms. In a particular embodiment, the alkynyl group is a C₂₋₆alkynyl group. Non-limiting examples of alkynyl groups are ethynyl and propargyl. In certain contexts, the term “alkynyl” may be understood to represent a monovalent radical or a divalent radical.

[0093] As used herein, the term “aryl” refers to aromatic monocyclic or multicyclic, e.g., bicyclic and tricyclic, hydrocarbon ring systems, consisting only of

hydrogen and carbon and containing from 6-20 carbon atoms, or 6-10 carbon atoms, particularly 6 carbon atoms, where the ring systems may be partially saturated. Aryl groups include, but are not limited to, groups such as phenyl, tolyl, xylyl, anthryl, naphthyl and phenanthryl. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin). In certain contexts, the term “aryl” may be understood to represent a monovalent radical (*e.g.*, $-\text{C}_6\text{H}_5$), a divalent radical (*e.g.*, $-\text{C}_6\text{H}_4-$), or a trivalent, tetravalent, or pentavalent radical.

[0094] As used herein, the term “heteroaryl” refers to a stable monocyclic or bicyclic ring of up to 7 atoms in each ring, wherein at least one ring is aromatic and contains from 1 to 4 heteroatoms selected from the group consisting of O, N and S. Heteroaryl groups within the scope of this definition include but are not limited to: acridinyl, carbazolyl, cinnolinyl, quinoxalinyl, pyrazolyl, indolyl, benzotriazolyl, furanyl, thienyl, benzothienyl, benzofuranyl, quinolinyl, isoquinolinyl, oxazolyl, isoxazolyl, indolyl, pyrazinyl, pyridazinyl, pyridinyl, pyrimidinyl, pyrrolyl, tetrahydroquinoline. As with the definition of heterocycle below, “heteroaryl” is also understood to include the N-oxide derivative of any nitrogen-containing heteroaryl. In cases where the heteroaryl substituent is bicyclic and one ring is non-aromatic or contains no heteroatoms, it is understood that attachment is via the aromatic ring or via the heteroatom containing ring, respectively. In certain contexts, the term “heteroaryl” may be understood to represent a monovalent radical (*e.g.*, $-\text{C}_4\text{H}_3\text{O}$), a divalent radical (*e.g.*, $-\text{C}_4\text{H}_2\text{O}-$), or a higher order radical.

[0095] As used herein, the term “alkoxy” refers to an alkyl groups, having from 1 to 10 carbon atoms, attached to the remainder of the molecule *via* an oxygen atom. Alkoxy groups may have 1-8 carbon atoms, 1-6 carbon atoms, 1-4 carbon atoms, 1-2 carbon atoms, or 1 carbon atom. The alkyl portion of an alkoxy group may be linear, cyclic, or branched, or a combination thereof. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy, butoxy, cyclopentyloxy, and the like. An alkoxy group can also be represented by the following formula: $-\text{OR}^i$, where R^i is the “alkyl portion” of an alkoxy group.

[0096] The terms “halogen” and “halo” refer to a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as “fluoroalkyl”, are meant to include monofluoroalkyl and perfluoroalkyl. For example, the term “ C_{1-6} fluoroalkyl” is meant to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, and the like. Non

limiting examples of fluoroalkyl include $-\text{CF}_3$, and $-\text{CF}_2\text{CF}_3$. Similarly, terms such as “fluoroalkoxy”, are meant to include monofluoroalkoxy and perfluoroalkoxy.

[0097] As used herein, the term “cyano” refers to the $-\text{CN}$ moiety, wherein the carbon and nitrogen are connected by a triple bond. Molecules and molecular
5 fragments comprising a cyano group may be referred to as nitriles.

[0098] The alkyl, alkenyl, alkoxy, aryl and heteroaryl groups described above can be “unsubstituted” or “substituted.” The term “substituted” is intended to describe moieties having substituents replacing a hydrogen on one or more atoms, *e.g.* C, O or N, of a molecule. Such substituents can independently include, for example, one or more of
10 the following: straight or branched alkyl (preferably $\text{C}_1\text{-C}_5$), cycloalkyl (preferably $\text{C}_3\text{-C}_8$), alkoxy (preferably $\text{C}_1\text{-C}_6$), thioalkyl (preferably $\text{C}_1\text{-C}_6$), alkenyl (preferably $\text{C}_2\text{-C}_6$), alkynyl (preferably $\text{C}_2\text{-C}_6$), heterocyclic, carbocyclic, aryl (*e.g.*, phenyl), aryloxy (*e.g.*, phenoxy), aralkyl (*e.g.*, benzyl), aryloxyalkyl (*e.g.*, phenyloxyalkyl), arylacetamidoyl, alkylaryl, heteroaralkyl, alkylcarbonyl and arylcarbonyl or other such
15 acyl group, heteroarylcarbonyl, or heteroaryl group, $(\text{CR}'\text{R}'')_{0-3}\text{NR}'\text{R}''$ (*e.g.*, $-\text{NH}_2$), $(\text{CR}'\text{R}'')_{0-3}\text{CN}$ (*e.g.*, $-\text{CN}$), $-\text{NO}_2$, halogen (*e.g.*, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, or $-\text{I}$), $(\text{CR}'\text{R}'')_{0-3}\text{C}(\text{halogen})_3$ (*e.g.*, $-\text{CF}_3$), $(\text{CR}'\text{R}'')_{0-3}\text{CH}(\text{halogen})_2$, $(\text{CR}'\text{R}'')_{0-3}\text{CH}_2(\text{halogen})$, $(\text{CR}'\text{R}'')_{0-3}\text{CONR}'\text{R}''$, $(\text{CR}'\text{R}'')_{0-3}(\text{CNH})\text{NR}'\text{R}''$, $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{O})_{1-2}\text{NR}'\text{R}''$, $(\text{CR}'\text{R}'')_{0-3}\text{CHO}$, $(\text{CR}'\text{R}'')_{0-3}\text{O}(\text{CR}'\text{R}'')_{0-3}\text{H}$, $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{O})_{0-3}\text{R}'$ (*e.g.*, $-\text{SO}_3\text{H}$, $-\text{OSO}_3\text{H}$),
20 $(\text{CR}'\text{R}'')_{0-3}\text{O}(\text{CR}'\text{R}'')_{0-3}\text{H}$ (*e.g.*, $-\text{CH}_2\text{OCH}_3$ and $-\text{OCH}_3$), $(\text{CR}'\text{R}'')_{0-3}\text{S}(\text{CR}'\text{R}'')_{0-3}\text{H}$ (*e.g.*, $-\text{SH}$ and $-\text{SCH}_3$), $(\text{CR}'\text{R}'')_{0-3}\text{OH}$ (*e.g.*, $-\text{OH}$), $(\text{CR}'\text{R}'')_{0-3}\text{COR}'$, $(\text{CR}'\text{R}'')_{0-3}(\text{substituted or unsubstituted phenyl})$, $(\text{CR}'\text{R}'')_{0-3}(\text{C}_3\text{-C}_8 \text{ cycloalkyl})$, $(\text{CR}'\text{R}'')_{0-3}\text{CO}_2\text{R}'$ (*e.g.*, $-\text{CO}_2\text{H}$), or $(\text{CR}'\text{R}'')_{0-3}\text{OR}'$ group, or the side chain of any naturally occurring amino acid; wherein R' and R'' are each independently hydrogen, a
25 $\text{C}_1\text{-C}_5$ alkyl, $\text{C}_2\text{-C}_5$ alkenyl, $\text{C}_2\text{-C}_5$ alkynyl, or aryl group.

[0099] The present disclosure of chemical compounds should be construed in congruity with the laws and principals of chemical bonding. For example, it may be necessary to remove a hydrogen atom in order accommodate a substituent at any given location. Furthermore, it is to be understood that definitions of the variables (*i.e.*, “R
30 groups”), as well as the bond locations of the generic compounds of the invention will be consistent with the laws of chemical bonding known in the art. It is also to be understood that all of the compounds of the invention described above will further include bonds between adjacent atoms and/or hydrogens as required to satisfy the valence of each atom. That is, bonds and/or hydrogen atoms are added to provide the following number

of total bonds to each of the following types of atoms: carbon: four bonds; nitrogen: three bonds; oxygen: two bonds; and sulfur: two-six bonds.

[0100] The compounds of this invention may include asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (e.g., all enantiomers, stereoisomers, rotamers, tautomers, diastereomers, or racemates) are included within the scope of this invention. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis. Furthermore, the structures and other compounds and moieties discussed in this application also include all tautomers thereof. Compounds described herein may be obtained through art recognized synthesis strategies.

[0101] It will also be noted that the substituents of some of the compounds of this invention include isomeric cyclic structures. It is to be understood accordingly that constitutional isomers of particular substituents are included within the scope of this invention, unless indicated otherwise. For example, the term "tetrazole" includes tetrazole, 2*H*-tetrazole, 3*H*-tetrazole, 4*H*-tetrazole and 5*H*-tetrazole.

[0102] The salts of the compounds described herein include acid addition salts and base addition salts. In a one embodiment, the salt is a pharmaceutically acceptable salt of the compound of Formula I. The term "pharmaceutically acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically-acceptable. Suitable pharmaceutically acceptable acid addition salts of the compounds of the invention may be prepared from an inorganic acid or an organic acid. Examples of such inorganic acids include, without limitation, hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Examples of appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, arylaliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which include, without limitation, formic, acetic, propionic, succinic, glycolic, gluconic, maleic, embonic (pamoic), methanesulfonic, ethanesulfonic, 2-hydroxyethanesulfonic, pantothenic, benzenesulfonic, toluenesulfonic, sulfanilic, mesylic, cyclohexylaminosulfonic, stearic, algenic, β -hydroxybutyric, malonic, galactic, and galacturonic acid. Suitable pharmaceutically-acceptable base addition salts of compounds of the invention include, but are not limited to, metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from *N,N'*-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine,

ethylenediamine, N-methylglucamine, lysine and procaine. All of these salts may be prepared by conventional means from the corresponding compound of the invention by treating, for example, the compound of the invention with the appropriate acid or base.

[0103] The compositions described herein can be administered to a system
5 comprising cells or tissues, as well as to a human subject (*e.g.*, a patient) or an animal subject.

[0104] The compositions of the present invention can be administered in various dosage forms and strength, in a pharmaceutically effective amount or a clinically effective amount. In some embodiments, a composition described herein may be
10 referred to as a “combination.” In some embodiments, a composition described herein may be referred to as a “pharmaceutical composition.”

[0105] The pharmaceutical compositions for separate administration of both combination components, or for the administration in a fixed combination, *e.g.*, a single galenical composition comprising the combination, may be prepared in any manner
15 known in the art and are those suitable for enteral, such as oral or rectal, and parenteral administration to mammals (warm-blooded animals), including humans.

[0106] The pharmaceutical compositions described herein may contain, from about 0.1 % to about 99.9%, preferably from about 1 % to about 60 %, of the therapeutic agent(s). Suitable pharmaceutical compositions for the combination therapy for enteral or
20 parenteral administration are, for example, those in unit dosage forms, such as sugar-coated tablets, tablets, capsules or suppositories, or ampoules. If not indicated otherwise, these are prepared in a manner known *per se*, for example by means of various conventional mixing, comminution, direct compression, granulating, sugar-coating, dissolving, lyophilizing processes, or fabrication techniques readily apparent to those
25 skilled in the art. It will be appreciated that the unit content of a combination partner contained in an individual dose of each dosage form need not in itself constitute an effective amount since the necessary effective amount may be reached by administration of a plurality of dosage units.

[0107] The unit dosage forms of the present invention may optionally further
30 comprise additional conventional carriers or excipients used for pharmaceuticals. Examples of such carriers include, but are not limited to, disintegrants, binders, lubricants, glidants, stabilizers, and fillers, diluents, colorants, flavors and preservatives. One of ordinary skill in the art may select one or more of the aforementioned carriers with respect to the particular desired properties of the dosage

form by routine experimentation and without any undue burden. The amount of each carriers used may vary within ranges conventional in the art. The following references which are all hereby incorporated by reference disclose techniques and excipients used to formulate oral dosage forms. See *The Handbook of Pharmaceutical Excipients*, 4th edition, Rowe et al., Eds., American Pharmaceuticals Association (2003); and
5 *Remington: the Science and Practice of Pharmacy*, 20th edition, Gennaro, Ed., Lippincott Williams & Wilkins (2003).

[0108] As used herein, the term "pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media,
10 coatings, surfactants, antioxidants, preservatives (e.g., antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, and the like and combinations thereof, as would be known to those skilled in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack
15 Printing Company, 1990, pp. 1289- 1329). Except insofar as any conventional carrier is incompatible with the active ingredient, its use in the therapeutic or pharmaceutical compositions is contemplated.

[0109] The optimal dosage of each combination partner for treatment of a bacterial infection can be determined empirically for each individual using known
20 methods and will depend upon a variety of factors, including, though not limited to, the degree of advancement of the disease; the age, body weight, general health, gender and diet of the individual; the time and route of administration; and other medications the individual is taking. Optimal dosages may be established using routine testing and procedures that are well known in the art.

25 [0110] The amount of each combination partner that may be combined with the carrier materials to produce a single dosage form will vary depending upon the individual treated and the particular mode of administration. In some embodiments the unit dosage forms containing the combination of agents as described herein will contain the amounts of each agent of the combination that are typically administered when the
30 agents are administered alone.

[0111] The effective dosage of each of the combination partners employed in the combination of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, and the severity of the condition being treated. Thus, the dosage regimen of the

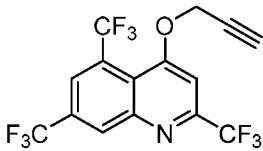
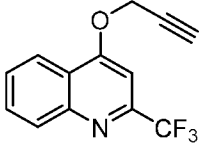
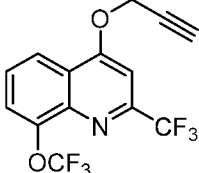
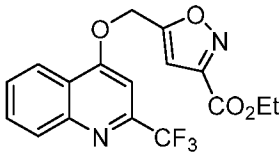
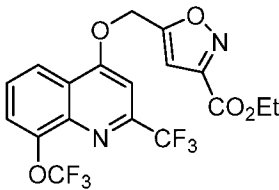
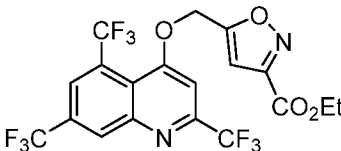
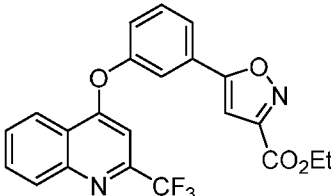
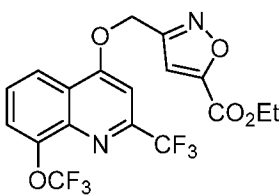
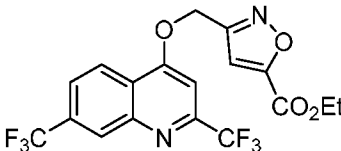
combinations described herein are selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient.

[0112] The effective dosage of each of the combination partners may require more frequent administration of one of the compound(s) as compared to the other
5 compound(s) in the combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of compounds, and one or more dosage forms that contain one of the combination of compounds, but not the other compound(s) of the combination.

Table 1(a): Effect of Aromatic Substitution on Inhibitory Activity

Compound	Structure	MIC (mg/L)	
		- oxacillin	+ oxacillin (16mg/L)
1		8	4
1a		>32	>32
1b		8	0.125
1c		2	0.5
1d		>64	>64
1e		16	16
1f		2	1
1g		>64	>64

Table 1(b): Effect of Modifications of the 4-Hydroxyl Group on Inhibitory Activity

Compound	Structure	MIC (mg/L)	
		- oxacillin	+ oxacillin (16mg/L)
2a		>128	>128
2b		>128	>128
2c		>128	>128
2d		>128	>128
2e		>128	>128
2f		>128	>128
2g		>128	>128
2h		>128	>128
2i		>128	>128

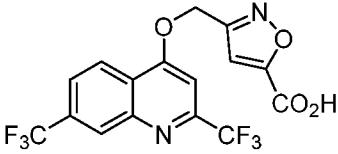
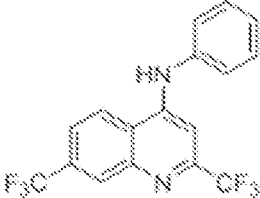
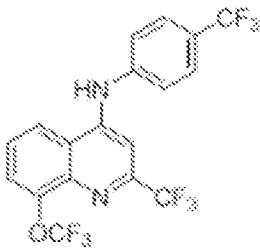
2j		2	2
3a		>128	2
3b		8	4

Table 2(a): MIC of different antibiotics at $\frac{1}{4} \times$ MIC of Compound 1 against USA300

Drug(A) (Target)	Compound 1 (4 mg/L)	MIC of Drug (A) (mg/L)	Interaction
Cephalothin (Cell wall)	-	16	Indifferent
	+	16	
Cefoxitin (Cell wall)	-	64	Synergistic
	+	16	
Cloxacillin (Cell wall)	-	32	Indifferent
	+	64	
Nafcillin (Cell wall)	-	2	Indifferent
	+	2	
Oxacillin (Cell wall)	-	128	Synergistic
	+	32	
Vancomycin (Cell wall)	-	2	Indifferent
	+	2	
Chloramphenicol (Protein Synthesis)	-	8	Indifferent
	+	8	
Daptomycin (pore formation/ cell membrane)	-	8	Indifferent
	+	8	
Levofloxacin (DNA syn)	-	0.5	Indifferent
	+	1	
Rifampicin (Transcription)	-	1	Indifferent
	+	1	

Table 2(b): MIC of different antibiotics at ¼ MIC of compound 1b against USA300

Drug(A) (Target)	Compound 1b (0.5mg/L)	MIC of Drug (A) (mg/L)	Interaction
Cephalothin (Cell wall)	-	16	Indifferent
	+	16	
Cefoxitin (Cell wall)	-	64	Synergistic
	+	16	
Cloxacillin (Cell wall)	-	32	Indifferent
	+	0.125	
Nafcillin (Cell wall)	-	2	Synergistic
	+	0.5	
Oxacillin (Cell wall)	-	128	Synergistic
	+	8	
Vancomycin (Cell wall)	-	2	Indifferent
	+	2	
Chloramphenicol (Protein Synthesis)	-	8	Indifferent
	+	4	
Daptomycin (pore formation/ cell membrane)	-	8	Indifferent
	+	2	
Levofloxacin (DNA syn)	-	1	Indifferent
	+	2	
Rifampicin (Transcription)	-	1	Antagonistic
	+	8	

Examples

[0113] Bacterial strains, strain construction and media: The strain used for compound screening was MRSA USA300, a community acquired MRSA strain. Also
 5 evaluated were clinical isolates of MRSA [Community-Acquired MRSA (20 strains), Hospital-Acquired MRSA (9 strains)] obtained from the Dartmouth Hitchcock Medical Centre, Lebanon NH for MIC studies (See FIG. 14). The bacterial cells were grown in Mueller Hinton Broth (Difco) supplemented with calcium sulphate (50mg/L) and magnesium sulphate (25mg/L) (MHC) for MIC studies and in Tryptone Soy broth (TSB)
 10 (Difco) for growth.

[0114] MRSA COL was used for assessing membrane integrity, permeability and cell wall integrity by fluorescence microscopy. Strains BCBPM073 and BCBPM162, expressing fusion of sfGFP-PBP2 and PBP4-yellow fluorescent protein, respectively,

were used to evaluate defects in the localization of PBP2 and PBP4, both enzymes involved in cell wall synthesis. For FtsZ localization, MRSA strain BCBAJ020, a COL derivative expressing FtsZ-CFP ectopically from the *spa* locus under the control of the *Pspac* promoter, was induced with 0.5mM IPTG. MRSA strain COLpPvra, expressing
5 sfGFP under the control of the promoter of the *vraRS* operon, was used to evaluate activation of the cell wall stress response.

[0115] MIC by broth Microdilution and Macrodilution: The MIC of compound 1 and compound 1b was determined with USA300 and other MRSA strains with and without sub-MIC oxacillin according to CLSI guidelines. The MIC is defined as
10 the lowest concentration of antibiotic inhibiting visible bacterial growth.

Example 1: Determination of time kill curves using of compound 1 and compound 1b

[0116] USA300 cells were grown to the exponential phase ($OD_{620}=0.5$ using 18 mm borosilicate glass tube in a Spectronic 20 spectrophotometer) in MHB and back diluted 100-fold. Samples were treated with DMSO as control, 4-32 mg/L of compound 1
15 (diluted in DMSO) or 1-8 mg/L of compound 1b. At specific time points (0, 2, 4, 6 and 24 hr), cells were washed free of compounds, dilutions were made and cells plated on regular TSB agar with no antibiotic to enumerate the CFU and further calculate the CFU/mL. Each data point represents mean and standard deviation from three replicate experiments. Two way analysis of variance test (ANOVA) using Bonferroni's multiple
20 comparisons test in GraphPad Prism was applied to test significance, with a P value of <0.05 considered significant. Bactericidal activity is calculated by comparing the final CFU/ml of a particular time point to the initial CFU/ml at time zero.

Example 2: Chequerboard analysis to test synergy of compound 1 and compound 1b with other antibiotics against MRSA

25 [0117] In order to determine the lowest effective concentration of oxacillin and other antibiotics when combined with compound 1, a chequerboard method was used as described previously (see Nair DR, Monteiro JM, Memmi G, et al. Characterization of a novel small molecule that potentiates beta-lactam activity against gram-positive and gram-negative pathogens. *Antimicrob Agents Chemother* 2015; **59**: 1876-85). Briefly,
30 two-fold dilutions of compound 1 or compound 1b starting at the highest concentration of 32 mg/L (4X MIC) were made in columns of 96 well plates while two-fold dilutions of oxacillin or other antibiotic compounds were made in rows to obtain different combinations of drug concentrations. MRSA USA300 was then added to microtiter wells

at a concentration of 1×10^5 CFU/ml, incubated at 37 °C for 24 hours without shaking, and the MIC defined according to the CLSI protocol. FIC_{AB} was defined as the FIC (Fractional Inhibitory Concentration) of drug A in the presence of drug B; FIC_{BA} was defined as the FIC of drug B in the presence of drug A, with FIC index (FICI) defined as FIC_{AB} / FIC_{BA} . Synergy was defined as a FICI of less than or equal to 0.5, an index between 0.5 and 4 suggests indifference (no interaction) and an index of greater than 4 is antagonistic.

Example 3: Assessing the toxicity of compound 1

[0118] Four methods were used to assess cellular toxicity. First, *C. albicans* cells were exposed to 20X MIC of compound 1 (as determined in USA300) to assess direct toxicity on eukaryotic cells based on a loss of viability. Second, we exposed 4% sheep red blood cells to 4X MIC of compound 1 and monitored for lysis at OD540 in an in vitro hemolysis assay to assess the generalized membrane perturbation properties of compound 1. Third, release of lactate dehydrogenase from human bronchial epithelial cells (CFBE) exposed to compound 1 was monitored. In brief, CFBE cells were maintained in RPMI 1640 medium (Sigma, St Louis MO) with 10% fetal bovine serum and grown with 5% CO₂ in an incubator at 37 °C. When confluent, cells in the flask were released with trypsin, collected and counted. Compound 1 at pre-determined concentrations (16X, 32X, 64X MIC) in serum free media was added to epithelial cells and incubated for 24 hrs at 37 °C. Triton X-100 at 2% final concentration was used as a positive control. On the following day, 100 µl of the cytotoxicity reagents (Promega, CytoTox 96[®] NonRadioactive Cytotoxicity Assay kit), prepared according to the manufacturer's protocol, was added to each of the wells containing CFBE supernatant in rapid succession. The assay plates were then incubated at room temperature in the dark for twenty minutes and absorbance read at 490nm using a plate reader. Fourth, inhibition of the enzyme cytochrome P450 (CYP) by compound 1 in human liver microsomes was carried out by SRI Biosciences under the auspices of NIH Product Development Services. In brief, compound 1 was incubated with a cocktail of model CYP substrates specific for different CYP isoforms (phenacetin for 1A2, bupropion for 2B6, diflofenac for 2C9, mepehenytoin for 2C19, bufuralol for 2D6, testosterone and midazolam for 3A4), human liver microsomes and cofactors for 20 minutes at 37 °C. Specific control inhibitors for different CYP isoforms (furafylline, thioTEPA, sulfaphenazole, nootkatone, quinidine, ketoconazole) were also included. Formation of metabolites was measured by LC-MS/MS and compared to control incubations with no compound 1 or inhibitor. A

decrease in metabolite formation in the presence of compound 1 indicated that the activity of the CYP isoform was inhibited under the condition used.

Example 4: Pharmacokinetics of compound 1

[0119] The in vitro metabolic stability of compound 1 was determined in human, rat, dog and mouse microsomes by SRI with NIH sponsor as delineated above. Briefly, compound 1 was incubated with active and heat inactivated human liver microsomes and co-factors at 37 °C. Aliquots were removed at 0, 15, 30, 60, 90 and 120 minutes and the amount of remaining compound 1 was determined using LC-MS/MS. The result was calculated as the percent of compound 1 remaining at a given time vs. t=0 min. The in vitro half-life was calculated as $t^{1/2} = 0.693/-k$, where k is the slope of the linear regression of the natural log of the percent remaining vs. time. Intrinsic clearance (CL_{int} , $\mu\text{l}/\text{min}/\text{mg}$) was calculated using the formula: $CL_{int} = (0.693/t^{1/2})/\text{protein concentration in incubation}$.

[0120] Bidirectional permeability of compound 1 into Caco-2 cells was determined as follows: A CacoReady™ plate (consisting of Caco-2 cells already plated on a HTS Transwell® 24-well plate) was purchased from ADMTEcell Inc. (Emeryville, CA). On the day of the experiment, compound 1 was prepared in transport buffer (pHs 6.0 and 7.4) and added to the appropriate compartment (apical or basal) of the Transwell plate. At selected time points (0.5, 1, 1.5 and 2 hours), aliquots were removed from the receiving compartment and analyzed by LC-MS/MS to determine the apparent permeability (P_{app} , $\times 10^{-6}$ cm/sec). Compound 1 was also incubated in the absence and presence of ketoconazole, a known inhibitor of the transporter protein P-gp. Control incubations consisting of ganciclovir (not permeable), diazepam (permeable), and 3H-digoxin, a known substrate of P-gp, in the absence and presence of ketoconazole were also included and analyzed by liquid scintillation counting. The Efflux Ratio (ER) was calculated using the determined P_{app} ($ER = P_{app, B-A}/P_{app, A-B}$). An $ER > 2$ in the absence of inhibitor indicates that the compound 1 is likely to be a substrate for P-gp. An $ER < 2$ or that is significantly reduced in the presence of inhibitor further confirms that the compound 1 is a substrate for P-gp. The % recovery of the test article was also determined. This was calculated by comparing the amount of compound 1 recovered during and at the end of the experiment with the amount of compound 1 added to initiate the experiment. Plasma protein binding to compound 1 was determined by equilibrium dialysis for 4 hrs at 37 °C using pooled human, Sprague Dawley rat, Beagle dog and BALB/C mouse plasma proteins.

Example 5: Enhanced efficacy of microbicidal activity of compound 1b compared to compound 1 on MRSA strain USA300

[0121] Both compound 1b and compound 1 show similar anti-MRSA activity on their own. However, compound 1b exhibited better combinatorial activity with oxacillin compared to compound 1. We compared the bactericidal activity of compound 1b with compound 1 in time kill experiments against actively growing USA300 cells. Compound 1 at 2X MIC (16 mg/L) showed a slow, steady and concentration-dependent bactericidal activity (Figure 1), resulting in a ~94% reduction of the initial bacterial inoculum within 24 hours. Compound 1b at 1X MIC (8 mg/L) showed a > 99.99% reduction of the initial bacterial load of USA300 by 24 hrs (Figure 2). Thus the analogue compound 1b exhibits the same MIC as compound 1 against MRSA USA300, but is more efficient than compound 1 in time-kill experiments with almost complete bactericidal activity.

Example 6: Compound 1 and compound 1b are synergistic with cell-wall active antibiotics against MRSA, but are indifferent or antagonistic with other classes of drugs

[0122] Compound 1 was shown to be more potent against USA300 in the presence of sub-MIC oxacillin (Table 1). Specifically, compound 1b was shown to be ten-fold more potent in combination with sub-MIC oxacillin. To examine if compound 1 or compound 1b can potentiate the activity of other classes of antibiotics, we investigated the effect of compound 1 and compound 1b on MRSA USA300 with other commonly used antibiotics including cell wall active agents (oxacillin, nafcillin, cloxacillin, cephalothin, cefoxitin, and vancomycin), an inhibitor of protein synthesis (chloramphenicol), a cell membrane active agent (daptomycin), a DNA gyrase/synthesis inhibitor (levofloxacin) and a transcriptional inhibitor (rifampicin). Using 1/4X MIC of compound 1 or compound 1b in chequerboard assays, [Tables 2(a) and 2(b)], we found that the MIC of oxacillin against USA300 dropped from 128 to 32 mg/L with compound 1 at 1/4X MIC, yielding a 4-fold potentiation in the activity of oxacillin. Compound 1 was also synergistic with cefoxitin. Replacing compound 1 with compound 1b led to a 16-fold reduction in the MIC of oxacillin from 128 to 8 µg/ml. This translated to a FICI of 0.5, indicating that the two drugs were synergistic. Compound 1b was also synergistic with cefoxitin and nafcillin. Compound 1 and compound 1b were additive at best with other cell wall active agents with a FICI slightly greater than 0.5. Combining levofloxacin or rifampicin with 1/4X MIC compound 1 yielded a FICI of 2.25 and 1.5, respectively,

indicating an indifferent relationship between these drugs. Similarly, compound 1b at ¼X MIC showed a FICI of 8 against rifampicin, indicating antagonism but was indifferent to levofloxacin.

Example 7: Macromolecular analysis

5 [0123] The effect of compound 1 on macromolecular synthesis was studied by monitoring the incorporation of ³H- or ¹⁴C-labeled precursors (5-[³H] thymidine, [³H] glucosamine hydrochloride, [³H] uridine and l-[¹⁴C] isoleucine) as described previously (see Schneider T, Kruse T, Wimmer R, et al. Plectasin, a fungal defensin, targets the bacterial cell wall precursor Lipid II. *Science* 2010; **328**: 1168-72). An overnight culture of *Staphylococcus simulans* 22 grown in MHC was diluted 50-fold into fresh medium and
10 cultured at 37 °C to an OD₆₀₀ of about 0.5. Cultures were then split into aliquots, diluted to an OD₆₀₀ of 0.1 and allowed to regrow to an OD₆₀₀ of 0.4. Subsequently, the respective labelled precursor was added to each culture (final concentration 1 µCi/ml); compound was added at 0.5X, 1X or 2X MIC, while another aliquot was run with 10X MIC of a
15 control antibiotic and one without any antibiotic. Control antibiotics were vancomycin (3.1 mg/L) to inhibit cell wall synthesis, tetracycline (0.4 mg/L) to inhibit protein synthesis, ciprofloxacin (0.3mg/L) to inhibit DNA synthesis and rifampicin (0.01µg/ml) to inhibit RNA synthesis. Incorporation of labelled precursors was monitored for up to 60 min. Macromolecules were precipitated with ice-cold TCA (10%) and incubated for at
20 least 30 min on ice before being filtered through glass microfiber filters (Whatman). Filters were washed with 5 ml of TCA (2.5%) containing 10 mM unlabeled metabolite, dried, counted and the data expressed as a percent inhibition of incorporation in comparison with a drug-free control.

Example 8: Transmission electron microscopy (TEM)

25 [0124] USA300 cells grown in MHC at 37 °C were treated with either compound 1 (2X MIC) or DMSO for 90 minutes. The cells were washed twice with phosphate buffered saline (PBS) and processed for EM by fixing with 10X volume of 2% glutaraldehyde-tannic acid (GTA)/1% paraformaldehyde in 0.1M Na Cacodylate buffer (pH 7.4) . Cells were further post-fixed in 1% OsO₄ in sodium cacodylate buffer pH 7.4,
30 embedded and serially dehydrated in ethanol. Samples were sectioned and stained with uranyl acetate and imaged using a JEOL TEM 1010 microscope at 100kV at 1200X or 15,000X magnification. Thirty fields of each strain with nearly equatorial cut surfaces were measured for cell wall thickness, and the results were expressed as a box and

whiskers plot. Statistical significance was determined using the unpaired two tailed t-test, with a *P* value of <0.05 considered significant.

Example 9: Fluorescence Microscopy

[0125] For fluorescence microscopy, strains were incubated overnight in TSB at 37 °C, supplemented with either erythromycin (10 µg/ml) or kanamycin (200µg/ml) as needed, back-diluted in fresh TSB and allowed to grow until mid-exponential phase (OD₆₀₀ ~ 0.6). Each culture was then divided into five flasks with compound 1 (in DMSO) added to three at 0.5X, 1X or 2X MIC and the two remaining flasks kept as controls with DMSO or TSB alone. Cultures were incubated for 30 minutes, after which the cells were pelleted, washed in PBS buffer and mounted on microscope slides with pads of 1% agarose in PBS. To analyze activation of the cell wall stress stimulon by compound 1, vancomycin at 1X MIC (3 mg/L) was used as a positive control. Displayed values of fluorescence were adjusted for each image for visualization purposes.

[0126] For staining, cells were incubated with FM 4-64 (2 mg/L), BODIPY FL vancomycin (2 mg/L) and Hoechst 33342 (4 mg/L) (all from Molecular Probes) for 5 min at room temperature with shaking and washed before being imaged. Cells were imaged using a Zeiss Axio Observer microscope equipped with a Photometrics CoolSNAP HQ2 camera (Roper Scientific) and Metamorph 7.5 software (Molecular Devices) or by Structured Illumination Microscopy (SIM) or laser widefield microscopy in an ELYRA PS.1 Microscope (Zeiss) with a sCMOS camera and 5 grating rotations for each channel. SIM images were reconstructed and analyzed with Zen Software (Zeiss). For quantification of the signal of fluorescent derivatives of PBP at the septa, five images were analyzed per condition using ImageJ. Only cells with a complete septum were analyzed. In order to assess *vraRS* activation, five images were analyzed per condition using ImageJ.

Example 10: Assay for membrane potential

[0127] The carbocyanine dye DiOC₂ (3,3'-Diethyloxacarbocyanine iodide, Life Technologies) was used as described previously (see Nair DR, Monteiro JM, Memmi G, et al. Characterization of a novel small molecule that potentiates beta-lactam activity against gram-positive and gram-negative pathogens. *Antimicrob Agents Chemother* 2015; 59: 1876-85) to assess membrane potential. *S. aureus* USA300 cultures were grown to the early exponential phase (OD₆₂₀=0.3) in MHB, incubated with 10 µM of DiOC₂ in PBS with 1% glucose at 24°C for 30 minutes in the dark and then transferred to a 384 well

plate. Cells were analyzed at Ex_{485nm}/Em_{680nm} using the Tecan M1000 plate reader. After establishing a baseline reading, CCCP (positive control) or compound 1 was added to the wells and the drop in red fluorescence (Em_{680nm}) was monitored over time. Significance was determined using the Kruskal Wallis test with Dunn's multiple comparisons in

5 GraphPad Prism.

Example 11: MIC of compound 1

[0128] Compound 1 (8-(trifluoromethoxy)-2-(trifluoromethyl)quinolin-4-ol) shows an MIC of 8 mg/L against MRSA USA300. The MIC of oxacillin against USA300 shows a 4-fold reduction from 128 mg/L to 32 mg/L in the presence of 4 mg/L of
10 compound 1. The MIC of compound 1 against a variety of CA-MRSA (20 strains) and HA-MRSA (8 strains) was in the range between 4-8 mgs/L and showed a 2-8 fold reduction in the presence of sub-MIC (16 mg/L) oxacillin (See Figure 14).

Example 12: Assessing toxicity of compound 1

[0129] We assessed the toxicity of compound 1 towards eukaryotic cells in
15 four different ways: (1) viability of *Candida albicans* at 20X MIC (based on data with USA300); (2) LDH release from exposed cultured human bronchial epithelial (CFBE) cells upon treatment with compound 1; (3) measurement of lysis of 4% sheep erythrocytes with compound 1 at varying concentrations (2X to 4X MIC against USA300) and (4) evaluating the *in vitro* inhibition of CYP using human liver
20 microsomes. Compound 1 was found to be non-toxic against *Candida albicans*; it also did not cause increased LDH release nor did it lyse RBCs when compared to the appropriate positive control such as SDS. Compound 1 did not affect a majority of the CYP isoforms tested with the exception of isoform 1A2, which showed a ~ 20% reduction in activity at 1 µM and 62% at 10 µM of compound 1.

25 **Example 13: Pharmacokinetics of compound 1**

[0130] The *in vitro* metabolic stability of compound 1 was determined using human, rat, dog and mouse liver microsomes. Compound 1 was not significantly metabolized by human, rat and dog liver microsomes (~90% remaining after 30 min) while the control compound midazolam did (1.4% remaining after 30 min). Even at 90
30 min, ~70-80% of compound 1 remained in the three microsomes above. This contrasts with mouse liver microsome where the metabolism was faster, with 50% remaining at 30 min and 17.4% at 90 min. The *in vitro* half-life (t_{1/2}) for 1 µM compound 1 was estimated to be 630 minutes in human microsomes, 495 min in rat microsomes and 165 min in dog

microsomes whereas the half-life was only 39 min in mouse liver microsomes. The intrinsic clearance (CL_{int}) of 1 μ M of compound 1 was 2.2, 2.8, 8.4 and 35.4 μ l/min/mg, respectively, for human, rat, dog and mouse liver microsomes; these data are consistent with a reasonable level of stability for compound 1 in human, rat and dog liver
5 microsomes, but compound 1 is rapidly cleared in mouse liver microsomes, with a half-life of 39 min and an intrinsic clearance of 35.4 μ l/min/mg. At both 1 and 10 μ M, compound 1 was permeable in the Caco-2 cells in both directions, with mean recoveries ranging from 77.7 to 93.4% in the apical-basal direction and 92.8 to 98.1% in the basal-apical direction, indicating that the compound was stable and non-specific binding was
10 not an issue in the conditions used for the experiment. The presence of the P-gp inhibitor ketoconazole did not have any impact on the permeability or efflux ratio, further confirming that compound 1 is not a substrate of P-gp. At 1 μ M concentration, compound 1 was ~89% bound to human, rat, dog and mouse plasma proteins at equilibrium.

15 ***Example 14: Effect of compound 1 on macromolecular synthesis in *S. simulans****

[0131] From our checkerboard data, we rationalized that compound 1 may affect both cell wall and DNA synthesis as well as having an effect on transcription. To verify these effects, macromolecular synthesis inhibition assays were performed with compound 1 and control antibiotics (ciprofloxacin, rifampicin, vancomycin and
20 tetracycline) as inhibitors of [3 H]thymidine, [3 H]uridine, [3 H]-glucosamine and [14 C]isoleucine uptake into DNA, RNA, cell wall and protein, respectively. In samples taken at 5, 15, 30, 45 and 60 minutes, compound 1 showed an interesting pattern of macromolecular inhibition. As shown in Figure 3, when cells were treated with increasing concentrations of compound 1 (0.5X, 1X and 2X MIC) for 30 minutes, a concentration-
25 dependent effect on DNA and cell wall synthesis was observed. In examining the effect of compound 1 on [3 H]-glucosamine incorporation over a period of an hour as seen in Figure 5, we observed that within 15 minutes of exposure to 0.5-1X MIC of compound 1, there was a 40% reduction in [3 H]-glucosamine incorporation into nascent cell wall as compared to untreated control. By 1 hour, the reduction in incorporation approached
30 ~95%. This level of inhibition was comparable to that of the positive control vancomycin. Exposure of cells to 1X MIC compound 1 also led to a 35% reduction in DNA synthesis within 15 minutes and 81% reduction, similar to the ciprofloxacin control, at the one-hour timepoint as seen in Figure 4. Transcription was inhibited by compound 1 to 36% of that of the control at 1 hour timepoint, however the reduction of [3 H]uridine incorporation into

RNA, in contrast to that of cell-wall and DNA precursors, was not concentration dependent. Thus, compound 1 appears to have specific effects on DNA and cell wall synthesis and an off target effect on transcription.

Example 15: Effect of compound 1 on the cellular morphology of MRSA strain

5 USA300 with TEM

[0132] To further assess the effect of compound 1 on cell wall morphology, we treated USA300 cells with 2X MIC compound 1 for 90 minutes, after which they were fixed, dehydrated and observed them under TEM. Evaluation of ~300 cells (Figures 6 and 7) from both untreated and compound 1 treated cells showed that half of the compound 1
10 treated cells (~55 %) had cross wall/septum formation with reduced or undetectable splitting compared to just 20% of untreated control cells. Measurements of the cell wall thickness of both untreated and treated cells, evaluated in up to 30 different fields, showed significantly thicker cell walls (30-42nm) in treated cells compared to untreated control (25-30nm) (Figure 8). Furthermore, treated cells displayed mesosome-like
15 membrane inclusions predominantly at the cross wall site in dividing cells but along the cell wall in non-dividing cells as seen in Figure 9. Additionally, in rare cases, branching and multiple cross walls without obvious cell separation and cell walls debris were observed in the sample treated with compound 1.

20 Example 16: compound 1 causes activation of cell wall stress stimulon (CWSS) and mislocalization of PBP2 without affecting PBP4 and FtsZ

[0133] Cell wall active antibiotics are known to activate the cell wall stress stimulon.¹⁴ Using a previously constructed COL strain (see Nair DR, Monteiro JM, Memmi G, et al. Characterization of a novel small molecule that potentiates beta-lactam activity against gram-positive and gram-negative pathogens. *Antimicrob Agents*
25 *Chemother* 2015; **59**: 1876-85) with the gene encoding a fast folding variant of GFP – sfGFP - fused to the promoter of the *vraSR* operon in its native chromosomal locus, we treated cells with compound 1 at 1X MIC for 30 minutes followed by fluorescence imaging. The cells exhibited a moderate activation of *vraSR* following treatment with compound 1, with the fluorescence signal doubling from 4521 ± 781 a.u., $n = 377$ in
30 untreated cells to 8246 ± 1178 a.u., $n = 406$ in exposed cells. However, activation of *vraSR* by the bona-fide cell wall active compound vancomycin was considerably stronger (29072 ± 7573 a.u., $n = 379$) (Fig. 10).

[0134] To elucidate the target of compound 1 in the cell wall synthesis pathway, we examined the localization of PBP2 and PBP4 with and without compound 1 (2X MIC) for 30 min (Figure 11, left panels). In untreated cells, PBP2 was localized at the septum in approximately one quarter of the population (23% of the cells, n = 593).

5 This number decreased three-fold to only 7% of the cells in the presence of compound 1 (n = 524). The localization of PBP4 remained unchanged with and without compound 1.

[0135] FtsZ is known to play a role in the localization of PBP2 to the septum in dividing cells. To determine if compound 1 interferes with FtsZ and hence PBP2 localization, we exposed a *S. aureus* strain containing CFP tagged with FtsZ to 0.5X- 2X
10 MIC of compound 1 for 15 minutes (Figure 5, right panels). In this case, localization of FtsZ was not severely affected by compound 1, with septal localization in 57% of the cells following compound 1 treatment (n= 477) and in 61% of the cells (n = 484) without treatment (Figure 11). The FtsZ division ring also appears to be normal in these cells. Thus the mislocalization of PBP2 is not mediated by FtsZ.

15 **Example 17: compound 1 has a concentration independent effect on the membrane of MRSA**

[0136] The mesosome-like formations near the cross wall of the cell seen in Figure 9 of the EM study prompted us to further examine the effect of compound 1 on the membrane. We did this in three different ways: (1) super resolution fluorescent
20 microscopy of unfixed MRSA COL cells stained with membrane dye FM 4-64; (2) ascertaining change in membrane potential with the dye DiOC₂ and (3) measuring ATP and potassium leakage upon treatment with compound 1. To examine membrane integrity, we incubated MRSA COL with 1X compound 1 for 30 min followed by staining with FM 4-64. To help localize the membrane, we also stained the cell wall with
25 bodipy-vancomycin to visualize the cell wall. As seen in Figure 12, membrane defects such as bulges in the membrane can be seen in compound 1 treated cells vs. untreated control. There was also a loss of membrane potential associated with treatment of USA300 with 2X–8X MIC of compound 1 (8-32 mg/L) as evidenced by a concentration independent loss in red fluorescence seen in Figure 13. This decrease in membrane
30 potential was significantly lower than our positive control, CCCP, a known proton translocator that shows a concentration dependent effect. Remarkably, treatment of USA300 with compound 1 did not result in either ATP or potassium leakage. Together these data suggest an off target effect of compound 1 on the cell membrane in MRSA.

Compound Synthesis and Characterization

[0137] Purchased solvent and reagents were used without purification unless indicated. 1,4-dioxane was refluxed over calcium hydride and then distilled in nitrogen atmosphere. Reactions were monitored by thin-layer chromatography (TLC) using commercial glass-backed silica gel plate (Silicycle TLG 60 F254). TLC spots were viewed under 254 nm and 280 nm UV lamp and stained by using cerium ammonia molybdate solution. Product purification was performed by automated flash chromatography on Biotage Isolara One using SNAP Ultra cartridges. ¹H NMR and ¹³C NMR spectra were obtained on Bruker Advance500 or 600 spectrometer. Chemical shifts for ¹H NMR are reported in the sequence of chemical shifts (ppm), integration, multiplicity and coupling constants (Hz). Abbreviations are used for the multiplicities: s: singlet, d: doublet, dd: doublet of doublet, t: triplet, q: quadruplet, m: multiplet, br s: broad singlet. High-resolution mass spectra results were obtained from Mass Spectrometry Laboratory of University of Illinois, Urbana Champaign.

Synthesis of compounds 1a-g

[0138] The 4-hydroxyquinoline analogues were prepared by the method reported by Kozikowski (Lilienkampff, A.; Mao, J.; Kozikowski, A., et al. *J. Med. Chem.* **2009**, *52*, 2109), with the exception of modifications in the use of catalysts to improve the yield.

[0139] **General Procedure A:** To a pressure tube containing ethyl 4,4,4-trifluoroacetoacetate (1.2 eq) and Eaton's reagent [7.5% wt. phosphorous pentoxide *p*-toluenesulfonic acid solution (~1 mL/0.3 mmol)], was added the corresponding aniline (1 eq). Then the pressure vessel was sealed and heated to 130 °C, and the reaction mixture was stirred overnight. After cooling to 0 °C, the reaction was poured into ice water (~5 mL/1 mmol) and the solution pH was adjusted to ~ 5 by the addition of saturated aqueous potassium carbonate. In situations when a pale yellow precipitate was formed, the solution was filtered and the residue was washed with water and cold ethanol, then dried in vacuo to yield the crude 4-hydroxyquinoline. Alternatively, the solution could be extracted with CHCl₃ (20 mL x 3) and dried over anhydrous Na₂SO₄. After filtration, the solvent was removed in vacuo to give the crude 4-hydroxyquinoline. Then the residue was purified by column chromatography using hexane–EtOAc as an eluent (30% of EtOAc) to give the desired products with yields ranging from 45% to 60%. Characterization data for compound 1 and 1a–e have been reported by Kozikowski.

[0140] 8-trifluoromethoxy-2-trifluoromethyl-4-quinolinol (**compound 1**):

Yield 51%. ¹H NMR (DMSO-d₆) δ 7.19 (1H, s), 7.74 (1H, apparent t, J = 8.4 Hz), 7.88 (1H, d, J = 7.2 Hz), 8.28 (1H, d, J = 8.4 Hz), 9.71 (1H, br s). ¹³C NMR (DMSO-d₆) δ 101.5 (q, J = 2.4 Hz), 119.9, 120.8 (q, J = 258 Hz), 122.4 (q, J = 273 Hz), 123.5, 123.7, 127.3, 128.8, 141.8 (q, 1.8 Hz), 144.5 (q, J = 36 Hz), 163.9. UV_{max}: 294 nm, 303 nm, 315 nm.

[0141] 2-trifluoromethyl-4-quinolinol (**1a**): Yield 48%. ¹H NMR (DMSO-d₆) δ 7.11 (1H, s), 7.76 (1H, apparent t, J = 7.8 Hz), 7.88 (1H, apparent t, J = 7.8 Hz), 7.91 (1H, d, J = 8.4 Hz), 8.10 (1H, d, J = 8.4 Hz) 9.51 (1H, br s). ¹³C NMR (DMSO-d₆) δ 100.8 (q, J = 1.8 Hz), 119.5, 120.5, 122.1 (q, J = 276 Hz), 123.7, 127.3, 129.8, 141.7, 144.5 (q, J = 36 Hz), 162.4.

[0142] 2,5,7-tris(trifluoromethyl)-4-quinolinol (**1b**): Yield 45%. ¹H NMR (DMSO-d₆) δ 7.10 (1H, s), 8.20 (1H, s), 8.65 (1H, s), 13.65 (1H, br s); ¹³C NMR (DMSO-d₆) δ 106.0 (q, J = 1.8 Hz), 117.6 (q, J = 36 Hz), 118.2 (q, J = 3.0 Hz), 122.2 (q, J = 276 Hz), 122.5 (q, J = 273 Hz), 123.4 (q, J = 276 Hz), 125.0 (q, J = 36 Hz), 127.1 (m), 129.9 (q, J = 36 Hz), 141.0, 144.7 (q, J = 36 Hz), 150.9.

[0143] 2,7-bis(trifluoromethyl)-4-quinolinol (**1c**): Yield 55%. ¹H NMR (DMSO-d₆) δ 7.21 (1H, s), 7.94 (1H, d, J = 7.8 Hz), 8.37 (1H, s), 8.42 (1H, d, J = 9.0 Hz). ¹³C NMR (DMSO-d₆) δ 105.5 (q, J = 2.4 Hz), 121.2, 123.2 (q, J = 273 Hz), 123.5, 123.7 (q, J = 270 Hz), 124.0 (q, J = 3 Hz), 127.8 (q, J = 4.2 Hz), 133.6 (q, J = 33 Hz), 147.6, 157.0 (q, J = 36 Hz), 161.9.

[0144] 8-methyl-2-trifluoromethyl-4-quinolinol (**1d**): Yield 45% ¹H NMR (DMSO-d₆) δ 2.63 (3H, s), 7.16 (1H, s), 7.49 (1H, apparent t, J = 8.4 Hz), 7.62 (1H, d, J = 7.2 Hz), 8.08 (1H, d, J = 8.4 Hz); ¹³C NMR (DMSO-d₆) δ 17.4, 100.3 (q, J = 2.4 Hz), 120.4, 121.2 (q, J = 276 Hz), 125.5, 127.4, 130.9, 131.6, 132.4, 135.3 (q, J = 36 Hz), 159.8.

[0145] 2,6-bis(trifluoromethyl)-4-quinolinol (**1e**): Yield 51%. ¹H NMR (DMSO-d₆) δ 7.19 (1H, s), 7.90 (1H, s), 8.08 (1H, d, J = 8.4 Hz), 8.17 (1H, s), 8.25 (1H, d, J = 7.2 Hz). ¹³C NMR (DMSO-d₆) δ 105.8 (q, J = 2.4 Hz), 107.5, 113.2, 120.7 (q, J = 3.6 Hz), 122.4 (q, J = 270 Hz), 122.5, 124.2 (q, J = 276 Hz), 126.3 (q, J = 3 Hz), 130.7 (q, J = 30 Hz), 131.4, 150.9 (q, J = 36 Hz).

[0146] 8-trifluoromethoxy-2-trifluoromethyl-6-ethynyl-4-quinolinol (**1f**): Yield 60%. ¹H NMR (DMSO-d₆) δ 8.64 (1H, s), 8.34 (1H, s), 8.15 (1H, s), 4.81 (1H, s). ¹³C NMR (DMSO-d₆) δ 81.6, 86.5, 120.0 (q, J = 1.8 Hz), 121.4 (q, J = 258 Hz), 123.8

(q, $J = 276$ Hz), 126.6, 130.1, 136.6, 140.5, 144.9, 147.7, 147.9 (q, $J = 1.5$ Hz), 148.2 (q, $J = 36$ Hz), 148.4. HRMS (ESI): calcd. for $C_{13}H_5F_6NO_2$ $[M+H]^+$ 322.0303, found 322.0301.

[0147] 7-trifluoromethyl-2-chloromethylene-4-quinolinol (**1g**): Yield 55%. 1H NMR ($CDCl_3$) δ 4.81 (2H, s), 6.33 (1H, s), 7.42 (1H, s), 7.78 (1H, d, $J = 9.6$ Hz), 8.10 (1H, d, $J = 8.4$ Hz). ^{13}C NMR ($DMSO-d_6$) δ 42.5, 48.0, 105.0, 110.7, 122.1 (q, $J = 4.2$ Hz), 122.4 (q, $J = 270$ Hz), 123.5, 124.6, 125.4 (q, $J = 2.4$ Hz), 127.2 (q, $J = 36$ Hz), 133.6, 137.7, 149.4, 176.8. HRMS (ESI): calcd. for $C_{11}H_8ClF_3NO_2$ $[M+H]^+$ 278.0196, found 278.0197.

10 *Synthesis of compounds 2a-c*

[0148] General Method B: To a round-bottom flask containing suitably substituted 4-hydroxyquinoline (1 eq) in acetone (12 mL/1 mmol, HPLC grade) was added anhydrous K_2CO_3 (4 eq), and then the mixture was refluxed in 1 h. Subsequently, KI (0.5 eq) and propargyl bromide (1.2 eq) were added, and then the reaction mixture was heated at reflux until the reaction was complete as judged by TLC (EtOAc–hexane 1:5 as an eluent). The reaction mixture was cooled, filtered, and the filtrate was evaporated in vacuo to give the crude product. The product was purified by automated flash chromatography using 10% EtOAc–hexane if it is to be assessed for antibiotic activity. Otherwise, the crude product can be used directly in the synthesis of isoxazole compounds. Characterizations of these following compounds have been reported by Kozikowski.

[0149] 2,5,7-Tris(trifluoromethyl)-4-propargyloxyquinoline (**2a**): Prepared by general method **B**, yield 74%. 1H NMR ($CDCl_3$) δ 2.69 (1H, s), 5.11 (2H, s), 7.10 (1H, s), 8.20 (1H, s), 8.65 (1H, s); ^{13}C NMR ($DMSO-d_6$) δ 53.4, 77.2, 80.1, 105.9 (q, $J = 1.8$ Hz), 106.1, 107.9, 113.5 (q, $J = 36$ Hz), 117.5 (q, $J = 3$ Hz), 122.2 (q, $J = 270$ Hz), 122.5 (q, $J = 276$ Hz), 124.4 (q, $J = 276$ Hz), 125.5 (q, $J = 30$ Hz), 127.1 (m), 129.9 (q, $J = 36$ Hz), 141.0, 144.7 (q, $J = 36$ Hz), 150.9.

[0150] 2-trifluoromethyl-4-propargyloxyquinoline (**2b**): Prepared by general method **B**, yield 80%. 1H NMR ($CDCl_3$) δ 2.69 (1H, s), 5.10 (2H, s), 7.10 (1H, s), 7.76 (1H, apparent t, $J = 7.8$ Hz), 7.85 (1H, apparent t, $J = 8.0$ Hz), 7.90 (1H, d, $J = 8.4$ Hz), 8.05 (1H, d, $J = 8.4$ Hz); ^{13}C NMR ($DMSO-d_6$) δ 13.4, 77.2, 80.1, 100.5 (q, $J = 2$ Hz), 119.1, 122.1 (q, $J = 276$ Hz), 122.3, 123.7, 127.3, 130.5, 141.7, 144.5 (q, $J = 36$ Hz), 162.4,

[0151] 8-trifluoromethoxy-2-trifluoromethyl-4-propargyloxyquinoline (**2c**):

Prepared by general method **B**, yield 77%. ¹H NMR (DMSO-*d*₆) δ 2.65 (1H, s), 5.09 (2H, s), 7.17 (1H, s), 7.77 (1H, apparent t, *J* = 8.4 Hz), 7.88 (1H, d, *J* = 7.2 Hz), 8.28 (1H, d, *J* = 8.4 Hz). ¹³C NMR (DMSO-*d*₆) δ 53.0, 77.1, 79.9, 101.9 (q, *J* = 2.4 Hz), 119.9, 120.8 (q, *J* = 258 Hz), 122.3 (q, *J* = 276 Hz), 123.0, 123.5 (m), 127.3, 130.8, 141.8 (q, *J* = 1.8 Hz), 144.5 (q, *J* = 36 Hz), 163.9.

Synthesis of compounds 2d-j

[0152] **General Method C:** To a round-bottom flask containing ethyl 2-chloro-2-(hydroxyimino)acetate (0.21 g, 1.5 mmol) and the corresponding acetylene intermediate prepared by general method **B** (0.15 g, 0.6 mmol), was subsequently added anhydrous Et₂O (20 mL). Then, a solution of Et₃N (0.25 mL, 1.8 mmol) in anhydrous Et₂O (10 mL) was added to the mixture via syringe pump over 6 h period and stirred overnight at room temperature. Finally, the reaction mixture was filtered, washed with Et₂O (2 × 15 mL), and the solvent was removed in vacuo to give the crude product. Then the crude product was purified by automated flash chromatography using 30% EtOAc-hexane to give products as white powders. Characterization of the following compounds has been reported by Kozikowski.

[0153] 5-[[[2-(Trifluoromethyl)-4-quinolinyloxy]methyl]-3-isoxazole-carboxylic Acid Ethyl Ester (**2d**): Prepared by method **C**, yield 71%. ¹H NMR (CDCl₃) δ 1.40 (3H, t, *J* = 7.2 Hz), 4.48 (2H, q, *J* = 7.2 Hz), 5.51 (2H, s), 6.88 (1H, s), 7.13 (1H, s), 7.66 (1H, apparent t, *J* = 7.8 Hz), 7.84 (1H, apparent t, *J* = 7.8 Hz), 8.19 (1H, d, *J* = 8.4 Hz), 8.25 (1H, d, *J* = 8.4 Hz). ¹³C NMR (CDCl₃) δ 14.1, 62.1, 62.6, 96.1 (q, *J* = 2 Hz), 104.3, 121.1, 121.3 (q, *J* = 276 Hz), 121.7, 128.2, 129.8, 131.7, 146.4, 148.9 (q, *J* = 36 Hz), 156.8, 159.5, 161.7, 167.7.

[0154] 5-[[[8-(trifluoromethoxy)-2-(trifluoromethyl)-4-quinolinyloxy]methyl]-3-isoxazolecarboxylic acid ethyl ester (**2e**): Prepared by method **C**, yield 60%. ¹H NMR (CDCl₃) δ 1.46 (3H, t, *J* = 7.2 Hz), 4.49 (2H, q, *J* = 7.2 Hz), 5.54 (2H, s), 6.94 (1H, s), 7.29 (1H, s), 7.67 (1H, apparent t, *J* = 8.4 Hz), 7.75 (1H, d, *J* = 7.8 Hz), 8.2 (1H, d, *J* = 8.4 Hz). ¹³C NMR (CDCl₃) δ 14.1, 61.4, 62.6, 97.8 (q, *J* = 2.4 Hz), 105.1, 119.9, 120.6 (q, *J* = 258 Hz), 122.7 (q, *J* = 276 Hz), 123.6, 127.6, 130.9, 141.7 (q, *J* = 1.8 Hz), 145.5 (q, *J* = 36 Hz), 156.8, 159.4, 161.7, 167.1.

[0155] 5-[[[2,5,7-Tris(trifluoromethyl)-4-quinolinyloxy]methyl]-3-isoxazolecarboxylic acid ethyl ester (**2f**)¹: Prepared by method **C**, 65%. ¹H NMR (CDCl₃) δ 1.45 (3H, t, *J* = 7.2 Hz), 4.48 (2H, q, *J* = 7.2 Hz), 5.57 (2H, s), 6.94 (1H, s),

7.41 (1H, s), 7.66 (1H, s), 8.28 (1H, s), 8.71 (1H, s). ¹³C NMR (CDCl₃) δ 14.1, 61.1, 63.7, 97.0, 105.9, 106.0 (q, J = 2 Hz), 107.5, 113.2 (q, J = 30 Hz), 118.2 (q, J = 3 Hz), 122.3 (q, J = 276 Hz), 122.5 (q, J = 270 Hz), 123.6 (q, J = 276 Hz), 125.0 (q, J = 36 Hz), 128.4 (m), 130.1 (q, J = 36 Hz), 150.9 (q, J = 36 Hz), 156.7, 159.0, 167.1.

5 **[0156] Synthesis of compound 2g:** To a round-bottom flask containing a solution of 3-hydroxyphenylacetylene (0.32 g, 2.7 mmol) in anhydrous THF (4 mL), was added successively 2.7 mL of 1 M *t*-BuOK in THF (2.7 mmol) and 4-chloro-2-(trifluoromethyl)quinoline (0.6 g, 2.6 mmol) in anhydrous THF (5 mL) dropwise. The reaction mixture was heated at reflux for 48 h. After cooling to room temperature, cold
10 H₂O (50 mL) was added and the solution was acidified to pH ~4 using 5% HCl, then the mixture was extracted with EtOAc (3 $\times$ 25 mL). The combined organic phases were washed with brine (20 mL) and dried with anhydrous Na₂SO₄. After filtration, the solvent was removed in vacuo to yield the crude phenyl acetylene substituted quinoline compound, which was used directly to prepare compound **2g** by general method **C**
15 without purification. The following compound has been reported by Kozikowski.

[0157] 5-[3-[[2-(Trifluoromethyl)-4-quinolinyloxy]phenyl]-3-isoxazolecarboxylic Acid Ethyl Ester (2g): Yield 55%. ¹H NMR (CDCl₃) δ 1.44 (3H, t, J = 7.2 Hz), 4.48 (2H, q, J = 7.2 Hz), 6.88 (1H, s), 7.00 (1H, s), 7.34 (1H, apparent t, J = 8.4 Hz), 7.53 (3H, m), 7.71 (1H, d, J = 7.8 Hz), 7.89 (1H, apparent t, J = 8.4 Hz), 8.24
20 (1H, d, J = 8.4 Hz), 8.43 (1H, d, J = 8.4 Hz). ¹³C NMR (CDCl₃) δ 14.2, 26.9, 62.4, 65.9, 100.3 (q, J = 2 Hz), 101.0, 118.6, 121.2 (q, J = 276 Hz), 121.7, 123.2, 123.7, 128.4, 129.2, 129.9, 131.6, 148.8, 148.9 (q, J = 34 Hz), 154.5, 157.2, 159.8, 162.9, 170.2.

[0158] Synthesis of compound 2h and 2i: To a round-bottom flask containing an aqueous solution of chloroacetaldehyde (0.3 mol / 47 mL), sodium acetate
25 (0.3 mol) and hydroxylamine hydrochloride (0.3 mol) were added. Then the reaction mixture was stirred for 30 min. After extraction of the reaction mixture with EtOAc (3 $\times$ 25 mL), the combined organic phases were washed with brine (20 mL) and dried over anhydrous Na₂SO₄. The solvent was removed in vacuo to furnish the crude intermediate compound. This residue was directly added to the solution of ethyl propionate (29.7 mg,
30 0.3 mmol) in anhydrous THF (5 mL). Subsequently, 10 mL of 10% sodium hypochlorite solution was added dropwise over 2 h, and the reaction mixture was stirred overnight. After cooling to room temperature, the reaction mixture was extracted with EtOAc (3 $\times$ 25 mL). The combined organic phases were washed with brine (20 mL) and dried over anhydrous Na₂SO₄. After filtration, the organic solvent was evaporated and the crude

product was purified by automated flash chromatography using a gradient eluent from hexane to 20% EtOAc-hexane. The isoxazole product was then reacted with the corresponding 4-hydroxyquinoline using general procedure **B** for the preparation of compound **2h** and **2i**.

5 **[0159]** 3-[[[8-trifluoromethoxy-2-trifluoromethyl-4-quinolinyloxy]methyl]-5-isoxazolecarboxylic Acid Ethyl Ester (**2h**): Yield 65%. ¹H NMR (CDCl₃) δ 1.46 (3H, t, J = 7.2 Hz), 4.49 (2H, q, J = 7.2 Hz), 5.54 (2H, s), 7.17 (1H, s), 7.66 (1H, apparent t, J = 7.8 Hz), 7.74 (1H, d, J = 7.8 Hz) 8.20 (1H, d, J = 9.6 Hz). ¹³C NMR (CDCl₃) δ 14.1, 53.7, 62.3, 62.7, 98.1 (q, J = 2 Hz), 108.4, 120.6 (q, J = 259 Hz), 122.6 (q, J = 276 Hz),
10 123.0, 127.5, 128.1, 141.7 (q, J = 2 Hz), 149.8 (q, J = 35 Hz), 156.3, 159.4, 161.8, 163.5. HRMS (ESI): calcd. for C₁₈H₁₂F₆N₂O₄ [M+H⁺] 434.0701, found 434.0704.

[0160] 3-[[[2,7-bis(trifluoromethyl)-4-quinolinyloxy]methyl]-5-isoxazolecarboxylic Acid Ethyl Ester (**2i**): Yield 71%. ¹H NMR (CDCl₃) δ 1.49 (3H, t, J = 7.2 Hz), 4.55 (2H, q, J = 7.2 Hz), 5.63 (2H, s), 7.31 (1H, s), 7.78 (1H, s), 7.81 (1H, d, J = 9.0 Hz), 8.42 (1H, d, J = 9.0 Hz), 8.52 (1H, s). ¹³C NMR (DMSO-*d*₆) δ 14.5, 62.8, 67.2, 73.7, 100.7 (q, J = 2 Hz), 123.6 (q, J = 276 Hz), 123.9 (q, J = 270 Hz), 124.8 (q, J = 3 Hz), 127.0, 127.3 (q, J = 4 Hz), 132.0 (q, J = 33 Hz), 146.9, 150.2 (q, J = 35 Hz), 152.6, 158.6, 159.7, 163.0. HRMS (ESI): calcd. for C₁₈H₁₂F₆N₂O₃ [M+H⁺] 418.0752, found 418.0750.

20 **[0161]** **Preparation of compound 2j**: To a round-bottom flask containing a solution of compound **1q** (5 mmol) in 6 mL methanol: water: THF (1:3:2), 0.15 g of sodium hydroxide was added. Then the reaction mixture was heated at reflux for 4 h, and then cooled to room temperature. The resulting mixture was acidified to pH ~6 with 1 M hydrochloric acid solution, then followed by extraction with EtOAc (3 $\times$ 25 mL). The
25 combined organic phase was washed with brine and dried over anhydrous Na₂SO₄. The organic solvent was evaporated in vacuo and the crude product was purified by automated flash chromatography using 15% EtOAc-hexane to give the carboxylic acid in 89% yield.

[0162] 3-[[[2,7-bis(trifluoromethyl)-4-quinolinyloxy]methyl]-5-isoxazolecarboxylic Acid (**1r**). ¹H NMR (DMSO-*d*₆) δ 5.79 (2H, s), 7.58 (1H, s), 7.71 (1H, s), 7.94 (1H, apparent t, J = 7.8 Hz), 8.39 (1H, s), 8.42 (1H, d, J = 9.0 Hz). ¹³C NMR (DMSO-*d*₆) δ 62.7, 99.6 (q, J = 2 Hz), 105.9, 121.2 (q, J = 276 Hz), 123.2, 123.5, 123.7 (q, J = 274 Hz), 124.0 (q, J = 3 Hz), 125.3, 127.8 (q, J = 4 Hz), 133.6 (q, J = 33 Hz), 147.6, 157.0 (q, J = 35 Hz), 159.6, 161.9, 166.5.

Synthesis of compounds 3a and 3b

[0163] **General Method D:** To a flame dried round-bottom flask containing 2 mL of 1,4-dioxane, was added successively 4-chloro-2,7-bis(trifluoromethyl)quinoline (1 eq), aniline (1 eq), palladium (II) acetate (5 mol%), DPEphos (10 mol%) and potassium phosphate (2.5 eq). Then the flask was capped with a rubber septum and purged with nitrogen, and heated to 110 °C. The reaction was heated for 18 h, and then cooled to room temperature. The resulting mixture was neutralized with 1 M hydrochloride acid solution and extracted with EtOAc (3 × 25 mL). Then the combined organic phase was washed with brine and dried over anhydrous Na₂SO₄. The organic solvent was removed by vacuum and the crude product was purified by automated flash chromatography with 25% EtOAc-hexane.

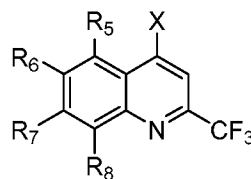
[0164] 4-aniliny-2,7-bis(trifluoromethyl)quinoline (**3a**): Yield 39%. ¹H NMR (CDCl₃) δ 5.61 (1H, br s), 6.91 (1H, s), 7.45 (4H, m), 7.71 (1H, d, *J* = 7.2 Hz), 7.91 (1H, d, *J* = 9.0 Hz), 8.47 (1H, s). ¹³C NMR (CDCl₃) δ 96.1 (q, *J* = 2 Hz), 120.3, 120.8 (q, *J* = 273 Hz), 122.1 (q, *J* = 276 Hz), 127.8 (q, *J* = 2.4 Hz), 128.4 (q, *J* = 3.6 Hz), 128.6, 129.1, 129.2, 132.0, 136.1 (q, *J* = 33 Hz), 138.8, 146.9, 150.9 (q, *J* = 35 Hz). HRMS (ESI): calcd. for C₁₇H₁₁F₆N₂ [M+H⁺] 357.0826, found 357.0828.

[0165] 4-(p-trifluoromethylaniliny)-8-trifluoromethoxy-2-trifluoromethylquinoline (**3b**): Yield 32%. ¹H NMR (CDCl₃) δ 7.17 (1H, s), 7.44 (3H, m), 7.61 (1H, apparent t, *J* = 9.6 Hz), 7.71 (3H, m), 7.98 (1H, d, *J* = 10.2 Hz). ¹³C NMR (CDCl₃) δ 104.2 (q, *J* = 2 Hz), 114.3, 119.8 (q, *J* = 270 Hz), 120.3, 125.3 (q, *J* = 270 Hz), 125.5, 125.9, 127.0, 129.0, 129.8, 130.2, 143.7, 145.9 (q, *J* = 1.8 Hz), 149.1 (q, *J* = 34 Hz), 150.7. HRMS (ESI): calcd. for C₁₈H₉F₉N₂O [M+H⁺] 440.0571, found 440.0568.

CLAIMS

We claim:

1. A composition comprising an antibiotic and a compound of formula (I), or a pharmaceutically acceptable salt thereof:



(I)

wherein

X is OR or NHR;

R is hydrogen or $R_1-R_2-(R_3)_n$;

10 R_1 is selected from C_{1-6} alkyl, C_{2-6} alkynyl, aryl and heteroaryl;

R_2 is selected from aryl and heteroaryl, or is absent;

each R_3 is independently selected from C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} fluoroalkyl, C_{1-6} fluoroalkoxy, halogen, cyano and $-CO_2R_4$;

R_4 is hydrogen or C_{1-6} alkyl;

15 R_5 , R_6 , R_7 and R_8 are independently selected from hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{1-6} fluoroalkyl and C_{1-6} fluoroalkoxy; and

n is 0-3;

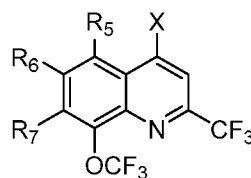
provided that at least one of R_5 , R_6 , R_7 and R_8 is C_{1-6} fluoroalkyl or C_{1-6} fluoroalkoxy.

20 2. The composition of claim 1, wherein R_8 is C_{1-6} fluoroalkoxy.

3. The composition of any one of claims 1-2, wherein R_5 , R_6 and R_7 are hydrogen, and R_8 is C_{1-6} fluoroalkoxy.

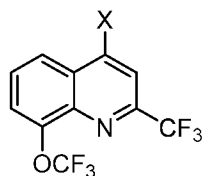
4. The composition of any one of claims 1-3, wherein R_8 is trifluoromethoxy.

5. The composition of claim 4, wherein the compound of formula (I) has the structure of formula (II), or a pharmaceutically acceptable salt thereof:



(II).

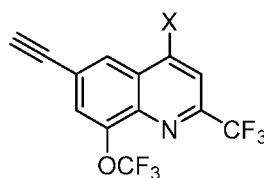
6. The composition of claim 5, wherein the compound of formula (II) has the structure of formula (IIa), or a pharmaceutically acceptable salt thereof:



5

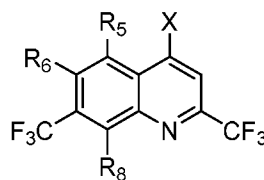
(IIa).

7. The composition of claim 5, wherein the compound of formula (II) has the structure of formula (IIb), or a pharmaceutically acceptable salt thereof:



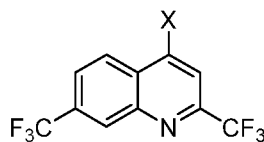
(IIb).

- 10 8. The composition of claim 1, wherein R_7 is C_{1-6} fluoroalkyl.
9. The composition of claim 8, wherein R_5 , R_6 and R_8 are independently selected from hydrogen and C_{1-6} fluoroalkyl.
10. The composition of claim 9, wherein R_5 , R_6 and R_8 are hydrogen.
11. The composition of claim 9, wherein R_5 is C_{1-6} fluoroalkyl and R_6 and R_8 are
15 hydrogen.
12. The composition of any one of claims 10-11, wherein said C_{1-6} fluoroalkyl is trifluoromethyl.
13. The composition of any one of claims 8-12, wherein R_7 is trifluoromethyl.
14. The composition of claim 13, wherein the compound of formula (I) has the
20 structure of formula (III), or a pharmaceutically acceptable salt thereof:



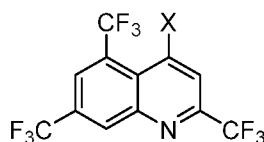
(III).

15. The composition of claim 14, wherein the compound of formula (III) has the structure of formula (IIIa), or a pharmaceutically acceptable salt thereof:



(IIIa).

16. The composition of claim 14, wherein the compound of formula (III) has the structure of formula (IIIb), or a pharmaceutically acceptable salt thereof:



(IIIb).

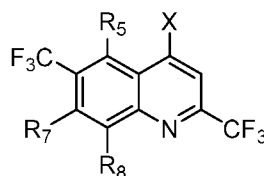
17. The composition of claim 1, wherein R₆ is C₁₋₆fluoroalkyl.

18. The composition of claim 17, wherein R₅, R₇ and R₈ are independently selected from hydrogen and C₁₋₆fluoroalkyl.

19. The composition of claim 18, wherein R₅, R₇ and R₈ are hydrogen.

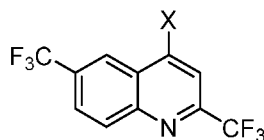
20. The composition of any one of claims 17-19, wherein R₆ is trifluoromethyl.

21. The composition of claim 20, wherein the compound has the structure of formula (IV), or a pharmaceutically acceptable salt thereof:



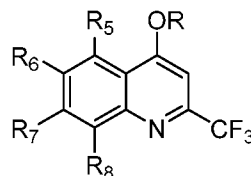
(IV).

22. The composition of claim 21, wherein the compound of formula (IV) has the structure of formula (IVa), or a pharmaceutically acceptable salt thereof:



(IVa).

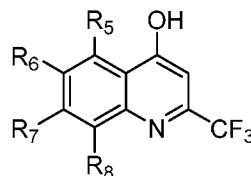
23. The composition of any one of claims 1-20, wherein X is OR and the compound has the structure of formula (V), or a pharmaceutically acceptable salt thereof:



5

(V).

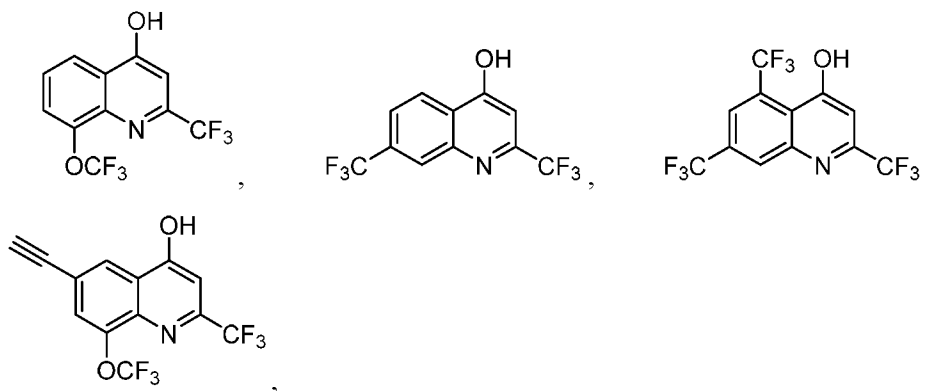
24. The composition of claim 23, wherein R is hydrogen and the compound of formula (V) has the structure of formula (Va), or a pharmaceutically acceptable salt thereof:



10

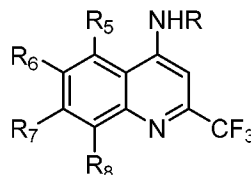
(Va).

25. The composition of claim 24, wherein the compound is selected from:



and pharmaceutically acceptable salts thereof.

26. The composition of any one of claims 1-20, wherein X is NHR and the compound has the structure of formula (VI):



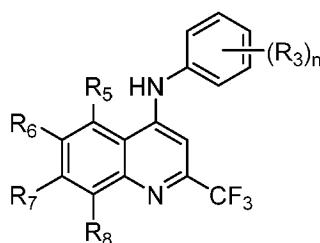
15

(VI).

27. The composition of claim 26, wherein R is $R_1-R_2-(R_3)_n$; and wherein
 R_1 is aryl or heteroaryl;
 R_2 is absent;
each R_3 is independently selected from C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} fluoroalkyl, C_{1-6} fluoroalkoxy, halogen and cyano; and
n is 0-3

28. The composition of claim 27, wherein R_1 is phenyl.

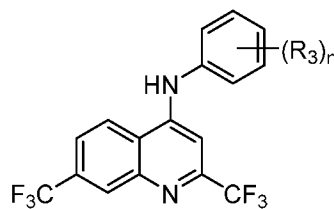
29. The composition of claim 28, wherein the compound of formula (VI) has the structure of formula (VII):



(VII).

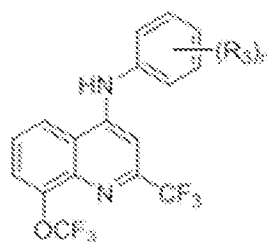
30. The composition of claim 29, wherein R_6 is hydrogen and R_5 , R_7 and R_8 are independently selected from hydrogen, C_{1-6} fluoroalkyl and C_{1-6} fluoroalkoxy.

31. The composition of claim 30, wherein the compound of formula (VII) has the structure of formula (VIIa):



(VIIa).

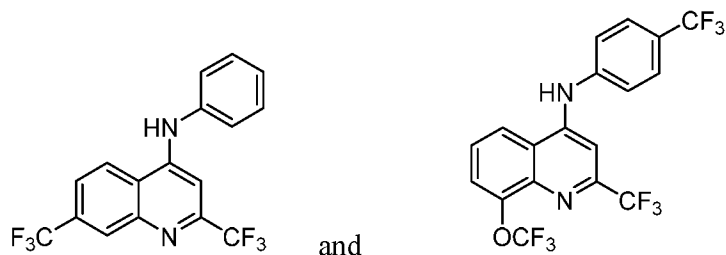
32. The composition of claim 30, wherein the compound of formula (VII) has the structure of formula (VIIb):



(VIIb).

33. The composition of any one of claims 29-31, wherein R_3 is trifluoromethyl and n is 0 or 1.

34. The composition of claim 33, wherein the compound is selected from:

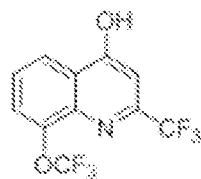


and pharmaceutically acceptable salts thereof.

35. The composition of any one of claims 1-34, wherein the antibiotic is selected from cefoxitin, nafcillin and oxacillin.

36. The composition of claim 35, wherein the antibiotic is cefoxitin.

37. The composition of claim 36, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

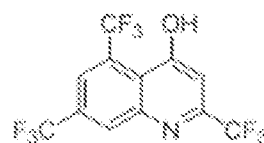
38. The composition of claim 36, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

39. The composition of claim 35, wherein the antibiotic is nafcillin.

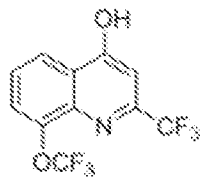
40. The composition of claim 39, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

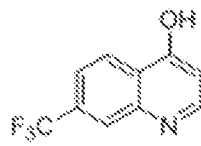
41. The composition of claim 35, wherein the antibiotic is oxacillin.

42. The composition of claim 41, wherein the compound is



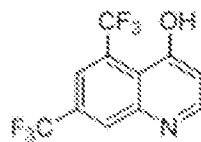
, or a pharmaceutically acceptable salt thereof.

43. The composition of claim 41, wherein the compound is



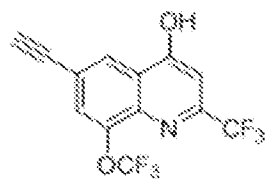
, or a pharmaceutically acceptable salt thereof.

- 5 44. The composition of claim 41, wherein the compound is



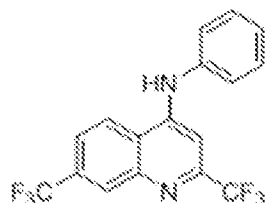
, or a pharmaceutically acceptable salt thereof.

45. The composition of claim 41, wherein the compound is



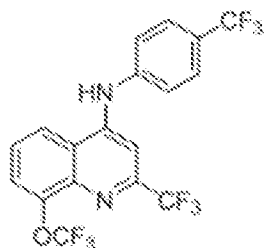
, or a pharmaceutically acceptable salt thereof.

46. The composition of claim 41, wherein the compound is



10 , or a pharmaceutically acceptable salt thereof.

47. The composition of claim 41, wherein the compound is



, or a pharmaceutically acceptable salt thereof.

48. A method of treating or preventing a bacterial infection comprising administering to a subject in need thereof a therapeutically-effective amount of the composition according to any one of claims 1-47.

49. A method of treating or a bacterial infection in a subject in need thereof,

5 comprising the steps of

- a. administering to the subject a compound of formula (I) according to any one of claims 1-47; and
- b. administering to the subject a therapeutically-effective amount of an antibiotic selected from cefoxitin, nafcillin and oxacillin.

10 50. A method of treating or a bacterial infection in a subject in need thereof, comprising the steps of

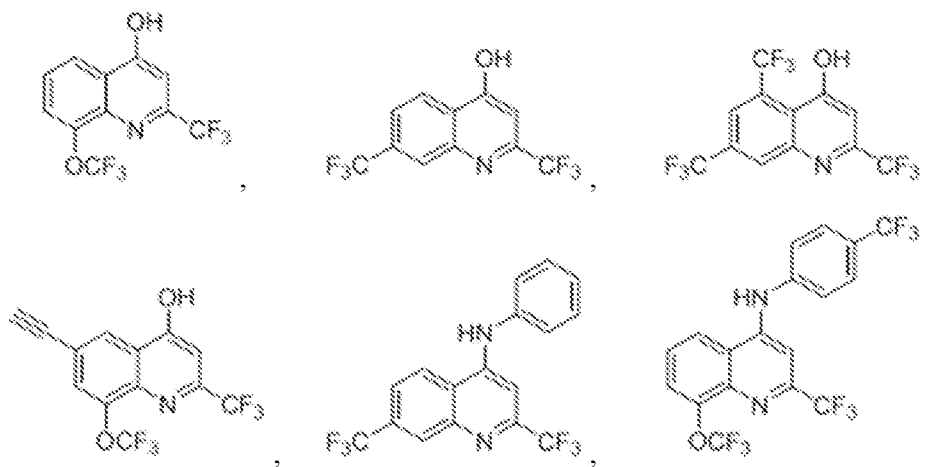
- a. administering to the subject a therapeutically-effective amount of an antibiotic selected from cefoxitin, nafcillin and oxacillin; and
- b. administering to the subject a compound of formula (I) according to any

15

one of claims 1-47.

51. The method of any one of claims 48-50, wherein the antibiotic is oxacillin.

52. The method of claim 51, wherein the compound is selected from the group of:



and pharmaceutically acceptable salts thereof.

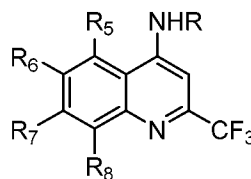
53. The method of any one of claims 48-52, wherein the bacterial infection is caused
20 by methicillin-resistant staphylococcus aureus.

54. The method of claim 53, wherein the methicillin-resistant staphylococcus aureus is a community-acquired strain.

55. The method of claim 53, wherein the methicillin-resistant staphylococcus aureus is a hospital-acquired strain.

56. The method of claim 53, wherein the bacterial infection is caused by the USA300 strain of methicillin-resistant staphylococcus aureus.

57. A compound of formula (VI), or a pharmaceutically acceptable salt thereof:



(VI)

wherein

R is $R_1-R_2-(R_3)_n$;

10 R₁ is aryl or heteroaryl;

R₂ is absent; and

each R₃ is independently selected from C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆fluoroalkyl, C₁₋₆fluoroalkoxy, halogen and cyano;

R₅, R₆, R₇ and R₈ are independently selected from hydrogen, C₁₋₆ alkyl, C₂₋₆

15 alkenyl, C₂₋₆ alkynyl, C₁₋₆fluoroalkyl and C₁₋₆fluoroalkoxy; and

n is 0-3.

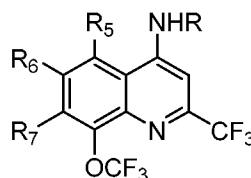
58. The compound of claim 57, wherein at least one of R₅, R₆, R₇ and R₈ is C₁₋₆fluoroalkyl or C₁₋₆fluoroalkoxy.

59. The compound of claim 57, wherein R₈ is C₁₋₆fluoroalkoxy.

20 60. The compound of any one of claims 57-59, wherein R₅, R₆ and R₇ are hydrogen, and R₈ is C₁₋₆fluoroalkoxy.

61. The compound of any one of claims 57-60, wherein R₈ is trifluoromethoxy.

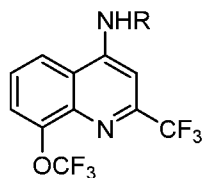
62. The compound of claim 61, wherein the compound of formula (VI) has the structure of formula (VIa), or a pharmaceutically acceptable salt thereof:



25

(VIa).

63. The compound of claim 62, wherein the compound of formula (VIa) has the structure of formula (VIb), or a pharmaceutically acceptable salt thereof:



5

(VIb).

64. The compound of claim 57, wherein R_7 is C_{1-6} fluoroalkyl.

65. The compound of claim 64, wherein R_5 , R_6 and R_8 are independently selected from hydrogen and C_{1-6} fluoroalkyl.

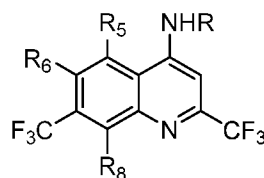
66. The compound of claim 65, wherein R_5 , R_6 and R_8 are hydrogen.

10 67. The compound of claim 65, wherein R_5 is C_{1-6} fluoroalkyl and R_6 and R_8 are hydrogen.

68. The compound of any one of claims 66-67, wherein said C_{1-6} fluoroalkyl is trifluoromethyl.

69. The compound of any one of claims 64-68, wherein R_7 is trifluoromethyl.

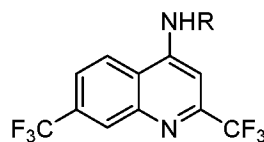
15 70. The compound of claim 69, wherein the compound of formula (VI) has the structure of formula (VIc), or a pharmaceutically acceptable salt thereof:



(VIc).

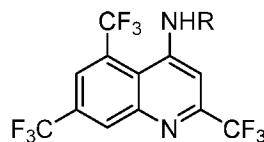
71. The compound of claim 70, wherein the compound of formula (VIc) has the structure of formula (VIId), or a pharmaceutically acceptable salt thereof:

20



(VIId).

72. The compound of claim 70, wherein the compound of formula (VIc) has the structure of formula (VIe), or a pharmaceutically acceptable salt thereof:



(VIe).

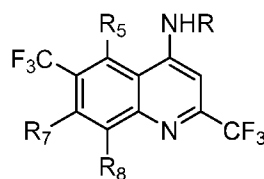
5 73. The compound of claim 57, wherein R_6 is C_{1-6} fluoroalkyl.

74. The compound of claim 73, wherein R_5 , R_7 and R_8 are independently selected from hydrogen and C_{1-6} fluoroalkyl.

75. The compound of claim 74, wherein R_5 , R_7 and R_8 are hydrogen.

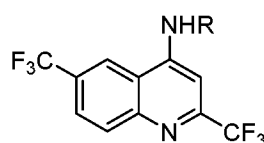
76. The compound of any one of claims 73-75, wherein R_6 is trifluoromethyl.

10 77. The compound of claim 76, wherein the compound of formula (VI) has the structure of formula (VI f), or a pharmaceutically acceptable salt thereof:



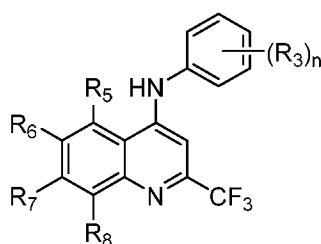
(VI f).

78. The compound of claim 77, wherein the compound of formula (VI f) has the
15 structure of formula (VIg), or a pharmaceutically acceptable salt thereof:



(VIg).

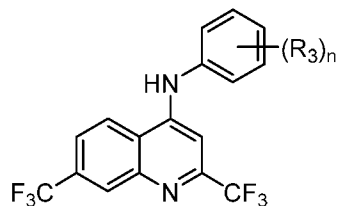
79. The compound of claim 57 having the structure of formula (VII):



(VII).

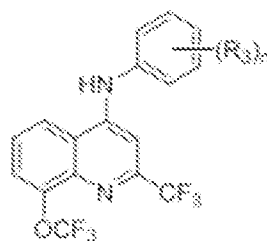
80. The compound of claim 79, wherein R_6 is hydrogen and R_5 , R_7 and R_8 are independently selected from hydrogen, C_{1-6} fluoroalkyl and C_{1-6} fluoroalkoxy.

81. The compound of claim 80, wherein the compound of formula (VII) has the structure of formula (VIIa):



(VIIa).

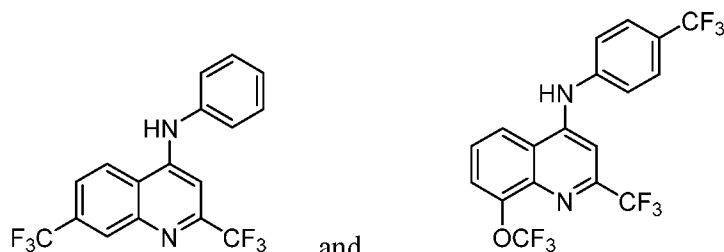
82. The compound of claim 80, wherein the compound of formula (VII) has the structure of formula (VIIb):



(VIIb).

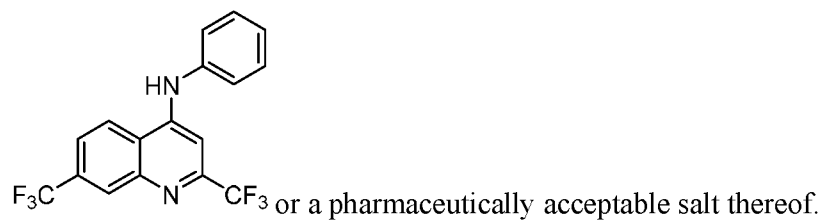
83. The compound of any one of claims 79-82, wherein R_3 is trifluoromethyl and n is 0 or 1.

84. The compound of claim 83, wherein the compound is selected from:

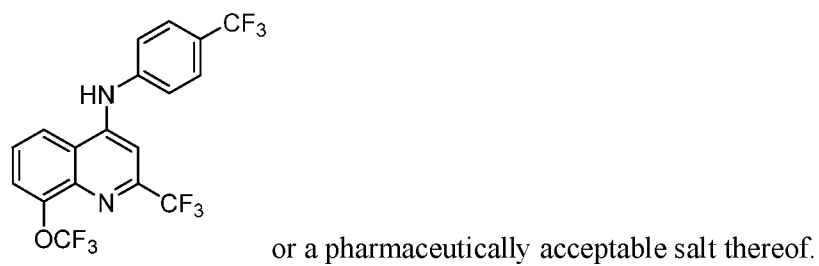


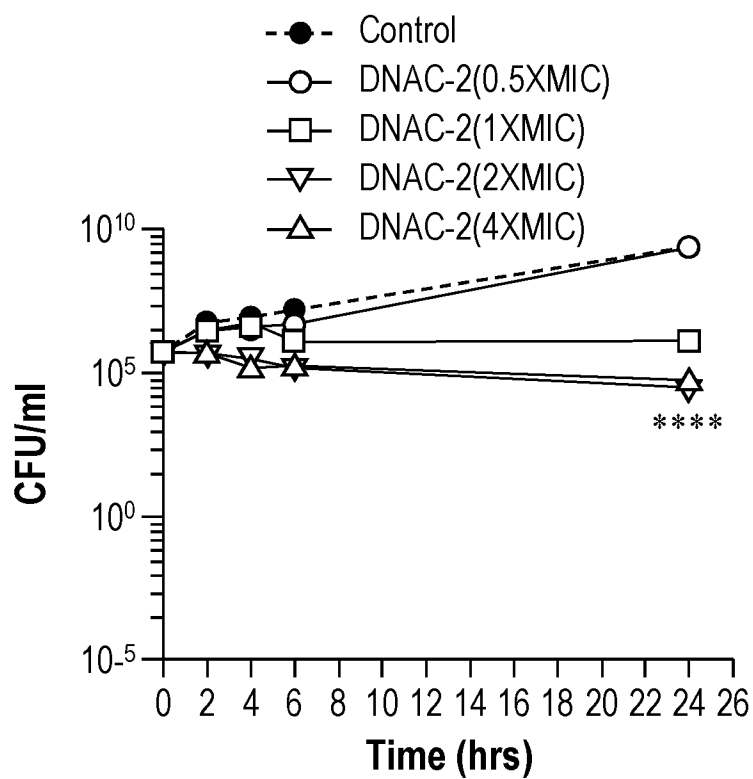
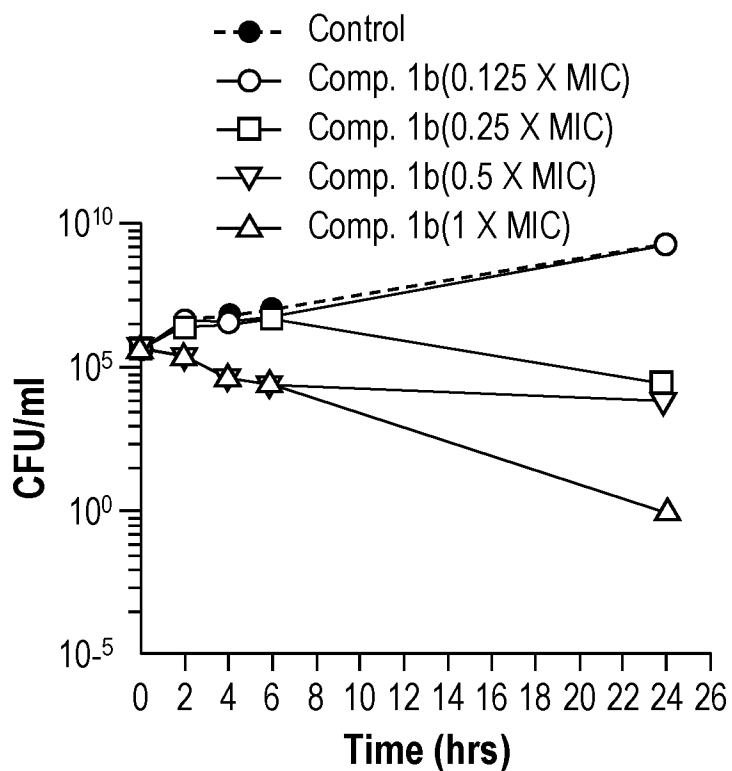
and pharmaceutically acceptable salts thereof.

85. The compound of claim 84, wherein the compound is:



86. The compound of claim 84, wherein the compound is:



**Fig. 1****Fig. 2**

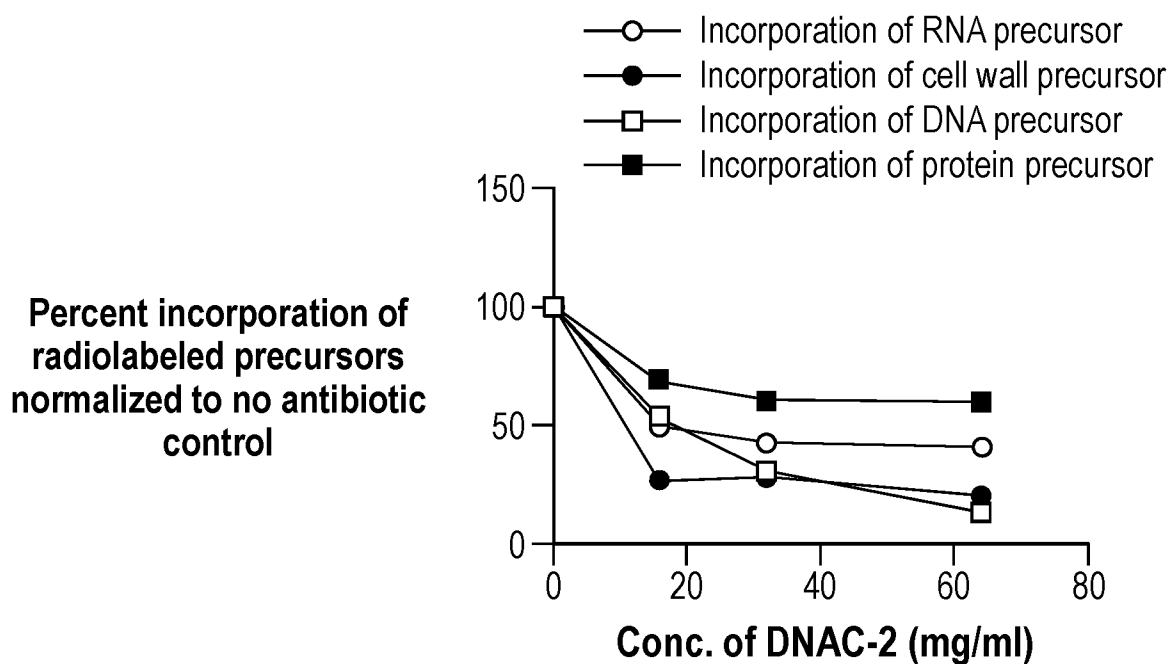
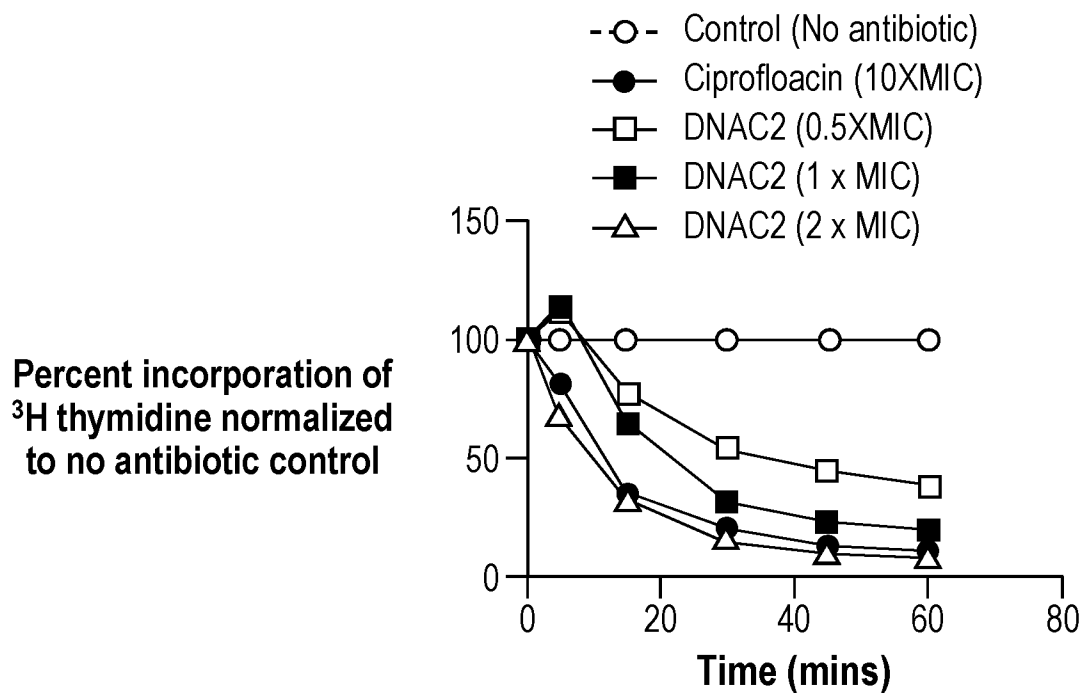
**Fig. 3****Fig. 4**

FIG. 5

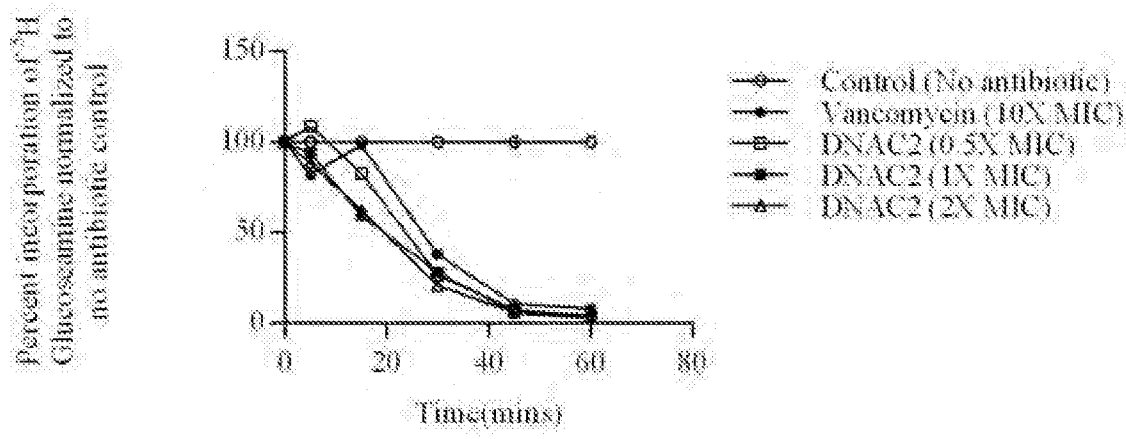
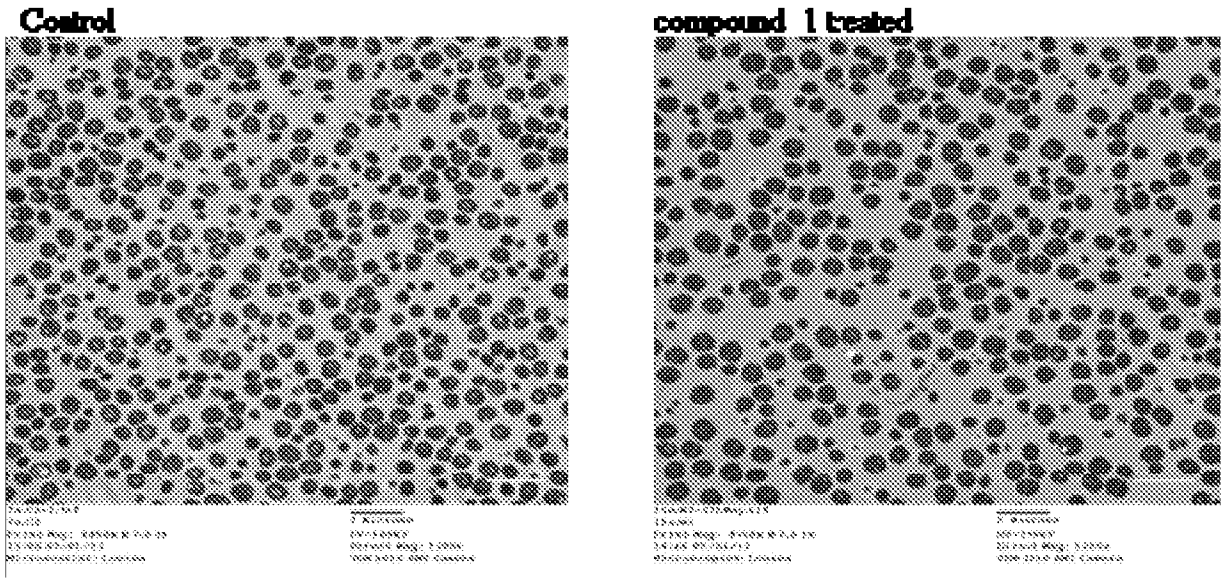


FIG. 6



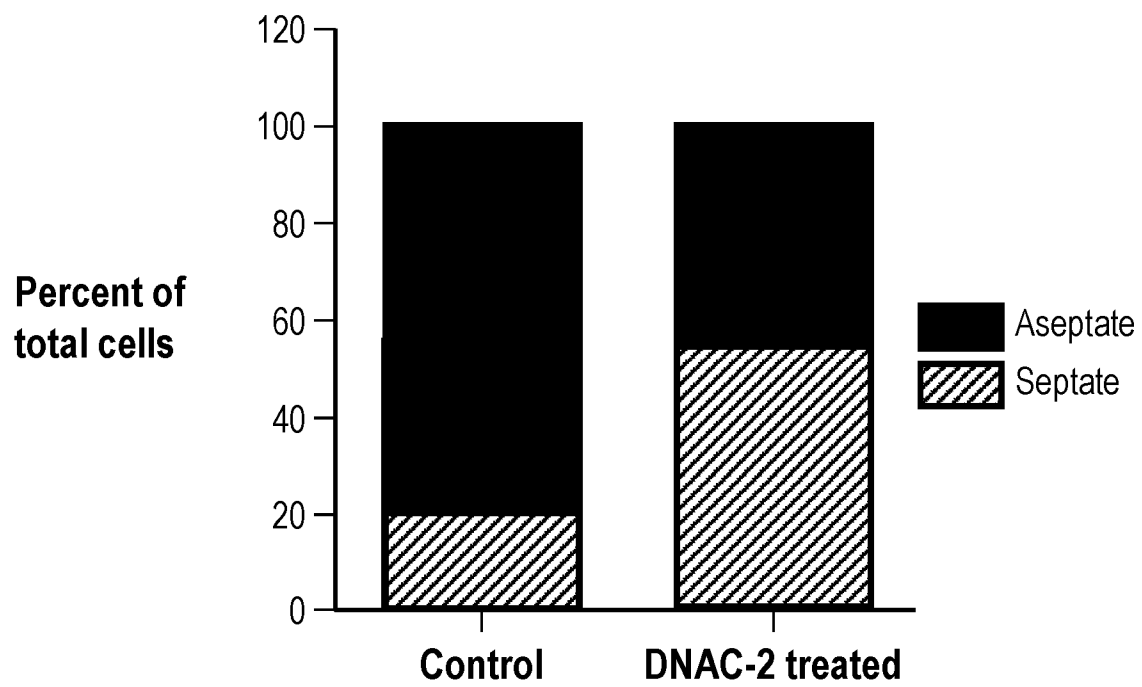


Fig. 7

FIG. 8

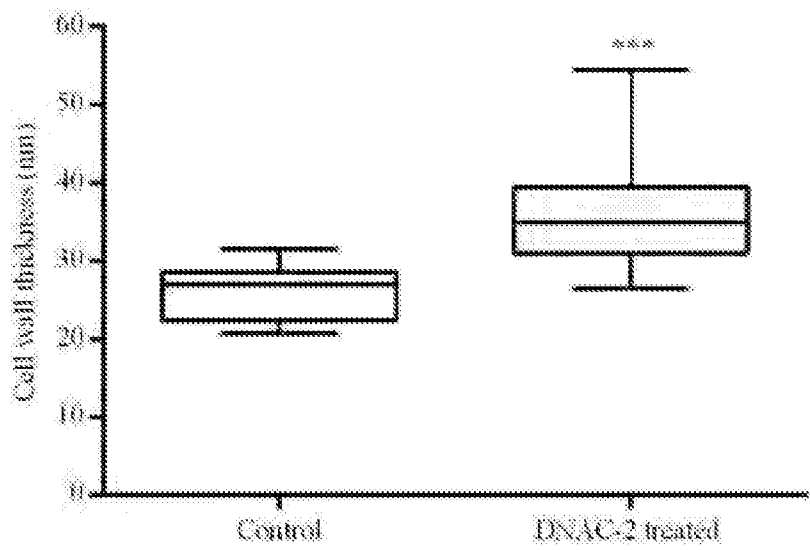
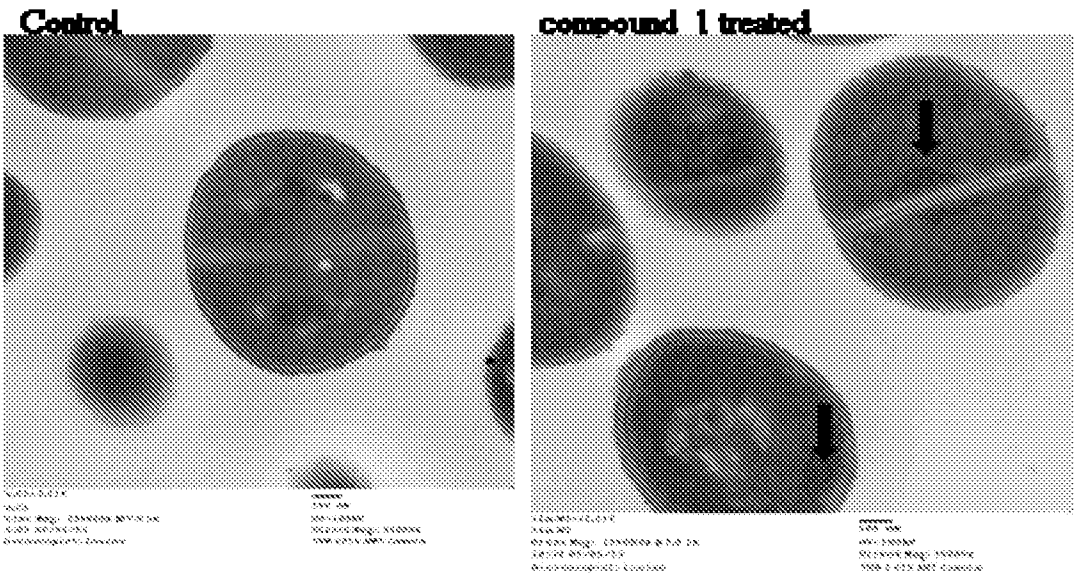


FIG. 9



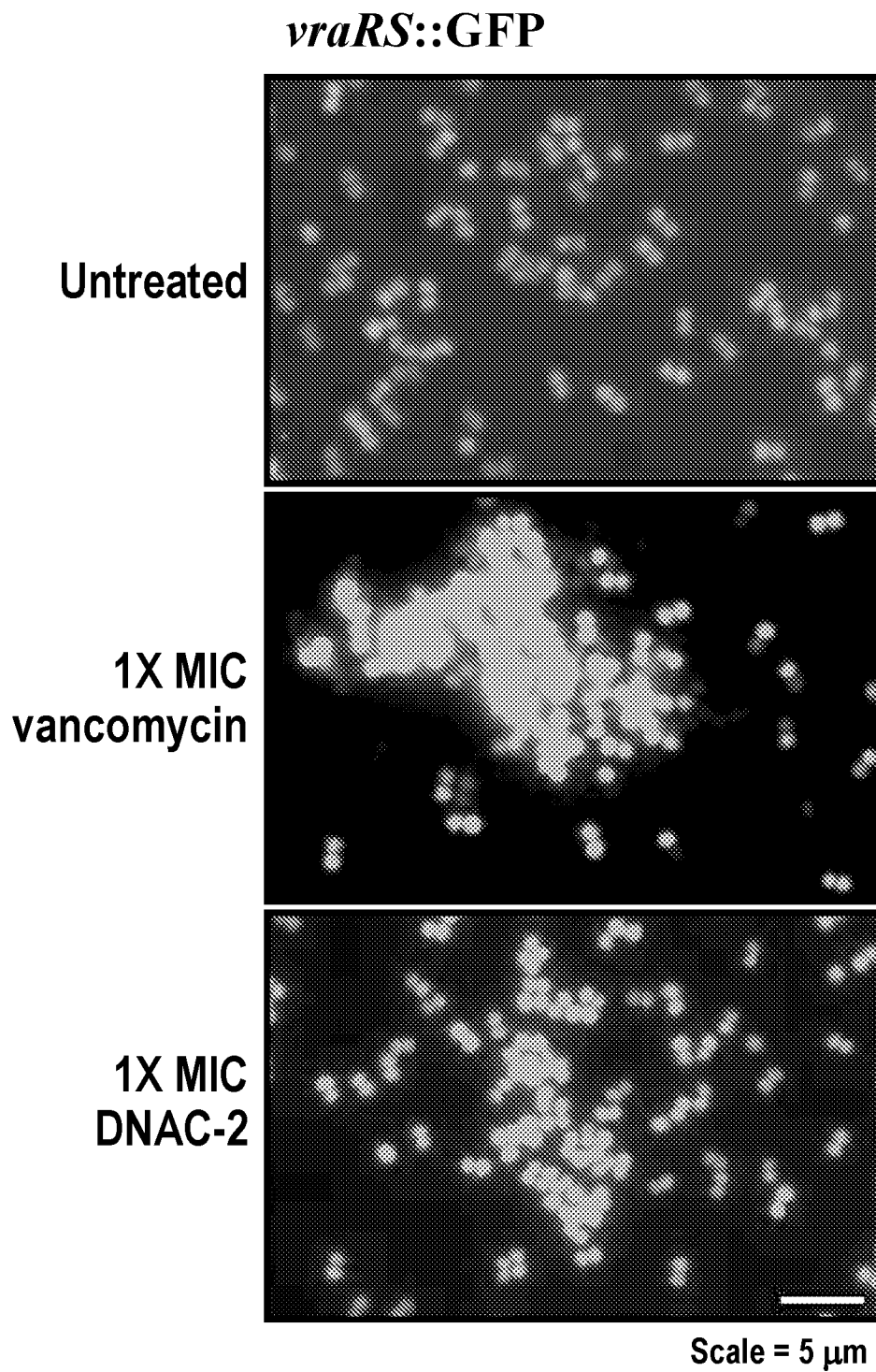


Fig. 10

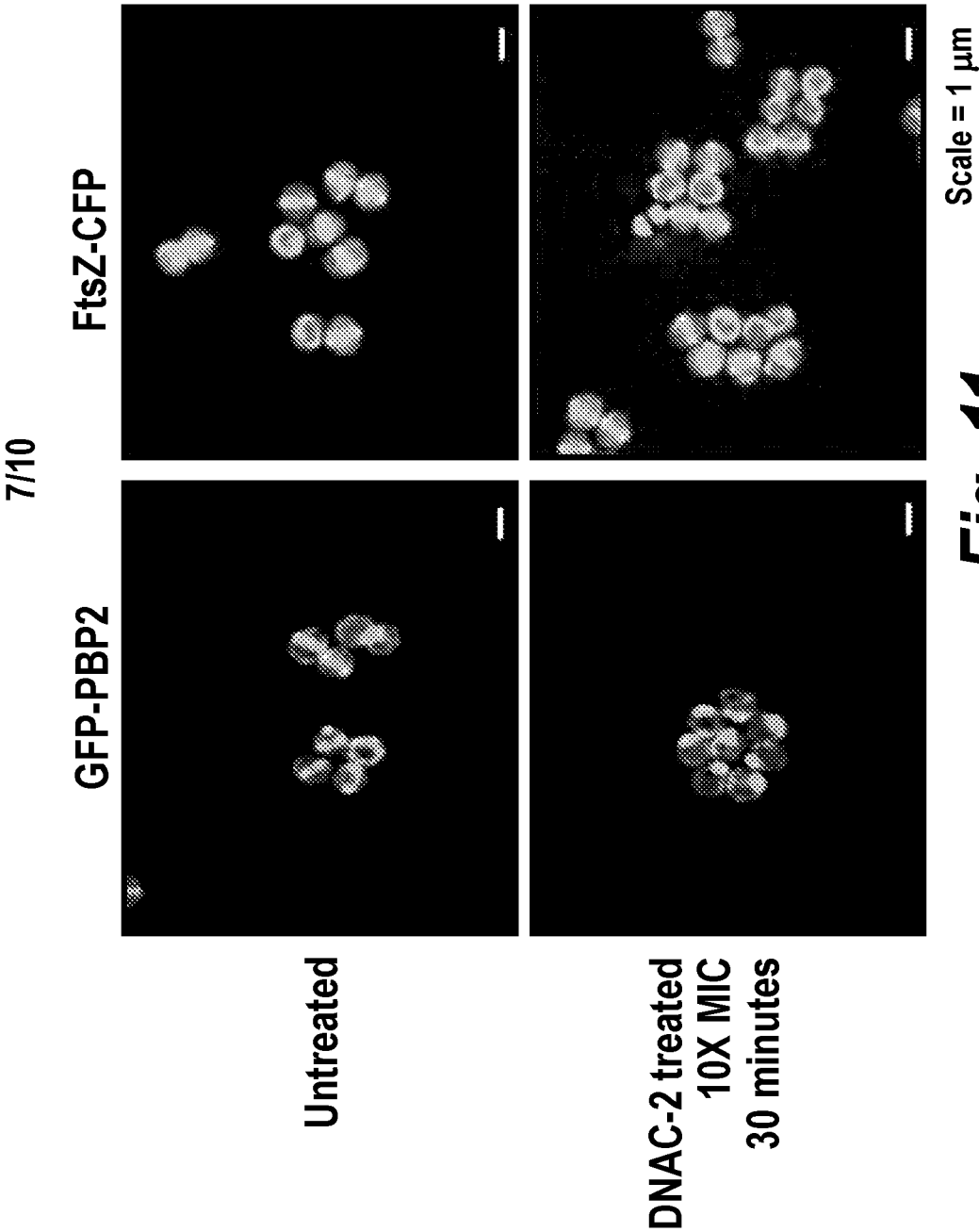


Fig. 11

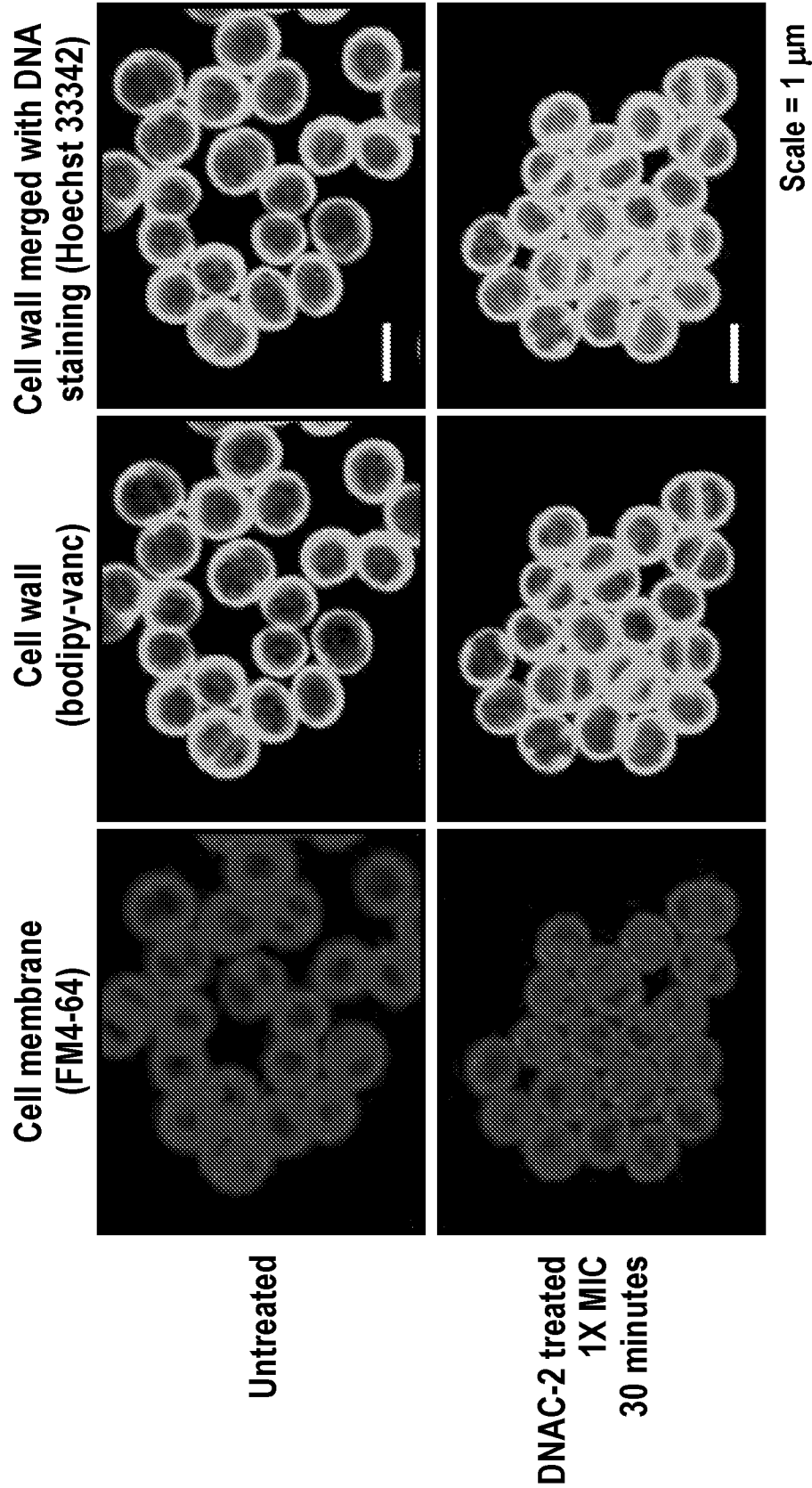


Fig. 12

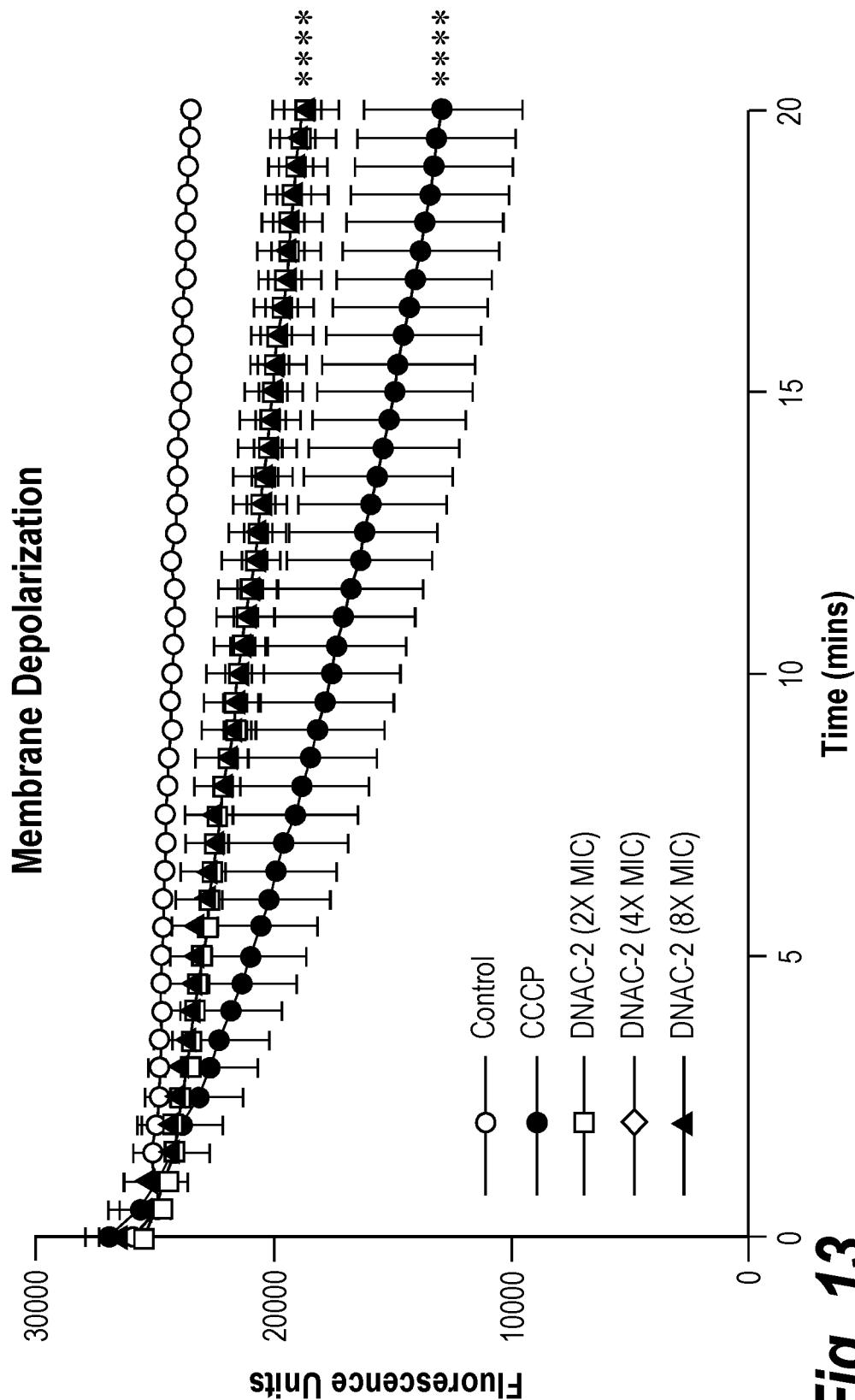


Fig. 13

Strain tested	Oxacillin (16mg/L)	MIC of DNAC-2 (48hrs)(mg/L)	Strain tested	Oxacillin (16mg/L)	MIC of DNAC-2 (48hrs)(mg/L)
CA-MRSA(1)	-	8	HA-MRSA(1)	+	N/A
CA-MRSA(1)	+	4	HA-MRSA(2)	-	8
CA-MRSA(2)	-	8	HA-MRSA(2)	+	4
CA-MRSA(2)	+	0.5	HA-MRSA(3)	-	4
CA-MRSA(3)	-	4	HA-MRSA(3)	+	4
CA-MRSA(3)	+	4	HA-MRSA(4)	-	4
CA-MRSA(4)	-	8	HA-MRSA(4)	+	4
CA-MRSA(4)	+	0.5	HA-MRSA(5)	-	8
CA-MRSA(5)	-	8	HA-MRSA(5)	+	4
CA-MRSA(5)	+	4	HA-MRSA(6)	-	4
CA-MRSA(6)	-	8	HA-MRSA(6)	+	8
CA-MRSA(6)	+	8	HA-MRSA(7)	-	4
CA-MRSA(7)	-	4	HA-MRSA(7)	+	4
CA-MRSA(7)	+	8	HA-MRSA(8)	-	4
CA-MRSA(8)	-	8	HA-MRSA(8)	+	4
CA-MRSA(8)	+	8	HA-MRSA(9)	-	4
CA-MRSA(9)	-	8	HA-MRSA(9)	+	4
CA-MRSA(9)	+	8	HA-MRSA(10)	-	4
CA-MRSA(10)	-	4	HA-MRSA(10)	+	4
CA-MRSA(10)	+	4			
CA-MRSA(11)	-	4			
CA-MRSA(11)	+	8			
CA-MRSA(12)	-	8			
CA-MRSA(12)	+	8			
CA-MRSA(13)	-	4			
CA-MRSA(13)	+	8			
CA-MRSA(14)	-	4			
CA-MRSA(14)	+	4			
CA-MRSA(15)	-	4			
CA-MRSA(15)	+	8			
CA-MRSA(16)	-	4			
CA-MRSA(16)	+	8			
CA-MRSA(17)	-	0.5			
CA-MRSA(17)	+	0.5			
CA-MRSA(18)	-	4			
CA-MRSA(18)	+	0.5			
CA-MRSA(19)	-	8			
CA-MRSA(19)	+	8			
CA-MRSA(20)	-	4			
CA-MRSA(20)	+	0.5			

Fig. 14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/016378

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/496; A61K 31/545; A61K 45/06; C07D 501/00; C07D 501/04; C07D 501/06 (2017.01)

CPC - A61K 31/496; A61K 31/545; A61K 45/06; C07D 501/00; C07D 501/04; C07D 501/06 (2017.01)

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)

See Search History document

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

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/0104513 A1 (THORNTON) 05 June 2003 (05.06.2003) entire document	1
Y	OHNMACHT et al. Antimalarials. 7. Bis(trifluoromethyl)-alpha-(2-piperidyl)-4-quinolinemethanols. Journal of Medicinal Chemistry, 14(10), 926-928, 1971. [retrieved on 24 April 2017]. Retrieved from the Internet: <http://pubs.acs.org/doi/abs/10.1021/jm00292a008> entire document	1
A	US 2014/0080810 A1 (EXELISIS INC) 20 March 2014 (20.03.2014) entire document	1
A	PUBCHEM, Substance Record for SID 132339749. Create Date: 2012-01-24. [retrieved on 28 February 2017]. Retrieved from the Internet: <https://pubchem.ncbi.nlm.nih.gov/substance/132339749>. entire document	1
A	PUBCHEM, Substance Record for SID 75721506. Create Date: 2009-06-11. [retrieved on 28 February 2017]. Retrieved from the Internet: <https://pubchem.ncbi.nlm.nih.gov/substance/75721506>. entire document	1



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

24 April 2017

Date of mailing of the international search report

22 MAY 2017

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/016378

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
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:
3. ☒ Claims Nos.: 4-7, 13-16, 23-56, 61-63, 69-72
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:

See Extra Sheet

Claim 1 has been analyzed subject to the restriction that the claim reads on a composition comprising an antibiotic, wherein the antibiotic is cefoxitin, and a compound of formula (I), or a pharmaceutically acceptable salt thereof; wherein X is OR; R is hydrogen; R5 is C1 fluoroalkyl, wherein the C1 fluoroalkyl is trifluoromethyl; R6, R7 and R8 is each independently hydrogen.

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. ☐ 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 claims Nos.:
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 claims Nos.:
1

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.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2017/016378

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3, 8-12, 17-22, 57-60, 64-68, and 73-86 are drawn to compositions comprising an antibiotic and a compound of formula (I) and compounds of formula (VI).

The first invention of Group I+ is restricted based on the proviso that at least one of R5, R6, R7 and R8 is C1-6 fluoroalkyl or C1-6 fluoroalkoxy; and is restricted to a composition comprising an antibiotic, wherein the antibiotic is cefoxitin, and a compound of formula (I), or a pharmaceutically acceptable salt thereof; wherein X is OR; R is hydrogen; R5 is C1 fluoroalkyl, wherein the C1 fluoroalkyl is trifluoromethyl; R6, R7 and R8 is each independently hydrogen; and compounds of formula (VI). It is believed that claim 1 reads on this first named invention and thus this claim will be searched without fee to the extent that it reads on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a composition comprising an antibiotic, wherein the antibiotic is cefoxitin, and a compound of formula (I), or a pharmaceutically acceptable salt thereof; wherein X is OR; R is hydrogen; R5 is C1 fluoroalkyl, wherein the C1 fluoroalkyl is trifluoromethyl; R6 is C1 alkyl; R7 and R8 is each independently hydrogen; and compounds of formula (VI). Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the antibiotic agent and compound variables X, R5, R6, R7, and R8.

The Groups I+ share the technical features of a composition comprising an antibiotic and a compound of formula (I), or a pharmaceutically acceptable salt thereof; and a compound of formula (VI), or a pharmaceutically acceptable salt thereof. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2014/0080810 A1 to Exelixis Inc. teaches a composition (Para. [0346]) comprising an antibiotic (Claims 1, and 82; Paras. [0055], [0350], [0351], [0355], and [0488]) and a compound having the core structure of formula (I) (Table 1, Pg. 72, Compound; 99, ...see shown structure...).

Additionally, Substance Record for SID 132339749 to PubChem teaches a compound of formula (I) wherein X is NHR; R is hydrogen; R5 is hydrogen, R6 is C1 fluoroalkoxy, R7 and R8 are each independently hydrogen (Pg. 3; ...see shown structure...).

Additionally, Substance Record for SID 75721506 to PubChem teaches a compound of formula (VI): wherein R is R1-R2-(R3)_n; R1 is aryl; R2 is absent; each R3 is absent; R5, R6, R7 and R8 are independently hydrogen; and n is 0 (Pg. 3; ...see shown structure...).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.