Title

Antibiotic compounds and compositions, and methods of screening thereof and uses for treating microbial infections

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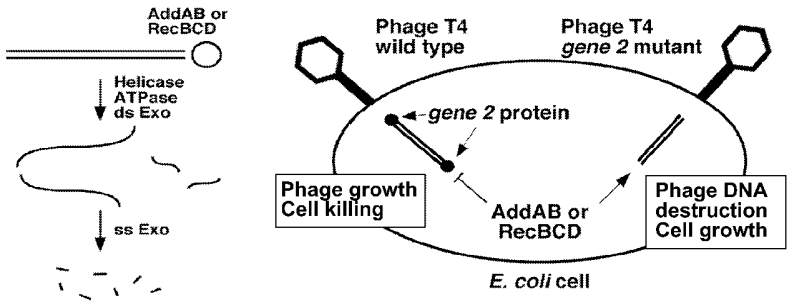

(57) Abstract: Disclosed herein are compounds and methods for inhibiting bacterial DNA repair enzymes, including AddAB and RecBCD helicase-nucleases. Pharmaceutical compositions and methods for treating a subject with an antibacterial agent are also disclosed herein.

WO 2013/142628 A2

ANTIBIOTIC COMPOUNDS AND COMPOSITIONS, AND METHODS FOR IDENTIFICATION THEREOF

Technical Field

[0001] The invention relates to compounds and compositions that inhibit bacterial DNA helicase, nuclease, or helicase-nuclease complex enzymes. In certain embodiments, in addition to inhibiting bacterial helicase, compounds and compositions described herein exhibit a dual functionality and inhibit bacterial DNA gyrase enzymes as well. Methods for identifying and using compounds and compositions described herein are also provided.

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Brief Description of the Drawings

[0003] Figure 1 describes the general principle of the cell-based screen for AddAB and RecBCD inhibitors, according to an embodiment of the present disclosure. *Left:* Activities of RecBCD and AddAB helicase-nucleases. Both enzymes (open circle) are active on linear duplex DNA (double lines). ds Exonuclease activity involves a combination of ATP-dependent DNA unwinding and endonucleolytic cuts. ss DNA intermediates are digested to short TCA-soluble oligonucleotides by the ss exonuclease activity. *Right:* RecBCD or AddAB nuclease activity blocks the growth of phage T4 *gene 2* mutants. Upon injection into *E. coli* cells, wild-type T4 DNA is

protected from AddAB and RecBCD nucleases by the *gene 2* protein bound to the linear duplex DNA ends in the virion; phages grow and the cells are killed. Unprotected T4 *gene 2* mutant DNA is digested by the nucleases; cells grow. Inhibition of AddAB or RecBCD is detected by lack of cell growth after T4 *gene 2* mutant infection.

[0004] Figure 2 describes how T4 *gene 2* mutant phage prevent the growth of *E. coli* lacking RecBCD enzyme but not growth of wild type, according to an embodiment of the present disclosure. *E. coli* strain V66 (*recBCD*$^+$; open symbols) and strain V67 (*recB21*; closed symbols) were infected with T4 *gene 2* triple non-nonsense mutant phage (MOI = 0.01; circles) or not (squares). Cultures were 0.1 ml in a 96-well plate, which was shaken at 37°C in an incubated plate reader. Each data point is the mean of 24 wells; SEM is within the size of the symbols. Similar results were found with *E. coli* expressing *H. pylori* AddAB.

[0005] Figure 3 shows general structures of five classes of AddAB and RecBCD inhibitors identified by screening, according to various embodiments of the present disclosure.

[0006] Figure 4 describes the inhibition of *H. pylori* AddAB and *E. coli* RecBCD nuclease activities by various compounds identified in the primary screen, according to embodiments of the present disclosure. ds Exonuclease activity of AddAB (filled circles) and RecBCD (open squares) was measured in the presence of the indicated concentration of compound and expressed as a percent of the activity in the absence of compound. Curves were fit by GraphPad software using the four-parameter logistic nonlinear regression model. For Compounds 1 and 4, the data are means +/- SEM with n = 3 or 4; for the other compounds, data are from one experiment.

[0007] Figure 5 describes the inhibition of *E. coli* RecBCD unwinding and Chi cutting activities by various compounds identified in the primary screen, according to embodiments of the present disclosure. DNA unwinding and cutting at Chi hotspots by RecBCD enzyme was assayed in the presence of the indicated concentration of compound. Unwinding is indicated by the amount of ss DNA and Chi cutting by the amount of Chi-dependent 1.46 kb ss DNA fragment ("Chi") produced from the 4.36 kb ds DNA substrate.

[0008] Figure 6 describes the inhibition of *H. pylori* AddAB and *E. coli* RecBCD nuclease activities by derivatives of Compounds 1 and 4, according to embodiments of the present disclosure. ds Exonuclease activity was measured in the presence of the indicated concentration of compound and expressed as a percent of the activity in the absence of compound.

[0009] Figure 7 describes the inhibition of *E. coli* Hfr recombination by selected compounds, according to embodiments of the present disclosure. The frequency of His^+ Str^R recombinants in matings between strains V66 (F^- *recBCD*$^+$ *hisG4 rpsL31*) and V1306 (Hfr PO44 *rpsL*$^+$ *his*$^+$) in the presence of compound (the concentration in μM as indicated for Compound 7 applies to all compounds) is expressed as a fraction of that in the absence of compound (9.3 % per viable Hfr cell). Data are from one experiment; similar results were obtained in two others.

[0010] Figure 8 describes the inhibition of phage λ recombination by selected compounds, according to embodiments of the present disclosure. The mean frequency of J^+ R^+ recombinants in λ crosses (1081 x 1082 and 1083 x 1084) in strain V66 in the presence of the indicated compound (concentration in μM as indicated for Compound 2 applies to all compounds) is expressed as a fraction of that without compound (6.9 ± 0.25 %; n = 4).

[0011] Figure 9 describes the inhibition of *E. coli* RecBCD nuclease, unwinding, and Chi cutting activities by compounds of structural class E, according to embodiments of the present disclosure. ds Exonuclease activity (*left panel*) and unwinding and Chi cutting activities (*right panel*) were measured in the presence of the indicated concentration of compound. ds Exonuclease activity is expressed as a percent of the activity in the absence of compound. Unwinding is indicated by the amount of ss DNA, and Chi cutting by the amount of Chi-dependent ss DNA fragment ("Chi").

[0012] Figure 10 shows that the AddAB DNA unwinding activity is not altered by certain compounds, according to embodiments of the present disclosure. DNA unwinding by AddAB enzyme was assayed in the presence of compound (50 μM). Unwinding is indicated by the amount of ss DNA (heavy arrow).

[0013] Figure 11 shows a screen for active derivatives of Compounds 1 and 4, according to embodiments of the present disclosure. *E. coli* strain V66 (*recBCD*$^+$) or strain V67 (*recB21*; RecBCD$^-$) (*left panels*) or strain V3065 (*addAB*$^+$) or strain V3069 (empty vector control; AddAB$^-$) (*right panels*) in the presence of the indicated compound (50 μM) were infected with T4 *gene 2* triple nonsense mutant (grey bar; MOI = 0.01) or not (black bar), and the optical density measured after ~20 h of incubation. Data are the mean and SEM of 4 wells in 2 independent experiments.

[0014] Figure 12 describes the inhibition of *H. pylori* AddAB and *E. coli* RecBCD nuclease activities by derivatives of Compounds 1 and 4, according to embodiments of the present disclosure. ds Exonuclease activity was measured in the presence of the indicated concentration of compound and expressed as a percent of the activity in the absence of compound. A separate experiment with RecBCD and 50 μM of Compound 1 derivatives showed the same pattern.

[0015] Figure 13 describes the inhibition of *E. coli* Hfr recombination by derivatives of Compounds 1 and 4, according to embodiments of the present disclosure. The frequency of His^+ Str^R recombinants in matings between strains V66 (F^- *recBCD*$^+$ *hisG4 rpsL31*) and V1306 (Hfr PO44 *rpsL*$^+$ *his*$^+$) in the presence of compound is expressed as a fraction of that in the absence of compound (4.4% per viable Hfr cell).

[0016] Figure 14 describes the inhibition of *E. coli* RecBCD nuclease activity by compounds of structural class E, according to embodiments of the present disclosure. ds Exonuclease activity was measured in the presence of compound (100 μM) and expressed as a percent of the activity in the absence of compound.

[0017] Figure 15 shows the minimum concentration of Compound 50 and norfloxacin required to inhibit the growth of *E. coli* strain V66 (*recBCD*$^+$) as measured by optical density. In triplicate, 100 μl of cells from an actively growing culture with the indicated titer (cfu/ml) were seeded into a 96-well plate. Compound was added to the indicated concentration, and the plate was incubated at 37°C for 18 hours. The reported optical densities are the means of 3-well sets. The MIC of Compound 50 is 1 μM, and that of norfloxacin 0.25 μM. "Uninoc" represents an uninoculated control.

[0018] Figure 16 shows the inhibition of *E. coli* growth by Compound 50 or norfloxacin. An overnight culture of strain V66 (*recBCD*$^+$) was diluted to 1 x 10^6 cells per ml and added to wells of a microtiter plate. After 1 hour of incubation at 37°C, norfloxacin or Compound 50 was added to the concentration indicated. The optical density was measured at the times indicated. Each data point represents the mean optical density of 3 wells; the values differed by less than 1%. At the end of the incubation period the number of viable cells in each 3-well pool was determined.

Except for the untreated control (1.4 x 10^9 colony forming units per ml), all cultures contained <200 colony forming units per ml.

[0019] Figure 17 shows that Compound 50 inhibits *E. coli* recombination in an Hfr cross, as measured by the relative frequency of His^+ Str^R recombinants in a cross between strain V66 (*hisG4 rpsL31*) and V1306 (Hfr PO44 *rpsL*$^+$ *hisG*$^+$). Cells were treated with the indicated concentration of compound for 45 minutes prior to the cross, made by mixing V66 and V1306 at a ratio of 10:1, incubating 30 min, vortexing, and plating for His^+ Str^R recombinants. Data are from a single experiment and are expressed relative to the untreated control, which had 12.4% recombinants per viable Hfr donor. The viability of V66 in the presence and absence of compound was indistinguishable.

[0020] Figure 18 shows a comparison of Compounds 1, 2, 50 and 51 in their inhibition of *E. coli* RecBCD ds exonuclease activity assayed as in Figure 12. Compounds 1, 50, and 51 inhibit more strongly than norfloxacin.

[0021] Figure 19 shows the effect of Compound 1 on the ciprofloxacin sensitization of an *E.coli* V66 wild type strain.

[0022] Figure 20 shows a dose response study of Compound 1 in the inhibition of *E. coli* RecBCD helicase unwinding activity.

[0023] Figure 21 shows a dose response study of Compound 50 in the inhibition of *E. coli* RecBCD and *H. pylori* AddAB nucleases.

[0024] Figure 22 shows a dose response study of the inhibition of *E. coli* RecBCD DNA unwinding activity and Chi cutting activity for Compound 50.

[0025] Figure 23 shows a dose response study of Compound 151 and Compound 148 for purified *Mycobacterium tuberculosis* AddAB enzyme.

[0026] Figure 24 shows the results of an *E. coli* p*recA::lacZ* reporter assay for the measurement of SOS induction by norfloxacin.

[0027] Figure 25 shows the results of an *E. coli* p*recA::lacZ* reporter assay for the measurement of SOS induction by H_2O_2 with or without compound 151.

[0028] Figure 26 shows the effects of AddAB inhibitors on the ability of *Helicobacter pylori* to colonize the stomach of mice.

[0029] Figure 27 shows the effects of RecBCD inhibitor compound 3 on the frequency of H_2O_2-induced mutation in *E. coli* strain V66.

[0030] Figure 28 shows the effects of RecBCD inhibitor compound 3 on the frequency of an H_2O_2-induced mutation to valine-resistance ($valine^R$) in *E. coli* strain V66. Data are the mean ± SEM (N = 16).

[0031] Figure 29 shows one embodiment of a method for the synthesis of compound 3.

Detailed Description

[0032] Disclosed herein are compounds and compositions useful as inhibitors of bacterial helicase-nuclease DNA repair enzymes. Compounds and compositions exhibiting a dual functionality are also described. In particular, in certain embodiments, the compounds and compositions detailed herein inhibit both bacterial DNA helicase-nuclease and bacterial DNA gyrase enzymes. Methods for indentifying compounds and compositions according to the present description are also provided. Moreover, because DNA helicase-nuclease and DNA gyrase functionality are important to bacterial infection in mammals, methods of inhibiting or treating bacterial infection are also disclosed.

I. Definitions

[0033] It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[0034] Unless defined otherwise, all technical and scientific terms used herein have the meanings that would be commonly understood by one of skill in the art. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Also, as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items. Furthermore, the term "about," as used herein when referring to a measurable value such as an amount of a compound, dose, time, temperature, and the like, is meant to encompass variations of 50%, 30%, 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[0035] It will be appreciated that the compounds, as described herein, may be substituted with substituents or functional moieties as described herein. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds, including but not limited to acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of the present disclosure, heteroatoms such as nitrogen may have hydrogen substituents

and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms.

[0036] "Alkyl" as used herein alone or as part of another group, refers to a straight, branched and/or cyclic hydrocarbon containing from 1 to 10 carbon atoms. In some embodiments, the alkyl employed in the invention contains 1 to 6 carbon atoms. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, cyclohexyl, 3-methylhexyl, 2,2- dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, and n-decyl.

[0037] "Lower alkyl" as used herein, is a subset of alkyl, in some embodiments preferred, and refers to a straight, branched and/or cyclic hydrocarbon group containing from 1 to 4 carbon atoms. Representative examples of lower alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, iso-butyl, and tert- butyl.

[0038] The term "alkyl" or "lower alkyl" is intended to include both substituted and unsubstituted alkyl or lower alkyl unless otherwise indicated and these groups may be substituted with groups selected from halo (e.g., haloalkyl), alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, aryl, arylalkyl, heterocyclo, heterocycloalkyl, hydroxyl, alkoxy (thereby creating a polyalkoxy such as polyethylene glycol), alkenyloxy, alkynyloxy, haloalkoxy, cycloalkoxy, cycloalkylalkyloxy, aryloxy, arylalkyloxy, heterocyclooxy, heterocyclolalkyloxy, aryl thioamido, haloalkylaryl thioamido, aryl amido, haloalkylaryl amido mercapto, alkyl-$S(O)_m$, haloalkyl- $S(O)_m$, alkenyl-$S(O)_m$, alkynyl- $S(O)_m$, cycloalkyl-$S(O)_m$, cycloalkylalkyl-$S(O)_m$, aryl-$S(O)_m$, arylalkyl-$S(O)_m$, heterocyclo-$S(O)_m$, heterocycloalkyl-$S(O)_m$, amino, carboxy, alkylamino, alkenylamino, alkynylamino, haloalkylamino, cycloalkylamino, cycloalkylalkylamino,

arylamino, arylalkylamino, heterocycloamino, heterocycloalkylamino, disubstituted-amino, acylamino, acyloxy, ester, amide, sulfonamide, urea, alkoxyacylamino, aminoacyloxy, nitro or cyano, where m = 0, 1, 2 or 3.

[0039] "Alkenyl" as used herein alone or as part of another group, refers to a straight, branched and/or cyclic chain hydrocarbon containing from 1 to 10 carbon atoms (or in lower alkenyl 1 to 4 carbon atoms) which include 1 to 4 double bonds in the normal chain. In some embodiments, the alkenyl employed in the invention contains 1 to 6 carbon atoms. Representative examples of alkenyl include, but are not limited to, vinyl, 2-propenyl, 3-butenyl, 2-butenyl, 4-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 2,4-heptadiene, and the like. The term "alkenyl" or "lower alkenyl" is intended to include both substituted and unsubstituted alkenyl and lower alkenyl unless otherwise indicated and these groups may be substituted with groups as described in connection with alkyl and lower alkyl above. Cycloalkenyl refers to a cyclic alkenyl group.

[0040] "Alkynyl" as used herein alone or as part of another group, refers to a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms (or in lower alkynyl 1 to 4 carbon atoms) which include 1 to 4 triple bonds in the normal chain. In some embodiments, the alkynyl employed in the invention contain 1 to 6 carbon atoms. Representative examples of alkynyl include, but are not limited to, 2-propynyl, 3-butynyl, 2- butynyl, 4-pentynyl, and 3- pentynyl. The term "alkynyl" or "lower alkynyl" is intended to include both substituted and unsubstituted alkynyl and lower alkynyl unless otherwise indicated and these groups may be substituted with the same groups as set forth in connection with alkyl and lower alkyl above.

[0041] "Cycloalkyl", as used herein alone or as part of another group, refers to groups having 3 to 10 carbon atoms. In some embodiments, the cycloalkyl

employed in the invention has 3 to 8 carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or hetercyclic moieties, may optionally be substituted with the same groups as set forth in connection with alkyl and lower alkyl above. The term “alkyl” includes cycloalkyl. The cycloalkyl may be a bicycloalkyl.

[0042] "Heterocycloalkyl" or "heterocycle", as used herein alone or as part of another group, refers to a non-aromatic 3-, 4-, 5-, 6-, 7-, or 8- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and four heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) the nitrogen and sulfur heteroatoms may be optionally oxidized, (ii) the nitrogen heteroatom may optionally be quaternized, and (iii) may form a spiro ring or be fused with a cycloalkyl, aryl, heterocyclic ring, benzene or a heteroaromatic ring. In some embodiments, the heterocycle employed in the invention has 3 to 10 carbon atoms. For example, the heterocycle may be a 4-(2-halophenylcarbamothioyl)piperazin-1-yl.

[0043] Representative heterocycles include, but are not limited to, 1,4-dioxa-8-azaspiro[4,5]decane, morpholine, azetidine, azepine, aziridine, diazepine, 1,3-dioxolane, dioxane, dithiane, furan, imidazole, imidazoline, imidazolidine, isothiazole, isothiazoline, isothiazolidine, isoxazole, isoxazoline, isoxazolidine, morpholine, oxadiazole, oxadiazoline, oxadiazolidine, oxazole, oxazoline, oxazolidine, piperazine, piperidine, pyran, pyrazine, pyrazole, pyrazolone, pyrazolidine, pyridine, pyrimidine, pyridazine, pyrrole, pyrroline, pyrrolidine, tetrahydrofuran, tetrahydrothiophene, tetrazine, tetrazole, thiadiazole, thiadiazoline, thiadiazolidine, thiazole, thiazoline, thiazolidine, thiophene, thiomorpholine, thiomorpholine sulfone, thiopyran, triazine,

triazole, trithiane, benzimidazole, benzothiazole, benzothiadiazole, benzothiophene, benzoxadiazole, benzoxazole, benzofliran, benzopyran, benzothiopyran, benzodioxine, 1,3-benzodioxole, cinnoline, indazole, indole, indoline, indolizine, naphthyridine, isobenzofuran, isobenzothiophene, isoindole, isoindoline, isoquinoline, phthalazine, purine, pyranopyridine, quinoline, quinolizine, quinoxaline, quinazoline, tetrahydroisoquinoline, tetrahydroquinoline, and thiopyranopyridine. These rings include quaternized derivatives thereof and may be optionally substituted with the same groups as set forth in connection with alkyl and lower alkyl above.

[0044] "Aryl" as used herein alone or as part of another group, refers to a monocyclic carbocyclic ring system or a bicyclic carbocyclic fused ring system having one or more aromatic rings. In some embodiments, the aryl employed in the invention has 3 to 14 carbon atoms.

[0045] Representative examples of aryl include azulenyl, indanyl, indenyl, naphthyl, phenyl, and tetrahydronaphthyl. The term "aryl" is intended to include both substituted and unsubstituted aryl unless otherwise indicated, and these groups may be optionally substituted with the same groups as set forth in connection with alkyl and lower alkyl above.

[0046] "Aryl alkyl" as used herein alone or as part of another groups refers to an aryl group, as defined herein, appended to the parent molecular moiety through an alkyl group, as defined herein. Representative examples of aryl alkyl include, but are not limited to, benzyl, 2- phenyl ethyl, 3-phenylpropyl, and 2-naphth-2-ylethyl.

[0047] "Heteroaryl" as used herein alone or as part of another group, refers to a cyclic or bicyclic, aromatic hydrocarbon in which one or more carbon atoms have been replaced with heteroatoms such as O, N, and S. If the heteroaryl group

contains more than one heteroatom, the heteroatoms may be the same or different. In some embodiments, the heteroaryl employed in the invention have 3 to 14 carbon atoms. Examples of heteroaryl groups include pyridyl, pyrimidinyl, imidazolyl, thienyl, furyl, pyrazinyl, pyrrolyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, indolyl, isoindolyl, indolizinyl, triazolyl, pyridazinyl, indazolyl, purinyl, quinolizinyl, isoquinolyl, quinolyl, quinolinyl, phthalazinyl, naphthyridinyl, quinoxalinyl, isothiazolyl, and benzo[b]thienyl. In some embodiments, heteroaryl groups are five and six membered rings and contain from one to three heteroatoms independently selected from O, N, and S. The heteroaryl group, including each heteroatom, can be unsubstituted or substituted with from 1 to 4 substituents, as chemically feasible. For example, the heteroatom N or S may be substituted with one or two oxo groups, which may be shown as =O. For example, the heteroaryl group may be a 4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

[0048] "Alkoxy" (or "alkyloxy"), or "thioalkyl", as used herein alone or as part of another group, refers to an alkyl or lower alkyl group appended to the parent molecular moiety through an oxygen or sulfur atom. In some embodiments, the alkoxy or thioalkyl group contains 1-10 carbon atoms. In other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 carbon atoms. In still other embodiments, the alkyl group contains 1-6 carbon atoms. In yet other embodiments, the alkyl group contains 1-4 carbon atoms. Representative examples, of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert- butoxy, neopentoxy and n-hexoxy. Representative examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, and n-butylthio.

[0049] "Halo" as used herein alone or as part of another group, refers to any suitable halogen, including -F, -Cl, -Br, and -I.

[0050] "Amine" or "amino group", as used herein alone or as part of another group, refers to the radical $-NH_2$. An "optionally substituted" amine refers to $-NH_2$ groups wherein none, one or two of the hydrogen(s) is replaced by a suitable substituent. Disubstituted amines may have substituents that are bridging, i.e., that form a heterocyclic ring structure that includes the amine nitrogen.

[0051] "Aminoalkyl group" is intended to mean the radical $-NHR_3$, where R_3 is an alkyl group.

[0052] "Haloalkyl", as used herein alone or as part of another group, refers to an alkyl group having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, and trifluoromethyl.

[0053] The terms “therapeutically useful” and “therapeutically effective” refer to a dose or amount of a compound or composition that causes a detectable change in biological or chemical activity, such as a detectable change in the inhibition of a bacterial helicase (e.g, a RecBCD and/or AddAB enzyme), a bacterial gyrase, and/or in bacterial growth. The terms "therapeutically useful" and "therapeutically effective" can designate an amount that maintains a desired physiological state, i.e., reduces or prevents significant decline and/or promotes improvement in the condition or disease of interest. For example, a therapeutically effective or useful amount of a compound or composition described herein would be an amount that inhibits, slows or eliminates growth of bacteria in a subject. As is generally understood in the art, therapeutically effective dosages will vary depending on the administration routes, symptoms and body weight of the patient but also depending upon the compound being administered.

[0054] The terms "active" or "biologically active" or "biological activity" refer to a compound or composition capable of inhibiting a bacterial helicase (e.g, RecBCD and/or AddAB activity, either the helicase or the nuclease activity or a combination of both) and/or a bacterial gyrase, to affect growth of a bacterium, such as *E. coli*. In some embodiments, an active or biologically active compound or composition as described herein as may have an IC_{50} of less than about 100 micromol/liter (100 μM), less than about 50 micromol/liter (50 μM), less than about 10 micromol/liter (10 μM), or less than about 1 micromol/liter (1 μM). As used herein, the IC_{50} is the concentration (μM) of compound or composition that results in 50% inhibition of enzyme activity (e.g. for RecBCD, AddAB, or bacterial gyrase activity) or cell growth or other cellular activity (e.g. for bacterial viability or recombination studies).

[0055] Further, an active or biologically active agent or composition as described herein may be alternatively or additionally characterized as an agent or composition that that inhibits growth of bacteria in a subject. For example, an active or biologically active agent or composition as described herein may be characterized as an agent or composition that causes a greater than 2-fold change, greater than 5-fold change, greater than 10-fold change, greater than 15-fold change, and greater than 20-fold change in bacterial growth, as compared to growth free from the agent.

[0056] In further embodiments, an active or biologically active agent, compound, or composition as described herein may be characterized as an agent, compound, or composition that selectively inhibits a bacterial helicase, such as RecBCD and/or AddAB. In other embodiments, an active or biologically active agent, compound, or composition as described herein may be characterized as an agent, compound, or composition that selectively inhibits a bacterial gyrase. In still further embodiments, an active or biologically active agent, compound, or composition as described herein

may be characterized as a dual function agent, compound, or composition that selectively inhibits both a bacterial helicase, such as RecBCD and/or AddAB, and a bacterial gyrase. For example, an active or biologically active agent or compound or composition as described herein may have a selective inhibition greater than about 2-fold, about 5-fold, about 10-fold, about 15-fold, and about 20-fold of bacterial helicase, such as RecBCD and/or AddAB, and/or a bacterial gyrase where the bacteria is present in a subject. In such examples, an active or biologically active agent or compound or composition as described herein may be characterized as an agent or composition that is substantially non-toxic to the subjects' cells, such as mammalian cells.

[0057] For purposes of the present disclosure, the terms "antibiotic" and/or "antibacterial" includes diseases, disorders, and conditions that are linked to the presence of at least some bacteria in a subject. Such diseases include, but are not limited to, community or nosocomial acquired infections, bacteremias, bacterium-related cutaneous, gastrointestinal and respiratory conditions, botulism, cholera, E. coli infection, Legionellosis, listeriosis, Lyme disease, pathogenic bacterial diseases, rickttsioses, salmonellosis, tuberculosis and zoonotic bacterial diseases.

[0058] Bacteria which may be affected by compounds and compositions disclosed herein include, for example, bacterial infections by both Gram-positive and Gram-negative bacteria, such as Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Morganella morganii, Salmonella serotypes including Enteritidis, Typhimurium and Newport, Enterococci, Shigella dysenteriae, Yersinia enterocolitica, Acinetobacter calcoaceticus, Francisella tularensis, Legionella pneumophila, Helicobacter pylori, Neisseria meningitides, Neisseria gonorrhoeae, Campylobacter jejuni, Vibrio cholera, Pseudomonas aeruginosa, Streptococcus,

Staphylococcus, pneumococcus, Mycobacterium tuberculosis, Borrelia burgdorferi, Bordetella pertussis, Legionella pneumophila, Clostridium difficile, Bacillus anthracis, and Haemophilus influenzae.

[0059] Bacteria-associated diseases further include those which involve antibacterial drug resistance, such as Methicillin-resistant Staphylococcus aureus (MRSA) infection.

[0060] The term "pharmaceutically acceptable" refers to materials approved by a regulatory agency, such as by a regulatory agency of a Federal or a state government, or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, including humans.

[0061] In the context of the present description, the term "carrier" refers to a diluent, adjuvant, excipient, stabilizer, vehicle, or any combination thereof, with which an active compound as described herein may be combined to provide a pharmaceutical composition suitable for administration to a subject.

[0062] The term “subject” refers to refers to an animal, including humans, in which an active compound as described herein will be therapeutically useful (e.g., selectively inhibit bacterial growth). The subject may be a veterinary subject, including birds and livestock.

[0063] The term "pharmaceutically acceptable salt(s)" as used herein refers to a salt form of a compound permitting its use or formulation as a pharmaceutical and which retains the biological effectiveness of the free acid and base of the specified compound and that is not biologically or otherwise undesirable. Examples of such salts are described in Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wermuth, C.G. and Stahl, P.H. (eds.), Wiley- Verlag Helvetica Acta, Zurich, 2002.

[0064] Examples of pharmaceutically acceptable salts, without limitation, include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, or tartaric acids, and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, or procaine. Examples of salts also include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogen phosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrates, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-l,4-dioates, hexyne-l,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ-hydroxybutyrates, glycollates, tartrates, methanesulfonates, ethane sulfonates, propanesulfonates, toluenesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates. In some embodiments, a pharmaceutically acceptable salt includes sodium, potassium, calcium, ammonium, trialkylarylammonium and tetraalkylammonium salts.

[0065] As used herein, "treatment" of a disease, disorder, or infection refers to, but is not limited to, prevention, retardation and prophylaxis of the disease, disorder, or infection.

II. Compounds

[0066] The compounds described herein may be single function (i.e., inhibit one or more bacterial DNA helicase, nuclease, or helicase-nuclease complex or one or more bacterial DNA gyrase) or dual function (i.e., inhibit both one or more bacterial DNA helicase, nuclease, or helicase-nuclease complex while also inhibiting bacterial DNA gyrase). In specific embodiments, the compounds inhibit helicase enzymes selected from one or both of the RecBCD and AddAB families of helicase-nucleases. The AddAB and RecBCD helicase-nucleases are related enzymes prevalent among bacteria but not eukaryotes, and are instrumental in the repair of DNA double-strand breaks and in genetic recombination. The RecBCD class of enzymes and the closely related AddAB enzymes are bacterial helicase-nuclease complexes important for repair of broken DNA and for genetic recombination. Dillingham, M. S.; Kowalczykowski, S. C., *Microbiol Mol Biol Rev* **2008,** *72* (4), 642-71; Smith, G. R., *Annu. Rev. Genet.* **2001,** *35*, 243-274; Smith, G. R. , *Microbiol Mol Biol Rev* **2012**, 76, 217-228. Starting at a double-strand (ds) DNA end, these enzymes unwind DNA rapidly and highly processively while hydrolyzing ATP or another nucleoside triphosphate (Fig. 1). During unwinding, they also hydrolyze DNA by making endonucleolytic scissions at a rate dependent on the ratio of [ATP] to [Mg^{2+}], both of which are required for the helicase and nuclease activities. The AddAB and RecBCD enzymes are needed for successful bacterial infection of animals including mammals, and compounds and compositions described herein may be used as antibacterial agents. Several structural classes of inhibitors of the three-subunit *E. coli* RecBCD enzyme and the related two-subunit *Helicobacter pylori* AddAB enzyme are disclosed herein. Also disclosed herein are inhibitors of *Mycobacterium*

tuberculosis AddAB and RecBCD and inhibitors of *Mycobacterium smegmatis* AddAB and RecBCD. Moreover, the compounds described herein may be useful in further enzymatic, genetic, and physiological studies of these enzymes, both purified and in cells.

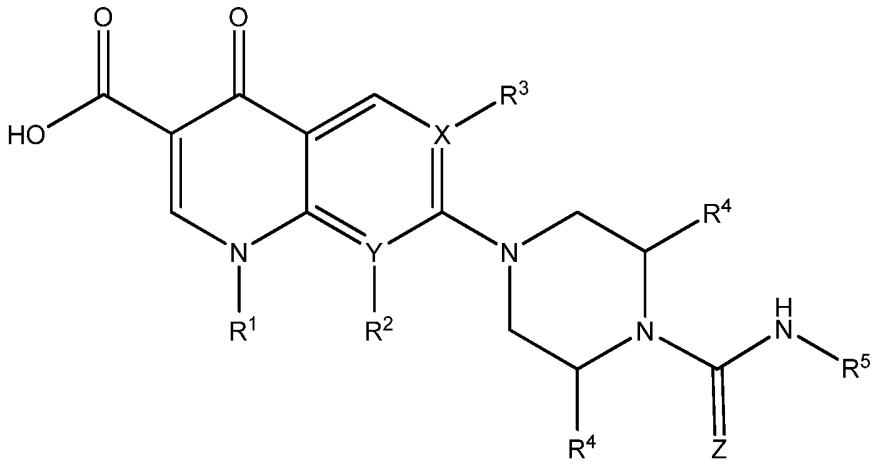
[0067] The RecBCD enzyme of *Escherichia coli* makes endonucleolytic scissions at especially high frequency at Chi sites (5' GCTGGTGG 3'), which as a consequence are hotspots of recombination. Ponticelli, A. S.; Schultz, D. W.; Taylor, A. F.; Smith, G. R., *Cell* **1985,** *41*, 145-151. The RecBCD and AddAB enzymes from other species similarly act at other short nucleotide sequences. Touzain, F.; Petit, M. A.; Schbath, S.; El Karoui, M., *Nat Rev Microbiol* **2011,** *9* (1), 15-26. The single-stranded (ss) DNA resulting from unwinding is a potent substrate for the enzymes' ATP-dependent ss nuclease, which, at least for the RecBCD enzyme of *E. coli*, produces a limit digest of primarily tetra- to hexanucleotides. Goldmark, P. J.; Linn, S., *J. Biol. Chem.* **1972,** *247*, 1849-1860. Because this class of enzymes is important for the repair of DNA double-strand (ds) breaks, mutants lacking them are deficient in infecting mammals. Without being bound by theory, this is likely because mammalian cells produce reactive oxygen species, such as hydrogen peroxide, that break the bacterial DNA upon infection. Buchmeier, N. A.; Lipps, C. J.; So, M. Y.; Heffron, F., *Mol Microbiol* **1993,** *7* (6), 933-6, *7*, 933–936; Buchmeier, N. A.; Libby, S. J.; Xu, Y.; Loewen, P. C.; Switala, J.; Guiney, D. G.; Fang, F. C., *J. Clin. Invest.* **1995,** *95* (3), 1047-53; Amundsen, S. K.; Fero, J.; Hansen, L. M.; Cromie, G. A.; Solnick, J. V.; Smith, G. R.; Salama, N. R., *Molec. Microb.* **2008,** *69*, 994-1007. Proper function of each subunit of RecBCD is required for normal nuclease activity, and small molecules that bind to or otherwise inhibit the functionality of one or more RecBCD subunits can inhibit the nuclease activity exhibited by the enzyme. In

AddAB, each subunit contains a nuclease domain; only AddA appears to have an active helicase domain, but its inactivation blocks all detectable nuclease activity (Kooistra, J.; Haijema, B. J.; Hesseling-Meinders, A.; Venema, G., *Molec. Microb.* ***1997,*** *23 (1), 13***7-49;** *A*mundsen, S. K.; Fero, J.; Salama, N. R.; Smith, G. R., *J. Biol. Chem.* **2009,** *284*, 16759-66; Haijema, B. J.; Meima, R.; Kooistra, J.; Venema, G., *J. Bacteriol.* **1996,** *178* (17), 5*130-7;* Sinha, K. M.; Unciuleac, M. C.; Glickman, M. S.; Shuman, S., *Genes & Development* **2009,** *23* (12)*, 1423-37).* Similar to RecBCD, each subunit of AddAB is required for normal nuclease activity, and small molecules that bind to or otherwise inhibit the functionality of one or more AddAB subunits can inhibit the nuclease activity exhibited by the enzyme, either directly or indirectly, and allow T4 *gene 2* mutant phage to grow, thereby blocking the growth of *E. coli.*

[0068] In further embodiments, compounds described herein inhibit bacterial DNA gyrase. For example, fluoroquinolone compounds capable of inhibiting DNA gyrase are detailed herein. In still other embodiments, the compounds described herein are capable of inhibiting both bacterial DNA helicase, such as for example a helicase selected from one or both of the RecBCD and AddAB families of helicases, and bacterial DNA gyrase. Such dual function (also referred to herein as "dual mechanism of action") compounds provide targeted inhibition of both helicase and gyrase enzymes within a single molecular moiety. Dual function compounds as described herein both (i) induce DNA damage through gyrase inhibition and (ii) block the repair of such damage, and thereby may provide significant additional functionality beyond single function actives providing helicase or gyrase inhibition alone. For example, dual function compounds described herein that exhibit activity against bacterial recombination and DNA repair proteins may not only serve as broad spectrum antibiotics, but may also act to combat antibiotic resistance by, for

example, reducing the rate of appearance of resistant mutants. Compounds according to the present description may also be co-administered with known antibiotics, such as, for example, known fluoroquinolones or other antibiotics that induce SOS response, as compounds providing a potent inhibitory effect on bacterial DNA helicase(s), such as RecBCD and/or AddAB, would be useful in treating microbial infections as a first line or augmentation therapy for treating both susceptible pathogens and reducing the emergence of drug resistant microorganisms.

[0069] In one embodiment, active compounds that inhibit bacterial helicase(s) are selected from a compound of structural class A (the "pyrimidopyridones"), according to Formula I:



Formula I

wherein R^1 is alkyl, aryl, or cycloalkyl;

R^2 is H, alkoxyl or halogen;

R^3 is H or halogen;

R^4 is H or alkyl;

R^5 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro;

X and Y are independently C or N; and

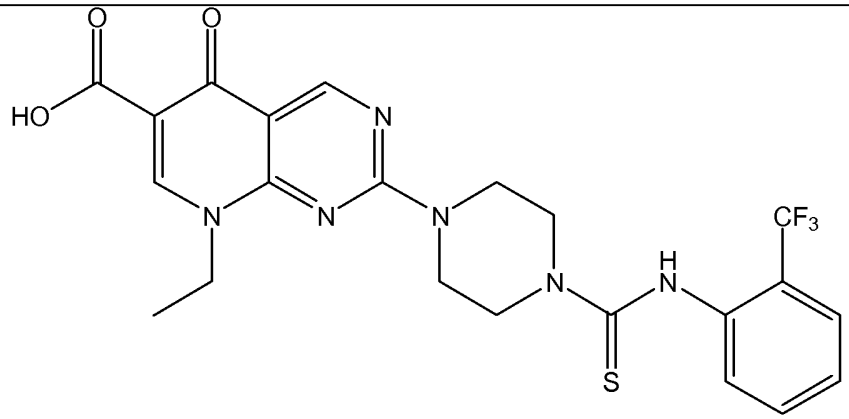
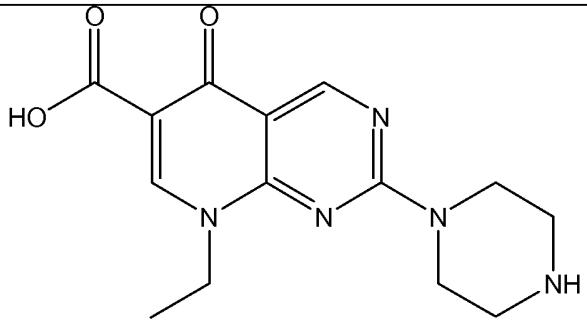
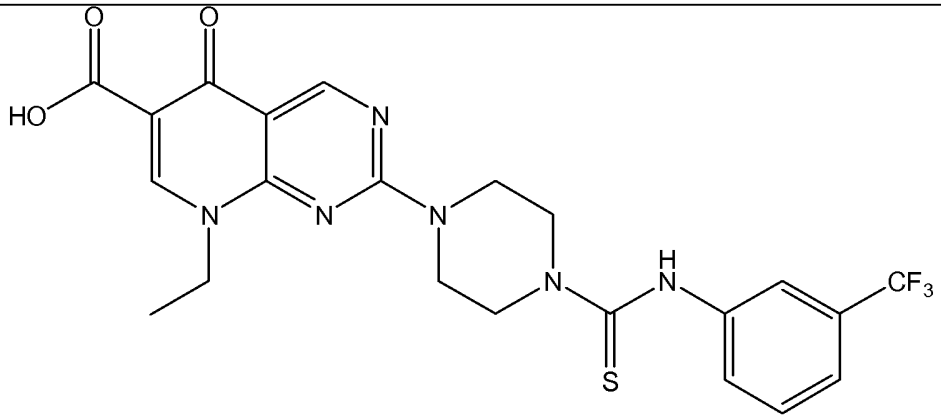
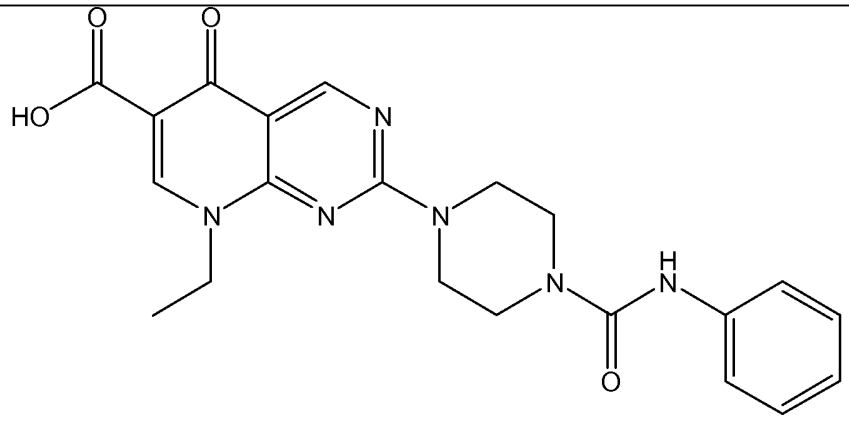
Z is O or S.

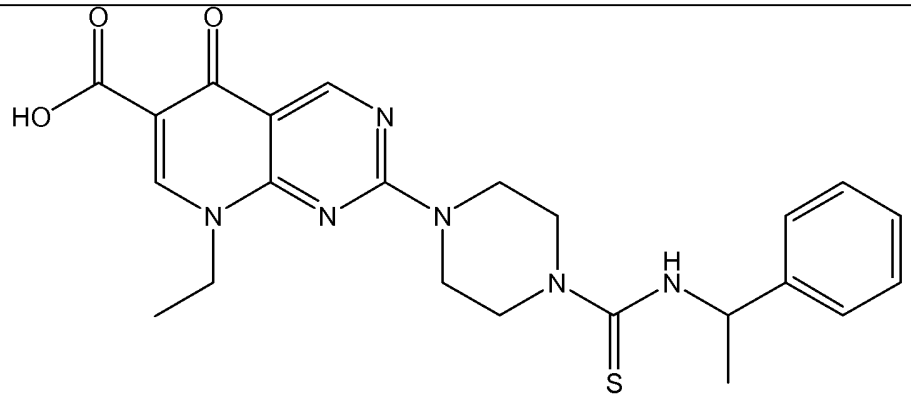
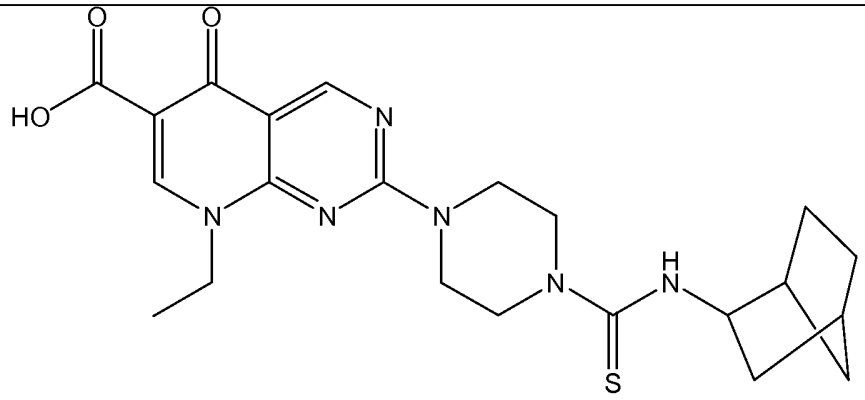
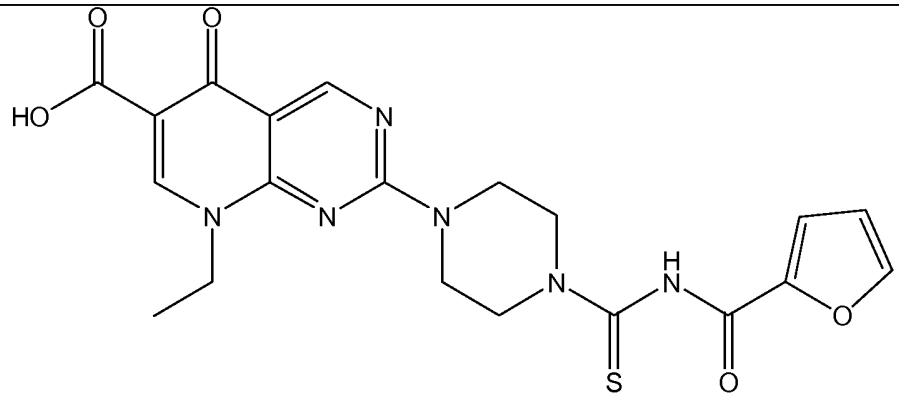
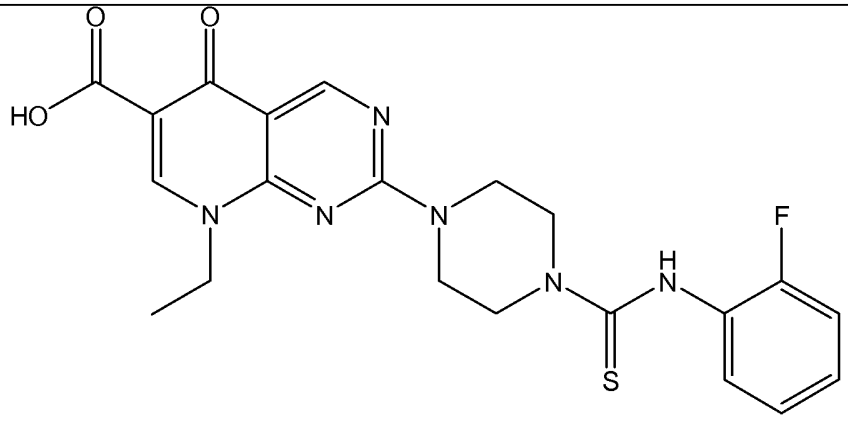
[0070] In particular embodiments of active compounds according to Formula I, R^1 is ethyl, X and Y are each N, R^4 is H, Z is S and R^5 is phenyl substituted with a CF_3 group. In an embodiment, the CF_3 group is in the ortho position. In an additional embodiment, the CF_3 group is in the meta position.

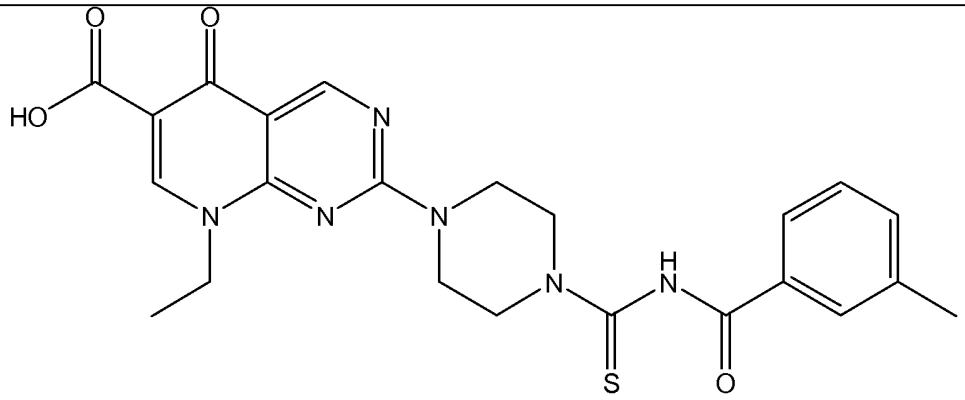
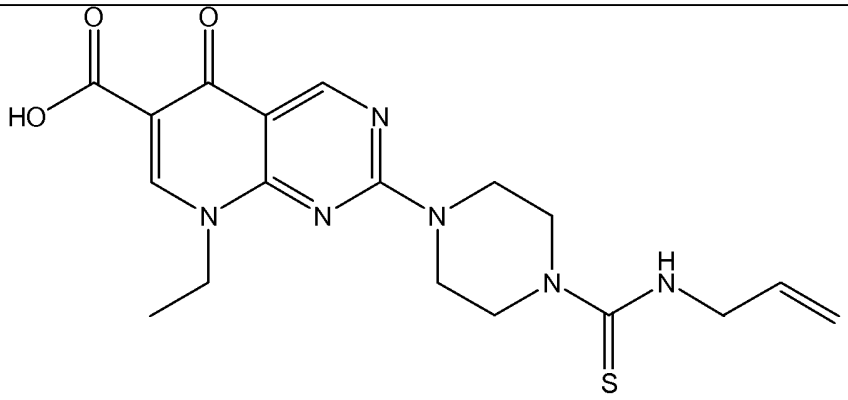
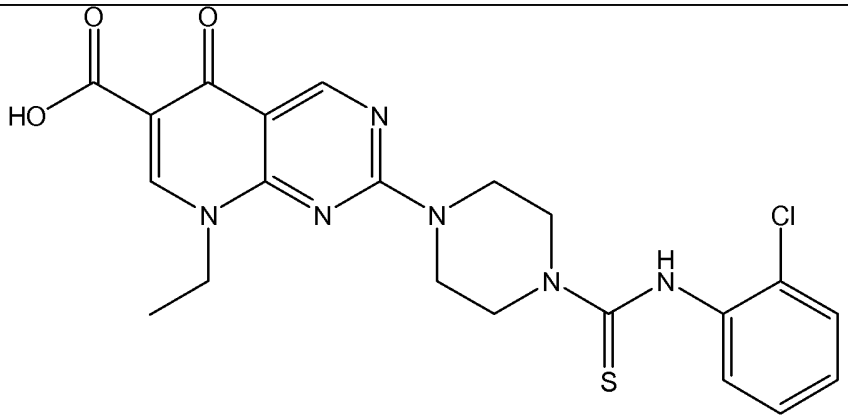
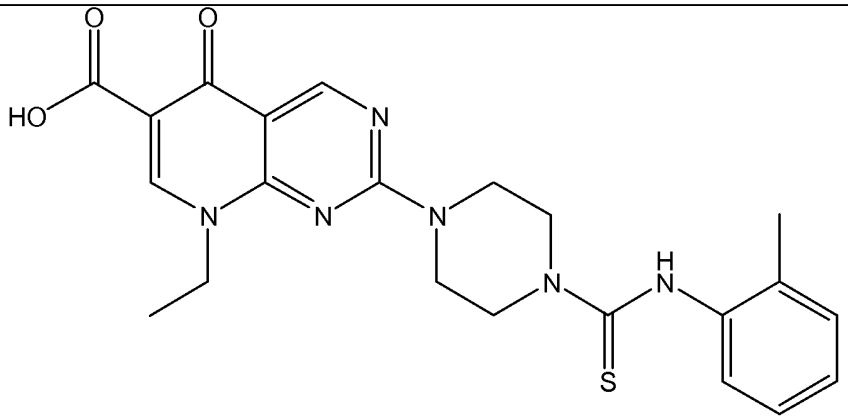
[0071] In certain embodiments, R^1 is ethyl, X and Y are each C, R^2 is hydrogen, R^3 is halogen, R^4 is H, Z is S and R^5 is phenyl substituted with a CF_3 group. In an embodiment, the CF_3 group is in the meta position.

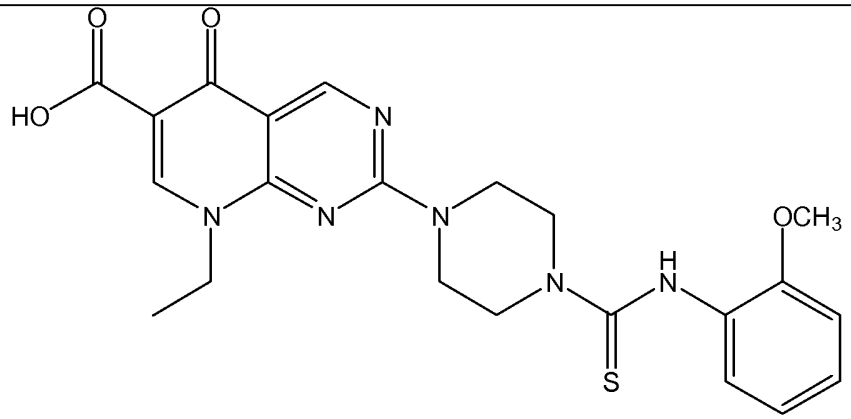
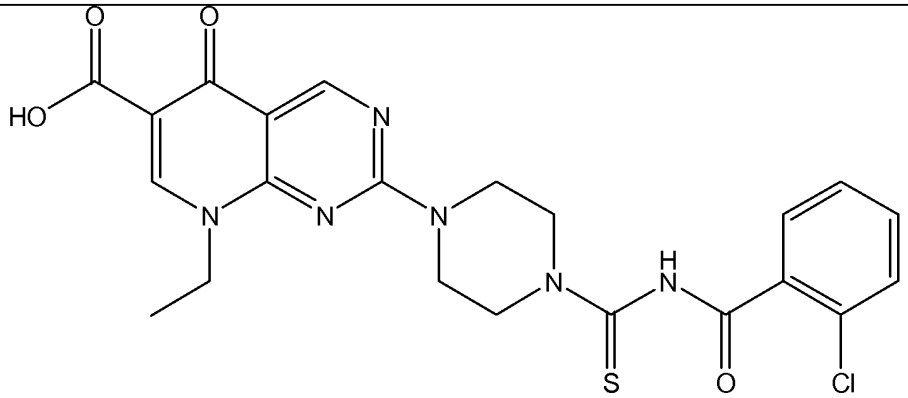
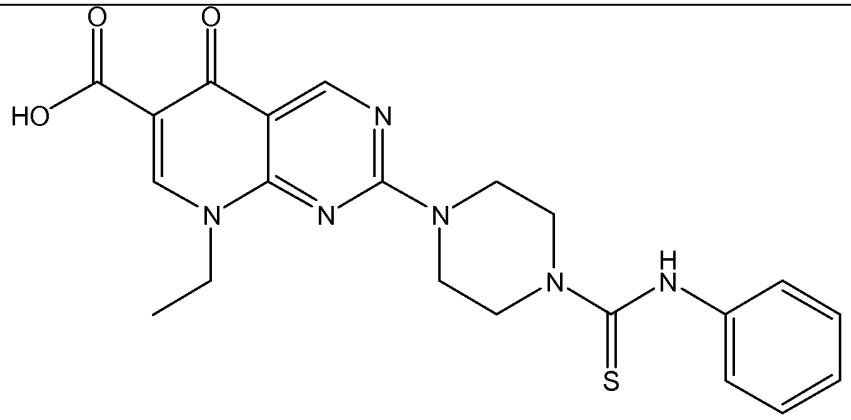
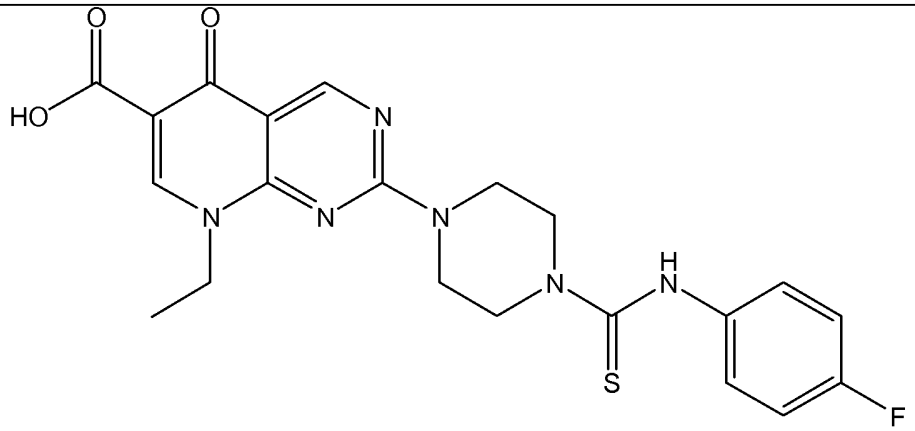
[0072] Compounds according to Formula I may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. Specific examples of compounds according to Formula I include the compounds in Table 1.

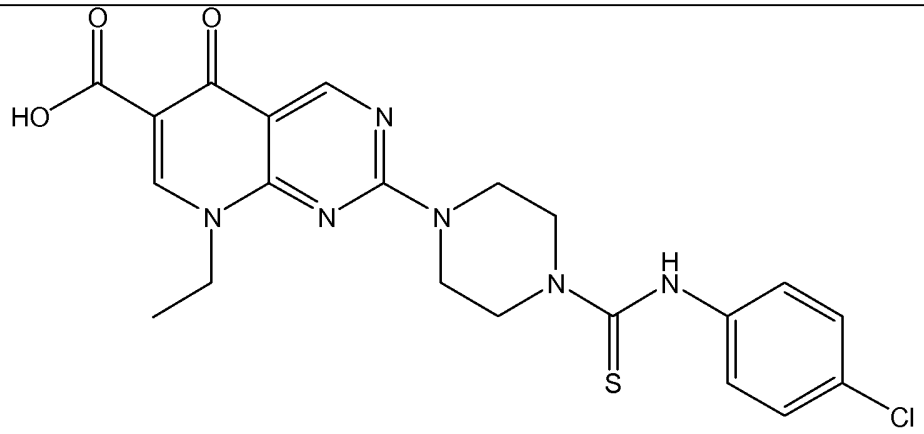
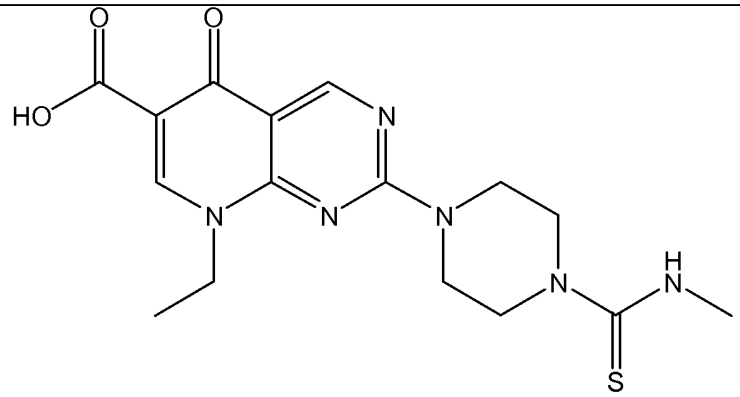
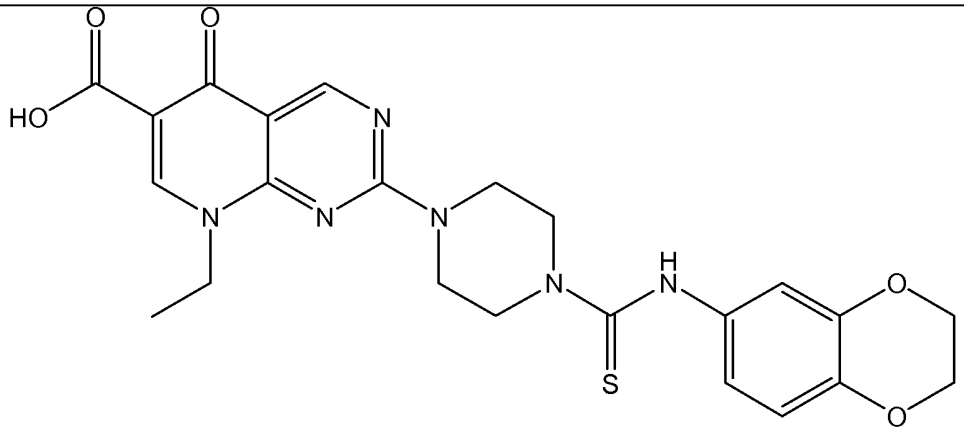
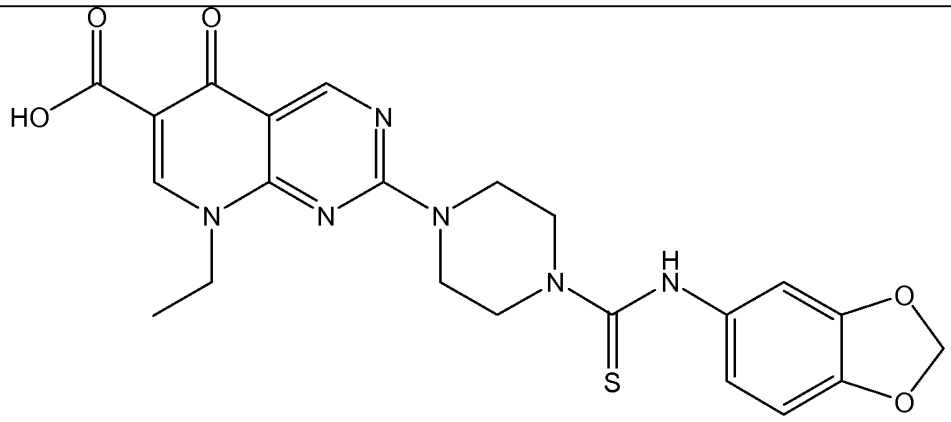
[0073] Table 1: compounds according to Formula I.

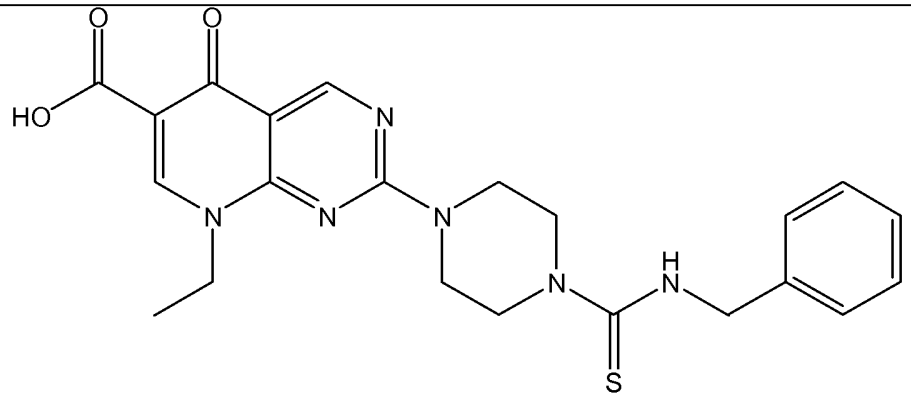
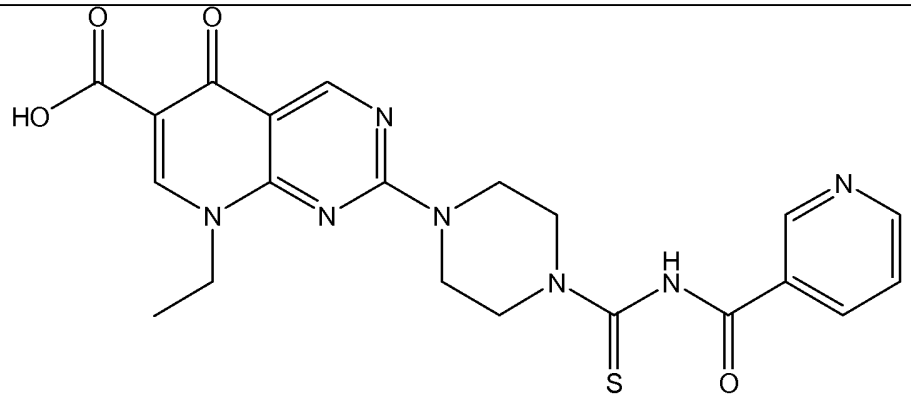
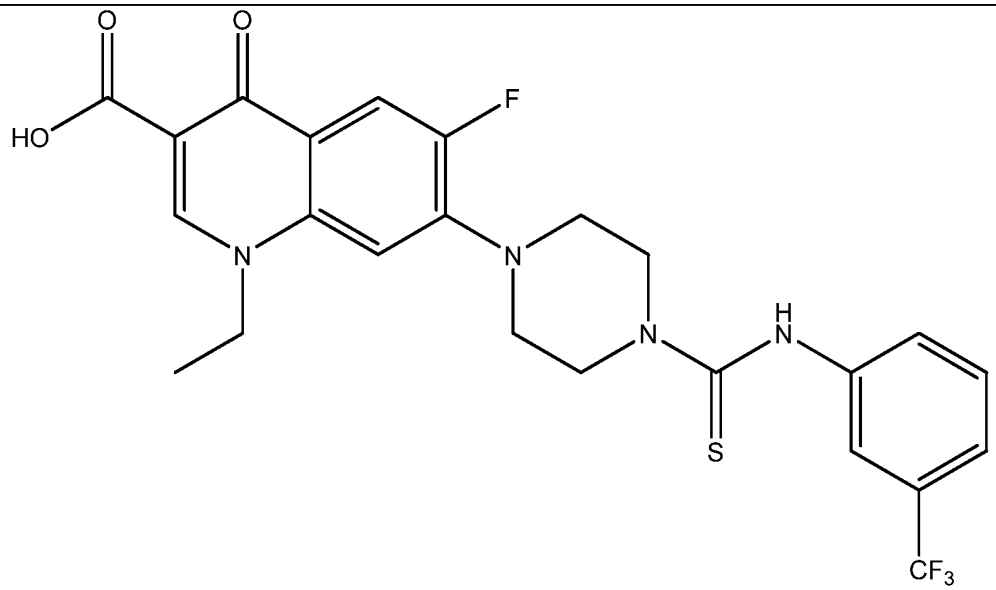
Compound 1	
Compound 2	
Compound 3	
Compound 19	

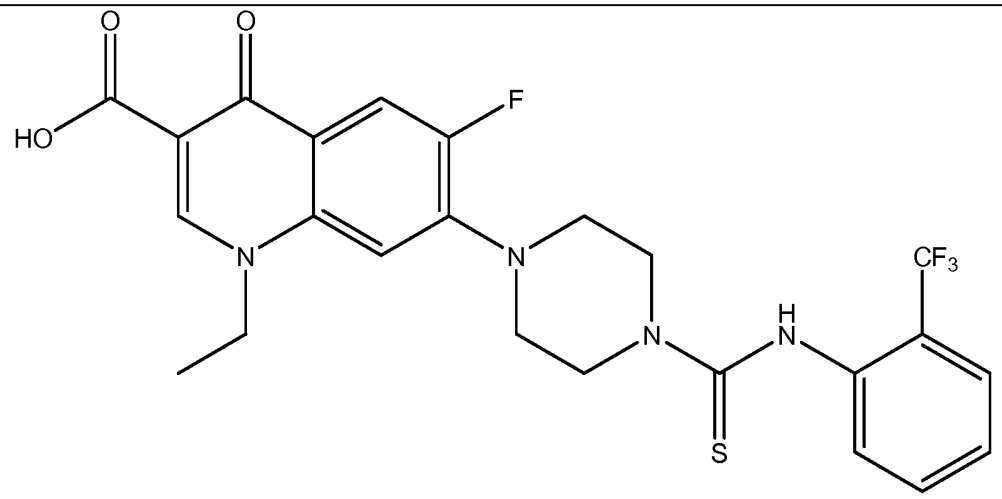
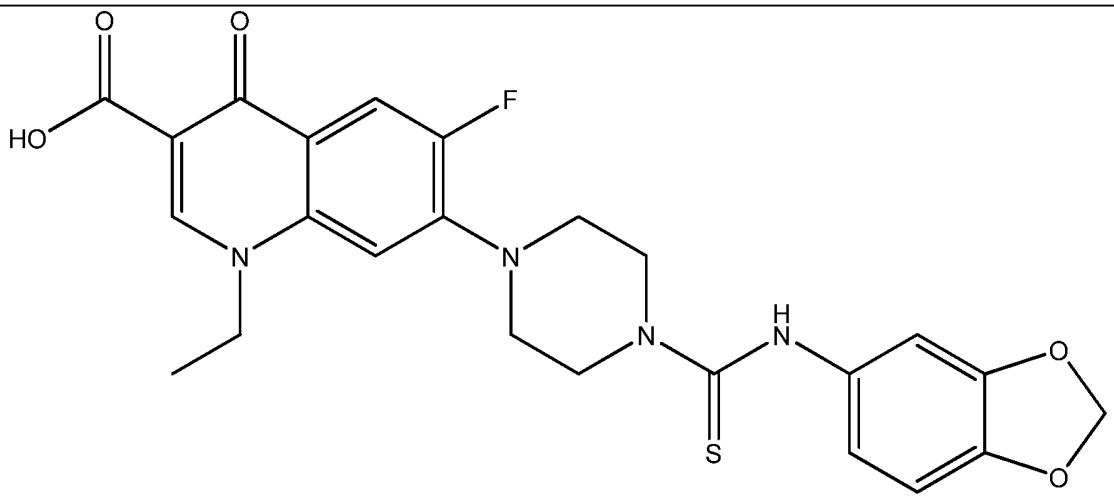
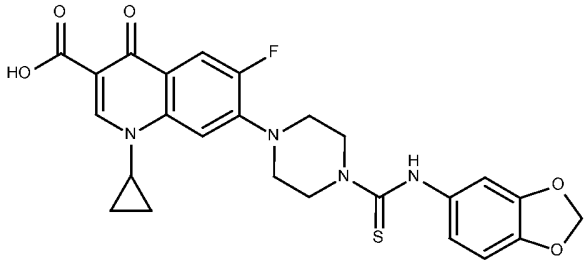
Compound 20	
Compound 21	
Compound 22	
Compound 23	

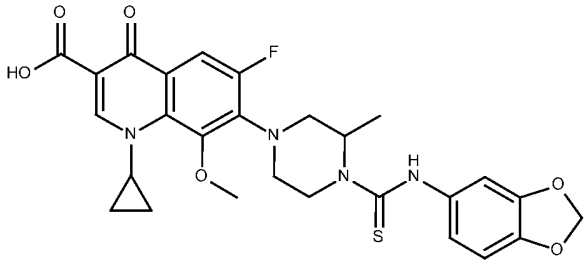
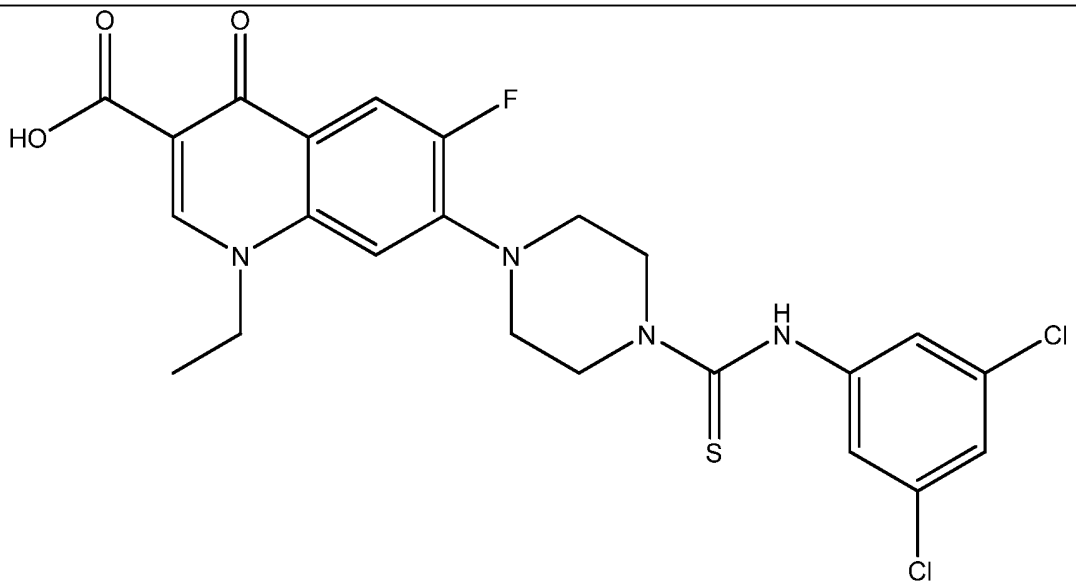
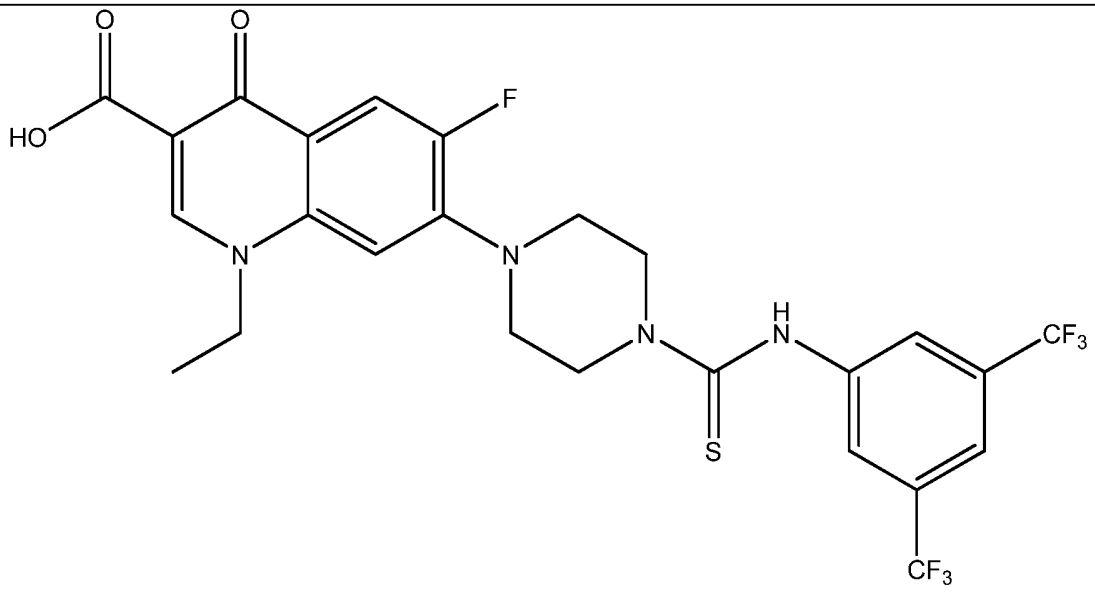
Compound 24	
Compound 25	
Compound 26	
Compound 27	

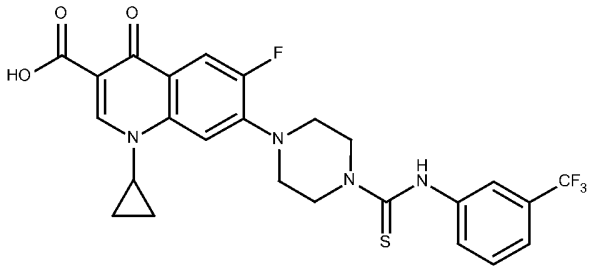
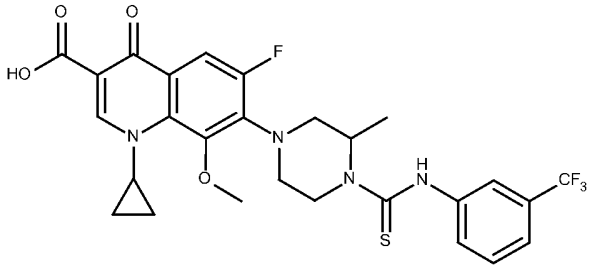
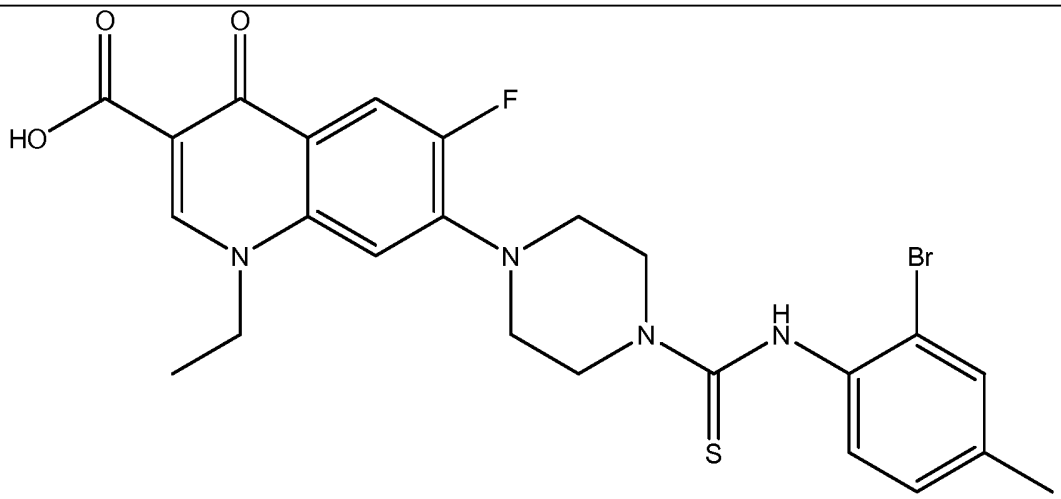
Compound 28	
Compound 29	
Compound 30	
Compound 31	

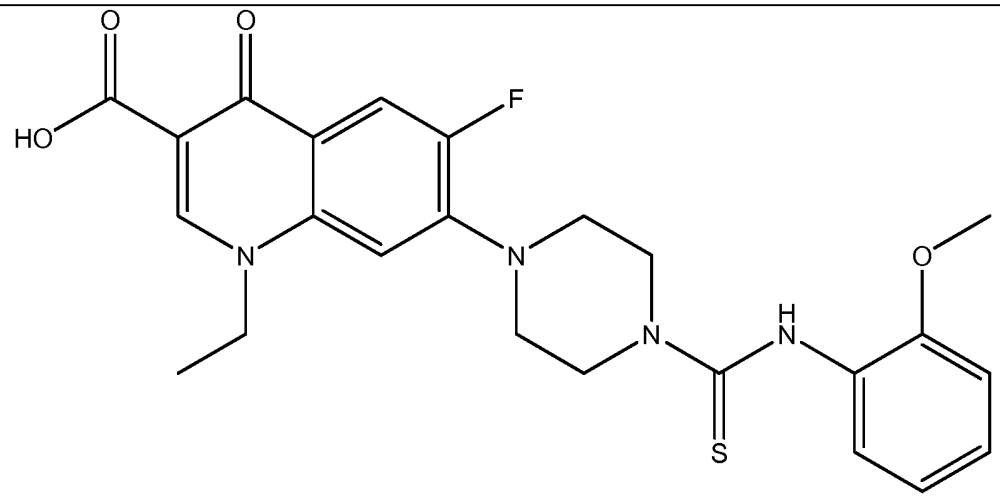
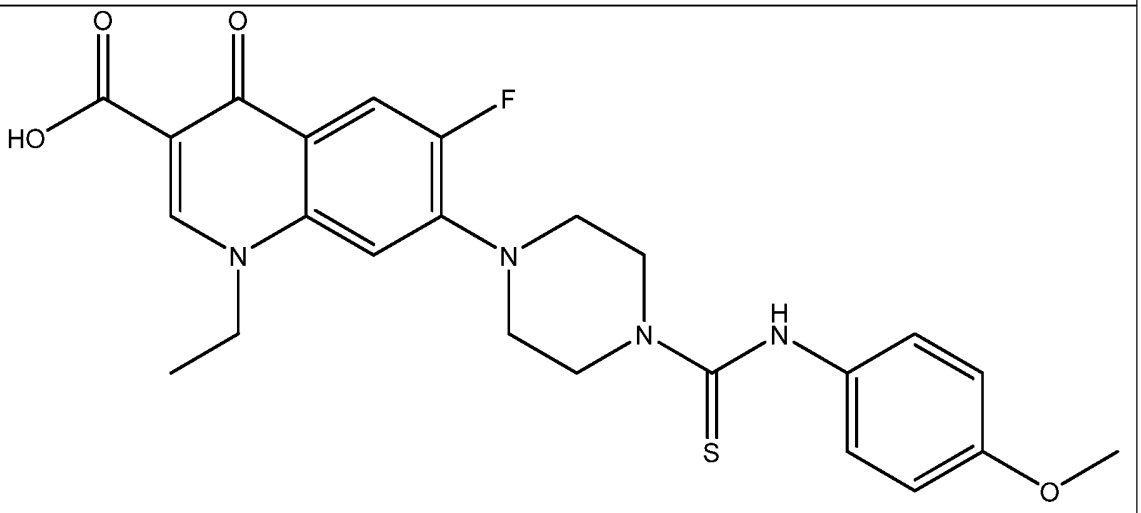
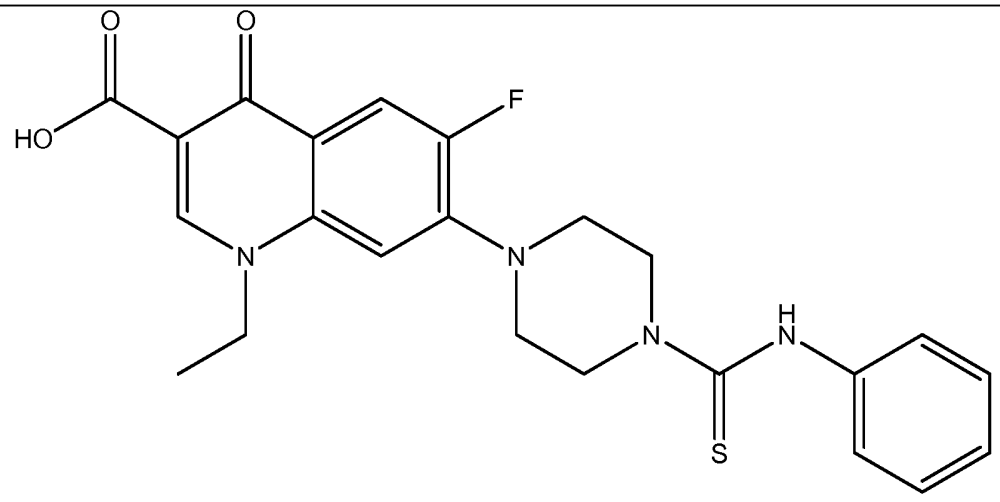
Compound 32	
Compound 33	
Compound 34	
Compound 35	

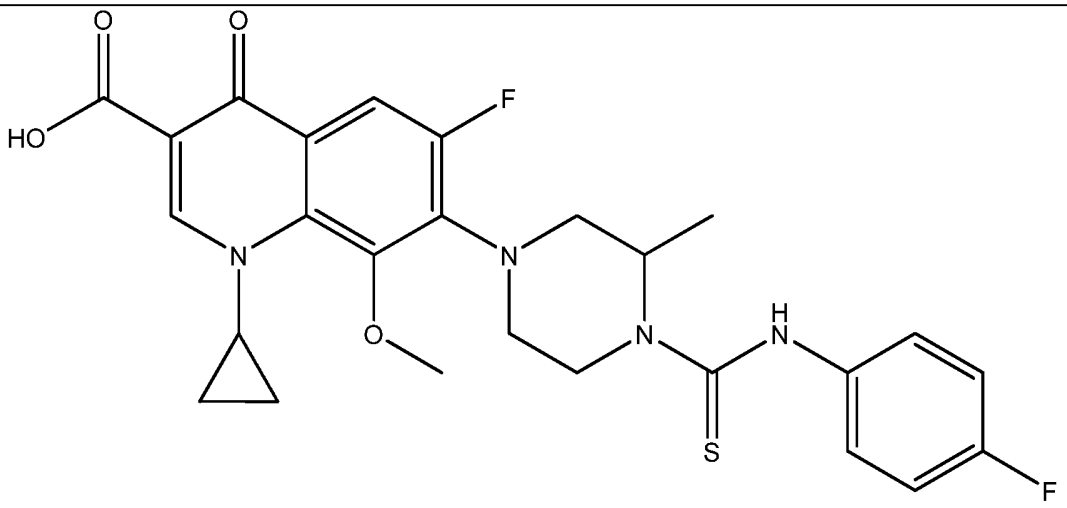
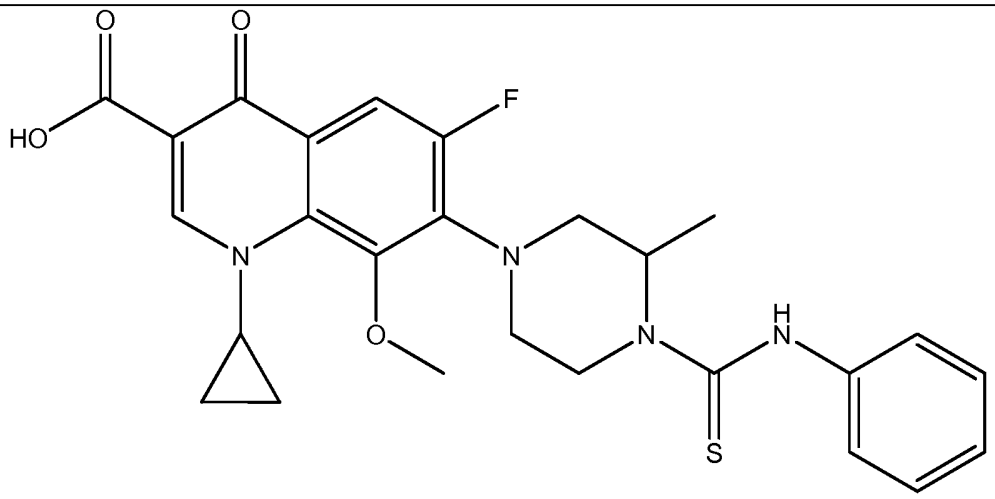
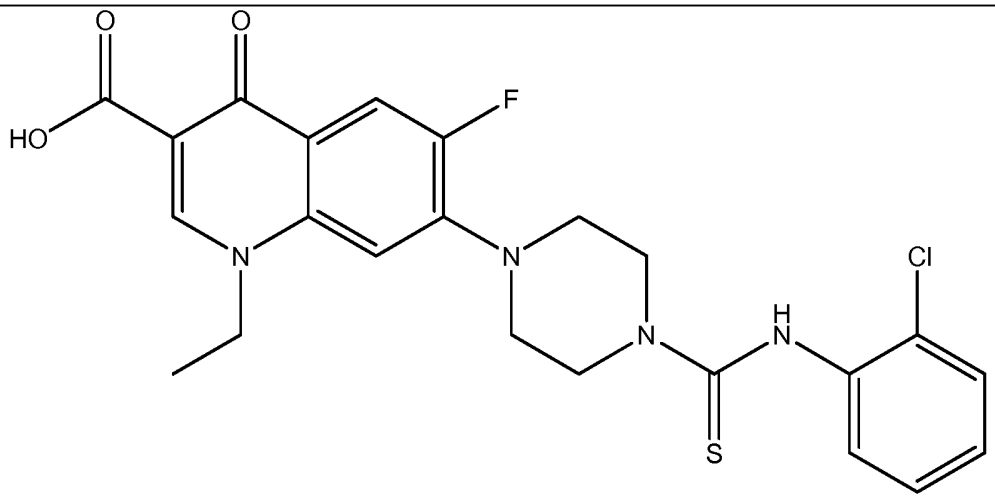
Compound 36	
Compound 37	
Compound 50	

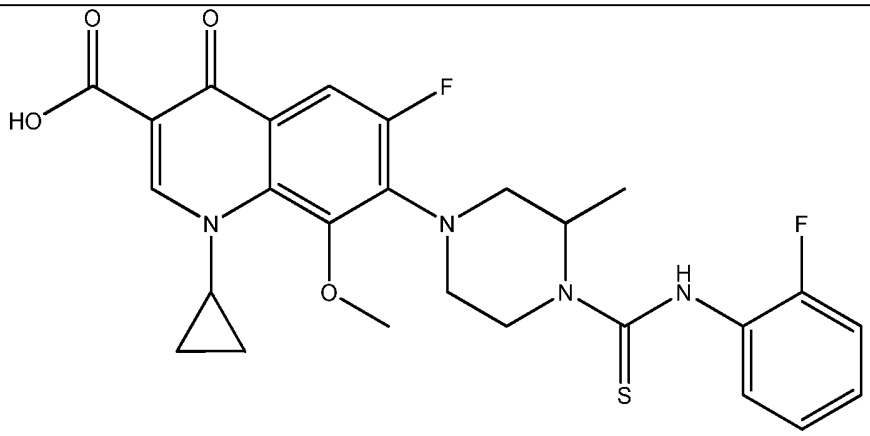
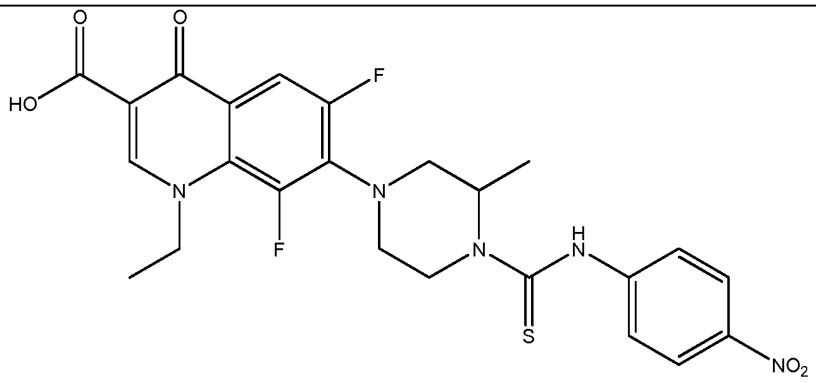
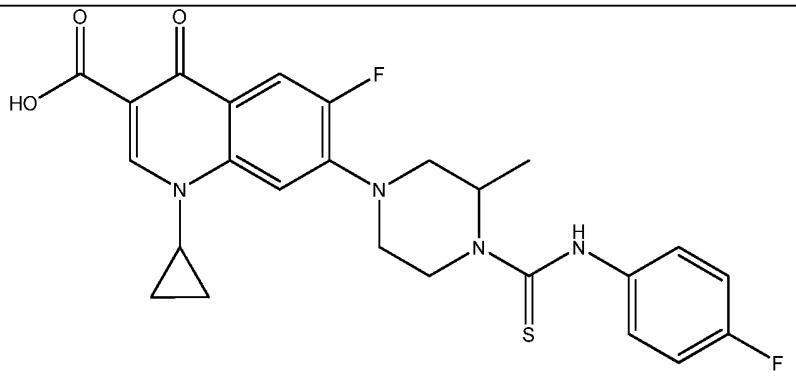
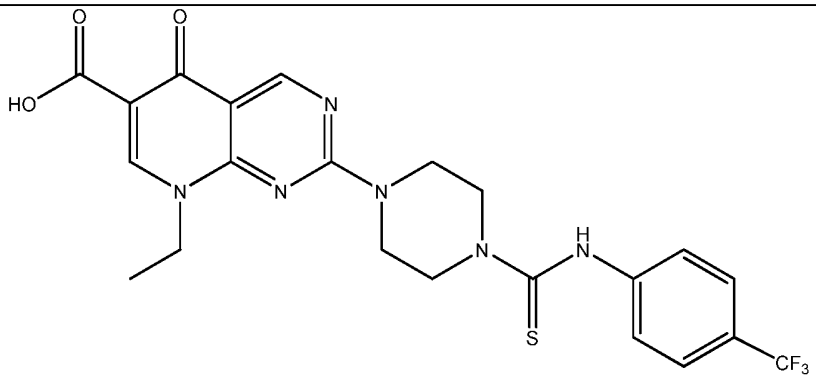
Compound 51	
Compound 52	
Compound 53	

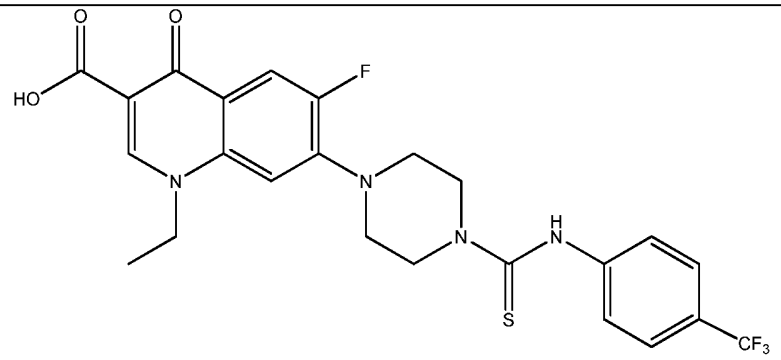
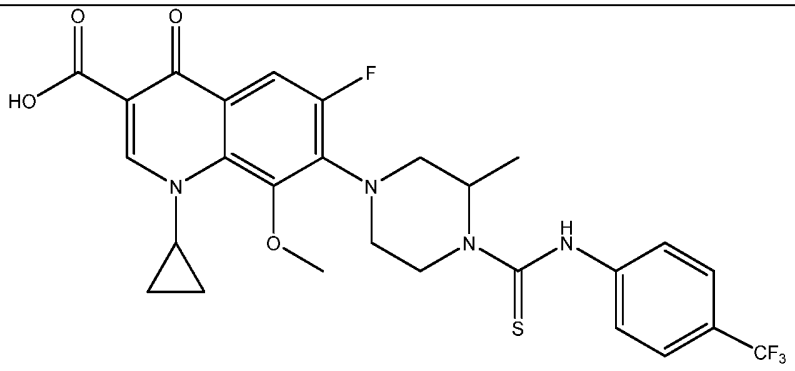
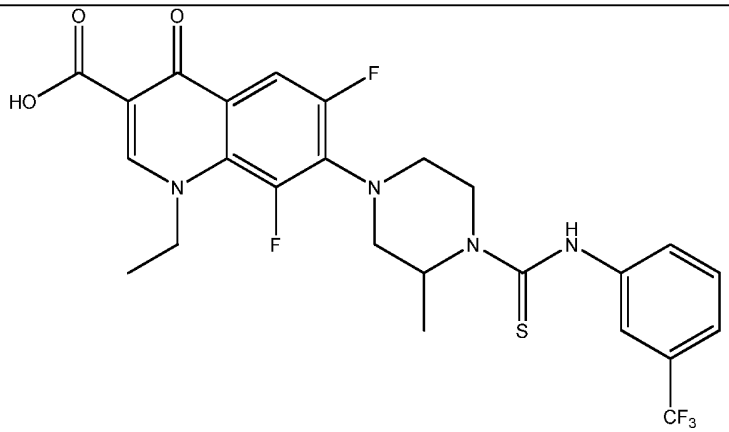
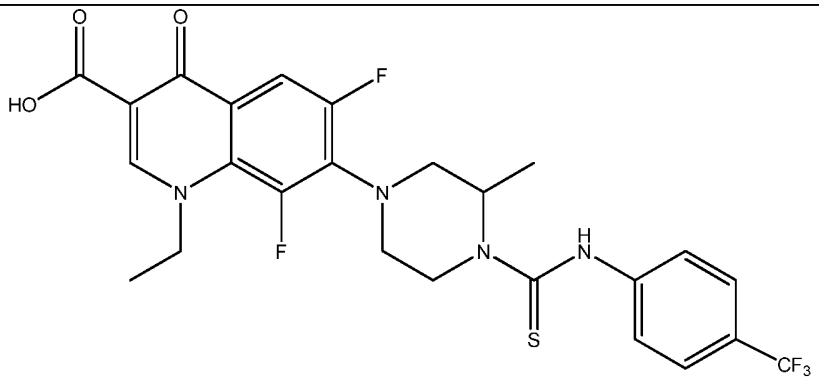
Compound 54	
Compound 55	
Compound 56	

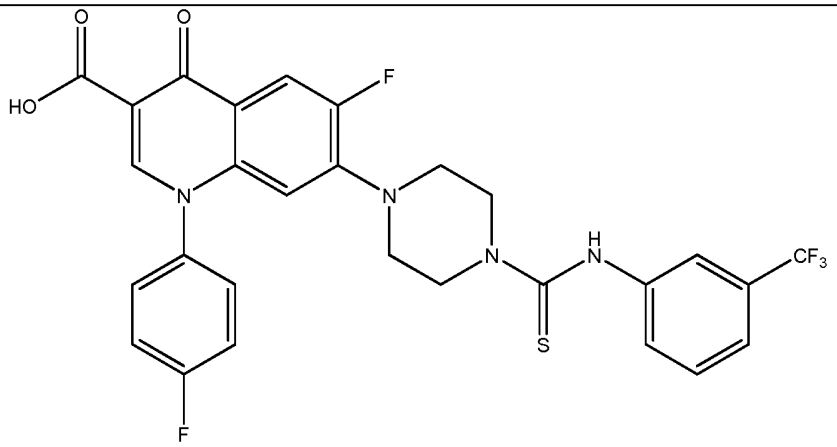
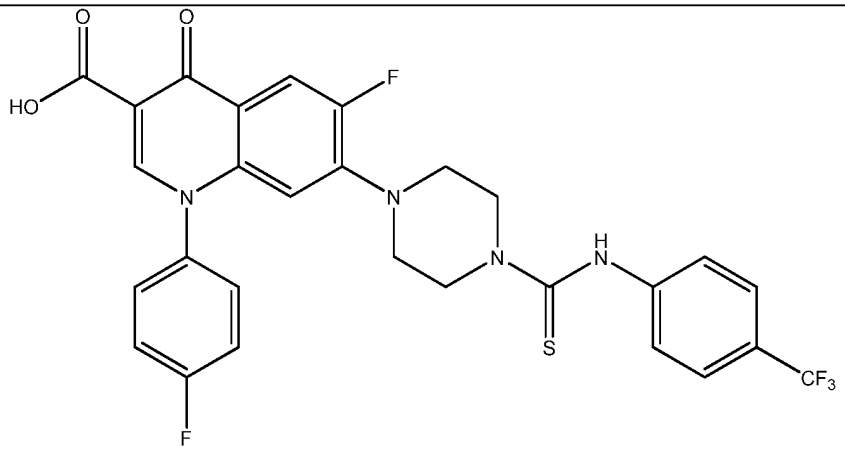
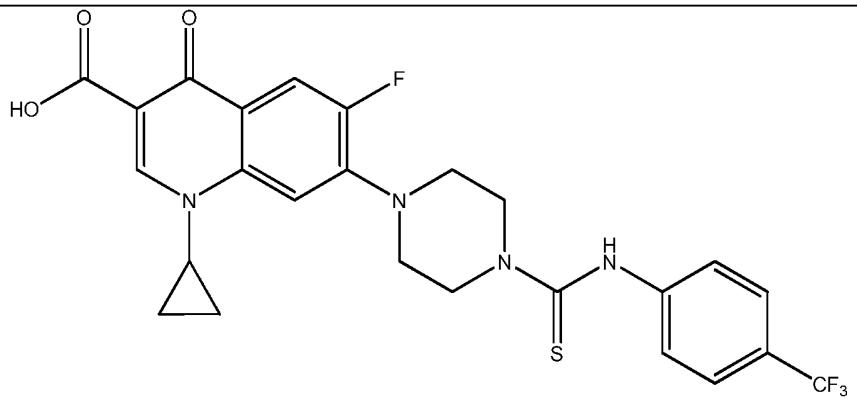
Compound 57	
Compound 58	
Compound 59	

Compound 60	
Compound 61	
Compound 62	

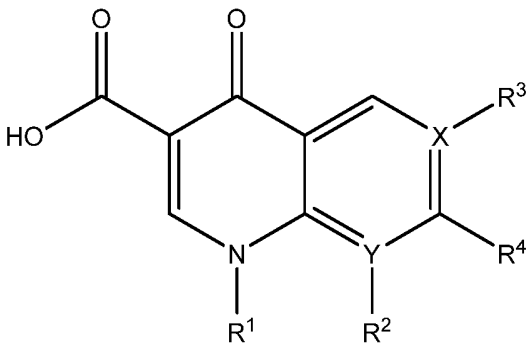
Compound 63	
Compound 64	
Compound 65	

Compound 66	
Compound 67	
Compound 68	
Compound 143	

Compound 144	
Compound 145	
Compound 146	
Compound 147	

Compound 148	
Compound 149	
Compound 150	

[0074] In certain embodiments, the active compounds as described herein are selected from compounds according to Formula Ia:



Formula Ia

wherein R^1 is alkyl, aryl, or cycloalkyl;

R^2 is H, alkoxyl or halogen;

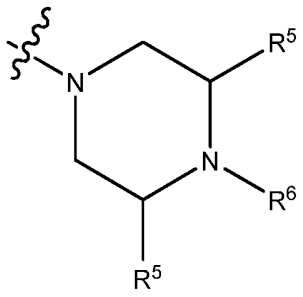
R^3 is H or halogen;

R^4 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, cycloalkyl, heteroaryl, alkyl heteroaryl, heterocyclyl, and heterocyclyl alkyl, any of which may be optionally substituted; and

X and Y are independently C or N.

[0075] In certain embodiments,

R^1 is alkyl, R^2 is H, R^3 is fluorine, X and Y are each C, and R^4 is

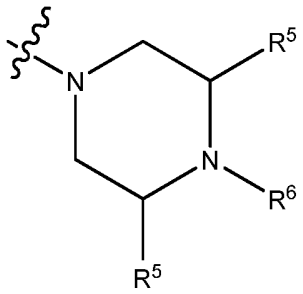


wherein R^5 is H or alkyl; and

R^6 is H, –C(=O)NH-R^7 or –C(=S)NH-R^7, wherein R^7 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally

substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro. For example in specific embodiments, R^7 is –C(=S)NH-phenyl wherein the phenyl is optionally substituted with a haloalkyl group. Examples of such embodiments of compounds of Formula Ia include Compound 50 and Compound 51.

[0076] In other embodiments of compounds according to Formula 1a, X and Y are each N, R^1 is alkyl, and R^4 is

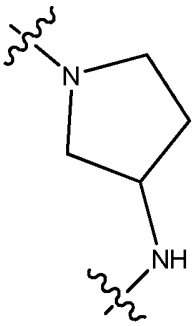


wherein R^5 is H or alkyl; and

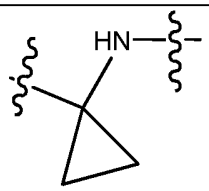
R^6 is H, –C(=O)NH-R^7 or –C(=S)NH-R^7, wherein R^7 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro. For example in specific embodiments, R^7 is –C(=S)NH-phenyl wherein the phenyl is optionally substituted with a haloalkyl group.

[0077] In further embodiment of compounds according to Formula Ia, R^4 may comprise one or more of the R^4 groups in Table 2.

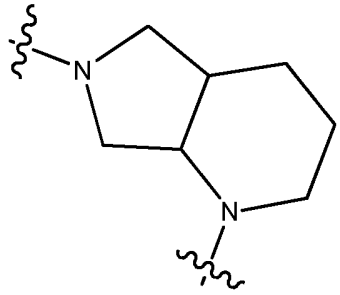
[0078] Table 2: Examples of R^4 groups.



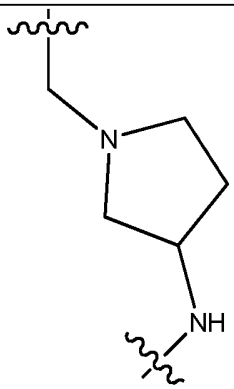
Group 1



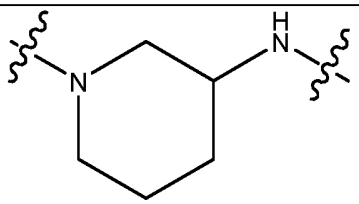
Group 2



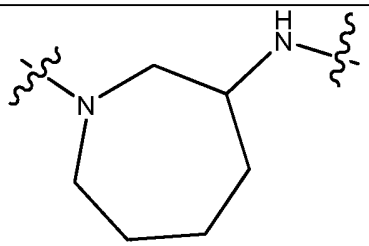
Group 3



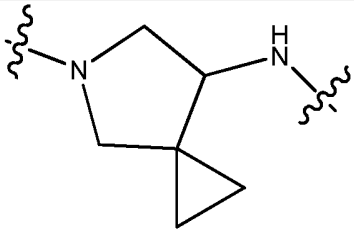
Group 4



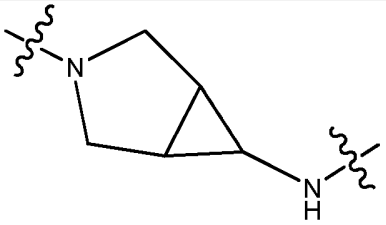
Group 5



Group 6



Group 7

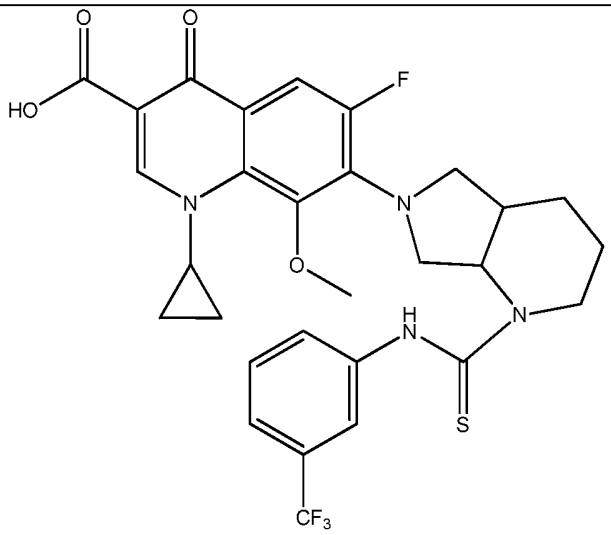
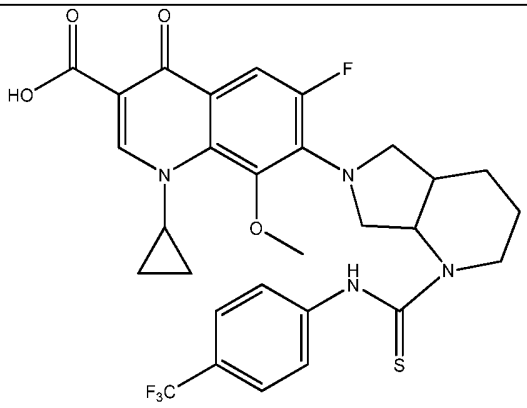
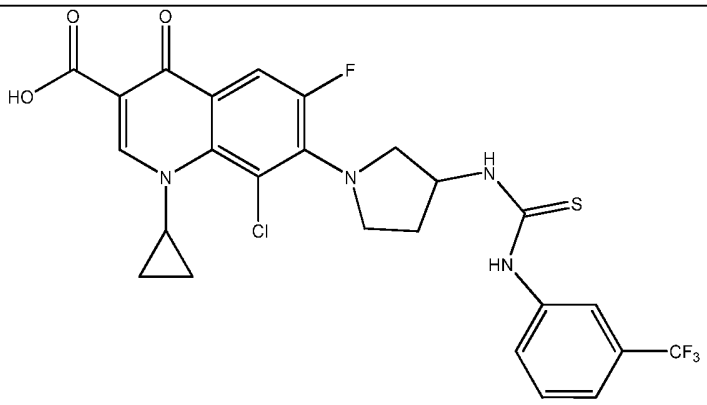
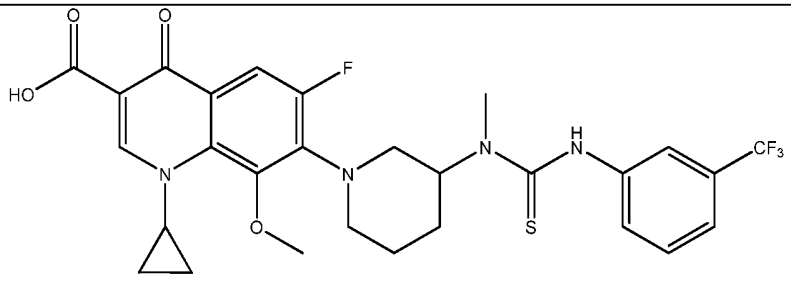


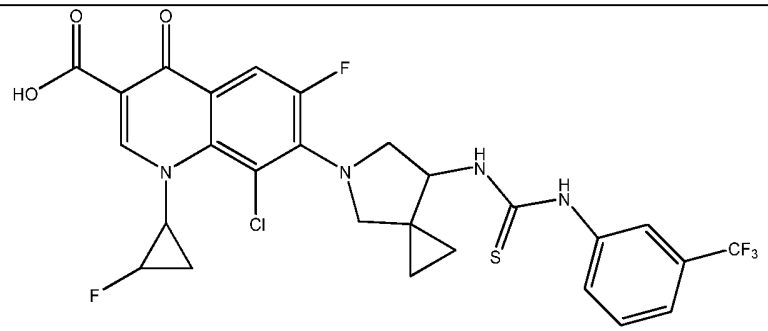
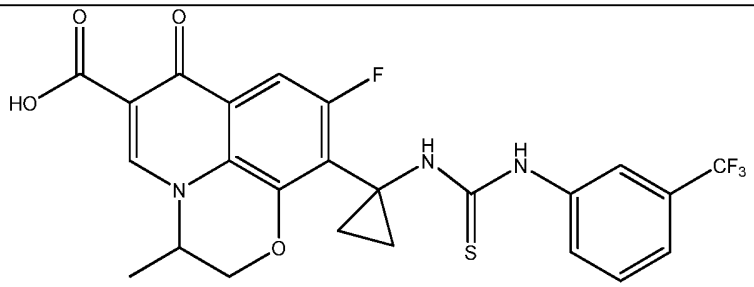
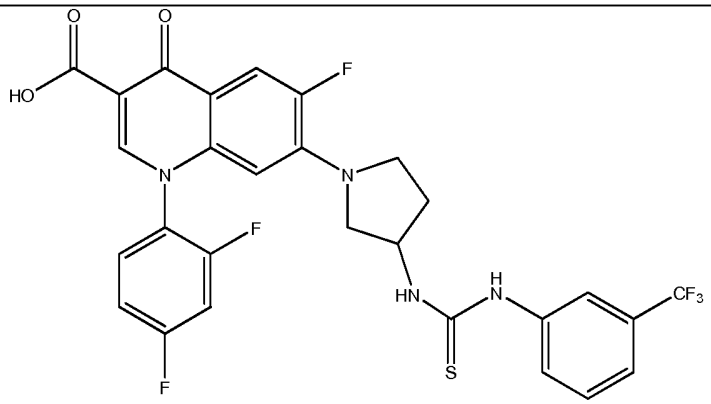
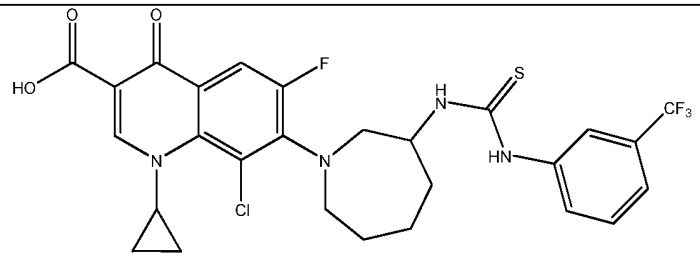
Group 8

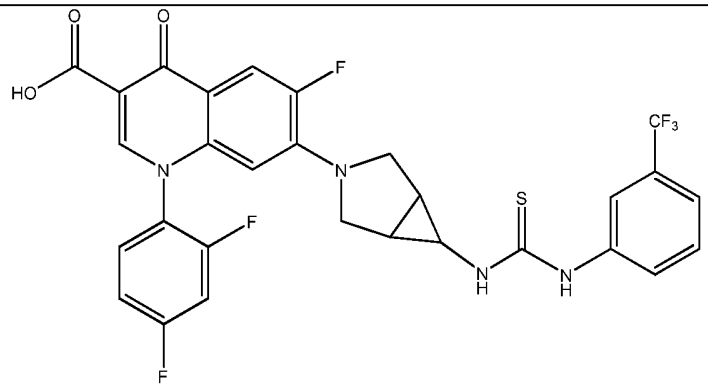
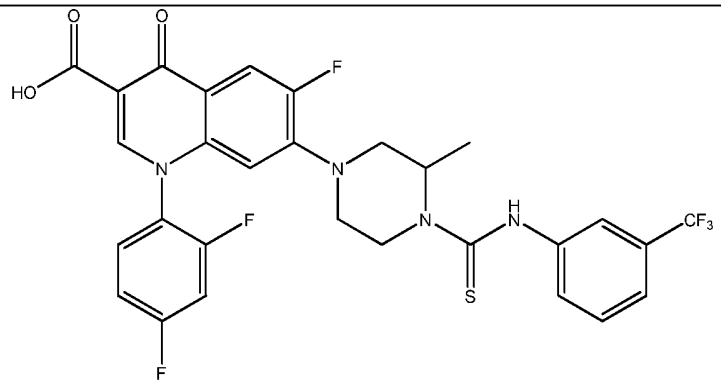
wherein each of the R^4 groups comprises at least two active nitrogen atoms and the R^4 groups may bond to Formula Ia at R^4 with one of the active nitrogen atoms. In certain embodiments, the other active nitrogen atom(s) may bound to H or –C(=O)NH-R^7 or –C(=S)NH-R^7, wherein R^7 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro. For example in specific embodiments, R^7 is –C(=S)NH-phenyl wherein the phenyl is optionally substituted with a haloalkyl group.

[0079] Compounds according to Formula Ia may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. Specific examples of compounds according to Formula Ia include the compounds in Table 3.

[0080] Table 3: Compounds according to Formula Ia.

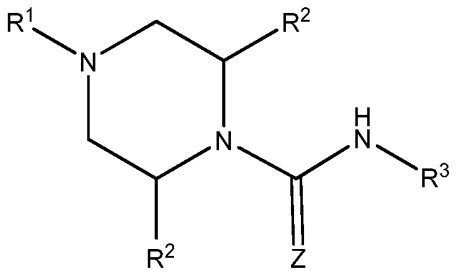
Compound 151	
Compound 152	
Compound 153	
Compound 154	

Compound 155	
Compound 156	
Compound 157	
Compound 157	

Compound 159	
Compound 160	

[0081] In certain embodiments, the compounds of Formula Ia inhibit bacterial DNA gyrase activity. In additional embodiments, the compounds of Formula Ia inhibit not only bacterial DNA gyrase, but also one or both of the nuclease and the helicase activity of a bacterial helicase, nuclease, or helicase-nuclease complex. For example, in such embodiments, compounds according to Formula Ia may inhibit the nuclease and/or helicase activities of a bacterial nuclease selected from the RecBCD and/or AddAB families of enzymes.

[0082] In other embodiments, active compounds as disclosed herein are selected from compounds according to Formula Ib:



Formula Ib

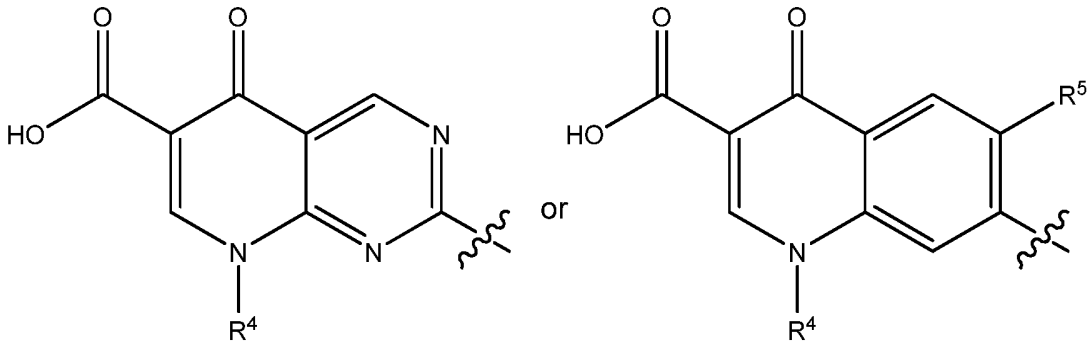
wherein R^1 is is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, cycloalkyl, heteroaryl, alkyl heteroaryl, heterocyclyl, and heterocyclyl alkyl, any of which may be optionally substituted;

R^2 is H or alkyl;

R^3 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro; and

Z is O or S.

[0083] In particular embodiments of compounds according to Formula Ib, R^1 is selected from a compound according to Formula Ic or Formula Id:



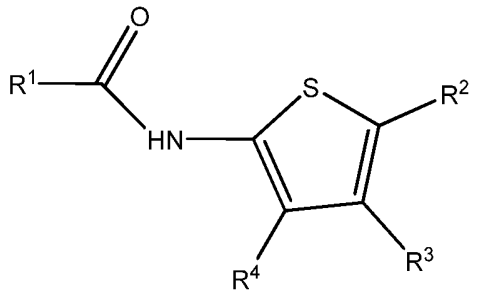
Formula Ic or Formula Id.

[0084] In specific embodiments of compounds according to Formula 1b, R^1 is selected from a compound according to Formula 1C, R^2 is H, Z is S, and R^3 is phenyl substituted with a CF_3 group. In such embodiments, the CF_3 group may be located in any of the ortho, para, or meta positions. Specific examples of such embodiments, include, for example, Compound 1 and Compound 3.

[0085] In other embodiments of active compounds according to Formula Ib, R^1 is selected from a compound according to Formula Id, R^2 is H, Z is S, R^3 is phenyl substituted with a CF_3 group, R^4 is alkyl, and R^5 is fluorine. In such embodiments, the CF_3 group may be located in any of the ortho, para, or meta position. Specific examples of such embodiments, include, for example, Compound 50 and Compound 51.

[0086] In particular embodiments, compounds according to Formula Ib inhibit the helicase and/or the nuclease activity of a bacterial nuclease, helicase, or helicase-nuclease complex. For example, in such embodiments, compounds according to Formula 1b may inhibit the nuclease and/or nuclease activity of a bacterial nuclease selected from the bacterial RecBCD and/or AddAB families of enzymes. In other embodiments, compounds of Formula Ib inhibit the helicase and/or the nuclease activity of a bacterial nuclease, such as, for example the nuclease and/or helicase activity of enzymes selected from one or both of the RecBCD and AddAB families of enzymes, in combination with inhibiting bacterial DNA gyrase activity.

[0087] In another embodiment, active compounds that inhibit bacterial helicase(s) are selected from a compound of structural class B (the “cyanothiophenes”), according to Formula II:



Formula II

wherein R^1 is aryl, cycloalkenyl, heteroaryl, optionally substituted with a substituent selected from at least one of the following: alkyl, aryl, nitro, -COOH, thioalkyl, thioalkylaryl and halogen;

R^2 is H or alkyl;

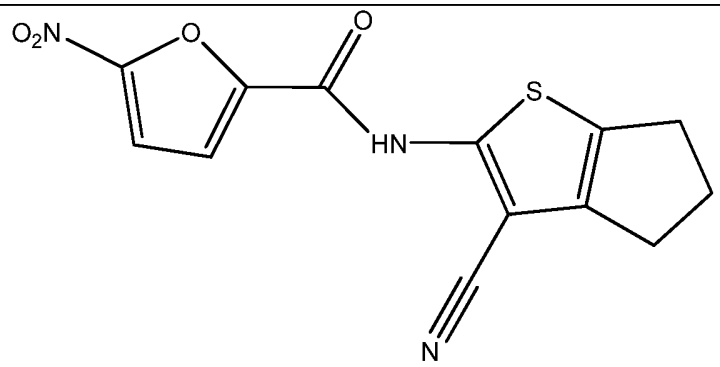
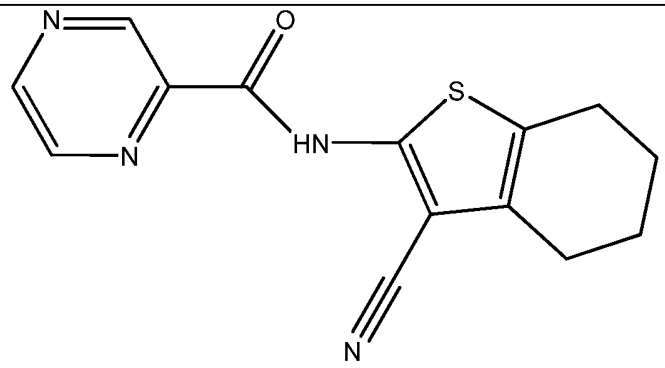
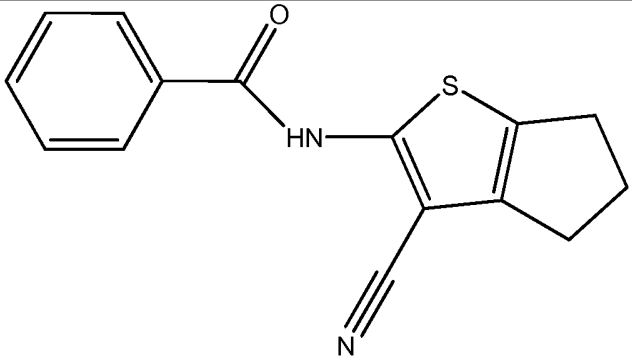
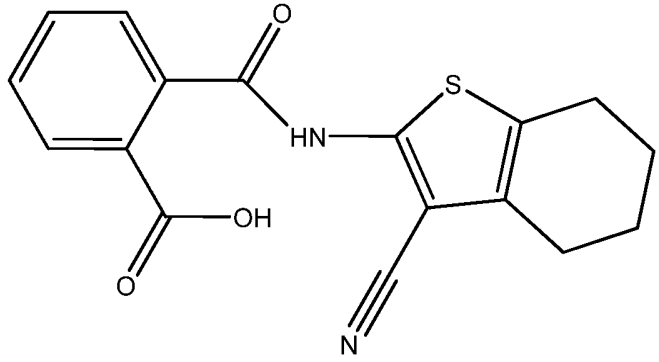
R^3 is H, alkyl, or aryl, each of which may be optionally substituted with an alkyl group, and wherein R^2 and R^3 together may be connected to form a cycloalkyl or heterocyclic group, which may be optionally substituted with an alkyl group; and

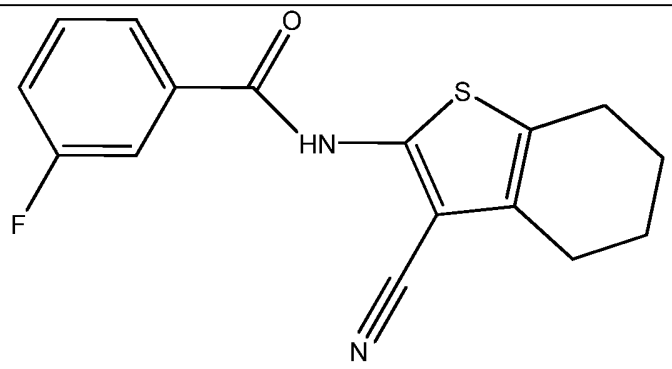
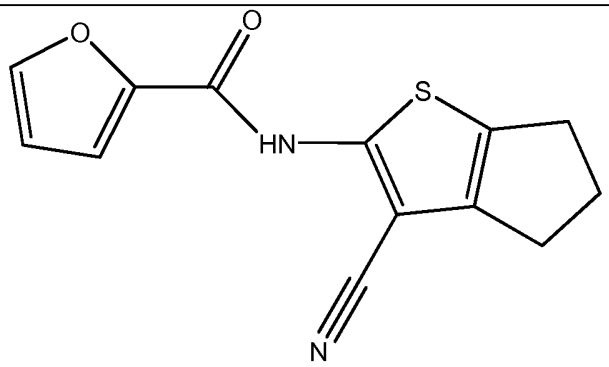
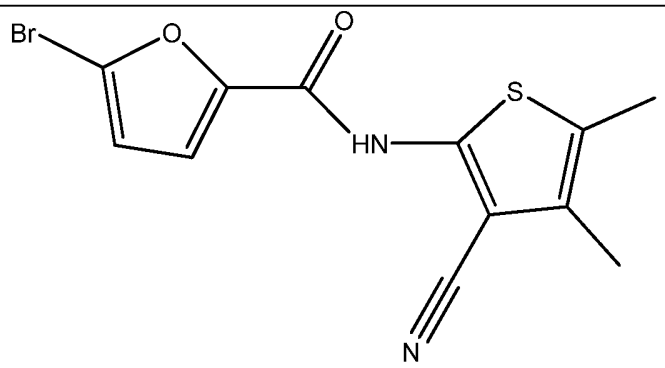
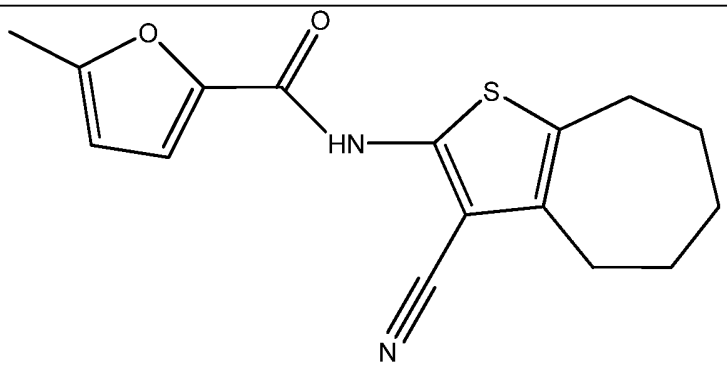
R_4 is CN, -COO-alkyl, -CO-NH_2, -CO-NH-alkyl, -CO-NH-heterocyclyl, -CO-NH-alkyl-heterocyclyl, or NH_2.

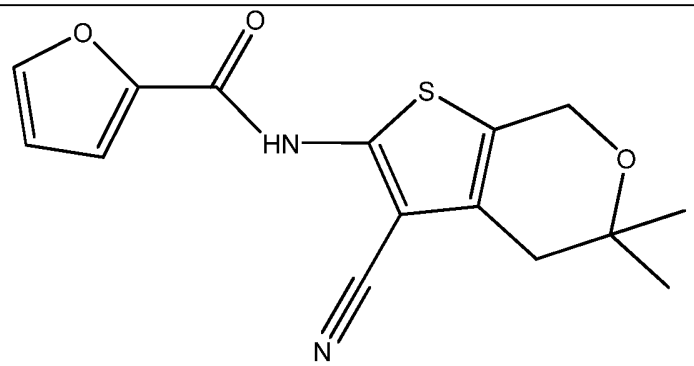
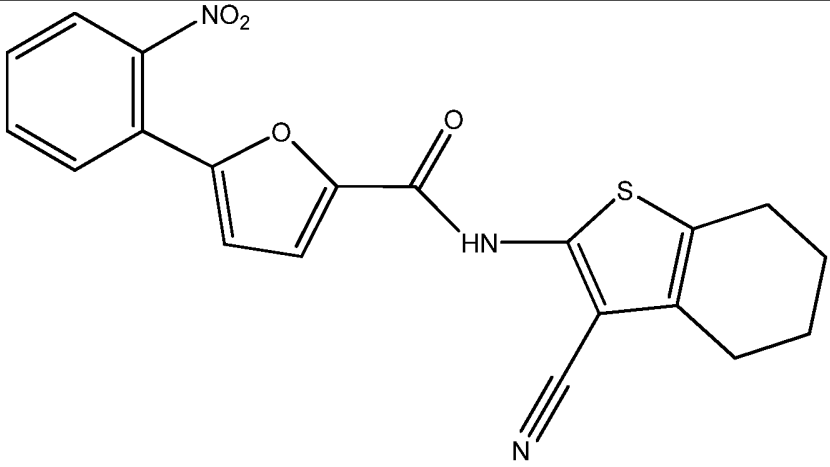
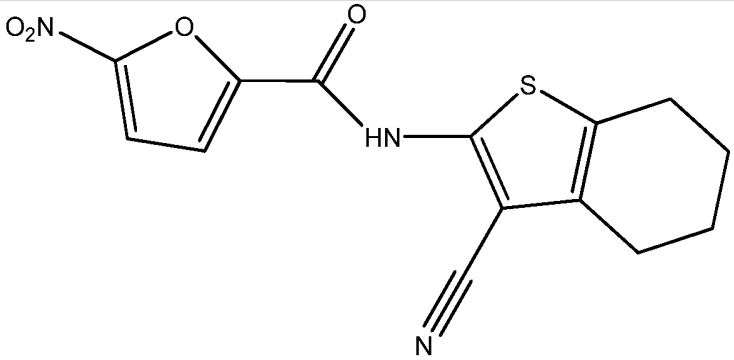
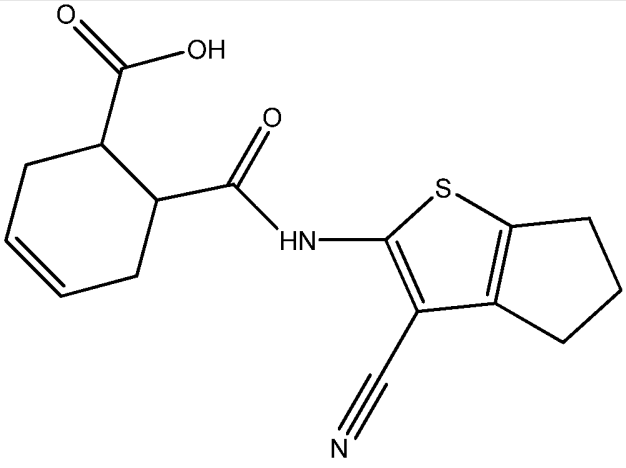
[0088] In particular embodiments of active compounds according to Formula II, R_1 is a nitro-substituted furan, and R^2 and R^3 together form a 5-membered cycloalkyl ring. In an embodiment, the furan is attached through the two position and the nitro is at the 5 position of the furanyl ring.

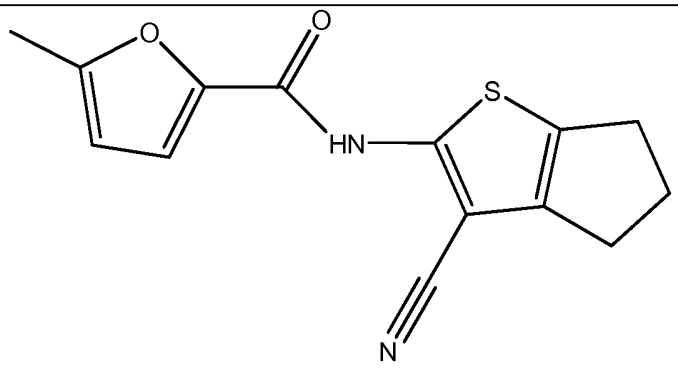
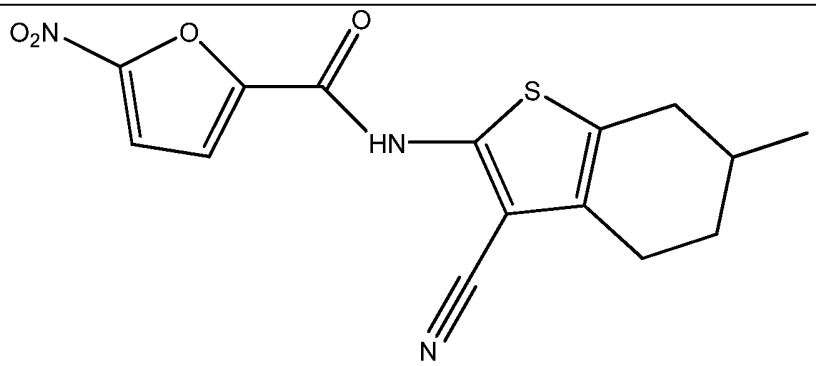
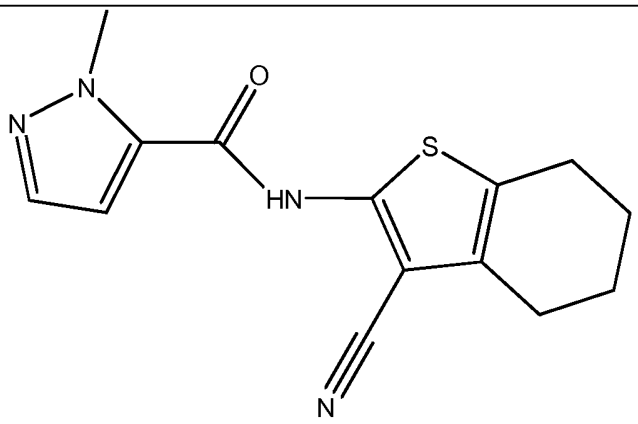
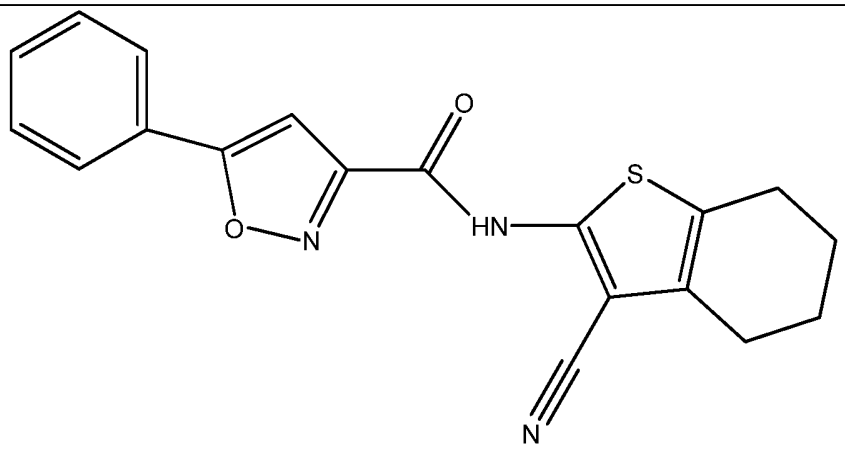
[0089] Compounds according to Formula II may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. Specific examples of compounds according to Formula II include the compounds in Table 4.

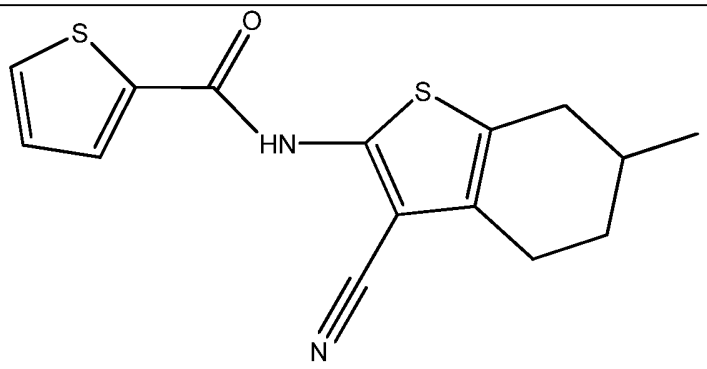
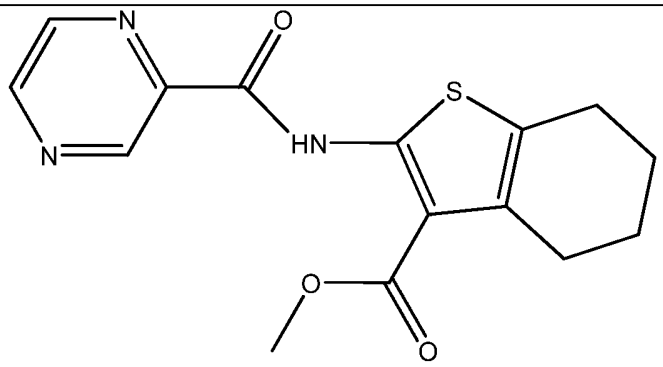
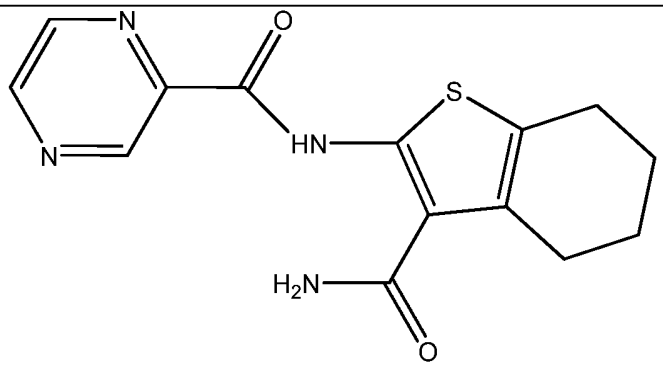
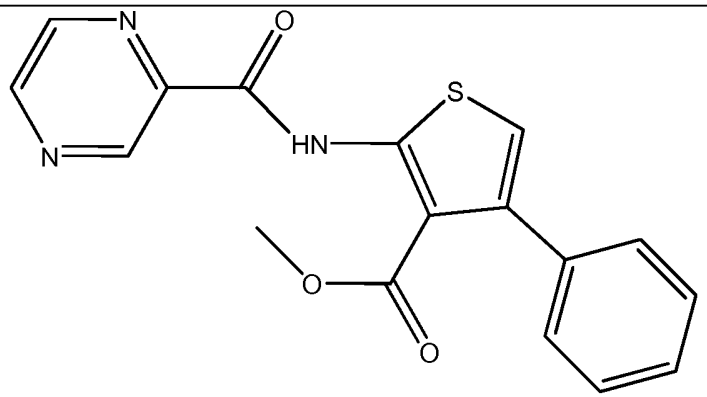
[0090] Table 4: Compounds according to Formula II.

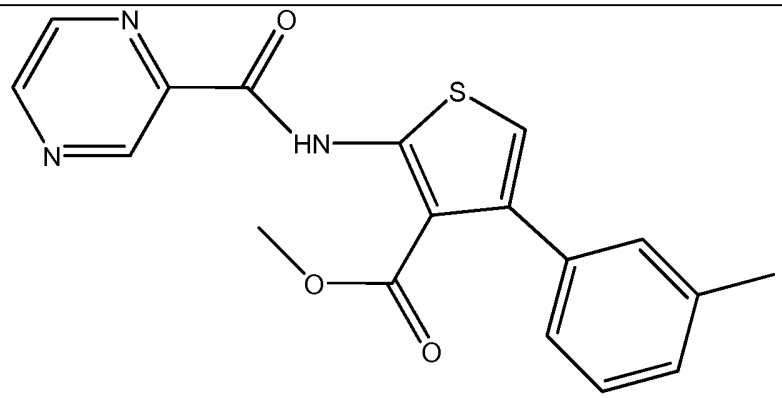
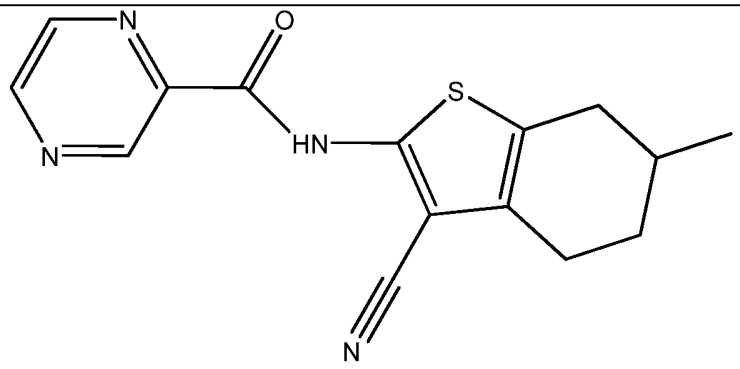
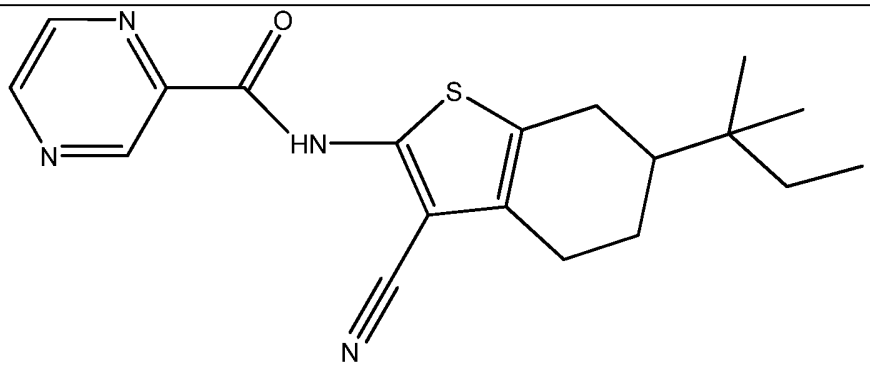
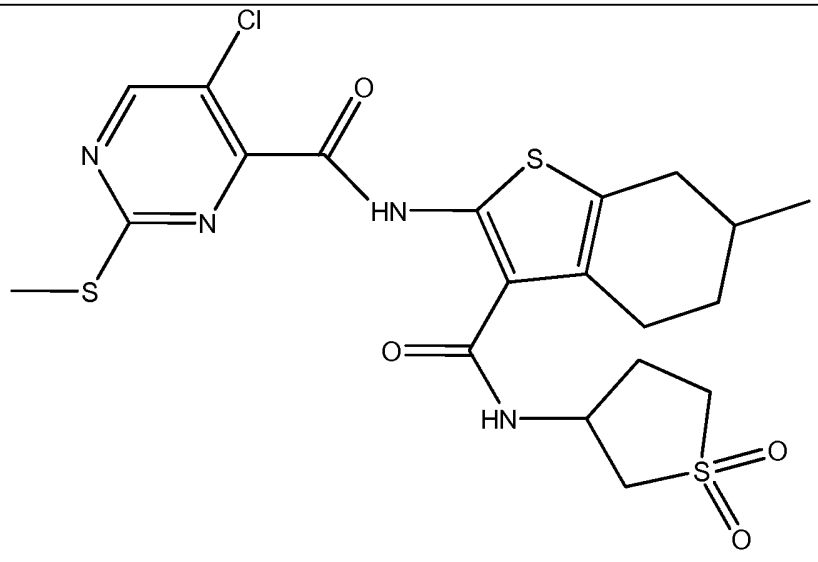
Compound 4	
Compound 5	
Compound 38	
Compound 39	

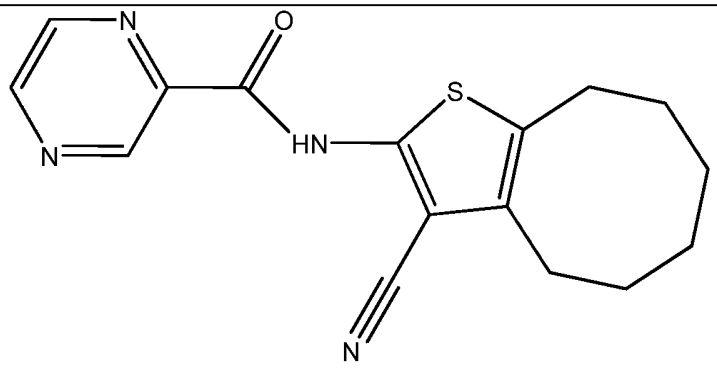
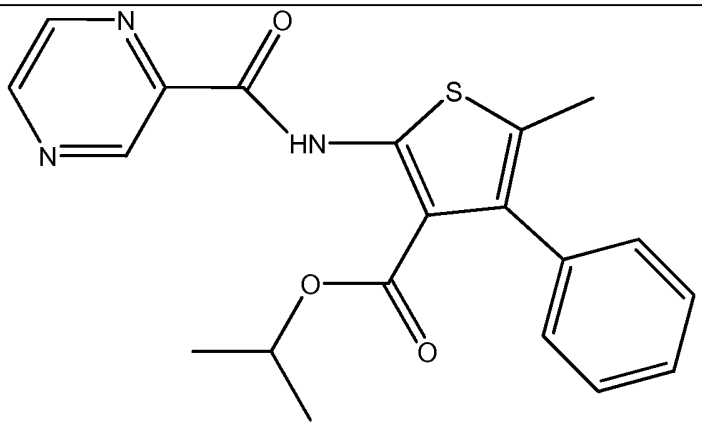
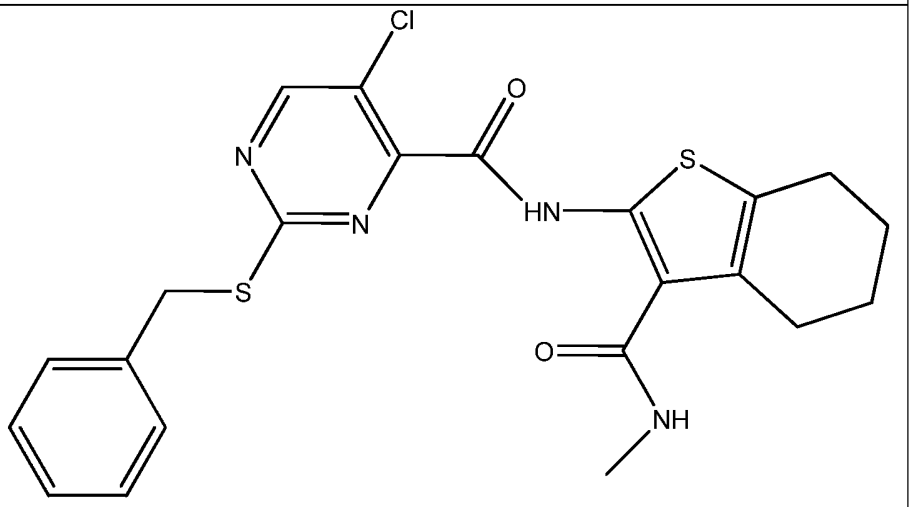
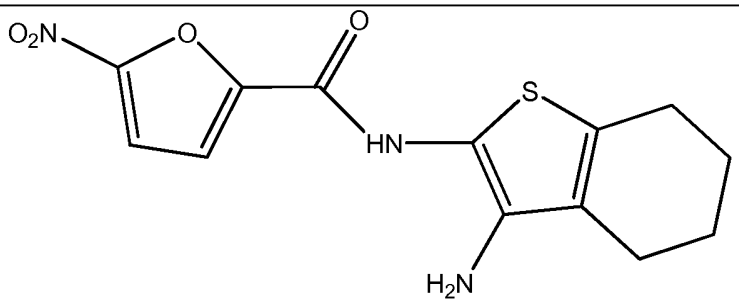
Compound 40	
Compound 41	
Compound 42	
Compound 43	

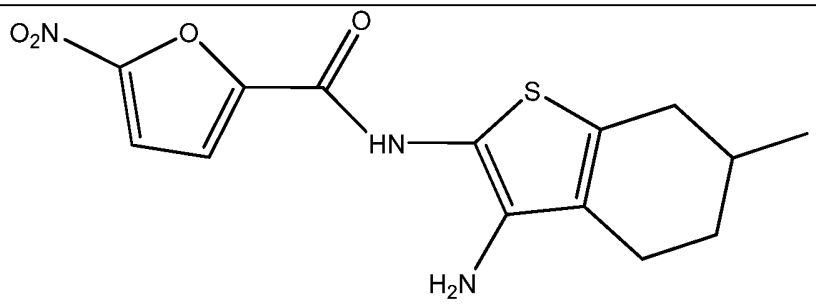
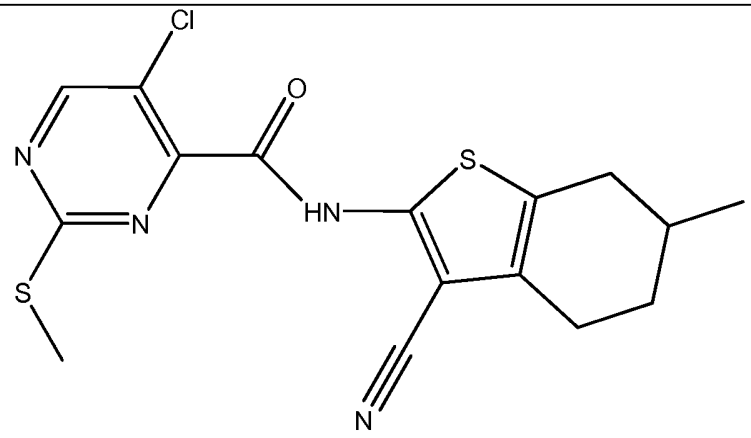
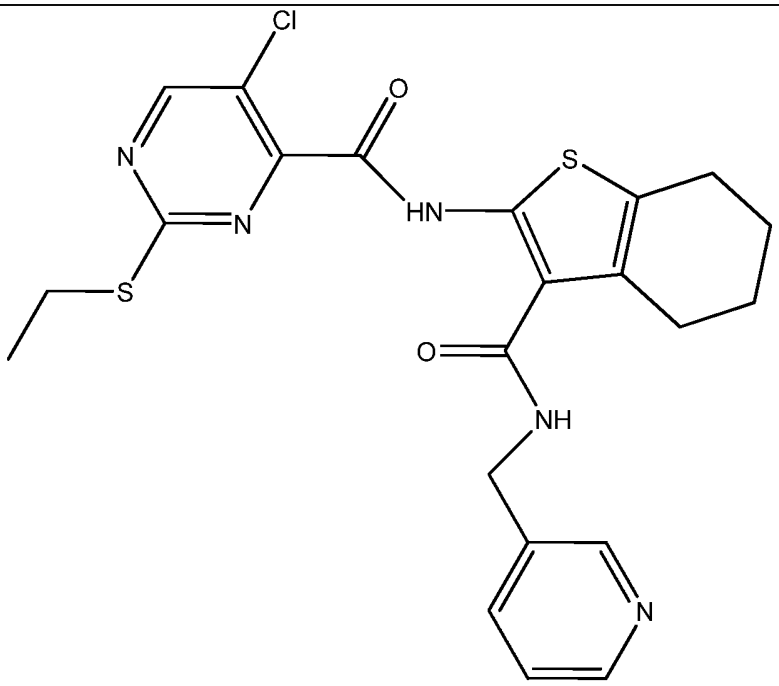
Compound 44	
Compound 45	
Compound 46	
Compound 47	

Compound 48	
Compound 49	
Compound 69	
Compound 70	

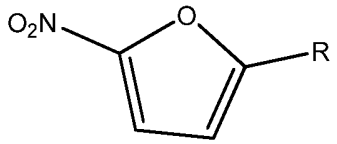
Compound 71	
Compound 72	
Compound 73	
Compound 74	

Compound 75	
Compound 76	
Compound 77	
Compound 78	

Compound 79	
Compound 80	
Compound 81	
Compound 82	

Compound 83	
Compound 84	
Compound 85	

[0091] In further embodiments, active compounds that inhibit bacterial helicase(s) are selected from a compound of structural class C (the "nitrofurans"), according to Formula III:

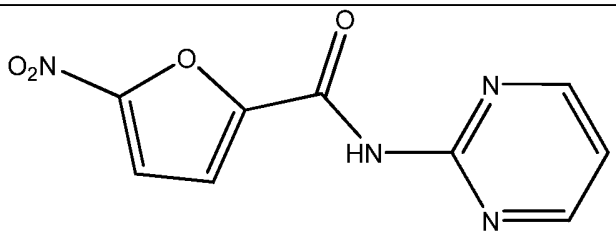


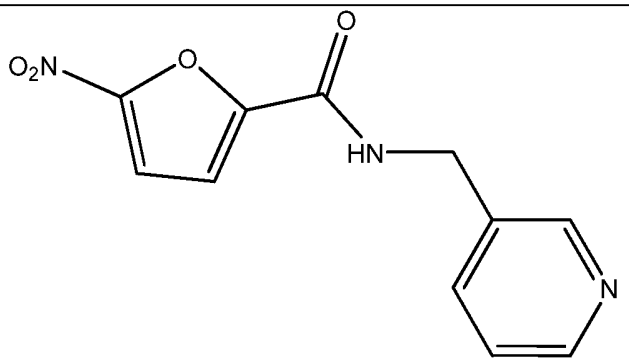
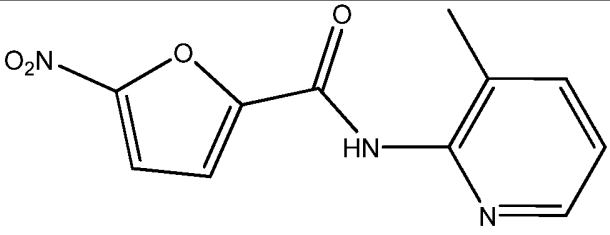
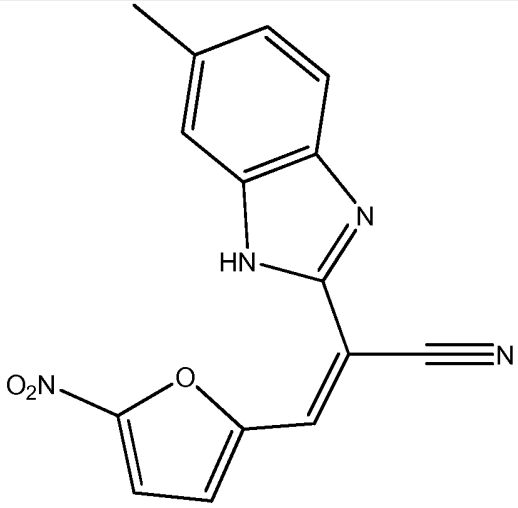
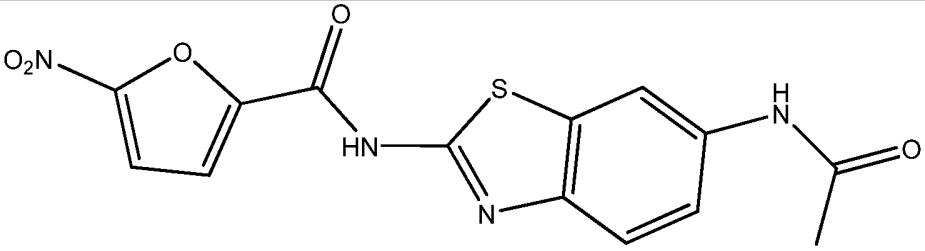
Formula III

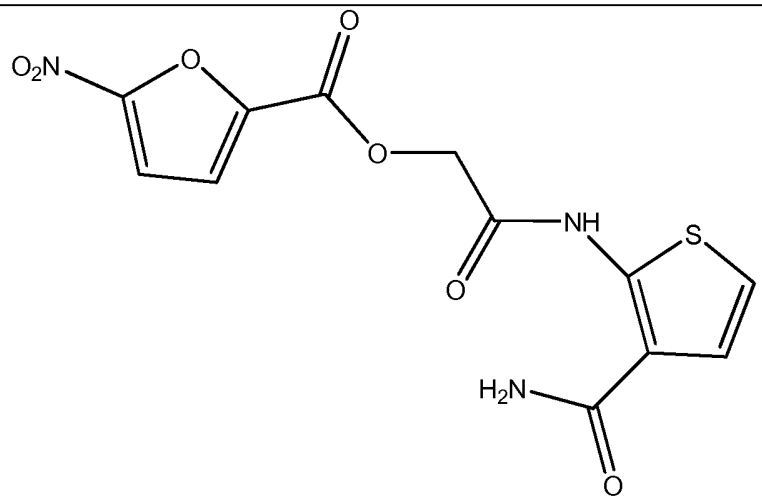
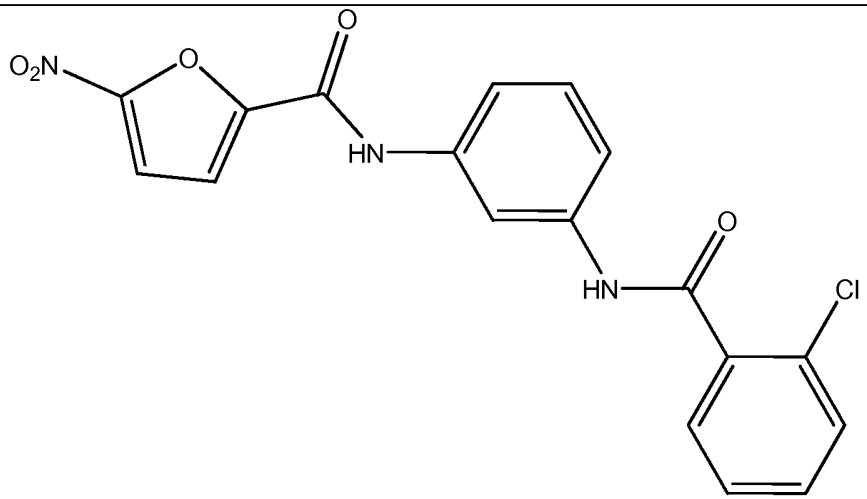
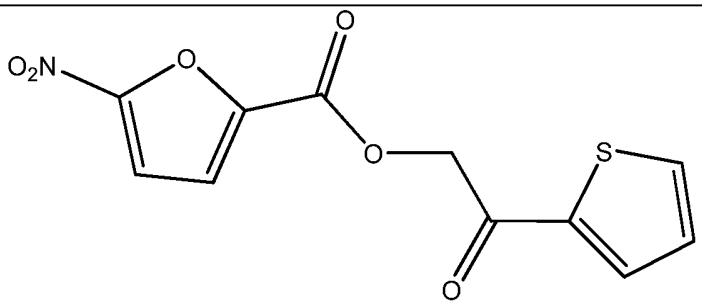
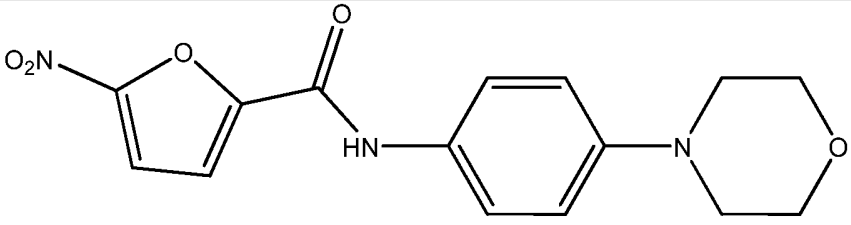
wherein R is selected from at least one of the following: -CO-O-alkyl heteroaryl, -CO-NH-heteroaryl, alkenyl heteroaryl, -CO-O-alkyl-CO-NH-heteroaryl, -CO-NH-aryl, and -CO-NH-alkyl aryl, any of which may be optionally substituted with a substituent selected from at least one of the following: C=O, N-CO-alkyl, CN, alkyl, $-CONH_2$, heterocyclyl or –NH-CO-haloaryl.

[0092] Compounds according to Formula III may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. Specific examples of compounds according to Formula III include the compounds in Table 5.

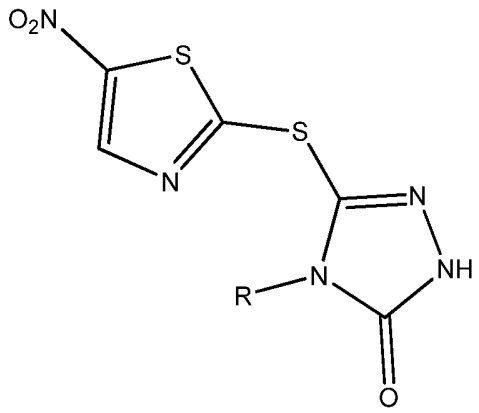
[0093] Table 5: Compounds according to Formula III.

Compound 6	

Compound 7	
Compound 8	
Compound 9	
Compound 10	

Compound 11	
Compound 12	
Compound 13	
Compound 14	

[0094] In further embodiments, active compounds that inhibit bacterial helicase(s) are selected from a compound of structural class D (the "nitrothiazoles"), according to Formula IV:

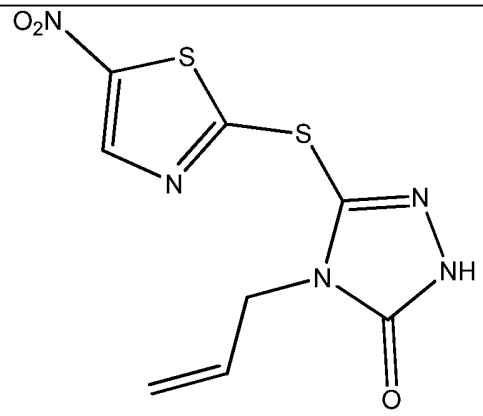


Formula IV

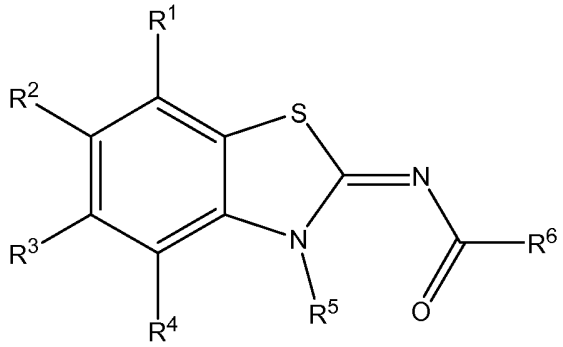
wherein R is an alkyl or alkenyl group.

[0095] Compounds according to Formula IV may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. An example of a compound according to Formula IV is compound 15 as shown in Table 6.

[0096] Table 6: Compound according to Formula IV.

Compound 15	

[0097] In further embodiments, active compounds that inhibit bacterial helicase(s) are selected from a compound of structural class E (the "iminobenzothiazoles"), according to Formula V:



Formula V

wherein R^1 is H;

R^2 is H, halo, alkyl, CONH-alkyl, nitro, CO_2-alkyl, SO_2-alkyl or SO_2NH_2;

R^3 is H;

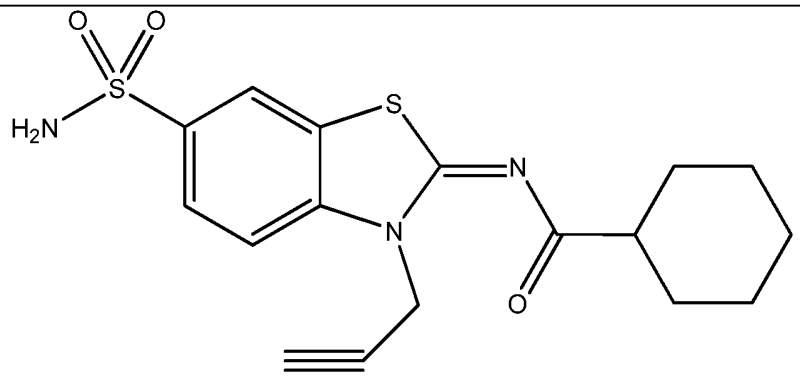
R^4 is H, halo, alkyl, or alkoxy;

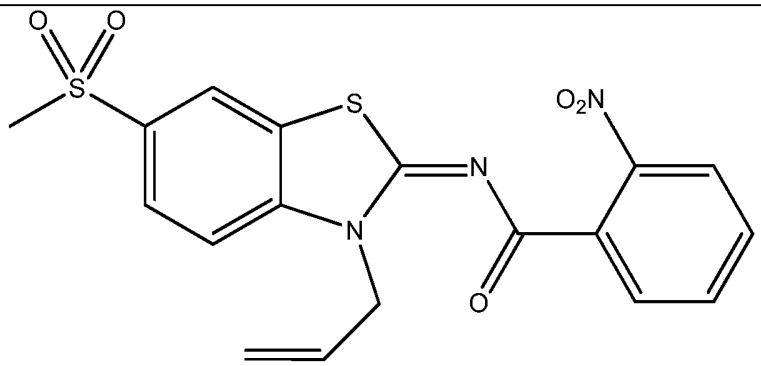
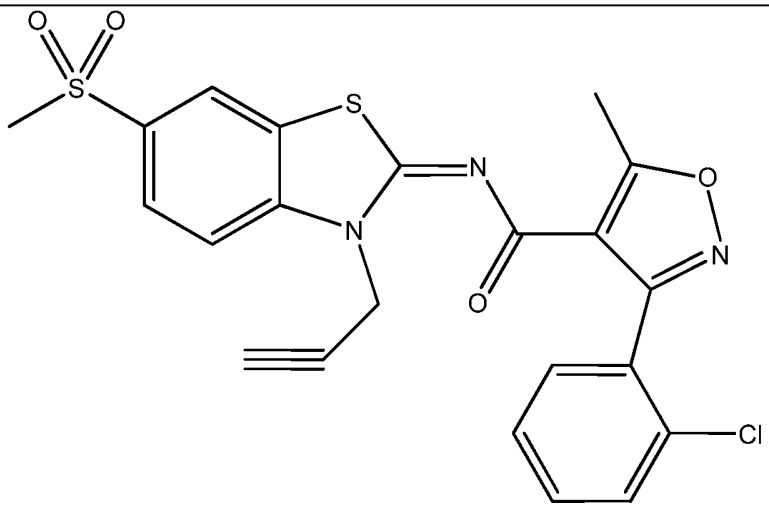
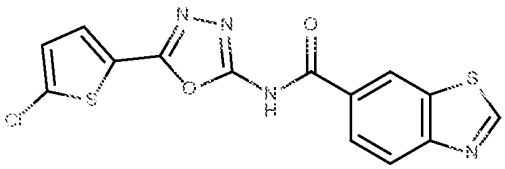
R^5 is alkyl, alkenyl, alkynyl, alkyl alkoxy, or alkyl-CO-alkoxy; and

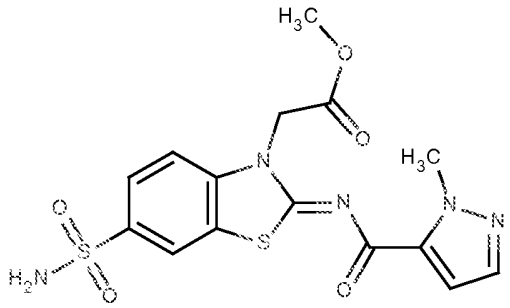
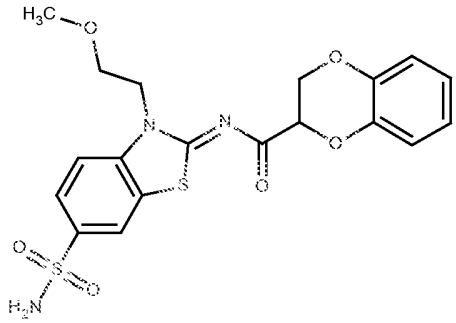
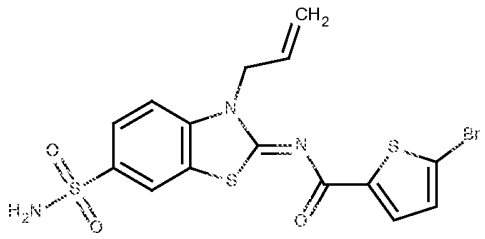
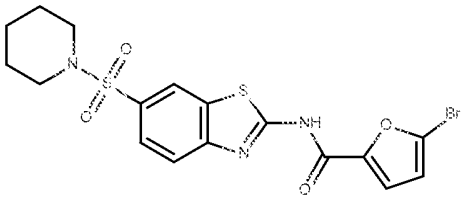
R^6 is aryl, alkyl aryl, alkenyl aryl, alkenyl heteroaryl, alkyl-SO_2-aryl, alkyl-O-aryl, aryl-SO_2-heterocyclyl, heteroaryl, heterocyclyl, cycloalkyl, diphenyl or heterocycloalkenyl, any of which may be optionally substituted with a substituent selected from at least one of the following: nitro, halo, alkyl, alkoxy, aryl, -CO, -CO_2-alkyl, CO-substituted heterocyclyl, methylenedioxy, SO_2-alkyl, or halophenyl-substituted heteroaryl.

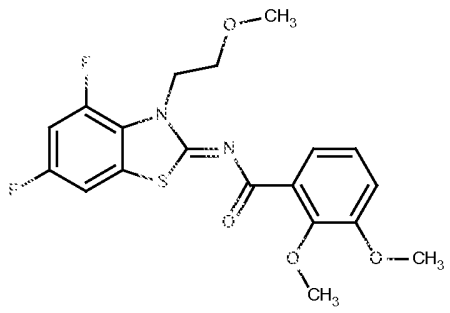
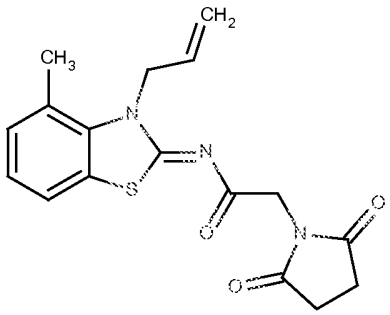
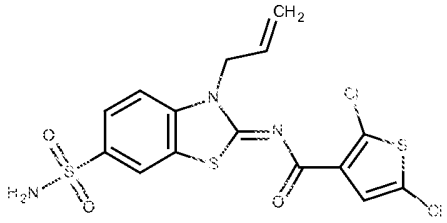
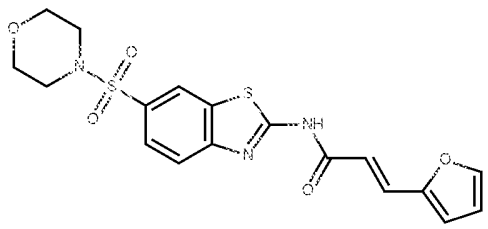
[0098] Compounds according to Formula V may be selected to exhibit an inhibitory effect on one or more bacterial DNA helicases, nucleases, or helicase-nuclease enzyme complexes, such as, for example, one or more enzymes selected from the RecBCD and AddAB families of enzymes. Examples of compounds according to Formula V include compounds shown in Table 7.

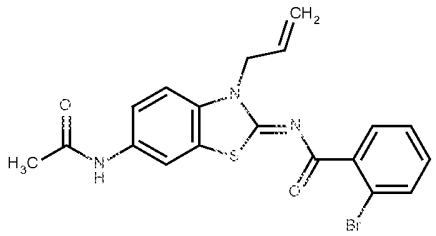
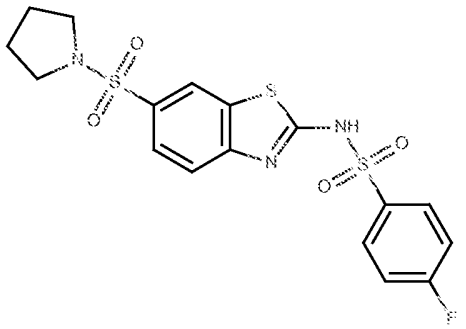
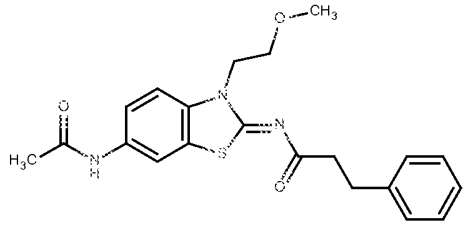
[0099] Table 7: Compounds according to Formula V.

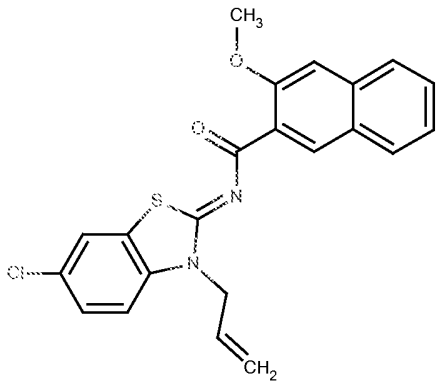
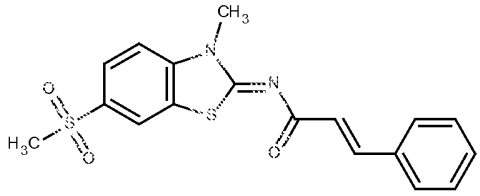
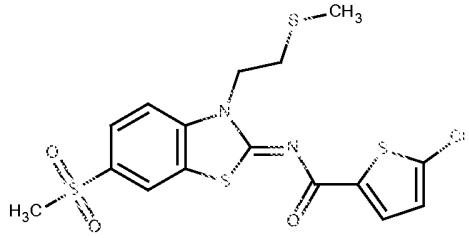
Compound 16	

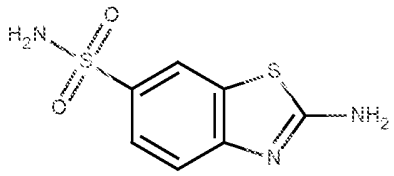
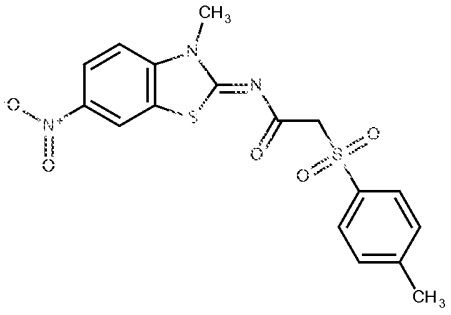
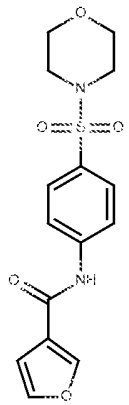
Compound 17	
Compound 18	
Compound 86	

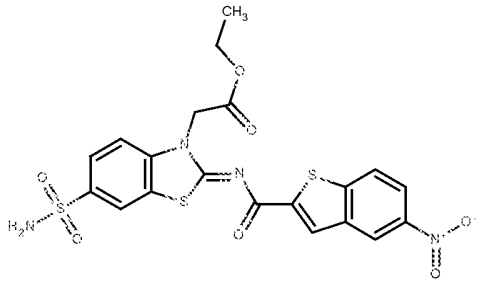
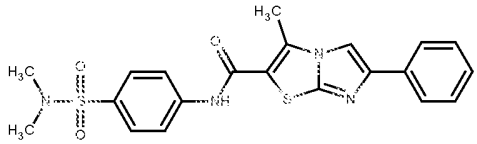
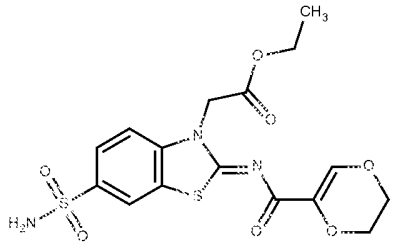
Compound 87	
Compound 88	
Compound 89	
Compound 90	

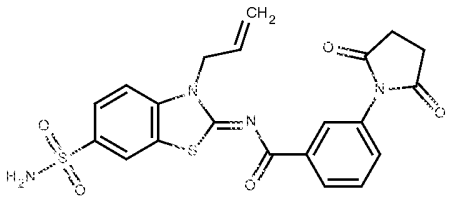
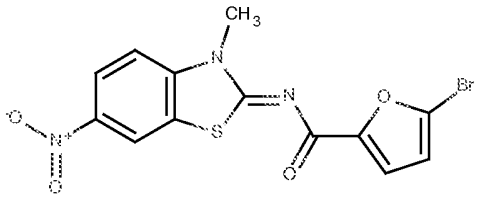
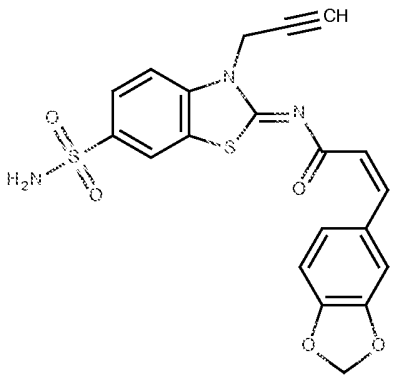
Compound 91	
Compound 92	
Compound 93	
Compound 94	

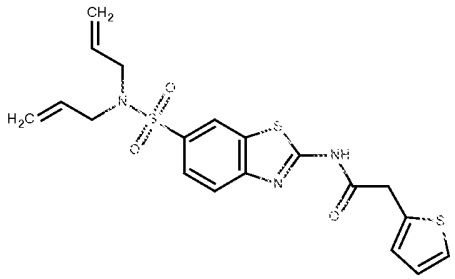
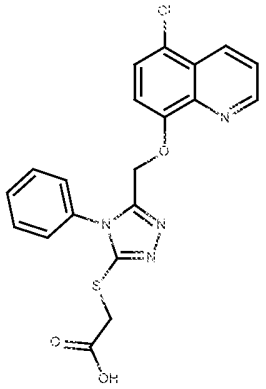
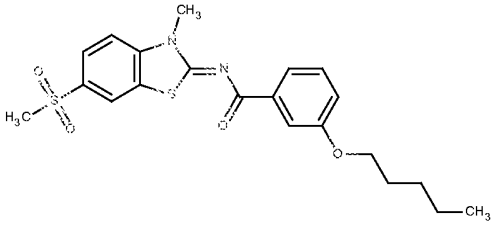
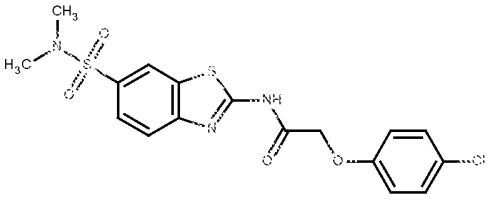
Compound 95	
Compound 96	
Compound 97	

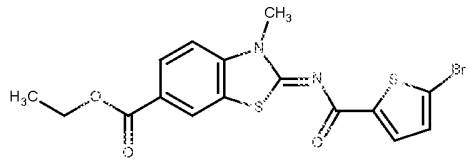
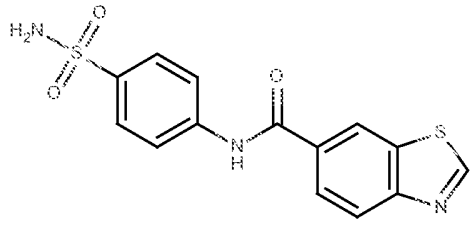
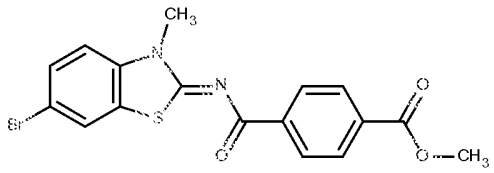
Compound 98	
Compound 99	
Compound 100	

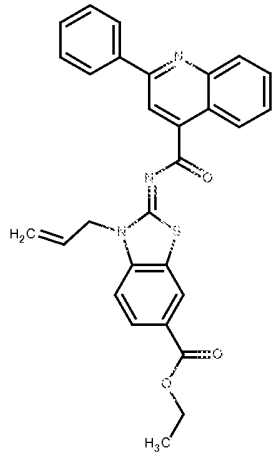
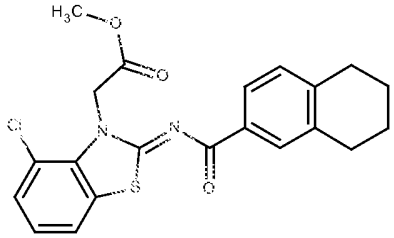
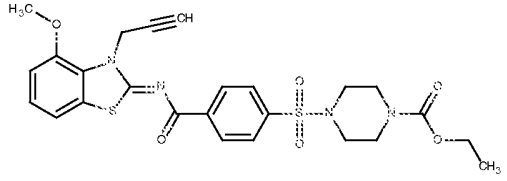
Compound 101	
Compound 102	
Compound 103	

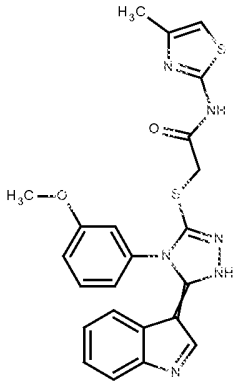
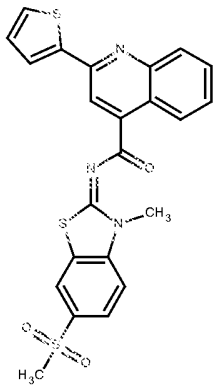
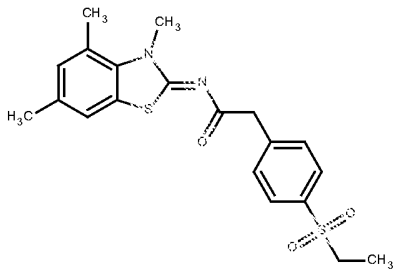
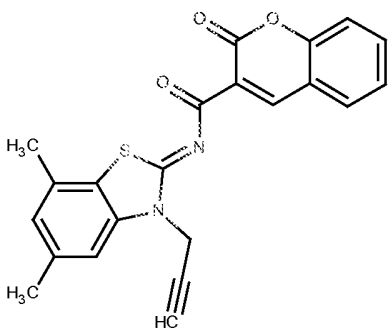
Compound 104	
Compound 105	
Compound 106	

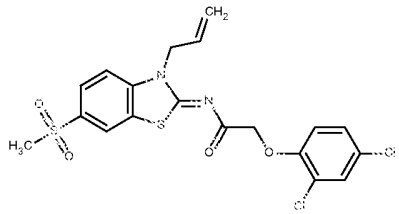
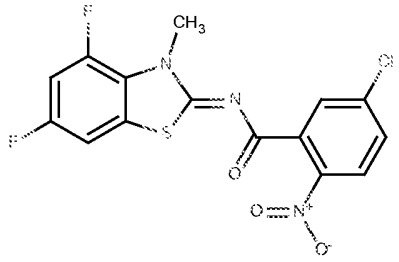
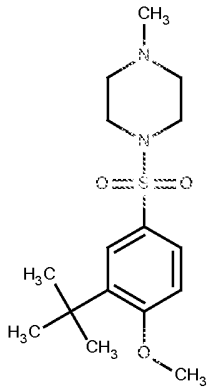
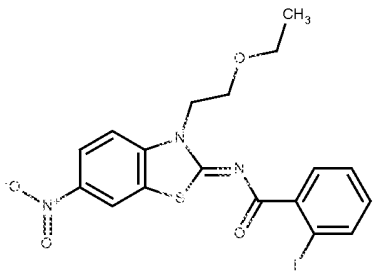
Compound 107	
Compound 108	
Compound 109	

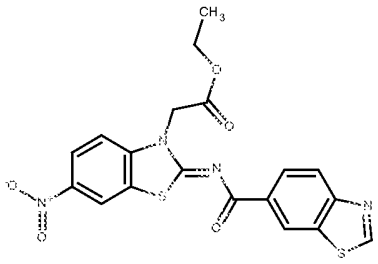
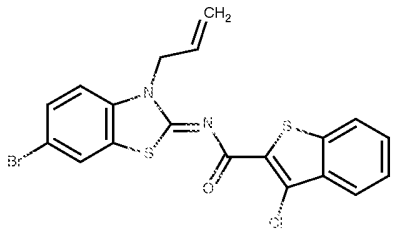
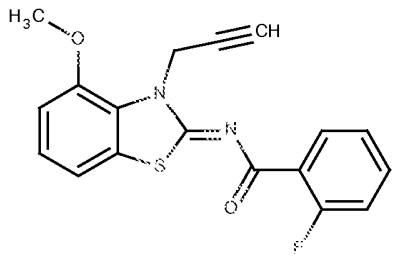
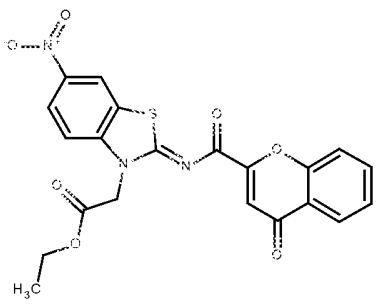
Compound 110	
Compound 111	
Compound 112	
Compound 113	

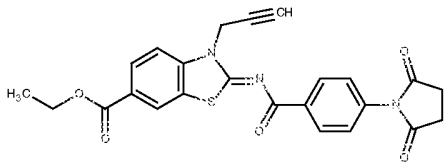
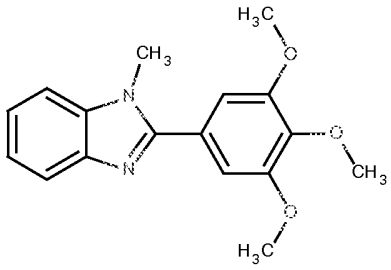
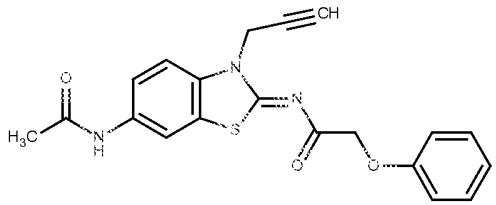
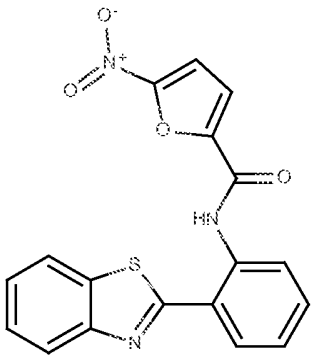
Compound 114	
Compound 115	
Compound 116	

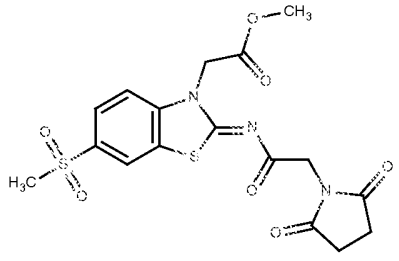
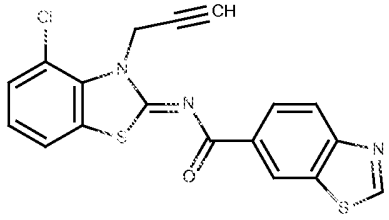
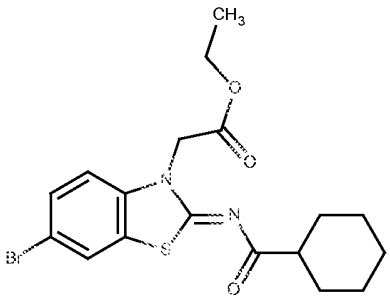
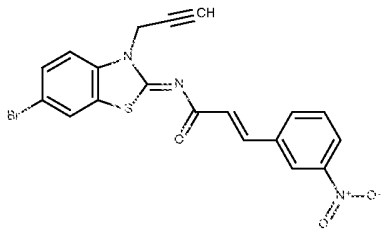
Compound 117	
Compound 118	
Compound 119	

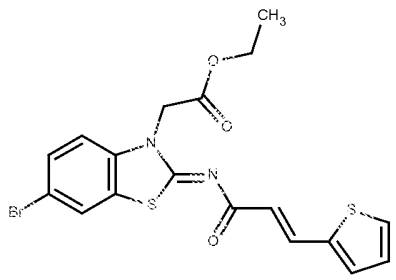
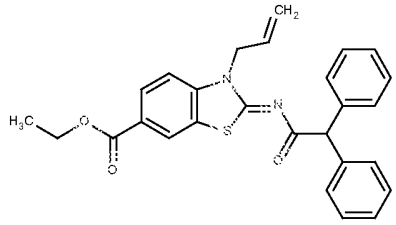
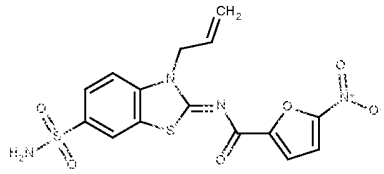
Compound 120	
Compound 121	
Compound 122	
Compound 123	

Compound 124	
Compound 125	
Compound 126	
Compound 127	

Compound 128	
Compound 129	
Compound 130	
Compound 131	

Compound 132	
Compound 133	
Compound 134	
Compound 135	

Compound 136	
Compound 137	
Compound 138	
Compound 139	

Compound 140	
Compound 141	
Compound 142	

[00100] It is to be understood that any pharmaceutically acceptable salts, esters, isomers or solvates of active compounds as described herein are contemplated and included within the scope of the present disclosure.

[00101] Exemplary compounds of the present invention may possess chiral or asymmetric carbon atoms (optical centers) or double bonds; the racemates, diastereomers, geometric isomers and individual optical isomers are all intended to

be encompassed within the scope of this disclosure. In embodiments, the compounds described herein can also include all isotopes of atoms occurring in the final compounds. Isotopes include those atoms having the same atomic number but different mass numbers. For example, isotopes of hydrogen include tritium and deuterium. Further, the compounds described herein may include tautomeric forms, such as keto-enol tautomers. Tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. For example, the tautomers of compounds of structure A, the pyrimidopyridones, are included.

[00102] In certain embodiments, the active compounds described herein may have an IC_{50} for a bacterial helicase selected from less than about 100 micromol/liter (100 μM), less than about 50 micromol/liter (50 μM), less than about 10 micromol/liter (10 μM), and less than about 1 micromol/liter (1 μM). In such embodiments, the bacterial helicase may be selected from one or both of a RecBCD and an AddAB helicase. In other embodiments, the active compounds described herein may be a dual function compound that exhibits an IC_{50} for a bacterial helicase selected from less than about 100 micromol/liter (100 μM), less than about 50 micromol/liter (50 μM), less than about 10 micromol/liter (10 μM), and less than about 1 micromol/liter (1 μM), and an IC_{50} for bacterial gyrase selected from less than about 100 micromol/liter (100 μM), less than about 50 micromol/liter (50 μM), less than about 10 micromol/liter (10 μM), and less than about 1 micromol/liter (1 μM). Again, in such embodiments, the bacterial helicase may be selected from one or both of a RecBCD and a AddAB helicase.

[00103] In further embodiments, an active compound as described herein may be characterized as an agent or composition that causes a measureable change in bacterial growth, viability, or survival. For example, active compounds as described

herein may be characterized as a compound that causes a greater than 2-fold change, greater than 5-fold change, greater than 10-fold change, greater than 15-fold change, and greater than 20-fold change in bacterial growth, viability or survival.

[00104] Without being bound by a particular theory, inhibitors of AddAB and RecBCD may be useful antibacterial drugs for at least two reasons. First, these enzymes are required for repair of DNA damage inflicted upon bacteria by their host cells upon infection. Salmonella *recB* mutants are much less able than wild type to kill a mouse (Buchmeier, N. A.; Lipps, C. J.; So, M. Y.; Heffron, F., *Mol Microbiol* **1993,** *7* (6), 933-6; Cirz, R. T.; Chin, J. K.; Andes, D. R.; de Crecy-Lagard, V.; Craig, W. A.; Romesberg, F. E., *PLoS Biol* **2005,** *3* (6), e176), and *H. pylori addAB* mutants less effectively colonize the mouse stomach than wild type (Amundsen, S. K.; Fero, J.; Hansen, L. M.; Cromie, G. A.; Solnick, J. V.; Smith, G. R.; Salama, N. R., *Molec. Microb.* **2008,** *69*, 994-1007). Second, RecBCD, and perhaps AddAB, is required for the induction of the SOS response to DNA damage, which includes the induction of mutagenic polymerases responsible for most induced mutations (McPartland, A.; Green, L.; Echols, H., Control of *recA* gene RNA in *E. coli*: regulatory and signal genes. *Cell* **1980,** *20*, 731-737). Inhibition of RecBCD and AddAB should thus lessen the evolution of bacteria resistant to the inhibitor, an important goal in current antibacterial drug therapy.

[00105] The AddAB and RecBCD class of enzymes is found in about 90% of all bacterial species whose genomes have been sequenced and reported (Cromie, G. A., *J Bacteriol* **2009,** *191* (16), 5076-84). Compound 1 and its derivatives may be especially effective, since, for example, they contain a pyrimidopyridone moiety that has been found to inhibit DNA gyrase and creates dsDNA breaks whose repair requires the RecBCD enzyme (Cirz, R. T.; Chin, J. K.; Andes, D. R.; de Crecy-

Lagard, V.; Craig, W. A.; Romesberg, F. E., *PLoS Biol* **2005,** *3* (6), e176). Therefore, administration of such compounds can lead to both DNA damage and the failure to repair it. In such embodiments, the compounds described herein provide a single-molecule capable of providing a combination of antibacterial functions.

III. Compositions

[00106] Pharmaceutical compositions are provided herein. Pharmaceutical compositions according to the present description include a pharmaceutically acceptable carrier and a therapeutically effective amount of an active compound according to the present description. The pharmaceutical compositions can take the form of, for example, solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, or suppositories. Examples of suitable pharmaceutical carriers are described in, for example, Remington's Pharmaceutical Sciences, by E.W. Martin. The pharmaceutical compositions disclosed herein may be prepared for administration by any suitable route known to the skilled artisan including, for example, intravenous, subcutaneous, intramuscular, intradermal, transdermal, intrathecal, intracerebral, intraperitoneal, intransal, epidural, pulmonary, and oral routes. Administration can be immediate or rapid, such as by injection, or carried out over a period of time, such as by infusion or administration of controlled or delayed release formulations.

[00107] Where pharmaceutical formulations are prepared for treating tissues in the central nervous system, administration can be by injection or infusion into the cerebrospinal fluid (CSF). Moreover, where pharmaceutical compositions are prepared for delivery to cells or tissues in the central nervous system, the pharmaceutical composition may be formulated to include one or more other carriers

or components capable of promoting penetration of the active compound or a derivative of the active compound across the blood-brain barrier.

[00108] When prepared for oral administration, the pharmaceutical compositions described herein may be prepared, for example, in capsules, tablets, caplets, lozenges, and aqueous suspensions or solutions. Pharmaceutical compositions described herein prepared for oral administration can be formulated using known carriers, including known fillers, diluents, excipients, binders, surfactants, suspending agents, emulsifiers, lubricants, sweeteners, flavorants, and colorants, suited to formulation of the desired dosage form. Additionally, pharmaceutical compositions as described herein can be prepared using formulation approaches that utilize encapsulation in liposomes, microparticles, microcapsules, receptor-mediated endocytosis (see, e.g., Wu et al. J. Biol. Chem. 262:4429-32, 1987), to facilitate delivery or uptake of the active compound.

[00109] Examples of pharmaceutically acceptable carriers include sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, and sesame oil. Aqueous carriers, including water, are typical carriers for pharmaceutical compositions prepared for intravenous administration. As further examples, saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, and ethanol. The composition, if desired, can also contain wetting or emulsifying agents, or pH buffering agents.

[00110] The pharmaceutical compositions described herein can be formulated using any of the active compounds described herein, including any pharmaceutically acceptable salts, esters, isomers or solvates thereof. In certain embodiments, the pharmaceutical compositions described herein include an active compound as described herein, and in alternative embodiments, the pharmaceutical compositions include two or more active compounds according to the present description. The amount of the one or more active compounds included in the pharmaceutical composition will vary, depending upon, for example, the nature and activity of the active compound(s), the nature and composition of the dosage form, and the desired dose to be administered to a subject.

[00111] In some instances, it can be desirable to administer the compositions described herein locally to the area in need of treatment. Local administration can be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (e.g., in conjunction with a wound dressing after surgery), by injection, by means of a catheter, by means of a suppository, or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes such as silastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site of bacterial infection.

[00112] In another embodiment, the agent can be delivered in a vesicle, in particular a liposome (see, e.g., Langer, Science 249:1527 33 (1990); Treat et al., In Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353 65 (1989); Lopez-Berestein, supra, pp. 317 27).

[00113] In yet another embodiment, the agent can be delivered in a controlled release system. In one embodiment, a pump can be used (see, e.g., Langer, supra;

Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see, e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, supra, Vol. 2, pp. 115 138 (1984)). Other controlled release systems are discussed in, for example, Langer (Science 249:1527 33 (1990)).

[00114] In addition to one or more active compounds as described herein and a pharmaceutical carrier, pharmaceutical compositions according to the present description may include one or more additional therapeutic or prophylactic agents. Examples of such agents include amifloxacin, cinoxacin, ciprofloxacin, danofloxacin, difloxacin, enoxacin, enrofloxacin, fleroxacin, irloxacin, lomefloxacin, miloxacin, norfloxacin, ofloxacin, pefloxacin, rosoxacin, rufloxacin, sarafloxacin, gatifloxacin, sparfloxacin, temafloxacin, tosufloxacin, tobramycin, colistin, azithromycin, amikacin, cefaclor (Ceclor), aztreonam, amoxicillin, ceftazidime, cephalexin (Keflex), gentamicin, vancomycin, imipenem, doripenem, piperacillin, minocycline, erythromycin, an aminoglycoside, a tetracycline, a sulfonamide, p-aminobenzoic acid, a diaminopyrimidine, a quinolone, a.beta-lactam, a.beta-lactam and/or a.beta-lactamase inhibitor, chloraphenicol, a macrolide, penicillins, cephalosporins,

corticosteroid, prostaglandin, linomycin, clindamycin, spectinomycin, polymyxin B, colistin, bacitracin, isoniazid, rifampin, ethambutol, ethionamide, aminosalicylic acid, cycloserine, capreomycin, a sulfone, clofazimine, thalidomide, a polyene antifungal, flucytosine, imidazole, triazole, griseofulvin, terconazole, butoconazole ciclopirax, ciclopirox olamine, haloprogin, tolnaftate, naftifine, or terbinafine, either individually or in any combination.

[00115] It should be understood, however, that a specific dosage and treatment regime for any particular subject or disease state will depend upon a variety of factors, including the age, body weight, general health, sex, diet, time of administration, nature of active compound(s), rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease and/or microorganism being treated. Moreover, determination of the amount of a pharmaceutical composition to be administered to a subject will depend upon, among other factors, the amount and specific activity of the active compound(s) included in the pharmaceutical composition and the use or incorporation of additional therapeutic or prophylactic agents or treatment regimes. Determination of therapeutically effective dosages may be based on animal model studies and is typically guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of bacterial growth in model subjects.

A non-limiting range for a therapeutically effective amount of the active compounds described herein is from about 0.001 mg/kg to about 100 mg/kg body weight per day. For example, pharmaceutical compositions according to the present description can be prepared and administered such that the amount of active compound according to the present description administered to a subject is selected from between about 0.001 mg/kg and about 50 mg/kg, between about 0.01 mg/kg and about 20 mg/kg,

between about 0.1 and about 10 mg/kg, and between about 0.1 mg/kg and about 5 mg/kg body weight per day.

IV. Methods of Identifying Active Compounds

[00116] Methods are also provided to identify agents that selectively inhibit bacterial RecBCD and/or AddAB activity. In one embodiment, the method generally comprises the steps of analyzing a candidate compound in a series of enzymatic and bacterial growth assays, and determining whether the candidate compound selectively inhibits the growth of bacteria.

[00117] In one embodiment, an assay disclosed herein is based on the ability of phage T4 *gene 2* mutants to grow in *E. coli* only if the RecBCD nuclease is inactivated by mutation (Oliver, D. B.; Goldberg, E. B., *J. Mol. Biol.* **1977,** *116*, 877-881) (Fig. 1). The nuclease, which resides in the RecB polypeptide (Yu, M.; Souaya, J.; Julin, D. A., *J. Mol. Biol.* **1998,** *283*, 797-808), is active only if the RecD subunit is present and only if the RecB helicase is active (Hsieh, S.; Julin, D. A., *Nucleic Acids Res.* **1992,** *20* (21), 5647-5653; Amundsen, S. K.; Taylor, A. F.; Chaudhury, A. M.; Smith, G. R., *Proc. Natl. Acad. Sci. USA* **1986,** *83*, 5558-5562). The RecB helicase in turn is highly active only in the presence of the RecC subunit (Masterson, C.; Boehmer, P. E.; McDonald, F.; Chaudhuri, S.; Hickson, I. D.; Emmerson, P. T., *J. Biol. Chem.* **1992,** *267* (19), 13564-13572).

[00118] In particular embodiments, the primary screen is an enzymatic assay to test candidate compounds for activity against AddAB activity in, for example, V3065 (*addAB*$^+$) and V3069 (vector control) strains of *E. coli,* in the presence of a T4 gene *2 am149* triple nonsense mutant phage. If a compound is confirmed to be active in the primary screen via retesting, the compound is further tested in a RecBCD enzymatic counterscreening assay and/or a bacterial viability counterscreening assay. The

RecBCD enzymatic counterscreen is similar to the primary screen, but uses, for example, V66 (*recBCD*$^+$) and V67 (*recB21*) strains of *E. coli* instead of the V3065/V3069 strains. It should be understood that, although the methods and screens provided herein are described in the context of certain materials, such as bacterial strains, the screens and methods are not limited to the specific materials and organisms described. For example, the methods and screens may utilize other bacterial strains to carry out the methods and obtain the desired information.

[00119] The bacterial viability counterscreen is similar to the primary screen, but without the presence of the T4 phage. Compounds which show adequate activity in these screens are further analyzed by running additional screens with purified enzymes (a titration assay with AddAB and a selectivity assay with RecBCD, for example), and a general cytotoxicity assay against the V3065 strain. In addition, dual function compounds as described herein may be screened by further assessing compounds that exhibit RecBCD and/or AddAB inhibition for activity against bacterial DNA gyrase.

[00120] A skilled artisan would understand that any strain of *E. coli* expressing *recBCD* or *addAB* genes from any species may be used in the primary screen. Similarly, any appropriate phage in any bacterial host may be used, and any phage derived from T4 or a related phage may be used.

[00121] In an embodiment, a compound may be deemed as active in the above-described screens, if it shows an inhibition greater than the average percent inhibition of the set of compounds screened. In other embodiments, a compound may deemed active if it exhibits an inhibition greater than the average percent inhibition of the set of compounds screened plus a significant increase in the standard deviation in the primary and confirmatory screens. For purposes of the

present disclosure a "significant increase" in the standard deviation may be selected from at least 1.5 times, at least 2 times, at least 2.5 times, and at least 3 times the standard deviation. In the counterscreen using RecBCD, a compound may be deemed active if it shows an inhibition greater than the average percent inhibition of all DMSO-only wells tested. In other embodiments, a compound may be deemed active if it exhibits an inhibition greater than the average percent inhibition of all DMSO-only wells plus a significant increase in the standard deviation. In particular embodiments of a counterscreen using *E. coli*, a compound may be deemed active if it shows an inhibition greater than the average percent inhibition of all DMSO-only wells tested plus a significant increase in the standard deviation. In specific embodiments, in the titration screen, a compound may be deemed active if it shows an IC_{50} < 10 μM. In further embodiments, in the RedBCD selectivity screen and the *E. coli* cytotoxicity screen, a compound may be deemed active if it shows an IC_{50} > 10 μM. In particular embodiments, the threshold values for deeming a compound to be active may be different than those listed above.

A compound may be further analyzed in genetic recombination assays, including an *E. coli* Hfr recombination assay, a phage λ recombination assay, and/or a Chi hotspot activity to help determine the specific type of inhibition exhibited (e.g., to differentiate the helicase vs. nuclease activity of the AddAB or RecBCD enzyme). The assays described herein may also be useful for applications outside of bacteria, for example, to assay in any way for inhibition of RecBCD and/or AddAB enzyme activity in cells. In an embodiment, the inhibition of RecBCD and/or AddAB may occur directly in a purified enzyme assay and intermediate assays may be skipped.

[00122] For purposes of providing a specific example of a screening process carried out according to an embodiment of the methods described herein, a detailed

description of such a process is provided. In particular, a high-throughput screen based on the ability of phage T4 *gene 2* mutants to grow in *Escherichia coli* only if the host RecBCD enzyme, or a related helicase-nuclease, is inhibited or genetically inactivated, has been developed. In embodiments described herein, this screen has been optimized for use in 1536-well plates and many small molecules have been screened as inhibiting the *Helicobacter pylori* AddAB enzyme expressed in an *E. coli recBCD* deletion strain.

[00123] Secondary screening utilized assays with cells expressing AddAB or RecBCD and a viability assay that measured the effect of compounds on cell growth without phage infection. From this screening campaign, a subset of compounds exhibiting efficacy and selectivity were tested for inhibition of purified AddAB and RecBCD helicase and nuclease activities and in cell-based assays for recombination; several were active in the 0.1 – 50 μM range in at least one assay. Compounds structurally related to two of these were similarly tested, and compounds active in the 0.1 – 50 μM range were identified.

[00124] Development of the Screens

[00125] Because phage T4 *gene 2* mutants grow in *E. coli* mutants lacking RecBCD nuclease activity but not in *E. coli* wild-type or other nuclease-deficient mutants (Oliver, D. B.; Goldberg, E. B., *J. Mol. Biol.* **1977,** *116*, 877-881), RecBCD evidently is the only nuclease that blocks this mutant phage's growth. Wild-type T4 phage are able to grow in wild-type *E. coli* presumably because the *gene 2* protein binds to the ends of the ds DNA in the phage virions and, upon injection of the DNA into the host cell, blocks the action of RecBCD (Fig. 1). As such, conditions were developed that allow *E. coli recB21* null mutants but not *recBCD*$^+$ cells to be lysed by T4 *gene 2* mutant phage.

[00126] After infection at a multiplicity of infection (MOI) of 0.01 in liquid culture, *E. coli recB21* cells increase in OD for about 2 h and then cease growth, presumably when the phage have multiplied sufficiently to infect and begin to lyse most or all of the cells (Fig. 2). Under these conditions, *recBCD*$^+$ cells grow about the same with or without phage infection. *E. coli* cells bearing a deletion of the *recBCD* genes and harboring a plasmid expressing the *H. pylori addAB*$^+$ genes also grow about the same with or without phage infection (Amundsen, S. K.; Fero, J.; Hansen, L. M.; Cromie, G. A.; Solnick, J. V.; Smith, G. R.; Salama, N. R., *Molec. Microb.* **2008,** *69*, 994-1007.) An inhibitor of RecBCD or AddAB nuclease would thus likely allow T4 *gene 2* mutant phage to block the growth of *recBCD*$^+$ (or *addAB*$^+$) cells, and an inhibitor specific for RecBCD or AddAB would not block the growth of uninfected cells. These criteria were used to screen for specific inhibitors of these two enzymes.

[00127] To screen large numbers of compounds in 0.1 ml cultures in 96-well plates, reproducible results were obtained by diluting freshly grown cells about 1:100

into LB broth containing compound and adding phage after 1 h of incubation. In every well *recB21* cells were lysed or failed to grow and in nearly all of the wells *recBCD*$^+$ cells grew to at least as high OD as without infection (*e.g.,* Fig. 2); however, in about 2% of the wells *recBCD*$^+$ cells were also lysed. Similar results were found with *E. coli* expressing *H. pylori* AddAB. The wells with lysed cells were found to contain revertants or pseudorevertants of the *gene 2* mutation, a nonsense mutation at codon 247 of 275 codons in the gene (NCBI NP_049754) (Miller, E. S.; Kutter, E.; Mosig, G.; Arisaka, F.; Kunisawa, T.; Ruger, W., *Microbiol. Molec. Biol. Rev.* **2003,** *67* (1), 86-156). To circumvent this problem, a phage was constructed with three nonsense mutations, at codons 247, 248, and 249. A phage with the deletion of *gene 2* was unable to be constructed, presumably because part of the *gene 2* protein is also required for packaging phage DNA (Wang, G. R.; Vianelli, A.; Goldberg, E. B., *J. Bacteriol.* **2000,** *182* (3), 672-9). Revertants of this triple nonsense phage, designated *gene 2 am149,* have not been observed in >10^3 assays without a compound added.

[00128] Screening of Large Libraries

[00129] To screen larger libraries, the assay was converted for use in 1536-well plates. The AddAB (strain V3605) phage assay was selected as the primary screen to test a total of 326,100 distinct chemical entities. All compounds were tested at 12 μM in singlicate. Primary screen results were reviewed, and 937 compounds that appeared nominally active ("hits") were advanced for secondary assay analysis; 52 of these compounds were unavailable from the NIH Molecular Libraries-Small Molecule Repository.

[00130] In the secondary assays, the 885 available compounds were first retested in triplicate in the primary screening assay to confirm activity. The same compounds

were also tested in triplicate for their effect on the viability of strain V3065 (*i.e.,* in the absence of phage) and also screened in triplicate in strain V66 in the presence of phage to determine RecBCD inhibition. From these efforts, 225 hits that appeared active in either the RecBCD or AddAB inhibition assays were advanced to titration assays.

[00131] In titration assays, compounds were tested in triplicate as 10-point titrations using the same protocols used for secondary assays: IC_{50} values were then determined. All HTS assays were determined to be robust, as each assay demonstrated Z'-scores greater than 0.8.

[00132] A summary of the entire screening effort, including summary statistics for all screening assays, is presented in Example 1.

[00133] Direct Enzyme Assays

[00134] From the screens above, seven compounds (Example 1) and five related compounds were chosen for direct tests with purified enzymes. These compounds, listed in Table 9 and shown generally in Fig. 3, form four general structural classes, designated here "pyrimidopyridones" (group A), "cyanothiophenes" (group B), "nitrofurans" (group C), and "nitrothiazole" (group D). An additional fifth class, the "iminobenzothiazoles" (group E) was also identified.

[00135] The ability of the compounds to inhibit the exonuclease activity of purified *E.coli* RecBCD and *H. pylori* AddAB enzymes was assayed initially. Compound concentrations from 0.2 μM to 500 μM were tested. IC_{50} values ranged from about 15 μM to over 100 μM (Fig. 4 and Table 9). For both enzymes, Compound 1 (group A) and Compound 4 (group B) were potent. In helicase assays, these compounds did not significantly inhibit AddAB (Fig. 10), but several inhibited RecBCD's helicase and Chi-cutting activities. Compound 4 inhibited both of these activities with an IC_{50}

of about 20 μM (Fig. 5), and three nitrofurans (Compounds 12, 14, and 8) inhibited with an IC_{50} of <500 μM. Compound 1 appeared to inhibit in a biphasic manner, by inhibiting both helicase and Chi-cutting activities at 50 μM, but at 500 μM it appeared to stimulate the helicase and to change the position of specific cuts.

[00136] To identify additional compounds, compounds related to Compound 1 and Compound 4 were tested. In the T4 *gene 2* mutant-sensitization assay, used for the initial screen, Compound 3 appeared to be RecBCD-specific and Compounds 34, 35, 36, and 39 appeared AddAB-specific; *i.e.,* growth of cells with each enzyme was inhibited only if cells were infected with T4 *gene 2*$^-$ phage (Fig. 11). Compound 2 inhibited growth of cells with either enzyme even without phage infection; thus, this compound appears to inhibit *E. coli* growth independently of RecBCD or AddAB. Compounds 25, 26, 37, 38, and 40, and to a lesser extent Compounds 20 and 21, also inhibited growth without phage infection, but only of cells with AddAB. This result may reflect the poorer growth of *E. coli* with AddAB than with RecBCD, perhaps because AddAB does not effectively utilize *E. coli* RecA but RecBCD does (Amundsen, S. K.; Fero, J.; Salama, N. R.; Smith, G. R., *J. Biol. Chem.* **2009,** *284*, 16759-66).

[00137] In direct tests of inhibition of the purified enzymes, it was found that most of the derivatives of Compound 1 inhibited RecBCD nuclease activity and several inhibited AddAB nuclease activity (Fig. 12). Notably, Compound 3 inhibited both enzymes in both cell-based and enzyme-based assays. Compound 2 did not inhibit either purified enzyme, a result consistent with its inhibition of cell growth independent of RecBCD or AddAB (Fig. 11). Such results serve to validate the cell-based screen.

[00138] Derivatives of Compound 4, e.g., Compounds 5, 44, and 45, inhibited the nuclease activity of both of the purified enzymes with IC_{50} of <100 μM (Figs. 6 and 12), From these nuclease assays, Compounds 3 and 5 appear to be potent inhibitors. The IC_{50} of Compound 3 is about 5 μM for RecBCD and about 25 μM for AddAB, and that of Compound 5 is about 30 μM for *H. pylori* RecBCD and about 15 μM for *H. pylori* AddAB (Fig. 6).

[00139] Assays of the RecBCD helicase and Chi-cutting activities showed that Compound 3 and Compound 5 inhibited these activities much like the parent compounds: Compound 3 inhibited in a biphasic way and Compound 5 in a monophasic way (IC_{50} of about 5 and about 30 μM, respectively); neither compound inhibited AddAB unwinding activity.

[00140] Inhibition of Intracellular Recombination

[00141] To explore further the ability of the assayed compounds to inhibit RecBCD or AddAB in cells, the ability of the compounds to inhibit *E. coli* Hfr-based recombination and phage λ recombination was analyzed. When tested at 100 or 200 μM, seven of the initial 12 compounds reduced Hfr recombinant frequencies by less than a factor of 2, but the remaining five compounds and four of the derivatives inhibited more, by factors up to about 200 (Table 9 and Figs. 7 and 13). For six of these latter nine, dose-response assays showed that Compounds 1, 2, 3, 6, 7, and 15 inhibited with IC_{50} of <1 μM (Fig. 7).

[00142] For Compounds 2, 6, and 7 this outcome was surprising, since these compounds did not significantly affect RecBCD nuclease, unwinding or Chi-cutting activities (Figs. 4, 5, and 12). These compounds at ≥40 μM inhibit cell growth without T4 *gene 2* mutant infection (Fig. 11; Table 9) and may inhibit an enzyme other than RecBCD required for recombination. For example, DNA gyrase, which is

required for RecBCD pathway recombination (Ennis, D. G.; Amundsen, S. K.; Smith, G. R., *Genetics* **1987,** *115*, 11-24) is inhibited by pyrimidopyridones such as Compound 2 (pipemidic acid) (Zweerink, M. M.; Edison, A., *Antimicrob. Agents Chemother.* **1986,** *29* (4), 598-601; Shen, L. L.; Pernet, A. G., *Proc. Natl. Acad. Sci. USA* **1985,** *82* (2), 307-11). In contrast Compounds 1, 3, and 15 inhibited Hfr recombination with IC_{50} of <1 μM (Fig. 7), as well as RecBCD nuclease, unwinding, or Chi-cutting activities, albeit at higher IC_{50} (Figs. 4, 5, 6, and 12). Compound 1 also inhibited Hfr recombination when AddAB replaced RecBCD in *E. coli* cells: 20 μM Compound 1 reduced the recombinant frequency to that of cells lacking AddAB or RecBCD (0.02% His^+ Str^R recombinants per Hfr donor cell).

[00143] To determine if inhibition of Hfr recombination was specific to RecBCD, the six compounds used in Fig. 7 for inhibition of Hfr recombination were tested by two alternative pathways, called RecE and RecF, that are activated by mutations (*sbc*) that suppress the recombination-deficiency of *recB recC* null mutants (Clark, A. J., *Annu. Rev. Genet.* **1973,** *7*, 67-86). Each compound inhibited recombination by the RecBCD (wild-type) pathway to a greater extent than recombination by the RecE or RecF pathway (Table 10). Two compounds, Compounds 2 and 15, significantly inhibited recombination by the latter pathways, by factors of 6 – 13.

[00144] Thus, these data suggest that these six compounds are not specific to RecBCD enzyme, but they may inhibit instead, or in addition, an enzyme required more stringently by the RecBCD pathway than by the other pathways. For example, DNA gyrase, which is inhibited by Compound 2 (Zweerink, M. M.; Edison, A., *Antimicrob. Agents Chemother.* **1986,** *29* (4), 598-601; Shen, L. L.; Pernet, A. G., *Proc. Natl. Acad. Sci. USA* **1985,** *82* (2), 307-11), may be the target of Compound 2, as suggested above. The effects of Compounds 1, 3, and 6 on purified RecBCD

(Figs. 4, 5, 6, and 12) and on intracellular recombination (Tables 9 and 10 and Figs. 7 and 13) indicate that at least these three compounds inhibit recombination by RecBCD in cells.

[00145] To extend the intracellular assays, the effects of the compounds on phage λ recombination dependent on RecBCD (the phages are *red gam* mutants) were analyzed. Results were obtained with Compounds 7, 6, 2, and 3, which inhibited λ recombination with IC_{50} of <15, 5, 0.6, and 5 μM, respectively (Fig. 8). These compounds also inhibited RecBCD-dependent Hfr recombination, as noted above. In λ crosses, the activity of the Chi hotspot, which regulates RecBCD activity (Smith, G. R., *Microbiol Mol Biol Rev* **2012**, 76, 217-228; Dillingham, M. S.; Kowalczykowski, S. C., *Microbiol Mol Biol Rev* **2008,** *72* (4), 642-71; Smith, G. R., *Annu. Rev. Genet.* **2001,** *35*, 243-274), is measured as the ratio of the recombinant frequency in a genetic interval with Chi to that in the same interval without Chi. This ratio is about 5 in wild-type *E. coli* and 1 in *recBCD* null mutants, meaning that Chi is inactive in the absence of RecBCD (Stahl, F. W.; Stahl, M. M., *Genetics* **1977,** *86*, 715-725). Three compounds, Compounds 2, 3, and 15, significantly lowered Chi hotspot activity to a value of about 3 (Table 9).

[00146] A class of compounds, here called "iminobenzothiazoles" or class E, was identified and tested for inhibition of *E.coli* RecBCD nuclease activity, and three were identified with an IC_{50} of <100 μM (Figs. 9 and 14). Their dose responses were unexpected. Compound 18 detectably inhibited at concentrations as low as about 2 μM; inhibition was about 50% at about 5 μM and remained at that level at concentrations as high as 500 μM. As their concentrations were raised, Compounds 16 and 17 inhibited more gradually than expected for single-site inhibition: activity decreased from about 90% to about 10% over a range of about 2.5 $\log_{10}$.

[00147] These results may reflect differential inhibition of the two helicases in RecBCD. Without being bound by theory, Compound 18 may inhibit only one of the helicases, with IC_{50} of about 2 μM, and this helicase may be responsible for only half of the nuclease activity measured. The other two compounds may inhibit this helicase with IC_{50} of about 10 μM and the other helicase with IC_{50} of about 100 μM, so that nuclease activity is inhibited only gradually as the concentration is raised. DNA unwinding and Chi-cutting activities were also inhibited by these three compounds (Fig. 9). The IC_{50} values, most readily quantified for Chi cutting, were about 50 μM.

[00148] Advantages of a cell-based screen

[00149] By using a cell-based assay, only compounds that entered *E. coli* cells sufficiently readily to inhibit the target enzyme, either the native RecBCD enzyme or the AddAB enzyme expressed from the *H. pylori* genes, or RecBCD or AddAB expressed from genes of any species, are able to be analyzed. In addition, these compounds must inhibit the enzyme in its natural environment, which might be markedly different from that of conditions normally employed to study the purified enzymes.

[00150] The assay disclosed herein is simple and inexpensive, since it employs only bacteria and phage, which are readily grown in large quantities, and reliable: Z' factors of ~0.9 were routinely observed (Table 8). In principle this assay is specific for RecBCD or related nucleases, such as AddAB, since activity of the critical reagent used – phage T4 *gene 2* mutants' lysis of *E. coli* cells (Fig. 1) – is detected only in *recBCD* mutants (Oliver, D. B.; Goldberg, E. B., *J. Mol. Biol.* **1977,** *116*, 877-881). Compounds that inhibit AddAB or RecBCD or both were identified. Certain compounds, Compound 1 (a "pyrimidopyridone") and Compound 4 (a

"cyanothiophene"), for example, were found to inhibit the ds exonuclease, DNA unwinding, and Chi-cutting activities of RecBCD and the ds exonuclease activity of AddAB (Figs. 4, 5, 6, and 12). Thus, this screen indeed revealed compounds of the desired type.

[00151] Identification of Potent Inhibitors of AddAB and RecBCD Enzymes

[00152] By direct nuclease assays in the presence of about 20 compounds structurally related to Compounds 1 and 4, additional inhibitors were identified. Compound 5 inhibits AddAB nuclease with an IC_{50} of about 15 μM, but like its parent compound (Compound 4), it does not detectably inhibit AddAB unwinding activity under the conditions used (Figs. 6, 10, and 12).

[00153] Like its parent compound (Compound 1), Compound 3 inhibited all of the activities of RecBCD tested, both with purified enzyme and with cell-based recombination assays (Figs. 4, 5, 6, 7, 8, 12, and 13; Tables 9 and 10). IC_{50} values were about 3 μM for nuclease and Chi-cutting with purified enzyme, about 0.3 μM for Hfr recombination and about 5 μM for phage λ recombination promoted by RecBCD; it also significantly reduced Chi hotspot activity in λ crosses (Table 9). It only marginally inhibits recombination by the *E. coli* RecE and RecF pathways, which do not employ RecBCD (Table 10). In the T4 *gene 2* mutant screen, it only slightly inhibits *E. coli* growth in the absence of phage but strongly inhibits in the presence of phage (Fig. 11).

[00154] The IC_{50} values of Compounds 1 and 3 were about 10 times lower in the intracellular assays for Hfr recombination than in assays with purified enzyme. Without being bound by theory, this result may reflect differences in the enzyme's environment during the assays, or it may reflect some activity of RecBCD not yet assayed, such as the loading of RecA protein after action at Chi (Anderson, D. G.;

Kowalczykowski, S. C., *Cell* **1997,** *90*, 77-86), that is even more sensitive to inhibition than the nuclease, DNA unwinding, and Chi-cutting. This result suggests that these or related compounds may be effective as antibacterial drugs.

[00155] The biphasic dose-response curve for inhibition of DNA unwinding and Chi-cutting by Compound 1 suggests a complex interaction between this compound and the RecBCD enzyme. At about 50 μM compound, unwinding and Chi-cutting were strongly inhibited, but at about 500 μM, the unwinding appeared to be stimulated and DNA was cut at novel positions (Fig. 5). Since RecBCD has two helicases and a nuclease involved in these activities, the compound may have differential effects on two or more primary activities. For example, one helicase may be simply inhibited at low concentrations, and the other stimulated or altered at high concentrations. The cutting of DNA at novel positions is reminiscent of the behavior of RecBCD enzyme from two mutants altered in the RecB helicase domain, *recB2732* (Y803H) and *recB2734* (V804E). These mutant enzymes cut not at Chi but at a position that depends on the length of the DNA substrate (Amundsen, S. K.; Taylor, A. F.; Reddy, M.; Smith, G. R., *Genes Dev* **2007,** *21* (24), 3296-307). Without being bound by a particular theory, an hypothesis is that the RecB nuclease cuts wherever it is on the DNA when the faster helicase, RecD, reaches the end of the substrate, and that the *recB* mutations sensitize the enzyme to a signal from Chi through RecC to stop RecD when RecBCD encounters Chi. Compound 1 may similarly sensitize the enzyme to signaling between RecD and RecB.

[00156] Searching for additional effective inhibitors related to Compound 1, Compound 2 was tested, which has the pyrimidopyridone part but not the benzene ring part of Compound 1 in general structure A (Fig. 3). Compound 2 did not detectably inhibit the nuclease activity of purified AddAB or RecBCD enzyme (Fig.

12), but it strongly inhibited Hfr and phage λ recombination (Figs. 7, 8, and 13). In the T4 *gene 2* mutant assay, it inhibited *E. coli* growth with or without addition of phage (Fig. 11). These results indicate that Compound 2 inhibits some cellular component other than RecBCD, such as DNA gyrase, which is known to be inhibited by Compound 2 (pipemidic acid) (Zweerink, M. M.; Edison, A., *Antimicrob. Agents Chemother.* **1986,** *29* (4), 598-601; Shen, L. L.; Pernet, A. G., *Proc. Natl. Acad. Sci. USA* **1985,** *82* (2), 307-11) and is required for cell growth (Gottesman, M. M.; Hicks, M. L.; Gellert, M., *J. Mol. Biol.* **1974,** *77*, 531-547) and for recombination (Ennis, D. G.; Amundsen, S. K.; Smith, G. R., *Genetics* **1987,** *115*, 11-24). These results suggest, in turn, that the benzene ring part of Compound 1 may function to inhibit RecBCD. Indeed, Compound 3, the *m*-trifluoromethyl isomer of Compound 1, is more potent than the *o*-trifluoromethyl parent compound in assays of both RecBCD and AddAB nuclease (Fig. 6).

V. Methods of Use

[00157] Methods for using the compounds and compositions described herein are described. In some embodiments, such methods include treating a bacterial infection by inhibition of bacterial DNA repair enzymes, including AddAB and RecBCD helicase-nucleases. In other embodiments, such methods include reducing bacterial survival based on inhibition of bacterial DNA repair enzymes, including AddAB and RecBCD helicase-nucleases. Selective inhibitors of bacterial DNA repair enzymes, including AddAB and RecBCD helicase-nucleases, may be useful as and may lessen the evolution of bacteria resistant to the inhibitor, an important goal in current antibacterial drug therapy. In further embodiments, the methods for treating a bacterial infection according to the present description further include inhibiting

bacterial DNA gyrase in addition or as an alternative to inhibiting one or more bacterial DNA helicases. Methods for the administration of the compounds and compositions described herein to a subject are also described herein.

[00158] The active compounds and compositions of the present invention are useful for treating a subject, including a mammal or other animal, infected with a microorganism, including bacteria. In particular embodiments, the methods described herein include selectively inhibiting one or more bacterial helicases selected from the RecBCD and/or AddAB families of helicases. In further embodiments, the methods described herein include inhibiting bacterial DNA gyrase in addition or as an alternative to inhibiting one or more bacterial helicases as described herein. In embodiments of the methods for using the compounds and compositions described herein to treat a bacterial infection in a subject, a therapeutically effective amount of one or more of the active compounds described herein is administered to the subject.

[00159] Methods for treating diseases or disorders associated with microorganisms including bacteria are also provided. In such methods, one or more active compound as described herein is provided and a therapeutically effective amount of the compound is administered to a subject suffering from the bacterial infection. In certain such embodiments, therapeutically effective amounts of two or more active compounds may be administered to the subject. The bacteria-associated disease or disorder treated by methods according to the present description may be selected from any of the bacteria-associated diseases or disorders described herein.

[00160] In each embodiment of the methods of use described herein, the active compound(s) used or administered may be selected from those described herein,

including any pharmaceutically acceptable salts, esters, isomers or solvates thereof. Moreover, the active compound(s) may be provided and delivered or administered in a pharmaceutical composition according to the present description. In embodiments of the methods described herein, exposure of cells to one or more active compounds or administration of one or more active compounds to a subject includes delivering a pharmaceutical composition as described herein using any suitable route of administration, technique or technology, including those described in association with the pharmaceutical compositions and methods detailed herein.

[00161] In some embodiments of the methods of use described herein, the active compounds may be delivered or administered locally to the area in need of treatment. Local administration can be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (e.g., in conjunction with a wound dressing after surgery), by injection, by means of a catheter, by means of a suppository, or by means of an implant. In another embodiment, the active compound(s) of the invention can be delivered in a vesicle, in particular a liposome (see, e.g., Langer, Science 249:1527-33, 1990; Treat et al, In Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-65, 1989; Lopez-Berestein, supra, pp. 317-27).

[00162] In yet other embodiments of the methods of use, active compound(s) and compositions can be delivered in a controlled release system. In one such embodiment, a pump can be used (see, e.g., Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201, 1987; Buchwald et al., Surgery 88:507, 1980; Saudek et al., N. Engl. J. Med. 321 :574, 1989). In another such embodiment, a polymeric controlled release system or formulation can be used (see, e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida, 1974;

Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macro mol. Sci. Rev. Macromol. Chem. 23:61, 1983; see also Levy et al, Science 228: 190, 1985; During et al, Ann. Neurol. 25:351, 1989; Howard et al, J. Neurosurg. 71:105, 1989). In yet another such embodiment, a controlled release system delivering the active compound(s) or composition can be placed in proximity of the therapeutic target, thus requiring a reduced systemic dose (see, e.g., Goodson, Medical Applications of Controlled Release, supra, Vol. 2, pp. 115-138, 1984). Other controlled release systems are discussed in, for example, the review by Langer (Science 249: 1527-1533, 1990).

[00163] The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology, recombinant DNA, genetics, immunology, cell biology, cell culture and transgenic biology, which are within the skill of the art.

[00164] The following examples are provided merely as illustrative of various aspects of the invention and shall not be construed to limit the invention. It is to be understood that the disclosed compositions and methods are not limited to the particular methodologies, protocols, and reagents described herein. In each instance, unless otherwise specified, standard materials and methods were used in carrying out the work described in the Examples provided.

Examples

Example 1

[00165] Compounds: For a 1536-well primary screen, the Molecular Libraries Small Molecule Repository (MLSMR) library was provided by the NIH's Roadmap: Molecular Libraries Initiative. The MLSMR library is a highly diversified collection of small molecules (more that 50% of compounds exhibit molecular weights between 350 and 410 g/mol) comprising both synthetic and natural products. Powders of compounds in Table 9 were obtained from ChemBridge Laboratories, except for Compounds 2, 3, and 7 (Vitas-M lab); Compounds 11 and 13 (Enamine); Compound 15 (Maybridge); Compound 12 (ChemDiv); and Compounds 16, 17 and 18 (Life Chemicals, Burlington, Canada). An additional library of about 18,400 compounds from Life Chemicals was obtained from Kineta, Inc. (Seattle, WA).

[00166] Results of the screening, including assay statistics, are summarized in Tables 8-10, below.

[00167] Table 8: Initial Screening Summary.

step	screen type	target	no. of cmpds tested	selection criteria	no. of selected cmpds	assay statistics Z'	S/B
1	primary screen	AddAB	326,100	inhibition >12.16%[a]	937	0.91 ±0.02	3.60 ±0.15
2	confirmation	AddAB	885	inhibition >12.16%[b]	256	0.84 ±0.02	2.55 ±0.07
3	counter-screen	RecBCD	885	inhibition >3.95%[c]	NA[e]	0.93 ±0.02	3.73 ±0.32
3b	counter-screen	*E. coli* V3065	885	inhibition >24.60%[d]	NA[e]	0.88 ±0.31	2.58 ±0.09
4a	titration	AddAB	225	IC_{50}<10 μM for AddAB and >10 μM for RecBCD and *E. coli*	7	0.91 ±0.01	2.61 ±0.06
4b	selectivity	RecBCD				0.93 ±0.01	3.46 ±0.07
4c	cytotoxicity	*E. coli*				0.89	2.58

		V3065		V3065		±0.02	±0.06

[00168] Notes for Table 8: [a] The primary screen hit cutoff was calculated at the average percent inhibition of all test compounds plus three times the standard deviation; [b] The hit cutoff calculated for the primary run was also applied to the confirmation run; [c] The hit cutoff calculated for the counterscreen was derived from the average percent inhibition of all DMSO-only wells tested plus three times the standard deviation; [d] The hit cutoff calculated for the counterscreen was derived from the average percent inhibition of all DMSO-only wells tested plus eight times the standard deviation; and [e] NA, not applicable.

[00169] Table 9: Properties of Active Compounds Found in Screens of AddAB and RecBCD.

Cmpd No.	Inhibition (approximate IC_{50}, μM)					Relative recombinant frequency[b]		
	E. coli (p*addAB*) growth with T4 *2*⁻ phage	AddAB nuclease	*E. coli* *recBCD*⁺ growth with T4 *2*⁻ phage	RecBCD nuclease	Inhibition of RecBCD Chi cutting	*E. coli* Hfr recombination at 200 μM compound	Phage λ recombination at 200 μM compound	Chi hotspot[c] activity at 200 μM compound
1	2.5	34	>40	13	10	16, 15	9, 14	4.1, 4.6
2	ND[e]	>100	ND	>100	ND	0.2, 0.4	9, 11	3.1, 3.5
3	ND	26	ND	5.1	ND	20, 10	18, 23	2.5, 2.1
4	18	96	>120	51	20	37, 21	ND	ND
5	ND	13	ND	33	>500	118, 121[d]	ND	ND
6	8.2	>100	>40	>100	>100	7, 5	0.4, 0.3	5.3, 5.2
7	9.7	>100	86	>100	>500	6, 7	5, 6	4.9, 3.8
8	10	>100	>120	>100	100	34, 42	ND	ND
9	4.9	>100	>120	>100	50	37, 43	ND	ND
10	>13	>100	>120	>100	>500	47, 51	ND	ND
11	4.8	>100	>120	>100	>500	65, 73	ND	ND
12	17	>100	>120	>100	20	47, 55	ND	ND
13	21	>100	>120	>100	>500	49, 63	ND	ND
14	ND	>100	ND	>100	200	49, 54	ND	ND
15	12	130	>120	>100	>500	0.6, 0.3	90, 50	3.1, 2.9
16	ND	ND	>100	10	50	64, 72[d]	ND	ND
17	ND	ND	>100	80	50	78, 67[d]	ND	ND
18	ND	ND	ND	3	50	57, 47[d]	ND	ND

[00170] Notes and references for Table 9: (no note for [a]); [b]Data, from two experiments for each compound, are the recombinant frequencies as a percentage of that without compound; For Hfr recombination these were 8.7 and 8.9% His^+ Str^R per viable Hfr cell in the two experiments, respectively; for λ recombination these were 11.2% and 3.4% J^+ R^+, respectively; [c]Chi hotspot activity, measured in crosses between λ phages 1081 x 1082 and 1083 x 1084 as in [1], is the frequency of recombinants in an interval with Chi to that in the same interval without Chi; In the absence of compounds, values were 5.1 and 4.9 in the two experiments, respectively; [d]Compound was 100 μM; recombinant frequencies in the absence of

compound were 7.6% and 8.4%; [e]ND, not determined; 1. Stahl, F. W.; Stahl, M. M., *Genetics* **1977,** *86*, 715-725.

[00171] Table 10: Pathway Specificity of Hfr Recombination Inhibitors.

Pathway	Genotype	Fold-reduction in recombinant frequency[a]						
		Null mutant[b]	Cmpd 15	Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 7	Cmpd 6
RecBCD	*recBCD*$^+$	1000	450	6.9	300	9	14	13
RecF	*recB21 recC22 sbcB14, C(D)*	1000	13	1.4	13	1.8	1.0	1.2
RecE	*recB21 recC22 sbcA23*	100	11	2.0	5.6	1.8	2.7	3.1

[00172] Notes and References for Table 10: [a]Recipient strains were grown and mated with donor strain V1306 (Hfr PO44) in LB plus the indicated compound (100 μM). Data are the mean factor of reduction (n = 3) in recombinant frequencies compared to those of the untreated control, which were for V66 (*recBCD*$^+$) 4.5, 10.2, and 11.1 %; for JC9387 (*recB21 sbcB14 sbcC* or *D*) 1.3, 2.2, 1.9%; and for JC8679 (*recB21 sbcA23*) 2.2, 2.1, 1.8%. SEM or range was <20% of the mean (n = 2 or 3). Strain JC9387 (*recB21 sbcB14*) presumably also carries an *sbcC* or *sbcD* mutation[2]. [b] The factor of reduction in the frequency of recombinants observed in crosses between an Hfr donor strain and a recombination-deficient null mutant compared to that in the corresponding *rec*$^+$ parent: for the RecBCD pathway V67 (*recB21*) compared to V66 [3]; for the RecF pathway JC8111 (*recF143*) compared to JC9387 [4]; for the RecE pathway N2510 (*recN262*) compared to JC8679.[5] 2. Lloyd, R. G.;

Buckman, C., *J. Bacteriol.* **1985,** *164*, 836-844; 3. Schultz, D. W.; Taylor, A. F.; Smith, G. R., *J. Bacteriol.* **1983,** *155*, 664-680; 4. Horii, Z.-I.; Clark, A. J., *J. Mol. Biol.* **1973,** *80*, 327-344; 5. Lloyd, R. G.; Buckman, C.; Benson, F. E., *J. Gen. Microbiol.* **1987,** *133*, 2531-2538.

[00173] Bacterial and Phage Strains. The *E. coli* strains used are listed in Table 11, and phage λ strains in Table 12.

[00174] Table 11: *E. coli* strains.

Strain	Genotype	Ref.
V66	*hisG4 argA21 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	1
V67	*recB21*::IS*186 hisG4 argA21 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	1
V1306	*thi-1 relA1* λ^- (Hfr PO44)	1
V2831	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^-	2
V3060	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- (λ DE3)	3
V3065	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^- (pSA405)	
V3069	*ΔrecBCD2731 <kan> hisG4 met recF143 rpsL31 galK2 xyl-5* F^- λ^- (pETDuet-1)	
JC8679	*thr-1 leuB6 ara-14 proA2 lacY1 tsx-33 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 recB21 recC22 sbcA23 supE44* F^- λ^-	4
JC9387	As JC8679 but *sbcA*$^+$ *sbcB15 sbcC* (*D*) *sup*$^+$ F^- λ^-	4
594	*lac-3350 galK2 galT22 rpsL179* F^- λ^-	5
C600	*thr-1 leuB6 thi-1 lacY1 tonA21 supE44 rfbD1* F^- λ^-	6

[00175] References for Table 11: 1. Schultz, D. W.; Taylor, A. F.; Smith, G. R., *J. Bacteriol.* **1983,** *155*, 664-680; 2. Amundsen, S. K.; Taylor, A. F.; Reddy, M.; Smith, G. R., *Genes Dev* **2007,** *21* (24), 3296-307; 3. Amundsen, S. K.; Fero, J.; Hansen, L. M.; Cromie, G. A.; Solnick, J. V.; Smith, G. R.; Salama, N. R., *Molec. Microb.* **2008,** *69*, 994-1007; 4. Gillen, J. R.; Clark, A. J., in *Mechanisms in Recombination*, Grell, R. F., Ed. Plenum Press: New York, 1974; pp 123-136; 5. Weigle, J., *Proc. Natl. Acad. Sci. USA* **1966,** *55*, 1462-1466; and 6. Appleyard, R. K., *Genetics* **1954,** *39*, 440-452.

[00176] Table 12: phage λ strains.

Strain	Genotype[a]	Source[a] or ref.
1081	*susJ6 b1453 cI857 χ+D123*	1, 7
1082	*b1453 χ+D123 susR5*	1, 7
1083	*susJ6 b1453 χ+76 cI857*	1, 7
1084	*b1453 χ+76 susR5*	1, 7
DE3	*imm21 Δnin5 Sam7 P_{lacUV5} gene 1* (T7 RNA polymerase)	Novagen

[00177] Notes and References for Table 12: [a] *b1453* is a deletion removing *red*, which encodes recombination proteins exo and beta, and *gam*, which encodes an inhibitor of RecBCD. These phage recombine by the *E. coli* RecBCD pathway. 1. Schultz, D. W.; Taylor, A. F.; Smith, G. R., *J. Bacteriol.* **1983,** *155*, 664-680; 7. Stahl, F. W.; Stahl, M. M., *Genetics* **1977,** *86*, 715-725.

[00178] Phage T4 wild type and gene *2 amN51* mutant are as described in Schultz, D. W.; Taylor, A. F.; Smith, G. R., *J. Bacteriol.* **1983,** *155*, 664-680. A derivative bearing three adjacent nonsense mutations is described herein. Stocks of T4 phage were grown in strain V67, which lacks RecBCD and does not suppress the nonsense mutation(s) so that the phage particles contain DNA not protected by gene 2 protein.

[00179] Plasmids and Oligonucleotide primers. Plasmids are listed in Table 13, and oligonucleotides in Table 14.

[00180] Table 13: Plasmids.

Plasmid	Vector and insertion site	*E. coli* or *H. pylori* insert	Source or ref.
pBR322		None	[8]
pETDuet-1		None	Novagen
pACYCDuet-1		None	Novagen
pSA21	pBR322, *Bam*HI	*E. coli recB*^{21}*CD*	[9]
pMR3	pBR322, *Bam*HI	*E. coli recBCD* wild type	[2]
pSA405	pETDuet-1, *Nde*I *Pst*I	*H. pylori addAB* wild type	[3]
pSA502	pACYCDuet-1, *Nco*I *Pst*I	*H. pylori recA* wild type	[3]
pSA520	pBR322, *Hin*dIII	T4 *gene 2 amN51* (W247*UAG)	This work
pSA524	pBR322, *Hin*dIII	T4 *gene 2 am149* (W247*UAG, A248*UAG, N249*UAA)	This work
pSA600	pBR322	None; bp 381 – 1624 deleted	This work
pSA607	pSA600, *Eco*RI *Bam*HI	*E. coli recBD* at *Bam*HI, *recC* at *Eco*RI	This work

[00181] References for Table 13: 8. Bolivar, F.; Rodriguez, R. L.; Greene, P. J.; Betlach, M. C.; Heyneker, H. L.; Boyer, H. W.; Crosa, J. H.; Falkow, S., *Gene* **1977,** *2*, 95-113; 9. Amundsen, S. K.; Taylor, A. F.; Chaudhury, A. M.; Smith, G. R., *Proc. Natl. Acad. Sci. USA* **1986,** *83*, 5558-5562; 2. Amundsen, S. K.; Taylor, A. F.; Reddy, M.; Smith, G. R., *Genes Dev* **2007,** *21* (24), 3296-307; and 3. Amundsen, S. K.; Fero, J.; Hansen, L. M.; Cromie, G. A.; Solnick, J. V.; Smith, G. R.; Salama, N. R., *Molec. Microb.* **2008,** *69*, 994-1007.

[00182] Table 14: Oligonucleotides.

Oligo number	Nucleotide sequence
OL2636	5’ CATATGAAGCTTGTCAGTGTTTGCTGCAAATACTCCCCATG 3’

	(SEQ ID NO: 1)
OL2637	5' CATATGAAGCTTCACCGTTCTCATTCACATGATATAC 3' (SEQ ID NO: 2)
OL2652	5' TGAAATCGCCCCGAAAGACTAGTAGTAAGTTGTGTTGATGCCACTTC AGC 3' (SEQ ID NO: 3)
OL2653	5' GCTGAAGTGGCATCAACACAACTTACTACTAGTCTTTCGGGGCGAT TTCA 3' (SEQ ID NO: 4)

[00183] Bacterial Growth Media. Luria-Bertani (LB) broth contains 1.0% (w/v) Tryptone (Difco), 0.5% yeast extract (Difco), and 0.5% NaCl. LB agar is LB broth with 1.5% agar (Difco). Cation-adjusted Mueller-Hinton broth was purchased from Becton-Dickinson. TB contains 1.0% Tryptone and 0.5% NaCl; for phage λ crosses, 0.1% maltose was added. BBL top and bottom agar contain TB with 0.75% and 1% agar, respectively. BBL-YE is BBL bottom agar supplemented with 0.2% yeast extract. For growth of strains with plasmids, media were supplemented with ampicillin (100 μg/ml) or chloramphenicol (15 μg/ml). Cultures were grown at 37°C.

[00184] Purified RecBCD and AddAB Enzyme Assays. Nuclease assays measured the formation of TCA-soluble radioactive material from phage T7 [^{3}H] DNA (2 μg/ml; 6 μM nucleotides) substrate in a 20 min incubation at 37°C (Eichler, D. C.; Lehman, I. R., *J. Biol. Chem.* **1977,** *252*, 499-503). AddAB assays were in 50 mM Tris-HCl (pH 8.5), 10 mM $MgCl_2$, polyvinylpyrrolidone (1 mg/ml), 1 mM DTT, and 50 μM ATP. RecBCD assays used the same condition but with 25 μM ATP. Compounds were diluted in DMSO and added to enzyme in assay buffer on ice; final DMSO concentration was 5.0% in each assay. DNA substrate was added, and after <5 min the reactions were started by transferring the samples to 37°C. Reactions were stopped by addition of calf thymus DNA to 0.2 mg/ml and TCA to 5%. After 10

min on ice, the mixtures were centrifuged for 5 min at 16,100 x g, and the soluble radioactive material determined in a scintillation counter.

[00185] Helicase assays measured the formation of ss DNA from 5' [^{32}P] pBR322 $\chi^{+}F$ (or χ^{o} control) DNA (0.1 nM molecules) linearized by digestion with *Hin*dIII enzyme. AddAB assays were in 25 mM Tris-acetate (pH 7.5), 2.0 mM $Mg(OAc)_2$, 5 mM ATP, 1.5 μM SSB and used 1 nM enzyme. RecBCD assays used the same conditions but with 0.15 nM enzyme and without SSB. Compounds were added to the reaction mixture containing all the reagents except ATP; final DMSO concentration was 2.5% for each assay. Reactions were started by addition of ATP and were at 37°C for 1 min (RecBCD) or 2 min (AddAB). Reactions were stopped by addition of 1/3 vol of stop buffer (2.5% SDS, 100 mM EDTA, 0.125% bromophenol blue, 0.125 % xylene cyanol FF, and 10% Ficoll), and the products subjected to electrophoresis in a 1.25% agarose gel at 5 V/cm for 2.5 h in TAE buffer (40 mM Tris base, 20 mM acetic acid, 1 mM EDTA). Gels were dried under vacuum, and the products detected by autoradiography or analyzed with a Typhoon Trio PhosphorImager (GE Lifesciences) and ImageQuant TL software (Amersham). With RecBCD, this assay also detects cutting of DNA at the Chi site $\chi^{+}F$, which produces a 1.46 kb fragment (Smith, G. R.; Kunes, S. M.; Schultz, D. W.; Taylor, A.; Triman, K. L., *Cell* **1981,** *24*, 429-436).

[00186] **Genetic Assays**. *E. coli* Hfr recombination, phage λ recombination, and Chi hotspot activity assays were conducted as described by Schultz, D. W.; Taylor, A. F.; Smith, G. R., *J. Bacteriol.* **1983,** *155*, 664-680.

[00187] To test for inhibition of Hfr recombination, recipient cells were grown in LB to an optical density (OD) of 0.25 at 650 nm. Compound was added and incubation continued until the OD reached 0.5 (typically 30 to 45 min later). An aliquot was

mixed with donor strain V1306 (Hfr PO44) in the ratio of one donor cell per ten recipient cells. After 8 min, the mixture was diluted 1:50 into LB with compound. After 20 additional min, cells were vortexed to separate mating pairs, diluted, and plated to select recombinants. Viability of the cells was not significantly affected by compounds during this 1.5 h incubation.

[00188] To test for inhibition of phage λ recombination and Chi hotspot activity, *E. coli* strain V66 (*recBCD*$^+$) was grown as above except in TB plus 0.1% maltose. Cells were infected with λ phages at an MOI of 5 each; cross 1 was λ 1081 x λ 1082, and cross 2 was λ 1083 x λ 1084. After 15 min, cells were diluted 1:100 into TB with compound at the same concentration, incubated at 37°C for 90 min, and treated with chloroform. Phage were titered on strain 594 (*sup*$^+$) for J^+ R^+ recombinants and on strain C600 (*supE44*) for total phage. Chi hotspot activity was measured as $\sqrt{(T1/C1)/(T2/C2)}$, where T1/C1 is the ratio of turbid (c^+) to clear (*cI857*) J^+ R^+ recombinants in cross 1 and T2/C2 is the same for cross 2 (Stahl, F. W.; Stahl, M. M., *Genetics* **1977,** *86*, 715-725).

[00189] Construction of T4 gene *2 am149* Triple Nonsense Mutant Phage. Phage T4 gene 2, including 851 bp 5' and 842 bp 3' of the ORF, was amplified from a lysate of phage T4 gene *2 amN51* by a PCR using oligonucleotides OL2636 and OL2637, Platinum Taq Polymerase (Invitrogen), and the manufacturer's suggested conditions. The product was purified on a QIAquick column (Qiagen), digested with *Hin*dIII (New England Biolabs), and ligated into *Hin*dIII-cleaved pBR322 to yield plasmid pSA520. The sequence of gene 2 in this plasmid was that of wild type except for 5' TAG 3' at codon 247 (5' TGG 3' in wild type). Two additional nonsense mutations were introduced into this gene at codons 248 (5' GCG 3' → 5' TAG 3') and 249 (5' AAC 3'→ 5' TAA 3') using a QuikChange reaction (Stratagene-Agilent

Technologies) and oligonucleotides OL2652 and OL2653 to yield plasmid pSA524. Strain V67 (*recB21*) transformed with this plasmid was grown in TB; about 1 x 10^6 cells were embedded in BBL top agar on an LB agar plate, and about 1 x 10^5 T4 wild-type phage spotted on this lawn. After overnight incubation at 37°C, phage were harvested, diluted, and plated on strain V67. About 100 small plaques, from a total of about 600 plaques, were transferred with toothpicks to a lawn of V67 and to a lawn of strain V66 (*recB*$^+$). Phage that grew on V67 but not on V66, about 10% of the total tested, were plaque-purified, grown in V67, and confirmed to contain the expected triple non-sense mutations. This complex mutation is designated gene *2 am149*.

[00190] Compound Screen in 96-well Format. A fresh overnight culture of strain V66 in LB was diluted 1:100 into LB broth with 0.1% DMSO and grown with aeration at 37°C to an OD_{650} of 0.05. Each well of a 96-well plate (Costar, Corning Inc.) was prepared by adding 10 μl of compound in 20% DMSO or the appropriate control (LB with 20% DMSO) and then 100 μl of the bacterial culture (containing about 2.5 x 10^6 cells) or uninoculated medium was added. The OD_{650} of each well was read on a VERSAmax microplate reader (Molecular Devices), and the plates were incubated without shaking at 37°C. After about 1 h the OD_{650} of the cultures was approximately 0.1, and 10 μl of a phage suspension containing 5 x 10^4 phage or 2.5 μg of chloramphenicol was added to the appropriate wells. The plates were incubated for approximately 20 h and the OD_{650} determined.

[00191] AddAB (Strain V3065) primary assay. All reagents were purchased from Sigma unless noted otherwise below. Prior to assay, strains V3065 (*addAB*$^+$) and V3069 (vector control) were grown at 37°C to an OD_{600} of 0.05 (about 2.5 x 10^7 cfu/mL). Three μL of assay buffer containing glycerol (0.1% v/v) and ampicillin (100

μg/mL) (Fisher) in Cation-adjusted Mueller Hinton Broth (Becton-Dickinson) were added to each well of a 1,536-well clear-bottom plate (Aurora, Nexus Biosystems). Sixty nL of test compound (12 μM final concentration), ciprofloxacin (0.95 μg/mL final concentration, as a control for complete inhibition), or DMSO alone (1.2% final concentration) were added to the appropriate wells; compounds and ciprofloxacin were in DMSO. One μL of strain V3065 (addAB$^+$) or strain V3069 (vector control) was dispensed into the appropriate wells, and plates were incubated for 60 min at 37°C. One μL of phage T4 gene *2 am149* mutant was dispensed to the appropriate wells at a multiplicity of infection (MOI) of 0.02. Plates were centrifuged, and after 18 h of incubation at 37°C the absorbance, as OD_{600}, was read on an Envision microplate reader (PerkinElmer). All data were normalized to that of the positive control (wells containing strain V3065, ciprofloxacin, and phage) and negative control (wells containing strain V3065, DMSO, and phage). This protocol was used for primary, secondary, and titration screening assays. The hit-cutoff used to qualify active compounds in the primary assay was calculated as the average percentage activity of all compounds tested plus three times their standard deviation (Hodder, P.; Cassaday, J.; Peltier, R.; Berry, K.; Inglese, J.; Feuston, B.; Culberson, C.; Bleicher, L.; Cosford, N. D.; Bayly, C.; Suto, C.; Varney, M.; Strulovici, B., *Anal. Biochem.* **2003,** *313* (2), 246-54).

[00192] The secondary or confirmation assay used the same conditions as the primary screening assay, except that plates were assessed in triplicate and results for each compound were reported as the average percentage activity of the three measurements, plus or minus the associated standard deviation. For titration experiments, assay protocols were identical to those described above, except that compounds were prepared in 10 point, 1:3 serial dilutions starting at a nominal test

concentration of 120 μM, and assessed in triplicate (Madoux, F.; Li, X.; Chase, P.; Zastrow, G.; Cameron, M. D.; Conkright, J. J.; Griffin, P. R.; Thacher, S.; Hodder, P., *Mol. Pharmacol.* **2008,** *73* (6), 1776-84).

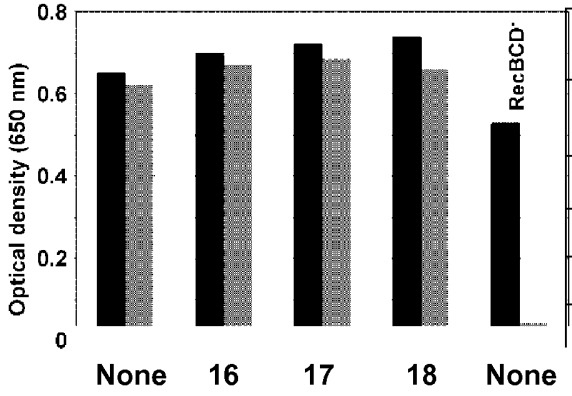
[00193] Bacterial Viability HTS assay. This assay was identical to the AddAB (strain V3065) screening assay, except that no phage was added to the wells. All data were normalized to that of the positive control (wells containing strain V3065 and ciprofloxacin) and negative control (wells containing strain V3065 and DMSO). This protocol was used for primary, secondary, and titration screening assays.

[00194] RecBCD (Strain V66) HTS assay. This assay was identical to the primary AddAB screening assay except that strains V66 (*recBCD*$^+$) and V67 (*recB21*) replaced strains V3065 and V3069, respectively. All data were normalized to that of the positive control (wells containing strain V66, ciprofloxacin, and phage) and negative control (wells containing strain V66, DMSO, and phage). This protocol was used for secondary and titration screening assays.

[00195] Table 15: Protocol for AddAB or RedBCD Screening in 1536-well plates.

Step	Operation	Condition	Comments
1	Medium dispensing	3 μL/well	Medium is Cation Adjusted Mueller Hinton Broth (CAMHB)
2	Compound addition	60 nL/well	Test concn, 12 μM; DMSO concn, 1.2%
3	Cell dispensing	1 μL/well	Bacteria grown in CAMHB to 2.5 x 10^7 cfu/mL
4	Incubation	60 min at 37°C	
5	Phage addition	1 μL/well	Phage concn, 5 x 10^5/mL; MOI, 0.02
6	Incubation	18 hr at 37°C	
7	Optical Density determination	Read plate	OD_{600} optimized absorbance read on PerkinElmer Envision

[00196] Table 16: Activity of Compounds of Structure E in T4 *gene 2* Mutant Screen and Hfr Crosses.



Compound (100 μM)	Relative Hfr recombinant frequency [b]
None	1.0
16	0.64, 0.72
17	0.78, 0.67
18	0.57, 0.47

[00197] Notes for Table 16: *Left:* Growth of strain V66 in the absence (black bars) or presence (gray bars) of phage T4 *gene 2* triple nonsense mutant, as in Fig. 2, in the presence of the indicated compounds (100 μM). "RecBCD$^-$" used strain V67 (*recB21*). Data are the mean of two wells; range is <5% of mean. *Right:* The frequency of His$^+$ Str^R recombinants in matings between strains V66 (F$^-$ *recBCD*$^+$ *hisG4 rpsL31*) and V1306 (Hfr PO44 *rpsL*$^+$ *his*$^+$) in the presence of compound is expressed as a fraction of that in the absence of compound (7.6 and 8.4 % per viable Hfr cell in the two experiments for which data are shown).

[00198] Further analysis of compound 50 is shown in Fig. 15-17. Fig. 15 shows the minimum concentration of compound 50 and norfloxacin required to inhibit the growth of *E. coli* strain V66 (*recBCD*$^+$). Fig. 16 shows the inhibition of *E. coli* growth by Compound 50 or norfloxacin, and Fig. 17 shows that compound 50 inhibits *E. coli* recombination in an Hfr cross.

Example 2

[00199] Compounds 1, 2, 50 and 51 were analyzed further. Fig. 18 shows their activity against *E.coli* RecBCD nuclease. In this experiment, the acid-soluble product formation is a measure of the nuclease activity of the RecBCD: more acid-soluble product indicates high nuclease activity. Compound 50 showed inhibition of both *E.coli* RecBCD and *H. pylori* AddAB nuclease activity at 100 μM and 50 μM (data not shown). Fig. 18 suggests that changes in the structure of the fluoroquinolone portion of the molecule does not affect helicase inhibition and that norfloxacin alone does not inhibit nuclease activity.

[00200] Fig. 19 shows the effect of compound 1 on the ciprofloxacin sensitization of an *E.coli* V66 wild type strain. In this assay, diluted bacteria grew overnight in the presence of Ciprofloxacin at the indicated concentration with 50 μM compound 1 or DMSO (1% final concentration). Fig. 19 suggests that inhibition of RecBCD enhances the antibacterial effects of fluoroquinolones such as ciprofloxacin. Compound 1 inhibits RecBCD but is a weak gyrase inhibitor. As can be seen from Fig. 19, bacteria were unable to grow in the presence of 5 ng/ml ciprofloxacin and 40 μM compound 1. The MIC for the inhibition of bacterial growth for compound 1 was 200 μM with a IC_{50} of 78 μM for *E. coli* V66 (data not shown). When mixed with ciprofloxacin at concentrations (40 μM) that generally have little effect on bacterial growth, compound 1 sensitized wild-type *E. coli* to ciprofloxacin and decreased the MIC of ciprofloxacin in this strain 2- to 4-fold. When compared to the DMSO control, 40 μM compound 1 sensitized V66 *E. coli* cells to ciprofloxacin and enhanced the effect of ciprofloxacin. This suggests that inhibition of RecBCD helicase results in an improved antibacterial activity for fluoroquinolones, and that development of, for example, a ciprofloxacin version of compound 1 or compound 50, may lead to improved antibacterial drugs when compared to conventional quinolones.

[00201] Fig. 20 shows a dose response study of compound 1 in the inhibition of RecBCD helicase and Chi cutting activities. Compound 1 appears to act upon RecBCD in a biphasic manner. The enzyme is effectively inactive at concentrations starting around 40 μM, which correlates with the sensitization of *E.coli* V66 strain to sub-inhibitory concentrations of ciprofloxacin in the presence of 40 μM compound 1 as seen in Fig. 19.

[00202] Fig. 21 shows a dose response study of compound 50 in the inhibition of *E. coli* RecBCD and *H. pylori* AddAB ds exonucleases.

[00203] Fig. 22 shows a dose response study of the inhibition of *E. coli* RecBCD DNA unwinding and Chi cutting activities for compound 50. This gel result was similar to that of compound 3, which is a weak gyrase inhibitor but inhibits RecBCD. This suggests that changing the quinolone portion of the molecule, to make it a more potent gyrase inhibitor, will still retain potency of the compound against DNA helicase.

Example 3

[00204] Several compounds as described herein were evaluated for their ability to inhibit bacterial DNA gyrase. Each of compounds 22, 30, 33, 35, 36, and 37 as described above were screened against *E. coli* gyrase to determine whether they are inhibitors of gyrase-catalysed supercoiling, and each of the compounds inhibited gyrase-catalyzed supercoiling at IC_{50} values below 200 μM, with compounds 22, 33, and 37 exhibiting IC_{50} values as low as 80 μM, and compound 35 exhibiting an IC_{50} as low as 40 μM.

Example 4

[00205] As shown in Table 17, compounds disclosed herein were assayed for their ability to inhibit *E. coli* RecBCD, *H. pylori* AddAB, *M. smegmatis* AddAB, and *M. smegmatis* RecBCD. The purified AddAB and RecBCD enzyme assays were performed as described in Example 1. The *H. pylori* AddAB, *M. smegmatis* AddAB and RecBCD, and *M. tuberculosis* AddAB enzymes were obtained from Seattle Structural Genomics Center for Infectious Disease (Seattle, WA).

[00206] Table 17: AddAB and RecBCD enzyme assays.

		Nuclease IC_{50}, [μM]			
Compound	**Laboratory Compound Name**	*E.coli* RecBCD	*H. pylori* AddAB	*M. smeg* AddAB	*M. smeg* RecBCD
1	104	13	34	-	-
3	104-34	4.6	16	2.4	5.5
143	104-64	4.7	6.6	7	30
30	104-26	20	46	-	-
51	Norf-104	26	79	-	-
50	Norf-34	3.2	10.7	31	4.5
144	Norf-64	3.7	16	3.2	37
57	Cipro-34	10	12	23	6
150	Cipro-64	3	-	6.6	4.9
64	Gati-26	1.2	16	-	-
58	Gati-34	1.8	3.8	3.4	2.1
145	Gati-64	1.2	-	4.5	12
151	Moxi-34	0.7	0.8	3.8	10
152	Moxi-64	-	-	1.8	5.4
148	Sara-34	0.65	0.8	3.3	7.2
149	Sara-64	0.58	0.7	0.6	2.6
146	Lome-34	6.3	8.4	1.2	20
147	Lome-64	-	-	3.1	12

[00207] With reference to Table 17, compounds 3, 26, 50, 148, and 151, among others, resulted in inhibition of the RecBCD and AddAB enzymes tested. More particularly, compound 3 had an IC_{50} of 4.6 μM for *E. coli* RecBCD, an IC_{50} of 16 μM for *H. pylori* AddAB, an IC_{50} of 2.4 μM for *M. smegmatis* AddAB, and an IC_{50} of 5.5 μM for *M. smegmatis* RecBCD. Compound 151 showed an IC_{50} of 0.7 μM for *E. coli*

RecBCD, an IC_{50} of 0.8 μM for *H. pylori* AddAB, an IC_{50} of 3.8 μM for *M. smegmatis* AddAB, and an IC_{50} of 10 μM for *M. smegmatis* RecBCD.

Example 5

[00208] Compounds 151 and 148 as described herein were evaluated for their ability to inhibit *M. tuberculosis* AddAB enzyme. The purified *M. tuberculosis* AddAB enzyme ds exonuclease assay was performed as described in Example 1. As shown by Fig. 23, both compounds inhibited *M. tuberculosis* AddAB, with compound 151 having an IC_{50} of 7.2 μM and compound 148 having an IC_{50} of 7.8 μM .

Example 6

[00209] An *E. coli* p*recA::lacZ* reporter assay was used for the measurement of SOS induction by norfloxacin and its dependence on RecBCD nuclease activity (Fig. 24). *E. coli* p*recA::lacZ* strains were GE94 (Weisemann et al., 1984) or *recB21* (null) or *recB1080* (nuclease-defective) mutant derivatives. Strains were grown at 37°C in LB broth to $OD_{650} \approx 0.4$, norfloxacin was added to the indicated concentration, and incubation continued 60 min, at which time the cultures were assayed for beta-galactosidase according to Weisemann et al. (1984). All cultures contained 2% DMSO, final concentration.

[00210] Norfloxacin and related fluoroquinolone antibiotics kill bacteria by damaging their DNA. These antibiotics cause DNA double strand breaks in bacterial chromosomes by inhibiting DNA gyrase. When there is a DNA break, RecBCD enzyme acts on the break and RecBCD activity induces a gene network to fix the DNA damage. This overall pathway is called the SOS response, and induction of this pathway is called SOS induction. SOS response increases the survival of the

bacteria when there is damage to their DNA in the presence of DNA damaging agents such as norfloxacin or hydrogen peroxide. Additionally, this repair pathway is very error prone and causes mutations that increase the chance of bacteria to develop resistance to antibiotics.

[00211] This network is activated by RecBCD enzyme and activation of this response can be measured by the expression of a reporter enzyme such as beta-galactosidase enyzme that is placed under the control of SOS inducible *recA* promoter. Increase in beta-galactosidase enzyme means increase in expression of SOS response genes. This way the activation of SOS response can be measured.

[00212] As can be seen in Fig. 24, when RecBCD enzyme is inactive as in null mutant (*recB21*) or nuclease mutant (*recB1080*), bacteria can't activate the SOS response in the presence of norfloxacin, resulting in no increase in beta-galactosidase expression. However, bacteria with fully functional RecBCD (RecBCD+) show a many-fold increase in SOS response in the presence of norfloxacin, as measured by increase in beta-galactosidase activity. Therefore, treatment with the antibiotic norfloxacin activates the SOS response in a RecBCD activity-dependent manner.

Example 7

[00213] An *E. coli* p*recA::lacZ* reporter assay was used for the measurement of RecBCD-dependant SOS induction with or without compound 151. *E. coli* p*recA::lacZ* strains were GE94 (Weisemann et al., 1984) or a *recB21* mutant derivative. Strains were grown at 37°C in LB broth to OD_{650} ≈ 0.4, the DNA-damaging agent H_2O_2 (hydrogen peroxide) was added to the indicated concentration with or without compound 151 (1 μM), and incubation continued 60 min, at which

time the cultures were assayed for beta-galactosidase activity according to Weisemann et al. (1984). All cultures contained 2% DMSO, final concentration.

[00214] As shown in Fig. 25, in the presence of hydrogen peroxide, bacteria with fully functional RecBCD ($RecBCD^+$) treated with compound 151 ($RecBCD^+$ + Compound 151) induced SOS response (empty circles) much less than the $RecBCD^+$ bacteria without compound 151 (filled circles). Bacteria without active RecBCD (squares with $recBCD^-$) were controls and showed no appreciable increase in beta-galactosidase activity. Accordingly, treatment the RecBCD inhibitor compound 151 blocks RecBCD-dependent activation of the SOS response.

Example 8

[00215] As shown by Fig. 26, AddAB inhibitors compound 50 and compound 4 impair the ability of *Helicobacter pylori* to colonize the stomach of mice. Mice were infected with 2.5×10^7 cfu of *H. pylori* by oral gavage in the presence or absence of 20 μM compound 50 or compound 4 in methylcellulose. The same dose of compound in methylcellulose or methylcellulose alone was administered by oral gavage daily for 5 days. The stomachs were removed on day 7, processed by mechanical disruption, and the number of *H. pylori* per g stomach determined by plating on Columbia blood agar. For Fig. 26, each circle represents a single mouse; open circles are placed at the limit of detection for the conditions of culture and represent mice from which no *H. pylori* were recovered; the black horizontal line indicates the geometric mean colonization of each group.

Example 9

[00216] As shown by Fig. 27, RecBCD inhibitor compound 3 reduces the frequency of H_2O_2-induced mutation in *E. coli*. H_2O_2 and other reactive oxygen species damage DNA and, in a RecBCD-dependent manner, induce the SOS pathway which includes mutagenic DNA polymerases. *E. coli* strain V66 (*recBCD*$^+$ valine-sensitive) was grown, as indicated, in the presence or absence of 25 μM compound 3 for 2 hr before the addition of 2 mM H_2O_2. The frequency of valine-resistant mutants in the culture at the times indicated was determined by plating on minimal media containing 100 ug/ml valine.

Example 10

[00217] Fig. 28 shows that compound 3, a RecBCD inhibitor, reduces the frequency of H_2O_2-induced mutation to valine-resistance (valine^R) in *E. coli*. H_2O_2 and other reactive oxygen species damage DNA and, in a RecBCD-dependent manner, induce the SOS pathway, which includes mutagenic DNA polymerases. Strain V66 (*recBCD*$^+$ valine-sensitive) was grown, as indicated, in the presence or absence of 25 μM compound 3 for 1 hr before the addition of 2 mM H_2O_2. The frequency of valine^R mutants in the culture was determined 1 hr later by plating on minimal media containing 100 ug/ml valine. The mean and standard error of the mean are shown for 16 separate cultures.

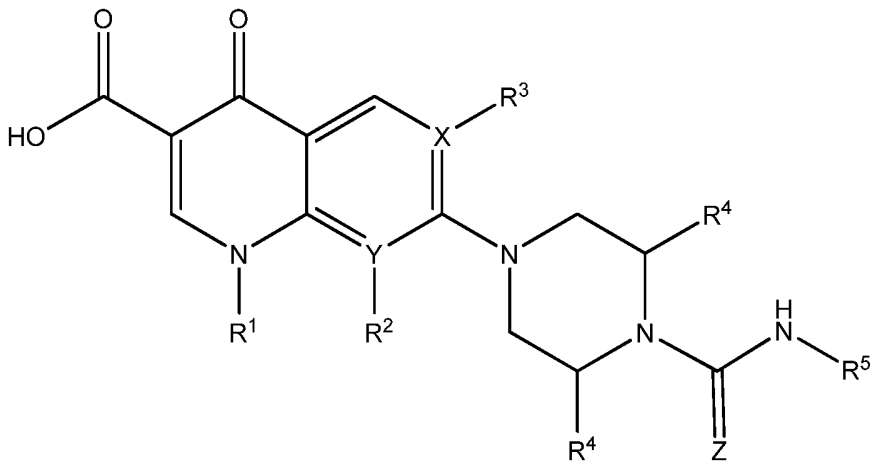
Example 11

[00218] Compounds disclosed herein may be synthesized according to a single step. As shown in Fig. 29, compound 3 is synthesized in a straightforward fashion in a single step using commercially available reagents. Briefly, to an oven dried 100 mL round bottom flask equipped with a magnetic stir bar was added pipemidic acid

(0.250 g; 0.824 mmol), 3(trifluoromethyl)phenyl isothiocyanate (0.167 g; 0.823 mmol), and sodium bicarbonate (0.083 g; 0.988 mmol), and the flask was flushed with Argon for 10 minutes. Dry N,N-dimethylformamide was added (40 mL) and the flask was once again flushed with Argon. The reaction mixture was allowed to stir for 15 hours at room temperature. After confirming the completion of reaction by TLC and LC-MS, the reaction was quenched by addition of 25 mL of a saturated NH_4Cl solution. The contents were transferred to a separatory funnel and extracted with ethyl acetate (3 x 25 mL). The organic extracts were combined and washed with water (3 x 25 mL), brine (1 x 25 mL), and dried over Na_2SO_4. Finally the solvents were evaporated on a rotary evaporator to give a crude solid. The crude material was then purified by flash chromatography (10% MeOH in CH_2Cl_2) to give 0.225 g (54% yield) of compound 3.

Claims

1. A compound which inhibits AddAB and/or RedBCD, the compound comprising an active compound according to one of Formulas I-V:



Formula I

wherein R^1 is alkyl, aryl, or cycloalkyl;

R^2 is H, alkoxyl or halogen;

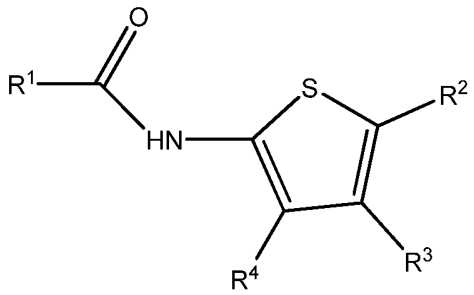
R^3 is H or halogen;

R^4 is H or alkyl;

R^5 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro;

X and Y are independently C or N; and

Z is O or S:



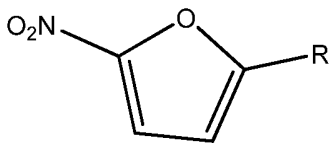
Formula II

wherein R^1 is aryl, cycloalkenyl, heteroaryl, optionally substituted with a substituent selected from at least one of the following: alkyl, aryl, nitro, -COOH, thioalkyl, thioalkylaryl and halogen;

R^2 is H or alkyl;

R^3 is H, alkyl, or aryl, each of which may be optionally substituted with an alkyl group, and wherein R^2 and R^3 together may be connected to form a cycloalkyl or heterocyclic group, which may be optionally substituted with an alkyl group; and

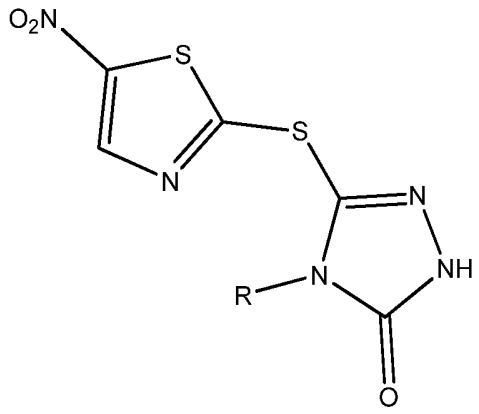
R_4 is CN, -COO-alkyl, -CO-NH_2, -CO-NH-alkyl, -CO-NH-heterocyclyl, -CO-NH-alkyl-heterocyclyl, or NH_2:



Formula III

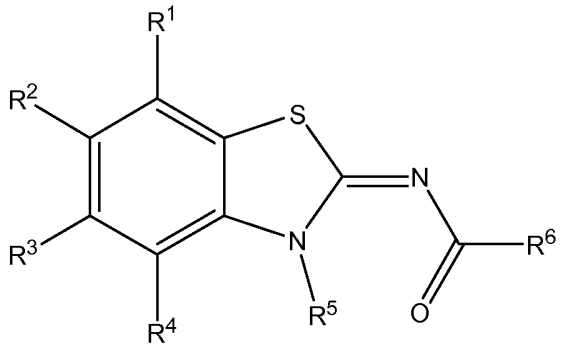
wherein R is selected from at least one of the following: -CO-O-alkyl heteroaryl, -CO-NH-heteroaryl, alkenyl heteroaryl, -CO-O-alkyl-CO-NH-heteroaryl, -CO-NH-aryl, and -CO-NH-alkyl aryl, any of which may be optionally substituted with a substituent

selected from at least one of the following: C=O, N-CO-alkyl, CN, alkyl, -$CONH_2$, heterocyclyl or –NH-CO-haloaryl:



Formula IV

wherein R is an alkyl or alkenyl group: or



Formula V

wherein R^1 is H;

R^2 is H, halo, alkyl, CONH-alkyl, nitro, CO_2-alkyl, SO_2-alkyl or SO_2NH_2;

R^3 is H;

R^4 is H, halo, alkyl, or alkoxy;

R^5 is alkyl, alkenyl, alkynyl, alkyl alkoxy, or alkyl-CO-alkoxy; and

R^6 is aryl, alkyl aryl, alkenyl aryl, alkenyl heteroaryl, alkyl-SO_2-aryl, alkyl-O-aryl, aryl-SO_2-heterocyclyl, heteroaryl, heterocyclyl, cycloalkyl, diphenyl or heterocycloalkenyl, any of which may be optionally substituted with a substituent selected from at least one of the following: nitro, halo, alkyl, alkoxy, aryl, -CO, -CO_2-alkyl, CO-substituted heterocyclyl, methylenedioxy, SO_2-alkyl, or halophenyl-substituted heteroaryl.

2. A compound according to claim 1, wherein the compound exhibits an IC_{50} of less than 100 μM against a bacterial DNA helicase.

3. A compound according to claim 3, wherein the bacterial DNA helicase is a helicase selected from an AddAB helicase and a RecBCD helicase.

4. A compound according to any one of claims 2–4, wherein the compound additionally exhibits an IC_{50} of less than 200 μM against DNA gyrase.

5. A compound according to claim 5, wherein the compound exhibits an IC_{50} of less than 175 μM against DNA gyrase.

6. A compound according to claim 6, wherein the compound exhibits an IC_{50} of less than 100 μM against DNA gyrase.

7. A compound according to claim 6, wherein the compound exhibits an IC_{50} of less than 80 μM against DNA gyrase.

8. A compound according to claim 6, wherein the compound exhibits an IC_{50} of less than 50 μM against DNA gyrase.

9. A compound according to any one of the preceding claims, wherein the compound is selected from any of compounds 1-160.

10. A pharmaceutical composition comprising one or more compounds according to any preceding claim and a pharmaceutically acceptable carrier or excipient.

11. A method of treating a microbial, bacterial or fungal infection in a subject, the method comprising administering to a subject having said infection therapeutically effective amount of one or more compounds according to any one of the preceding claims.

12. A method according to claim 10, wherein administering said one or more compounds to the subject comprises administering a pharmaceutical composition comprising said one or more compounds to the subject, wherein the pharmaceutical composition comprises said one or more compounds and a pharmaceutically acceptable excipient or carrier.

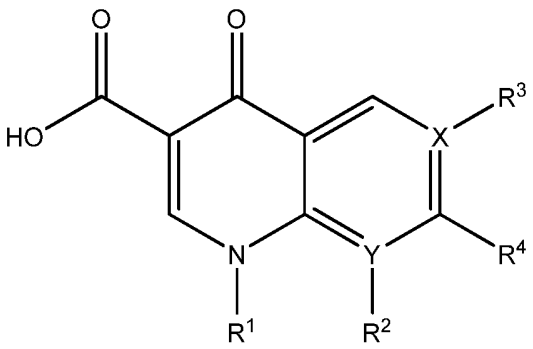
13. The method according to claim 11, wherein the method comprises treating a bacterial infection selected from bacterium-related cutaneous conditions, botulism, cholera, *E. coli* infection, Legionellosis, listeriosis, Lyme disease,

pathogenic bacterial diseases, rickettsioses, salmonellosis, tuberculosis and zoonotic bacterial diseases.

14. The method according to claim 11, wherein the method comprises treating an infection selected from infection by Gram-positive and Gram-negative bacteria, such as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Morganella morganii, Salmonella* serotypes including *Enteritidis, Typhimurium* and *Newport, Enterococci, Shigella dysenteriae, Yersinia enterocolitica, Acinetobacter calcoaceticus, Francisella tularensis, Legionella pneumophila, Helicobacter pylori, Neisseria meningitides, Neisseria gonorrhoeae, Campylobacter jejuni, Vibrio cholera, Pseudomonas aeruginosa, Streptococcus, Staphylococcus, pneumococcus, Mycobacterium tuberculosis, Borrelia burgdorferi, Bordetella pertussis, Legionella pneumophila, Clostridium difficile, Bacillus anthracis*, and *Haemophilus influenza*.

15. A method for treating a subject having a disease or disorder associated with bacterial infection, the method comprising administering to the subject a therapeutically effective amount of an inhibitor of at least one of AddAB or RecBCD, wherein said inhibitor is selected from a compound as defined in claim 1.

16. A compound which inhibits DNA gyrase, the compound comprising an active compound according to Formula Ia:



Formula Ia

wherein R^1 is alkyl, aryl, or cycloalkyl;

R^2 is H, alkoxyl or halogen;

R^3 is H or halogen;

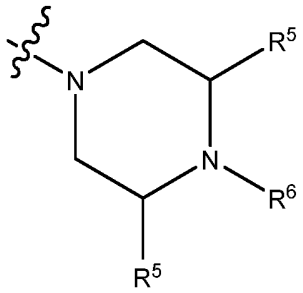
R^4 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, cycloalkyl, heteroaryl, alkyl heteroaryl, heterocyclyl, and heterocyclyl alkyl, any of which may be optionally substituted; and

X and Y are independently C or N..

17. A compound according to claim 16, wherein the compound is selected from one of Compound 151, Compound 152, Compound 153, Compound 154, Compound 155, Compound 156, Compound 157, Compound 158, Compound 159, and Compound 160.

18. A compound according to claim 16,

wherein R^1 is alkyl, R^2 is H, R^3 is fluorine, X and Y are each C, and R^4 is

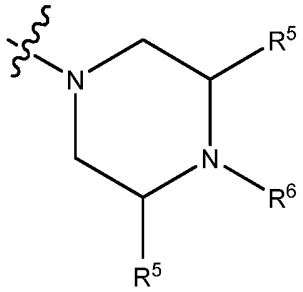


wherein R^5 is H or alkyl; and R^6 is –C(=O)NH-R^7 or –C(=S)NH-R^7, wherein R^7 is phenyl optionally substituted with a haloalkyl group.

19. A compound according to claim 18, wherein the compound is selected from one of Compound 50, Compound 51, Compound 144, Compound 145, Compound 146, Compound 147, Compound 148, Compound 149, and Compound 150.

20. A compound according to claim 16, wherein

X and Y are each N, R^1 is alkyl, and R^4 is



wherein R^5 is H; and R^6 is –C(=O)NH-R^7 or –C(=S)NH-R^7, wherein R^7 is phenyl optionally substituted with a haloalkyl group.

21. A compound according to claim 20, wherein the compound is selected from one of Compound 1, Compound 3, Compound 30, and Compound 143.

22. A compound according to claim 16, wherein the compound exhibits an IC_{50} of less than 100 μM against a DNA gyrase.

23. A compound according to any one of claims 16-22, wherein the compound exhibits an IC_{50} of less than 200 μM against a bacterial DNA helicase selected from an AddAB helicase and a RecBCD helicase.

24. A compound according to claim 23, wherein the compound exhibits an IC_{50} of less than 175 μM against a bacterial DNA gyrase.

25. A compound according to claim 24, wherein the compound exhibits an IC_{50} of less than 100 μM against a bacterial DNA gyrase.

26. A compound according to claim 25, wherein the compound exhibits an IC_{50} of less than 80 μM against a bacterial DNA gyrase.

27. A compound according to claim 26, wherein the compound exhibits an IC_{50} of less than 50 μM against a bacterial DNA gyrase.

28. A method of treating a microbial, bacterial or fungal infection in a subject, the method comprising administering to a subject having said infection therapeutically effective amount of one or more compounds according to any one of claims 16-27.

29. The method according to claim 28, wherein administering said one or more compounds to the subject comprises administering a pharmaceutical composition comprising said one or more compounds to the subject, wherein the pharmaceutical composition comprises said one or more compounds and a pharmaceutically acceptable excipient or carrier.

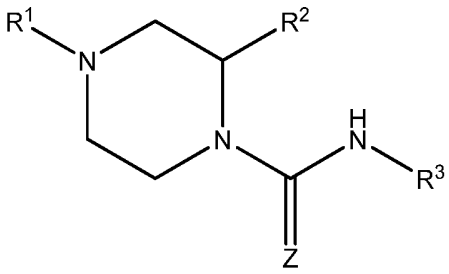
30. The method according to claim 28, wherein the method comprises treating a bacterial infection selected from bacterium-related cutaneous conditions, botulism, cholera, *E. coli* infection, Legionellosis, listeriosis, Lyme disease, pathogenic bacterial diseases, rickttsioses, salmonellosis, tuberculosis and zoonotic bacterial diseases.

31. The method according to claim 28, wherein the method comprises treating an infection selected from infection by Gram-positive and Gram-negative bacteria, such as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Morganella morganii,* Salmonella serotypes including *Enteritidis, Typhimurium* and *Newport*, *Enterococci, Shigella dysenteriae, Yersinia enterocolitica, Acinetobacter calcoaceticus, Francisella tularensis, Legionella pneumophila, Helicobacter pylori, Neisseria meningitides, Neisseria gonorrhoeae, Campylobacter jejuni, Vibrio cholera, Pseudomonas aeruginosa, Streptococcus, Staphylococcus, pneumococcus, Mycobacterium tuberculosis, Borrelia burgdorferi, Bordetella pertussis, Legionella pneumophila, Clostridium difficile, Bacillus anthracis*, and *Haemophilus influenza*.

32. A method for treating a subject having a disease or disorder associated with bacterial infection, the method comprising administering to the

subject a therapeutically effective amount of an inhibitor of at least one of AddAB or RecBCD, wherein said inhibitor is selected from a compound as defined in claim 16.

33. A compound which inhibits a bacterial DNA helicase, nuclease, or helicase-nuclease complex selected from an AddAB helicase-nuclease and a RecBCD helicase-nuclease, the compound comprising an active compound according to Formula 1b:



Formula Ib

wherein R^1 is is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, cycloalkyl, heteroaryl, alkyl heteroaryl, heterocyclyl, and heterocyclyl alkyl, any of which may be optionally substituted;

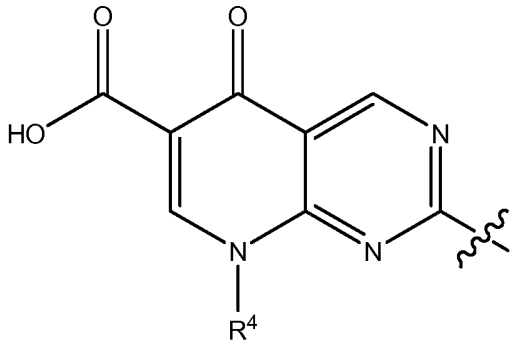
R^2 is H or alkyl;

R^3 is selected from at least one of the following: alkyl, alkenyl, aryl, alkyl aryl, -CO-aryl, -CO-alkyl aryl, cycloalkyl, heteroaryl, and -CO-heteroaryl, any of which may be optionally substituted with a substituent selected from at least one of the following: alkyl, haloalkyl, alkoxy, methylenedioxy, halogen, ethylenedioxy, and nitro; and

Z is O or S.

34. A compound according to claim 33,

wherein R^1 is selected from a compound according to Formula 1c



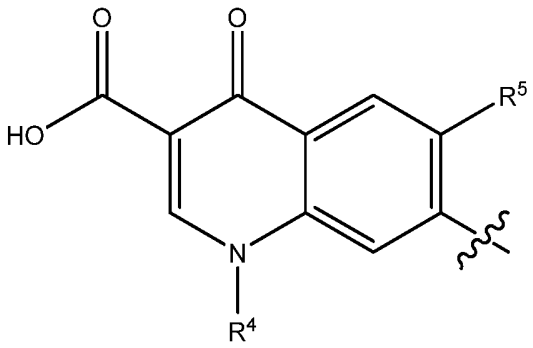
Formula Ic

and R^2 is H, Z is S, R^4 is alkyl and R^3 is phenyl substituted with a haloalkyl group.

35. A compound according to claim 34, wherein R^3 is phenyl substituted with a CF_3 group positioned in one of the ortho, para, and meta positions.

36. A compound according to claim 35, wherein the compound is selected from one of Compound 1, Compound 3, Compound 30, and Compound 143.

37. A compound according to claim 36, wherein R^1 is selected from a compound according to Formula Id



Formula Id

and R^2 is H, Z is S, R^3 is phenyl substituted with a CF_3 group, R^4 is alkyl, and R^5 is fluorine.

38. A compound according to claim 37, wherein R^3 is phenyl substituted with a CF_3 group positioned in one of the ortho, para, or meta positions.

39. A compound according to claim 38, wherein the compound is selected from one of Compound 50, Compound 51, Compound 144, Compound 145, Compound 146, Compound 147, Compound 148, Compound 149, and Compound 150.

40. A compound according to any one of claims 32-39, wherein the compound exhibits an IC_{50} of less than 200 μM against a bacterial DNA helicase, nuclease, or helicase-nuclease complex.

41. A compound according to claim 40, wherein the compound exhibits an IC_{50} of less than 175 μM against a bacterial DNA helicase, nuclease, or helicase-nuclease complex.

42. A compound according to claim 41, wherein the compound exhibits an IC_{50} of less than 100 μM against a bacterial DNA helicase, nuclease, or helicase-nuclease complex.

43. A compound according to claim 42, wherein the compound exhibits an IC_{50} of less than 80 μM against a bacterial DNA helicase, nuclease, or helicase-nuclease complex.

44. A compound according to claim 43, wherein the compound exhibits an IC_{50} of less than 50 μM against a bacterial DNA helicase, nuclease, or helicase-nuclease complex.

45. A compound according to any one of claim 33-44, wherein the compound exhibits an IC_{50} of less than 100 μM against a DNA gyrase.

46. A method of treating a microbial, bacterial or fungal infection in a subject, the method comprising administering to a subject having said infection therapeutically effective amount of one or more compounds according to any one of claims 33-45.

47. The method according to claim 46, wherein administering said one or more compounds to the subject comprises administering a pharmaceutical composition comprising said one or more compounds to the subject, wherein the pharmaceutical composition comprises said one or more compounds and a pharmaceutically acceptable excipient or carrier.

48. The method according to claim 46, wherein the method comprises treating a bacterial infection selected from bacterium-related cutaneous conditions, botulism, cholera, *E. coli* infection, Legionellosis, listeriosis, Lyme disease, pathogenic bacterial diseases, rickttsioses, salmonellosis, tuberculosis and zoonotic bacterial diseases.

49. The method according to claim 46, wherein the method comprises treating bacterial infections by both Gram-positive and Gram-negative bacteria, such as *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Morganella morganii, Salmonella* serotypes including *Enteritidis*, *Typhimurium* and *Newport*, *Enterococci, Shigella dysenteriae, Yersinia enterocolitica, Acinetobacter calcoaceticus, Francisella tularensis, Legionella pneumophila, Helicobacter pylori, Neisseria meningitides, Neisseria gonorrhoeae, Campylobacter jejuni, Vibrio cholera, Pseudomonas aeruginosa, Streptococcus, Staphylococcus, pneumococcus, Mycobacterium tuberculosis, Borrelia burgdorferi, Bordetella pertussis, Legionella pneumophila, Clostridium difficile, Bacillus anthracis*, and *Haemophilus influenzae*.

50. A method for treating a subject having a disease or disorder associated with bacterial infection, the method comprising administering to the subject a therapeutically effective amount of an inhibitor of bacterial DNA helicase-nuclease, wherein said inhibitor is selected from a compound as defined in any one of claims 33-46.

51. A method of identifying an inhibitor of AddAB activity, the method comprising:

testing a candidate compound for inhibition of AddAB activity in an bacterial strain expressing an active AddAB (*addAB*$^{+}$) in the presence of a T4 *gene 2* mutant phage, wherein a lack of growth of the bacterial strain is indicative of the inhibition of AddAB activity by the candidate compound.

52. The method of claim 51, wherein the bacterial strain is an *E. coli* bacterial strain.

53. The method of claim 52, wherein the bacterial strain is an *E. coli recBCD* mutant bacterial strain.

54. The method of claim 51, wherein the T4 *gene 2* mutant phage is a T4 *gene 2 am149* triple nonsense mutant phage.

55. The method of claim 51, wherein the active AddAB is an AddAB enzyme expressed in an *recBCD* mutant bacterial strain.

56. The method of claim 55, wherein the active AddAB is an AddAB enzyme expressed in an *E. coli recBCD* mutant strain.

57. A method of identifying an inhibitor of RecBCD activity, the method comprising:

testing a candidate compound for inhibition of RecBCD activity in an bacterial strain expressing an active RecBCD (*recBCD*$^{+}$) in the presence of a T4 *gene 2* mutant phage, wherein a lack of growth of the bacterial strain is indicative of the inhibition of RecBCD activity by the candidate compound.

58. The method of claim 57, wherein the bacterial strain is an *E. coli* bacterial strain.

59. The method of claim 58, wherein the bacterial strain is an *E. coli recBCD* mutant bacterial strain.

60. The method of claim 59, wherein the T4 *gene 2* mutant phage is a T4 *gene 2 am149* triple nonsense mutant phage.

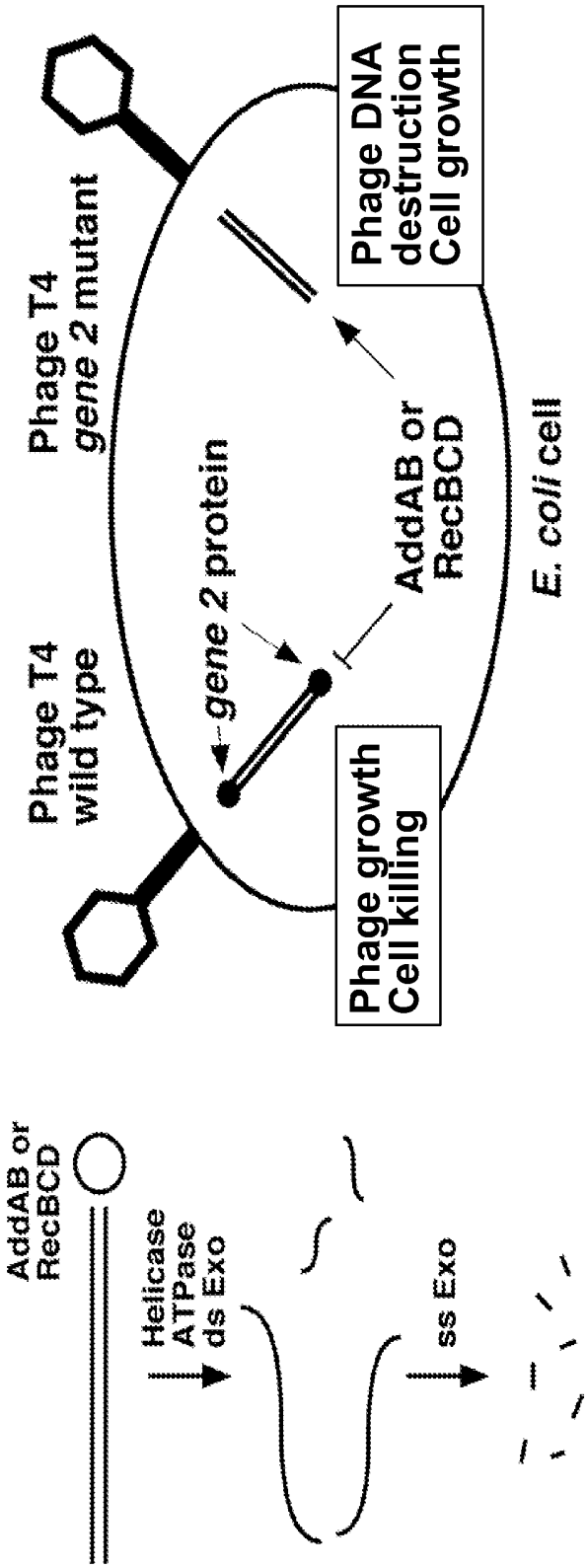

Phage T4
gene 2 mutant
Phage DNA
destruction
Cell growth
gene 2 protein
AddAB or
RecBCD
E. coli cell
Phage T4
wild type
Phage growth
Cell killing
AddAB or
RecBCD
Helicase
ATPase
ds Exo
ss Exo

Fig. 1

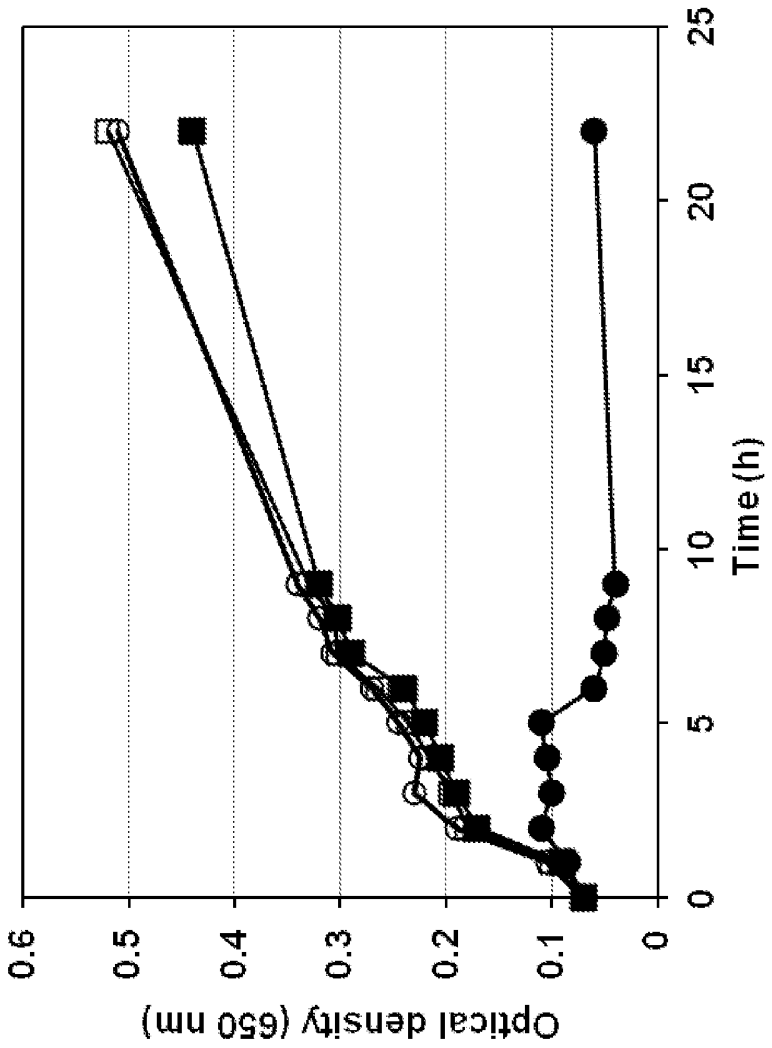


Fig. 2

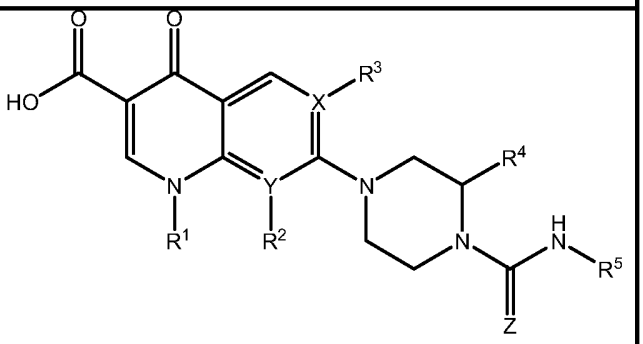
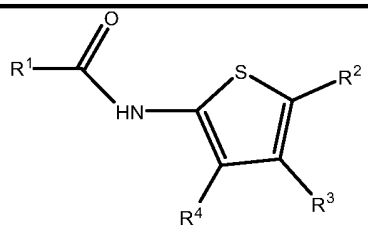
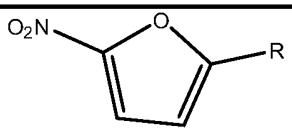
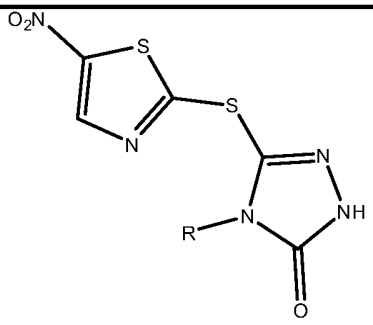
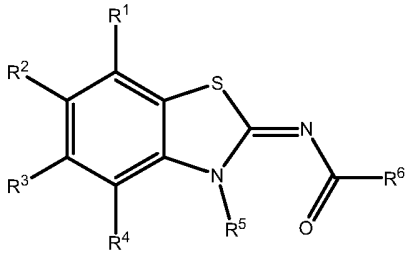
structural class	structure
A (the "pyrimidopyridones")	
B (the "cyanothiophenes")	
C (the "nitrofurans")	
D (the "nitrothiazoles")	
E (the "iminobenzothiazoles")	

Fig. 3

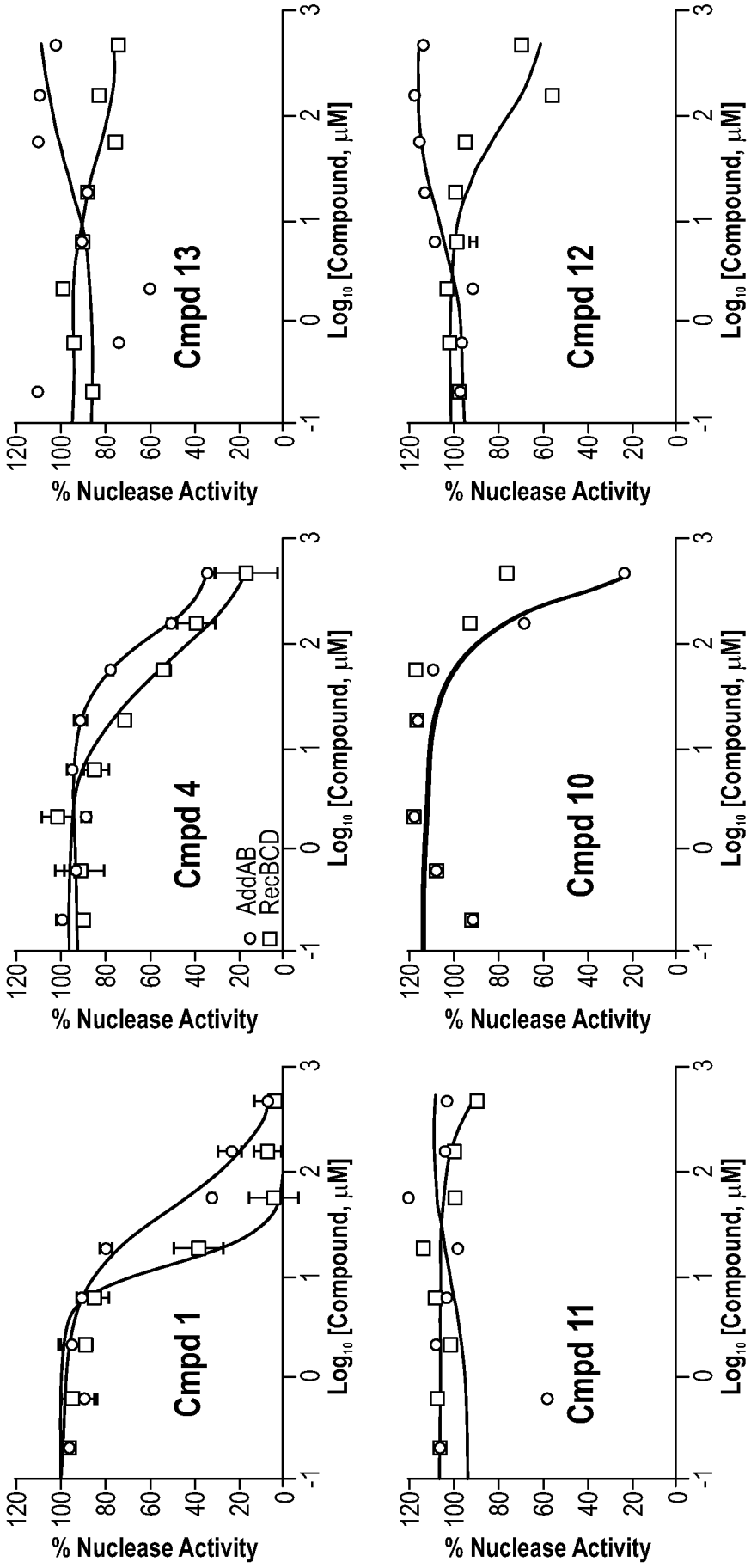


Fig. 4
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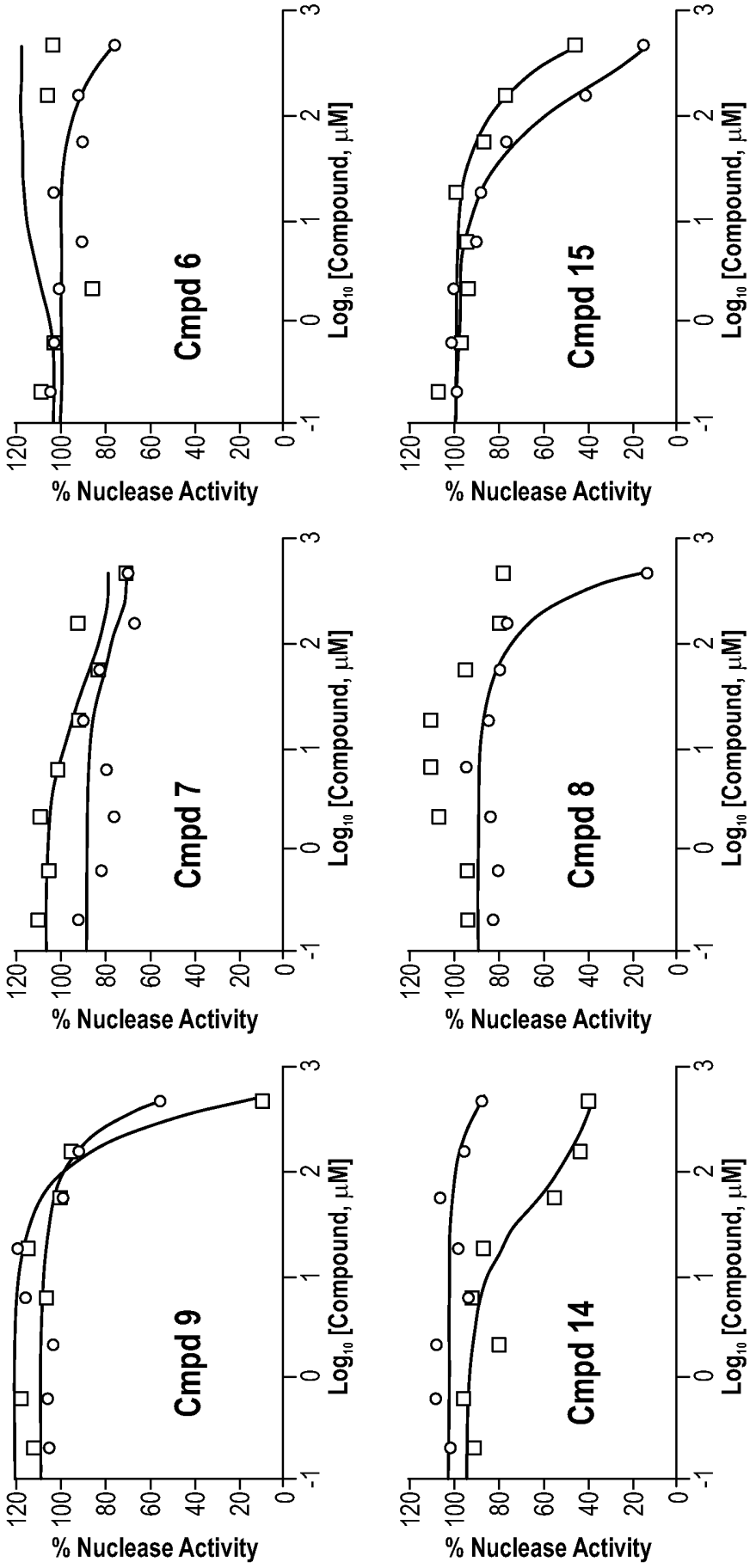

Fig. 4
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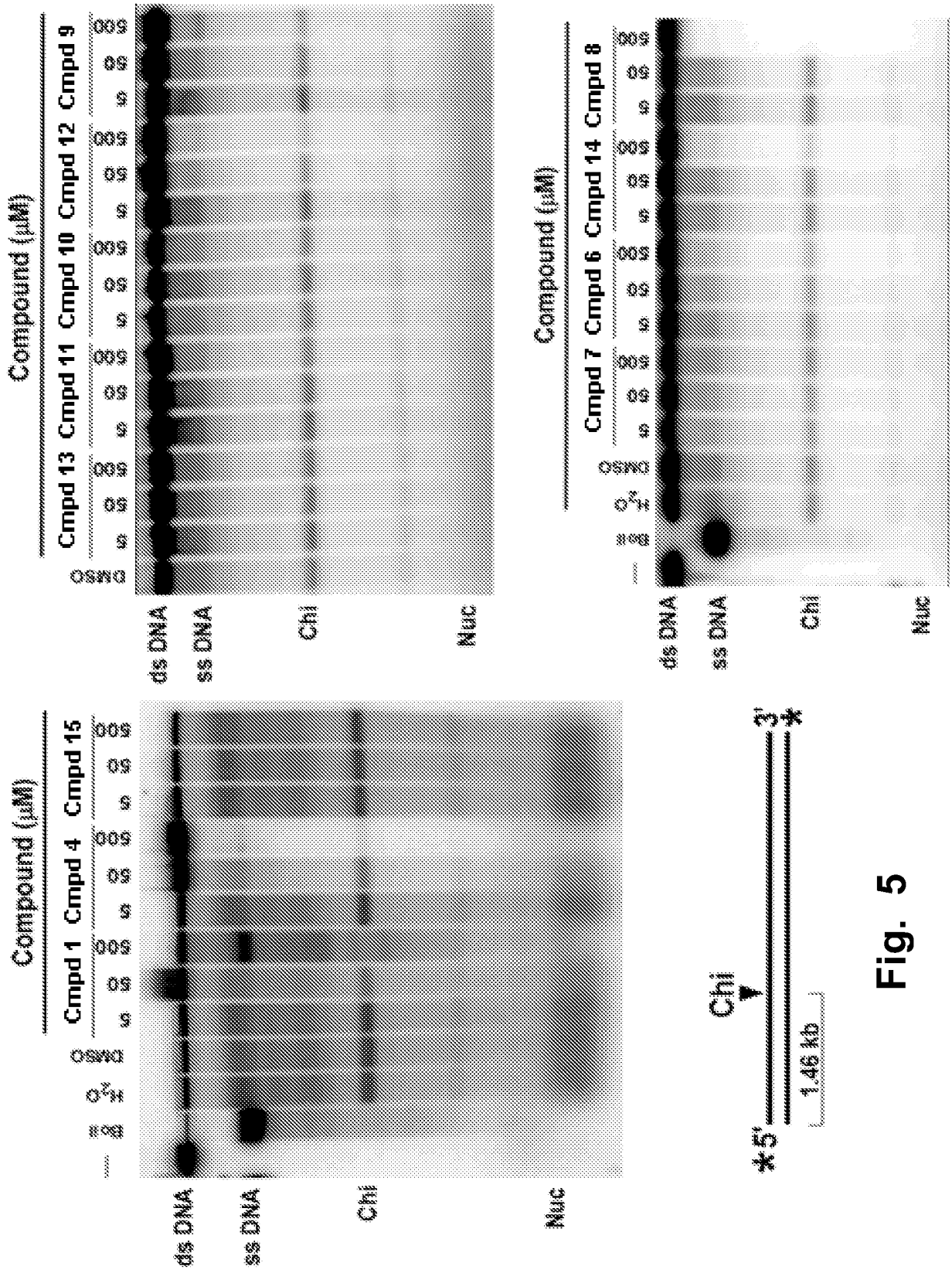

Compound (μM)
Cmpd 1
Cmpd 4
Cmpd 15
Cmpd 13
Cmpd 11
Cmpd 10
Cmpd 12
Cmpd 9
Cmpd 7
Cmpd 6
Cmpd 14
Cmpd 8
DMSO
ds DNA
ss DNA
Chi
Nuc
Chi
5'
3'
1.46 kb

Fig. 5

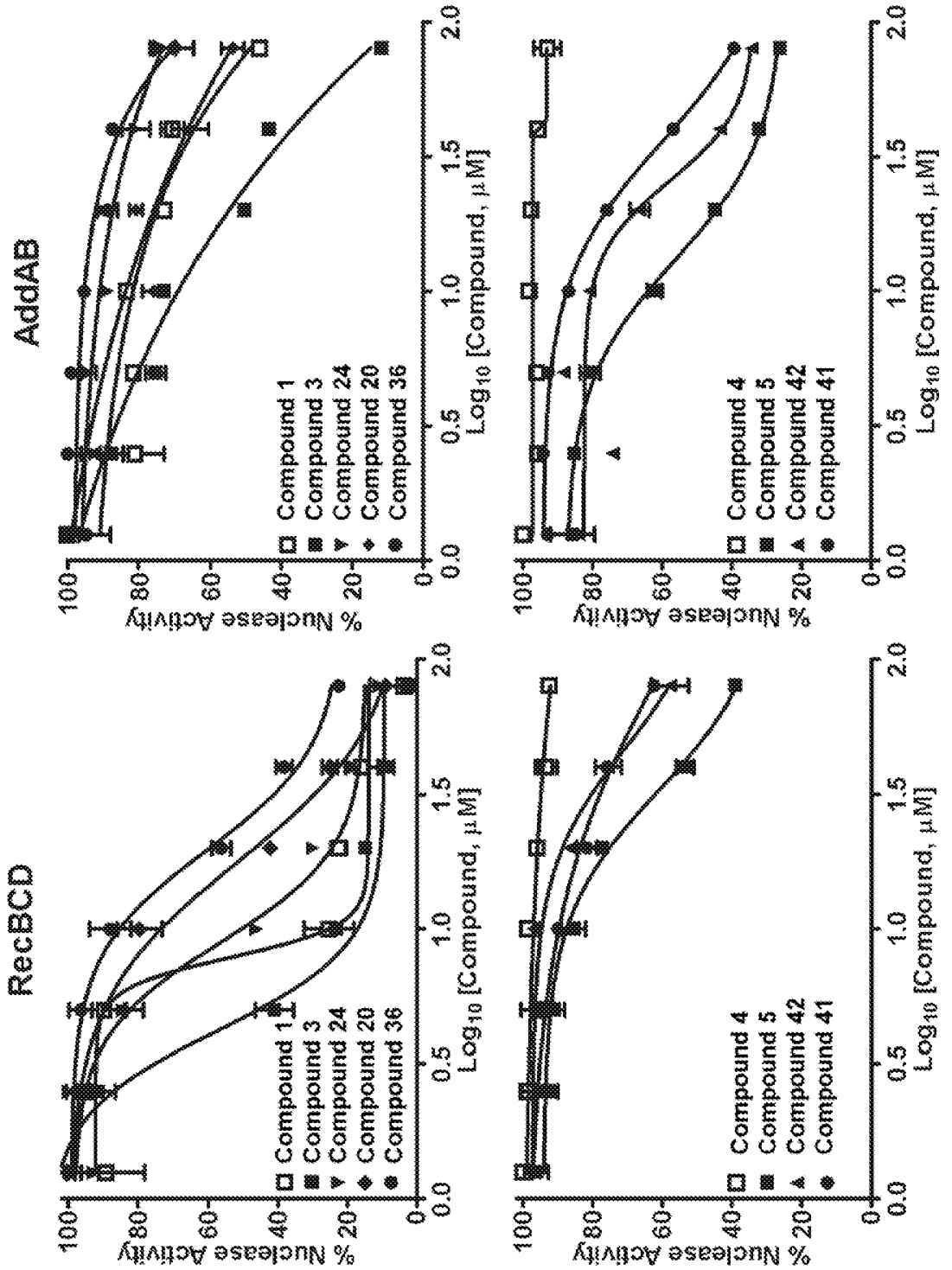

AddAB
RecBCD
% Nuclease Activity
Log10 [Compound, μM]
Compound 1
Compound 3
Compound 24
Compound 20
Compound 36
Compound 4
Compound 5
Compound 42
Compound 41

Fig. 6

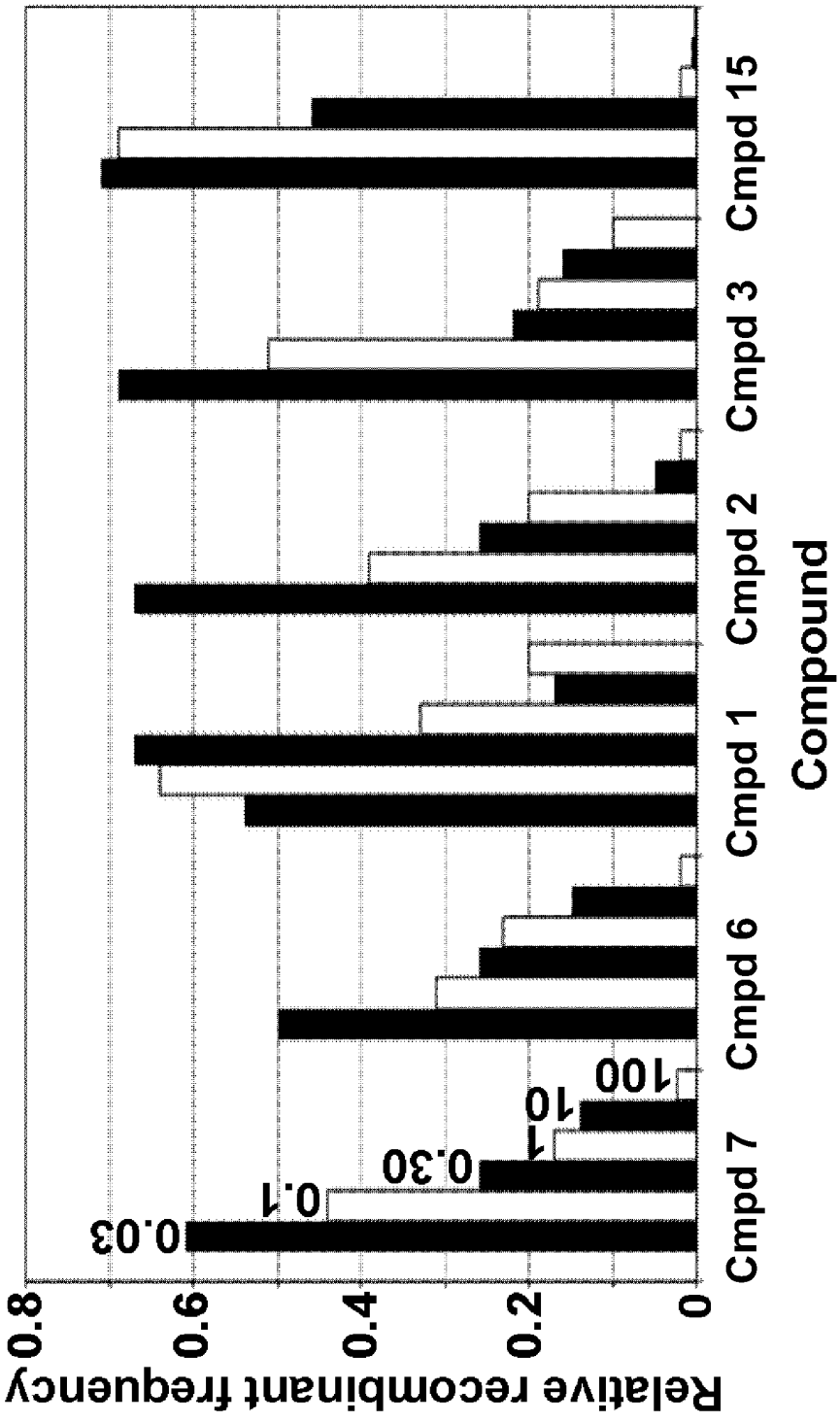

Relative recombinant frequency
0.8
0.6
0.4
0.2
0
0.03
0.1
0.30
1
10
100
Cmpd 7
Cmpd 6
Cmpd 1
Cmpd 2
Cmpd 3
Cmpd 15
Compound

Fig. 7

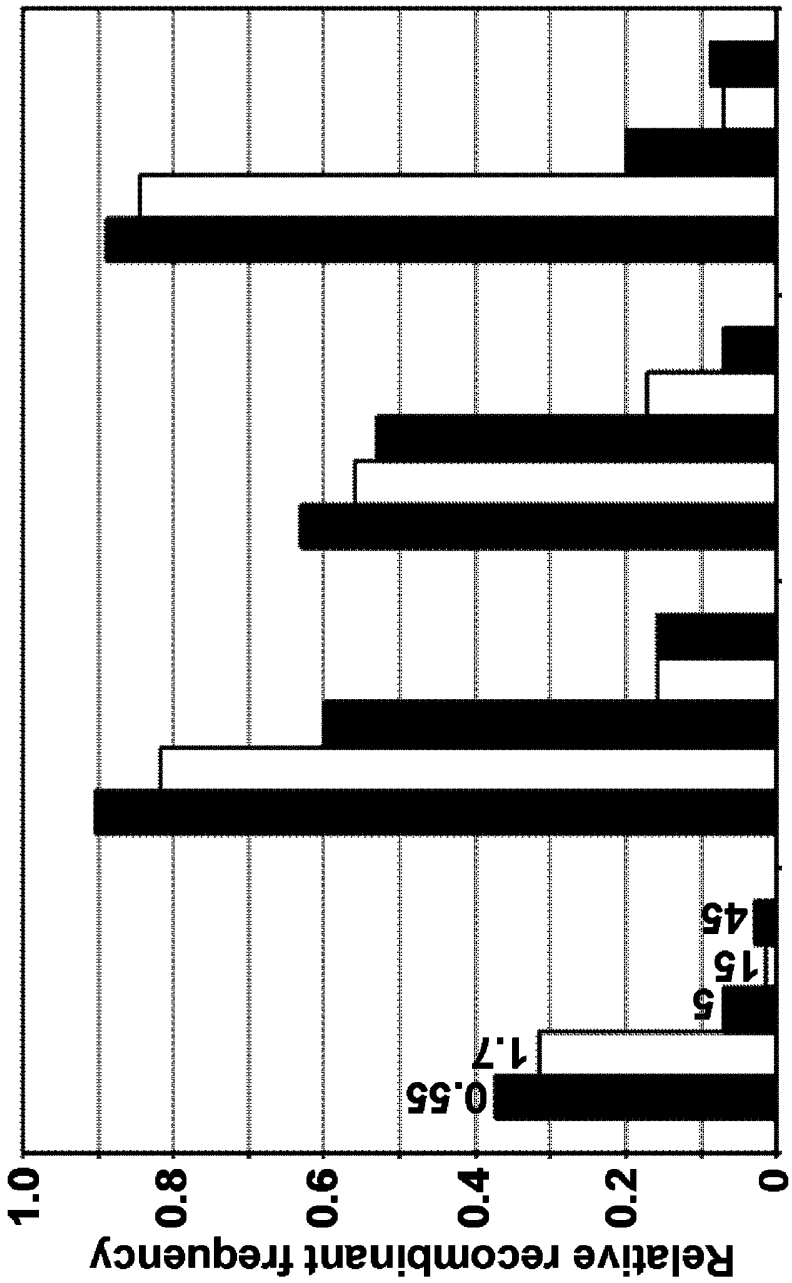

Relative recombinant frequency
1.0
0.8
0.6
0.4
0.2
0
0.55
1.7
5
15
45
Cmpd 2
Cmpd 3
Cmpd 7
Cmpd 6
Compound

Fig. 8

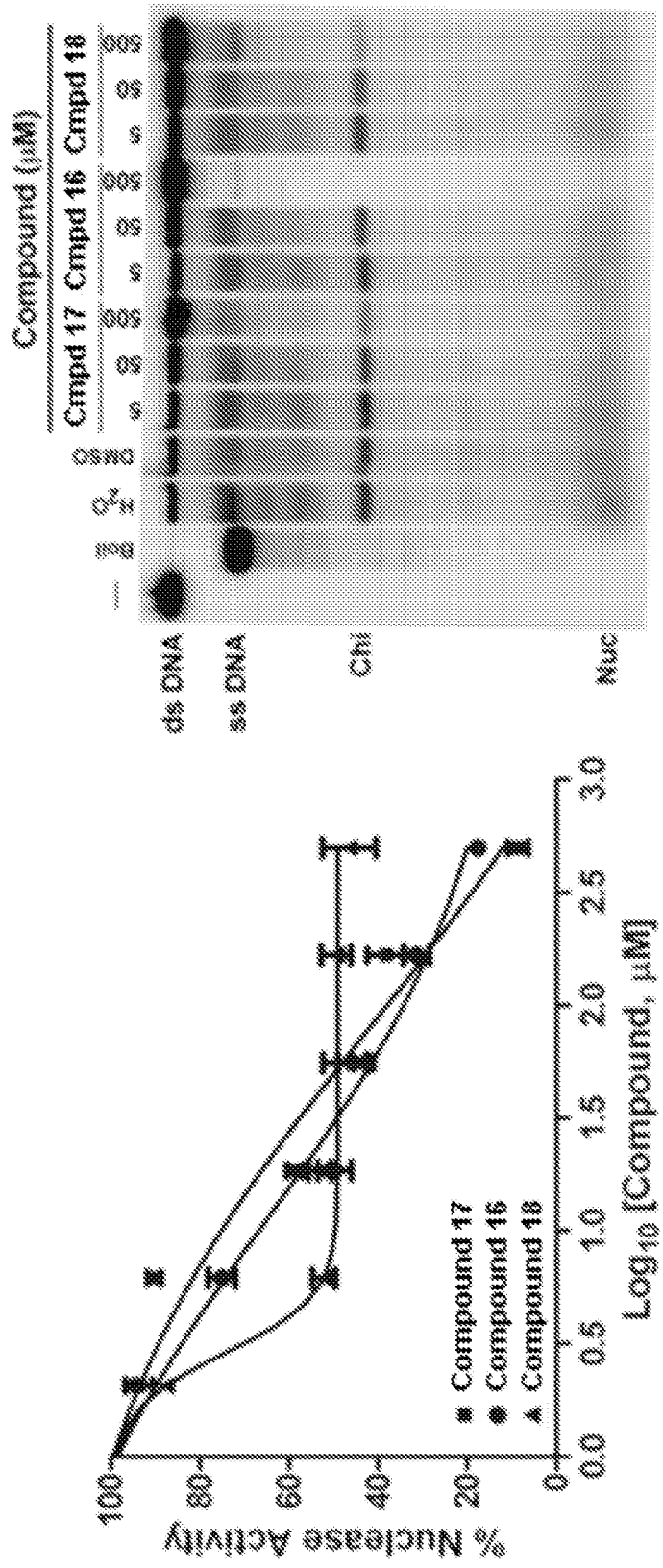

Compound (μM)
Cmpd 17
Cmpd 16
Cmpd 18
ds DNA
ss DNA
Chl
Nuc
% Nuclease Activity
Log10 [Compound, μM]
Compound 17
Compound 16
Compound 18

Fig. 9

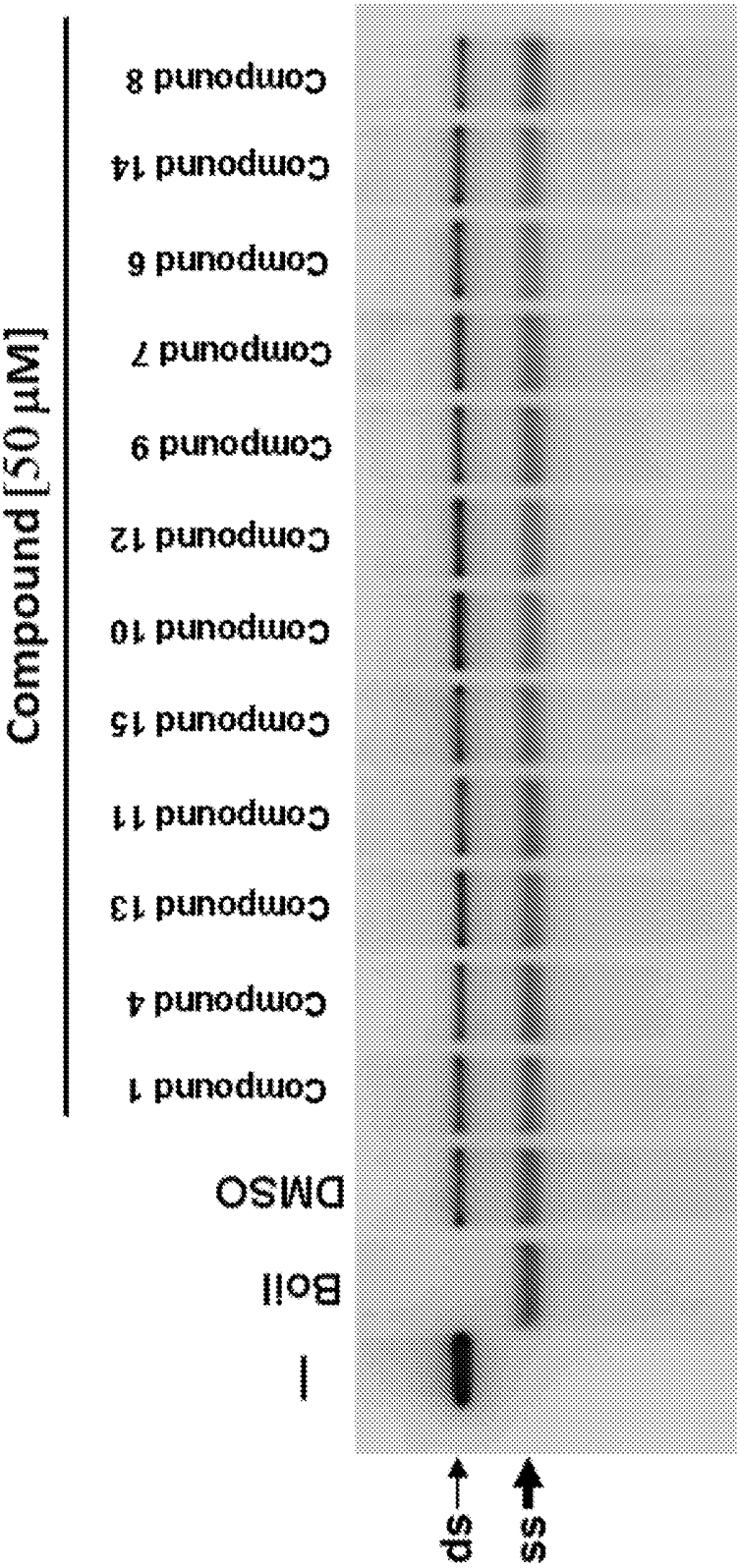
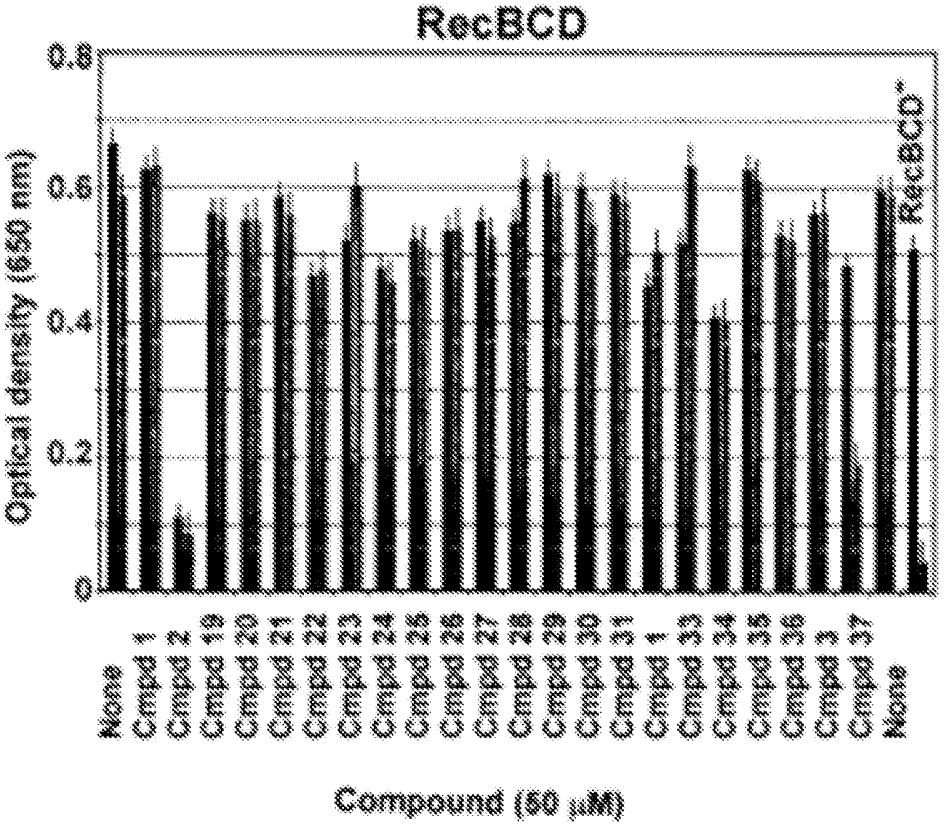

Compound [50 µM]
Compound 8
Compound 14
Compound 6
Compound 7
Compound 9
Compound 12
Compound 10
Compound 15
Compound 11
Compound 13
Compound 4
Compound 1
DMSO
Boil
ds
ss

Fig. 10



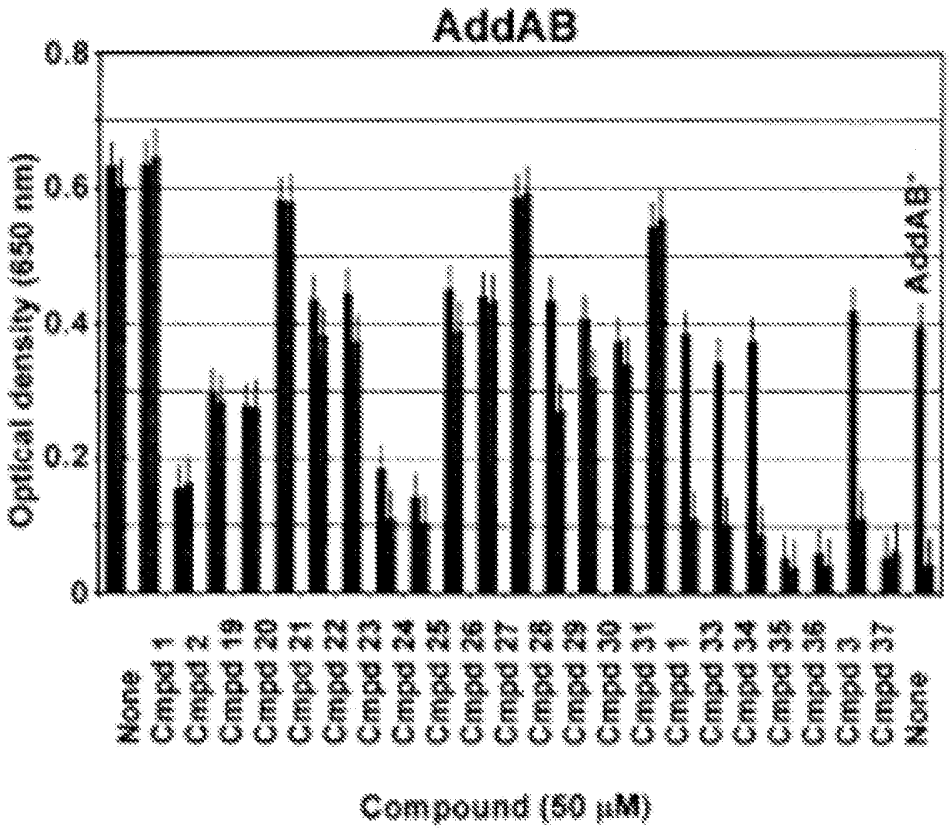


Fig. 11
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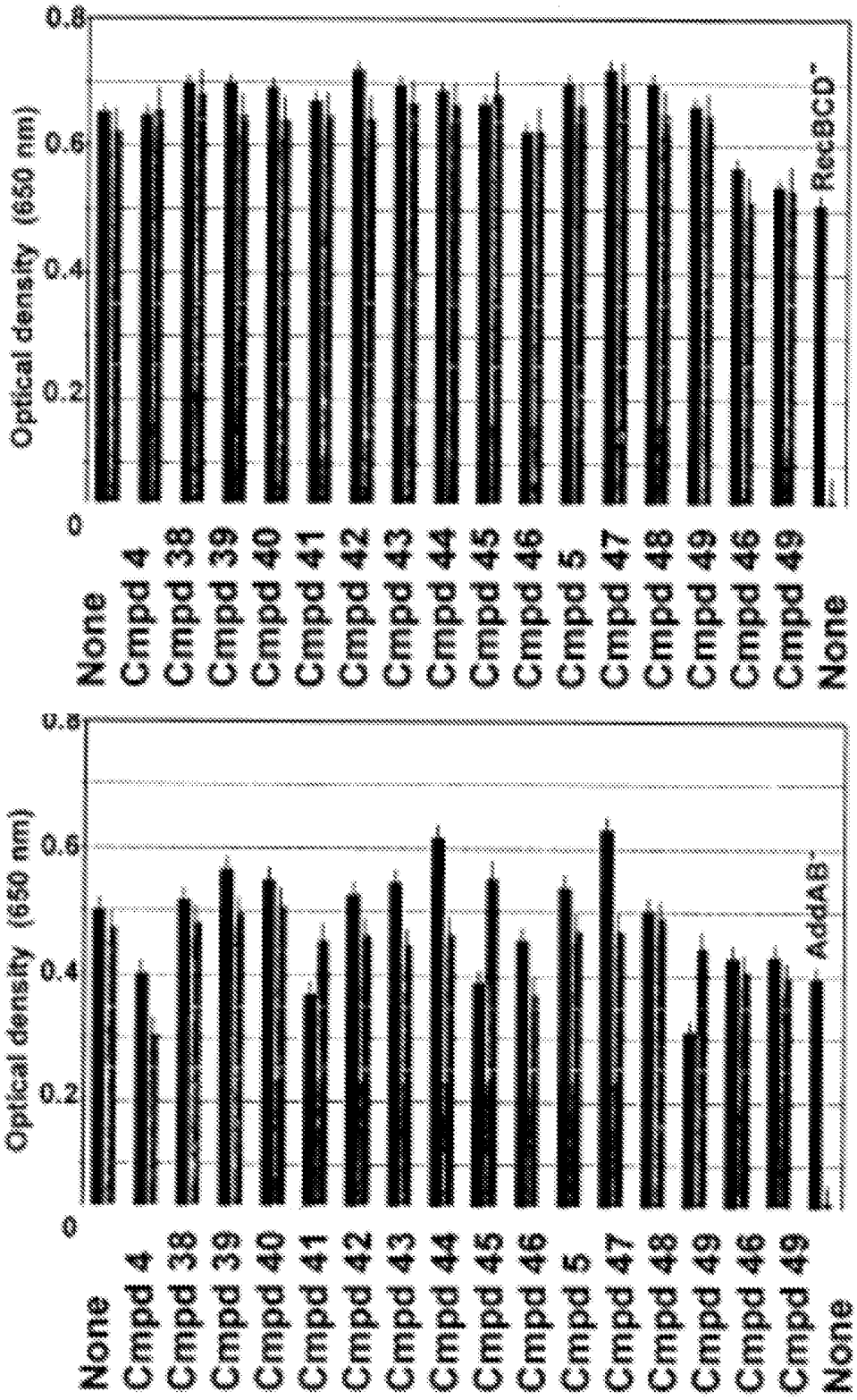

Fig. 11
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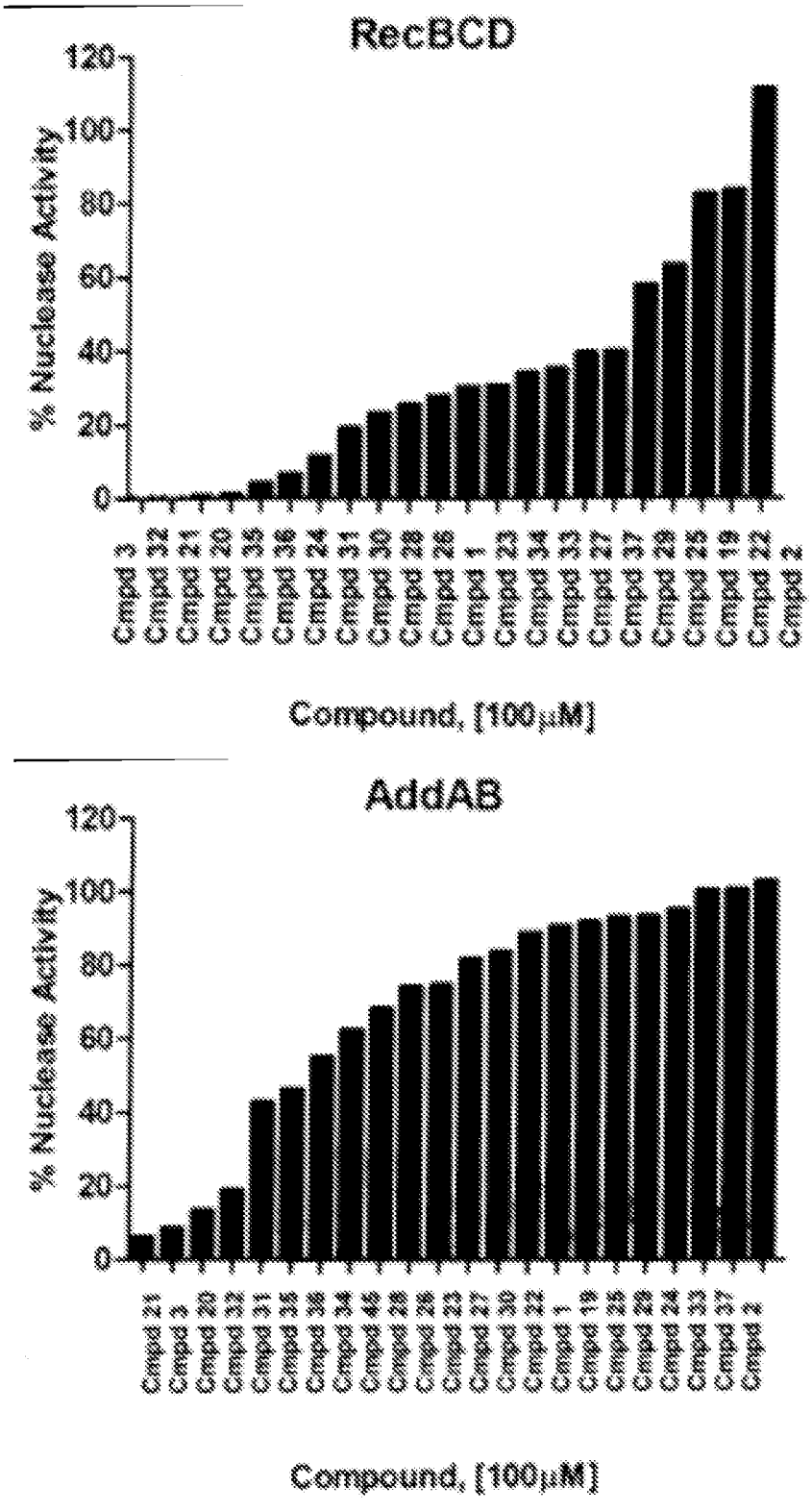

Fig. 12
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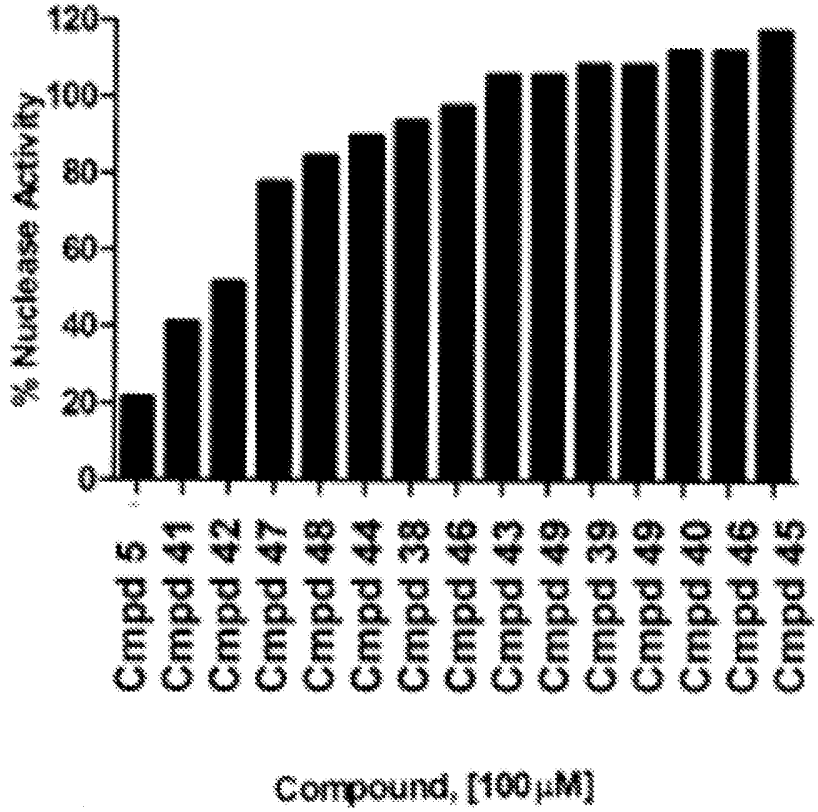

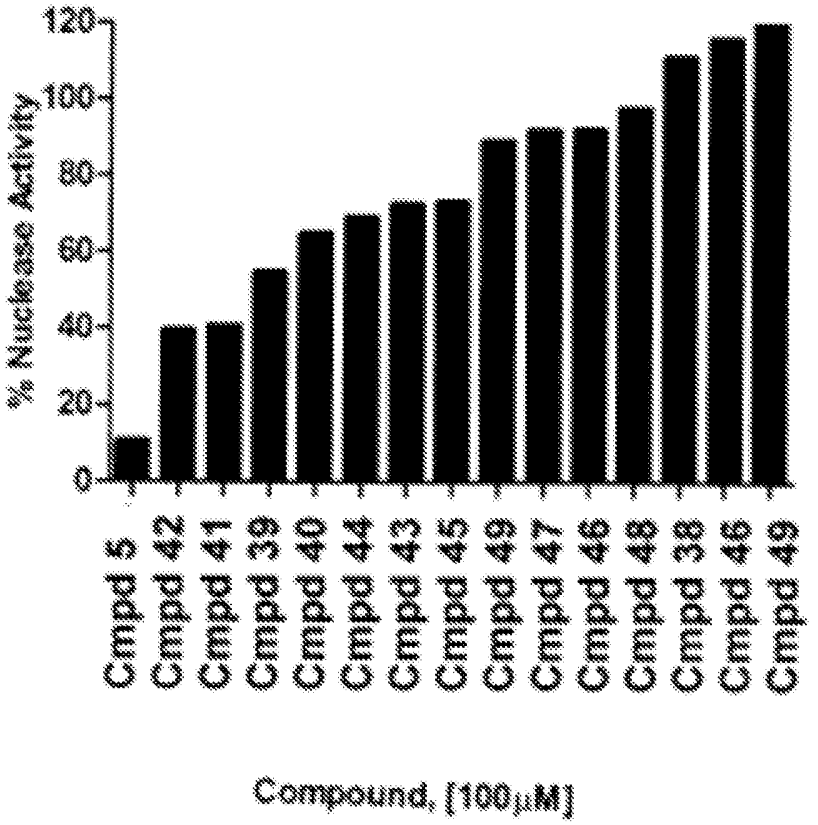

Fig. 12
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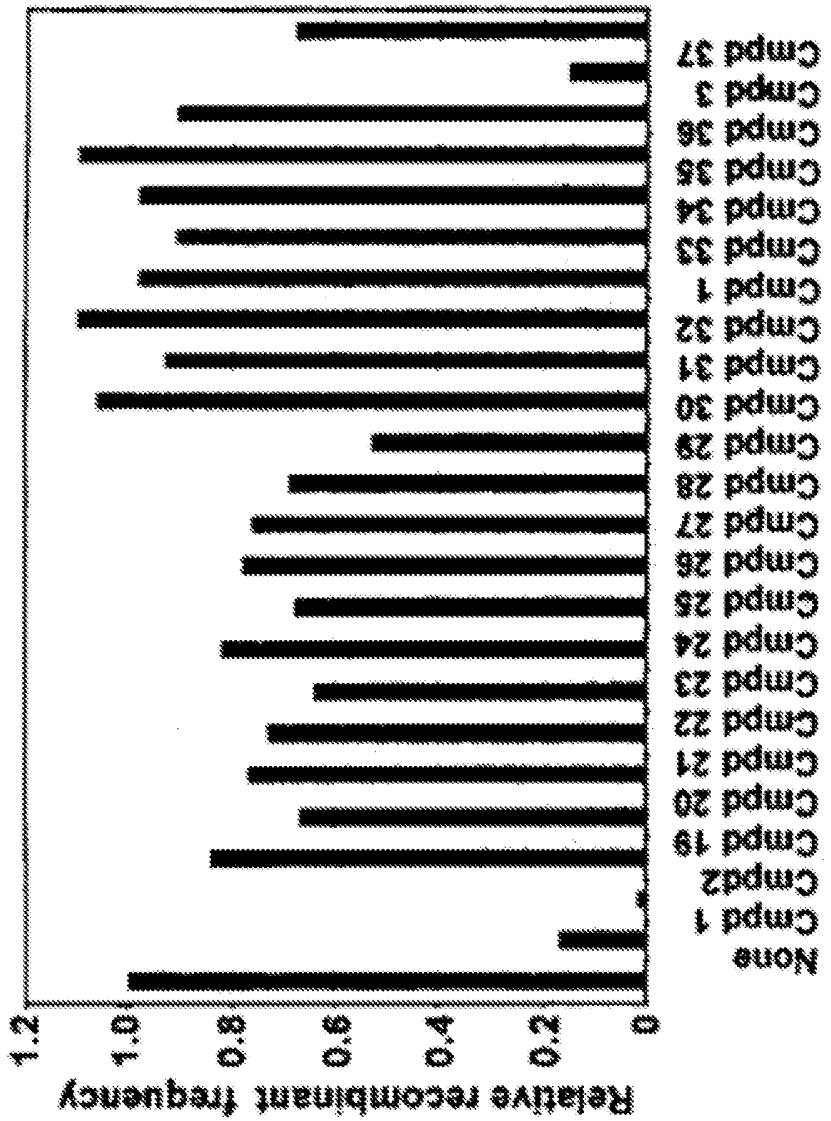


Fig. 13
(cont'd next page)

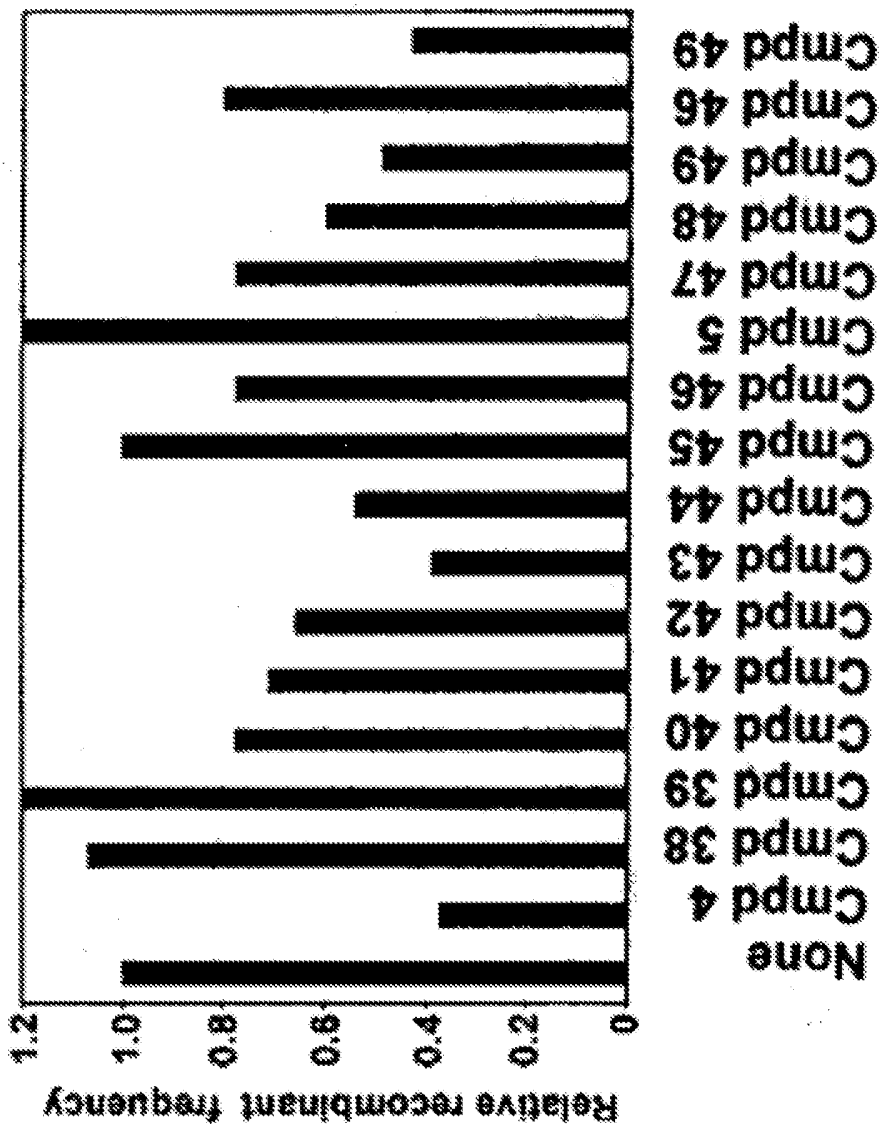


Fig. 13
(cont'd from previous page)

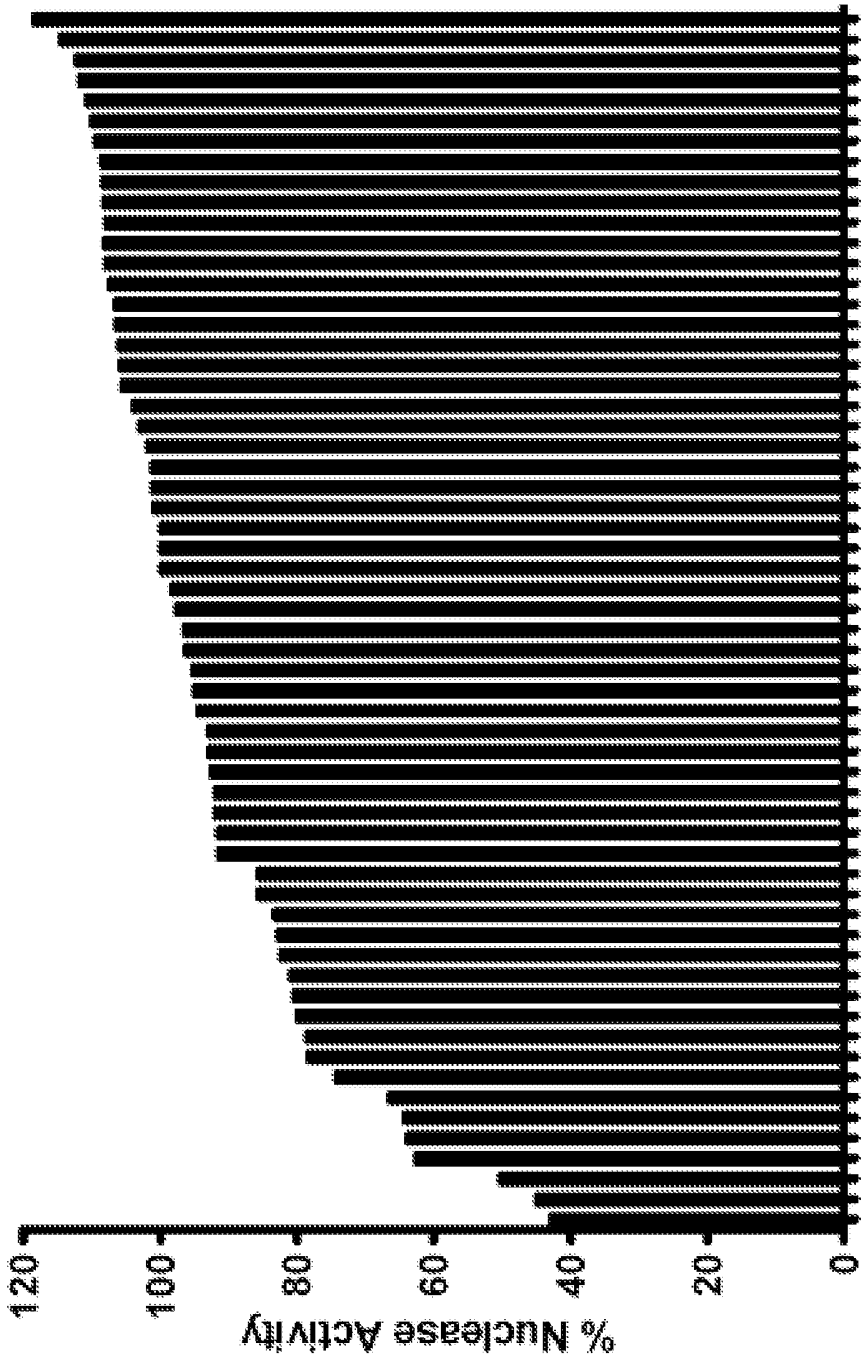
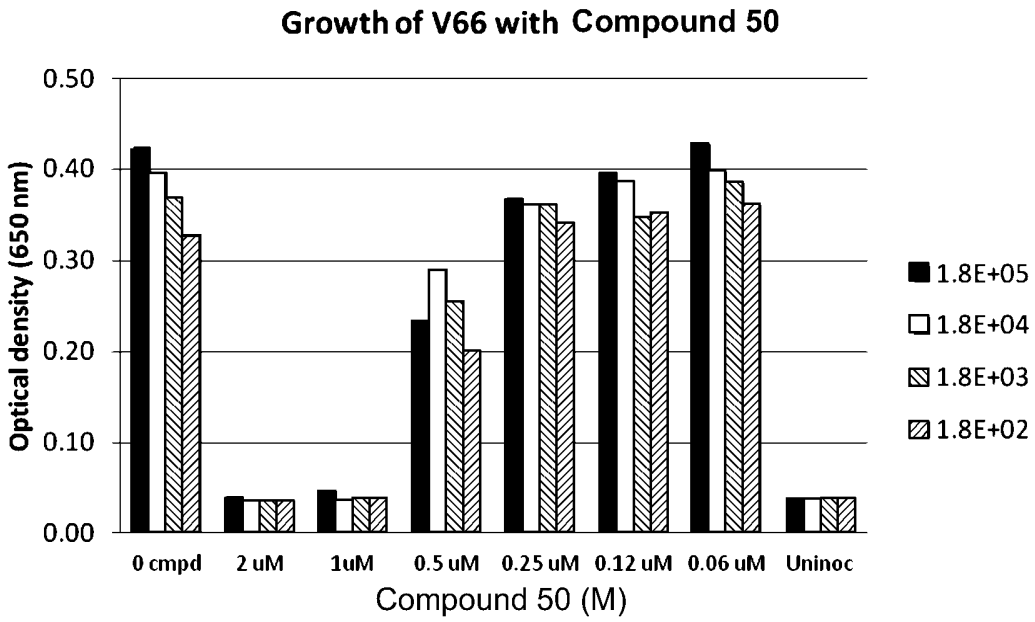


Fig. 14


Growth of V66 with Compound 50
Optical density (650 nm)
0.50
0.40
0.30
0.20
0.10
0.00
0 cmpd
2 uM
1uM
0.5 uM
0.25 uM
0.12 uM
0.06 uM
Uninoc
Compound 50 (M)
1.8E+05
1.8E+04
1.8E+03
1.8E+02

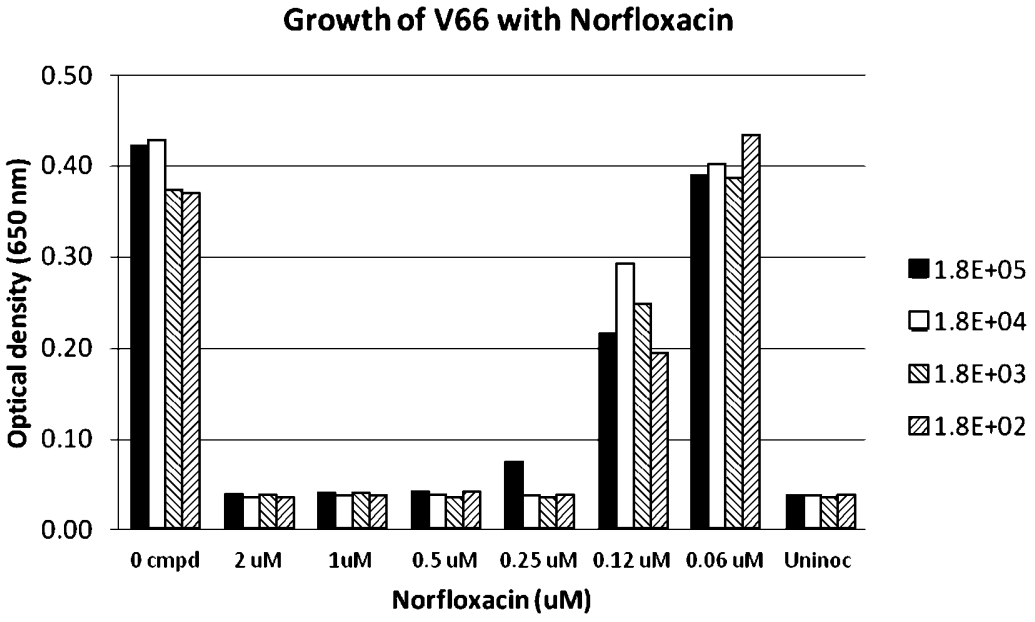
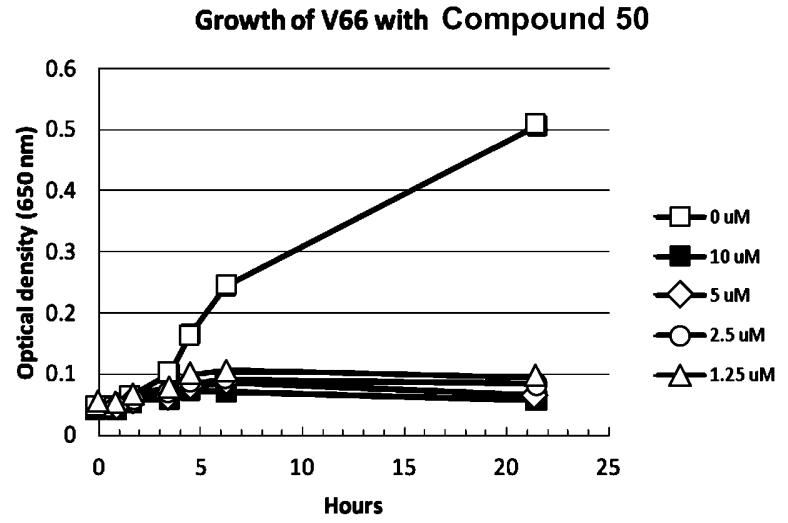

Growth of V66 with Norfloxacin
Optical density (650 nm)
0.50
0.40
0.30
0.20
0.10
0.00
0 cmpd
2 uM
1uM
0.5 uM
0.25 uM
0.12 uM
0.06 uM
Uninoc
Norfloxacin (uM)
1.8E+05
1.8E+04
1.8E+03
1.8E+02

Fig. 15


Growth of V66 with Compound 50
Optical density (650 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Hours
0 uM
10 uM
5 uM
2.5 uM
1.25 uM

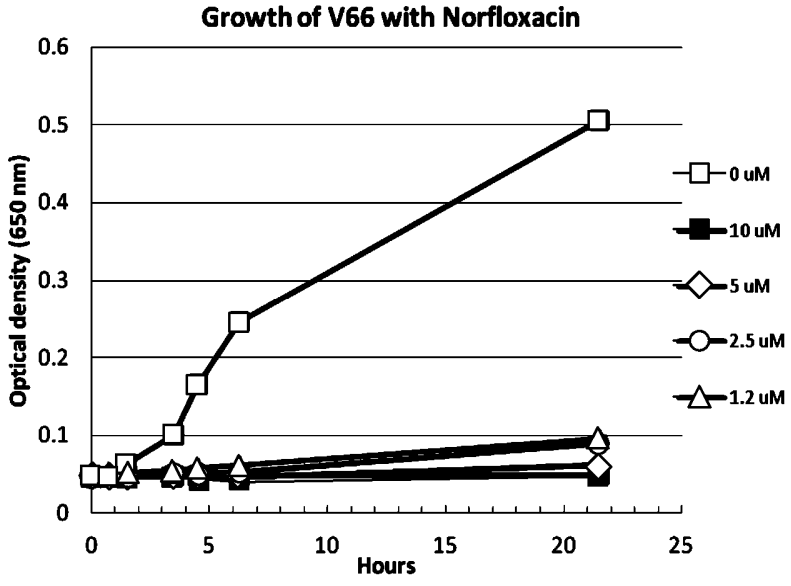

Growth of V66 with Norfloxacin
Optical density (650 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Hours
0 uM
10 uM
5 uM
2.5 uM
1.2 uM

Fig. 16

Concentration of Compound 50 (μM)	Relative recombinant frequency in Hfr cross
0	1.0
0.1	0.7
0.3	0.7
1.0	0.3
3.0	0.12
10	0.09
30	0.07

Fig. 17

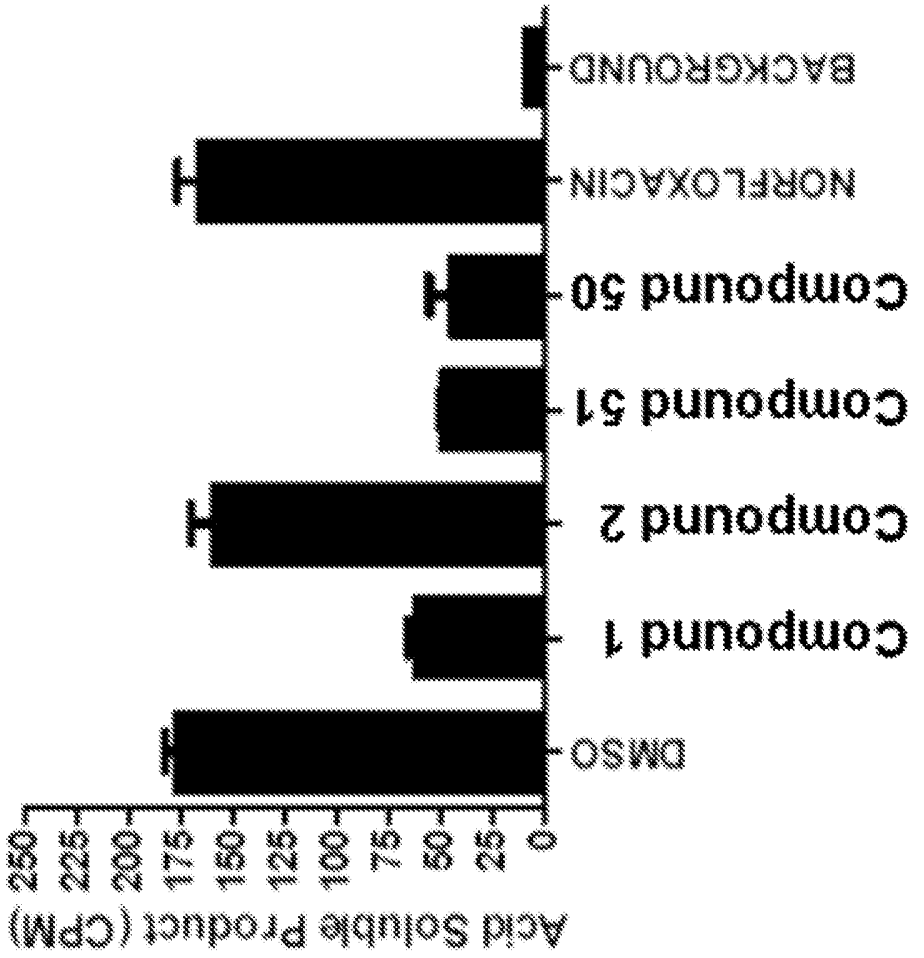

Acid Soluble Product (CPM)
250
225
200
175
150
125
100
75
50
25
0
DMSO
Compound 1
Compound 2
Compound 51
Compound 50
NORFLOXACIN
BACKGROUND
Compounds [100 μM]

Fig. 18

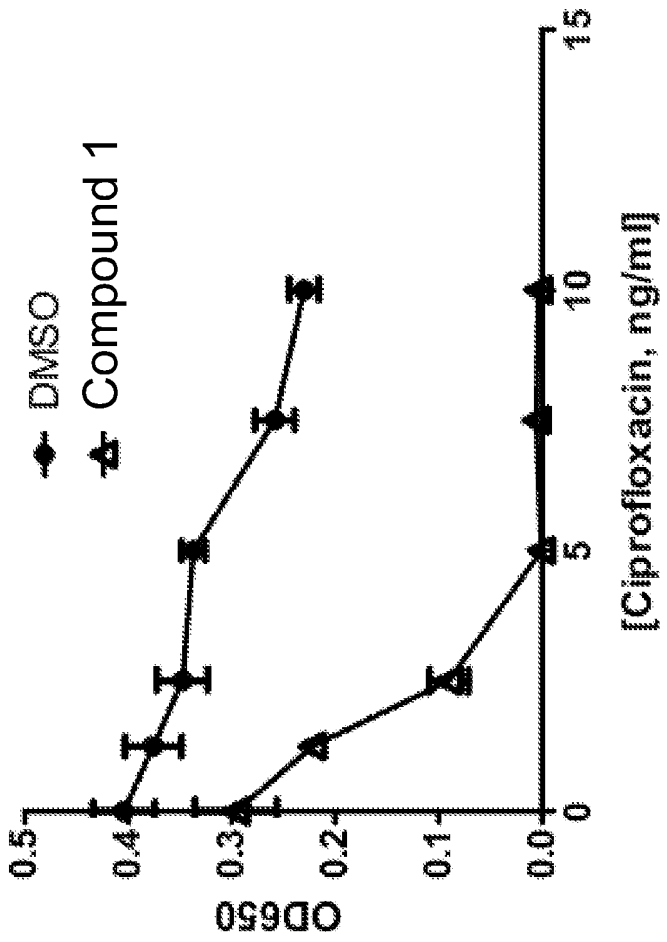

DMSO
Compound 1
OD650
0.5
0.4
0.3
0.2
0.1
0.0
0
5
10
15
[Ciprofloxacin, ng/ml]

Fig. 19

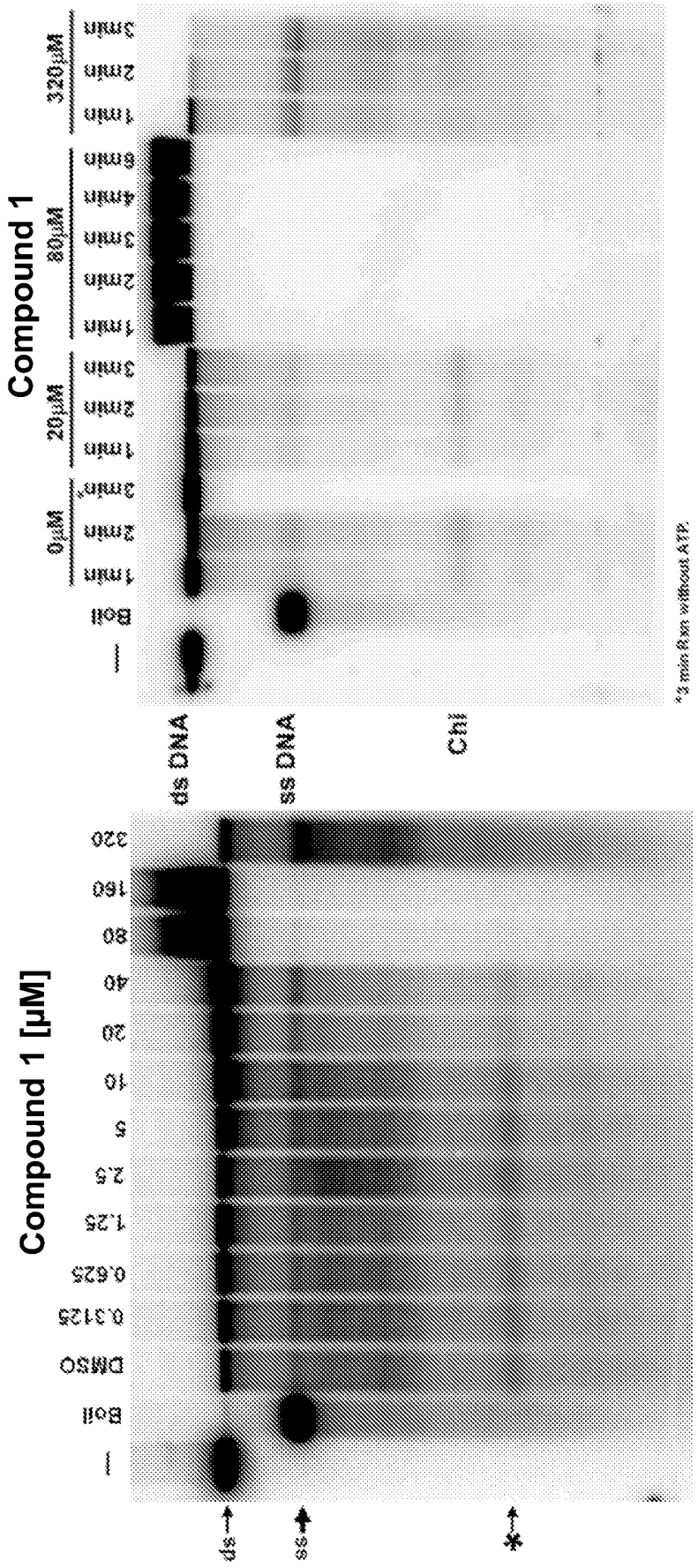

Compound 1
Compound 1 [µM]
ds DNA
ss DNA
Chi
BclI
DMSO
0µM
20µM
80µM
320µM
1min
2min
3min
4min
5min
*3 min 8 km without ATP
ds
ss
*

Fig. 20

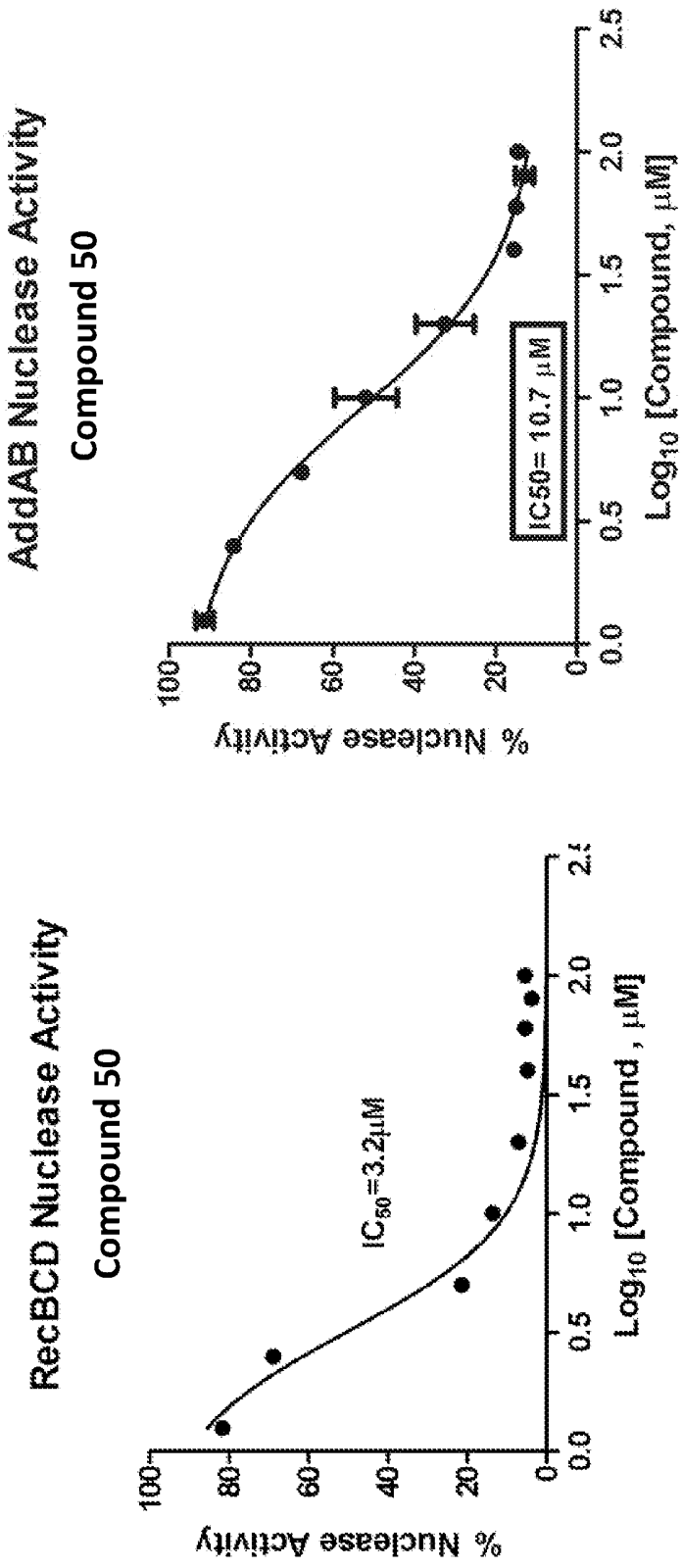

AddAB Nuclease Activity
Compound 50
% Nuclease Activity
Log10 [Compound, μM]
IC50= 10.7 μM
RecBCD Nuclease Activity
Compound 50
% Nuclease Activity
Log10 [Compound , μM]
IC50=3.2μM

Fig. 21

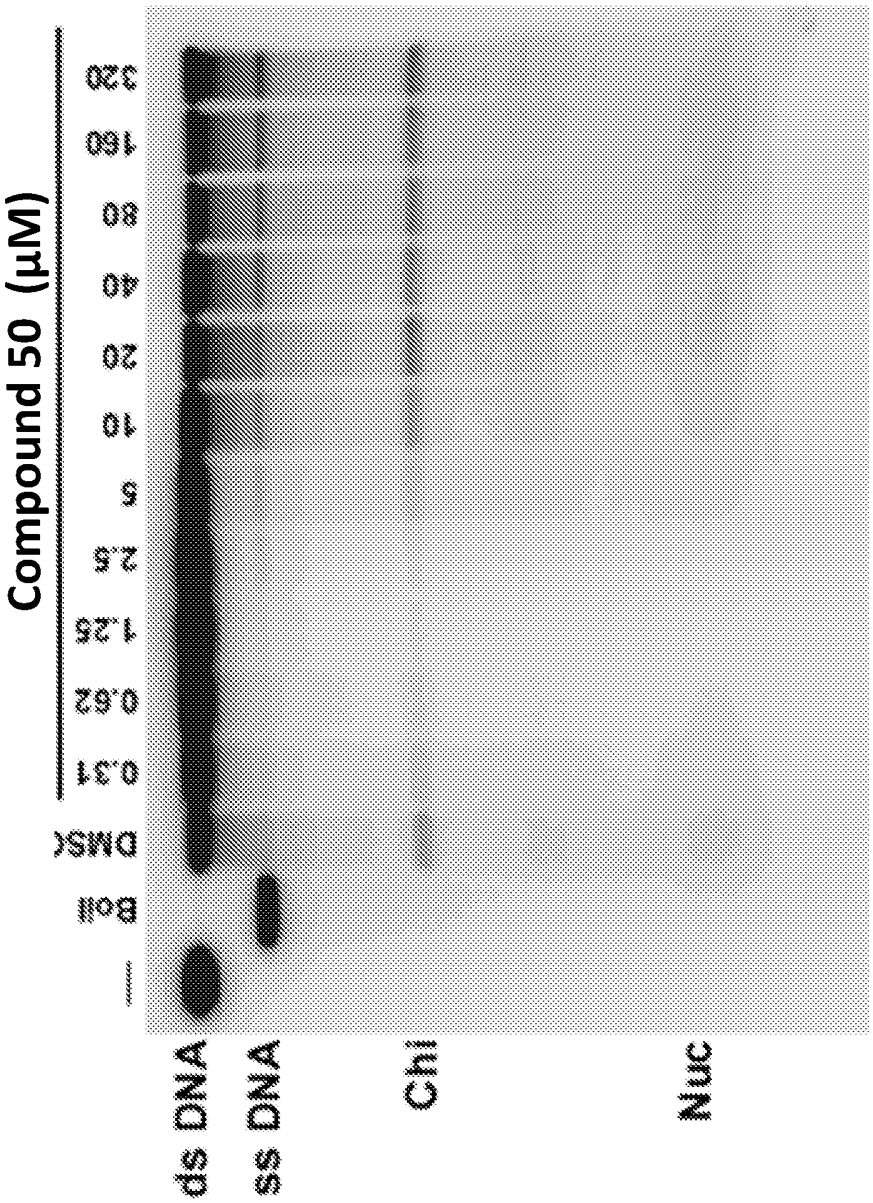
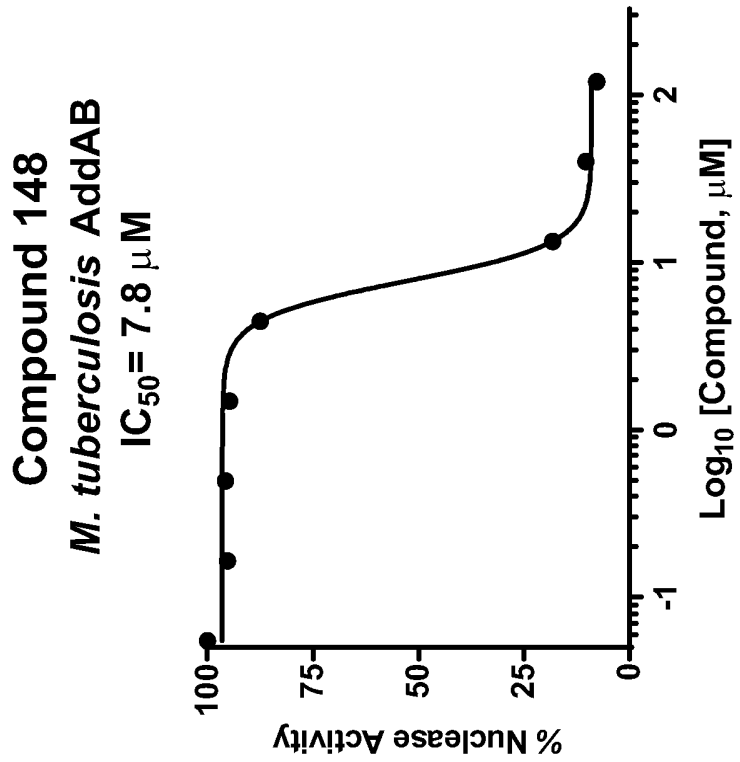

Compound 50 (μM)
—
Boil
DMSO
0.31
0.62
1.25
2.5
5
10
20
40
80
160
320
ds DNA
ss DNA
Chi
Nuc

Fig. 22


Compound 148
M. tuberculosis AddAB
IC50= 7.8 μM
% Nuclease Activity
100
75
50
25
0
-1
0
1
2
Log10 [Compound, μM]

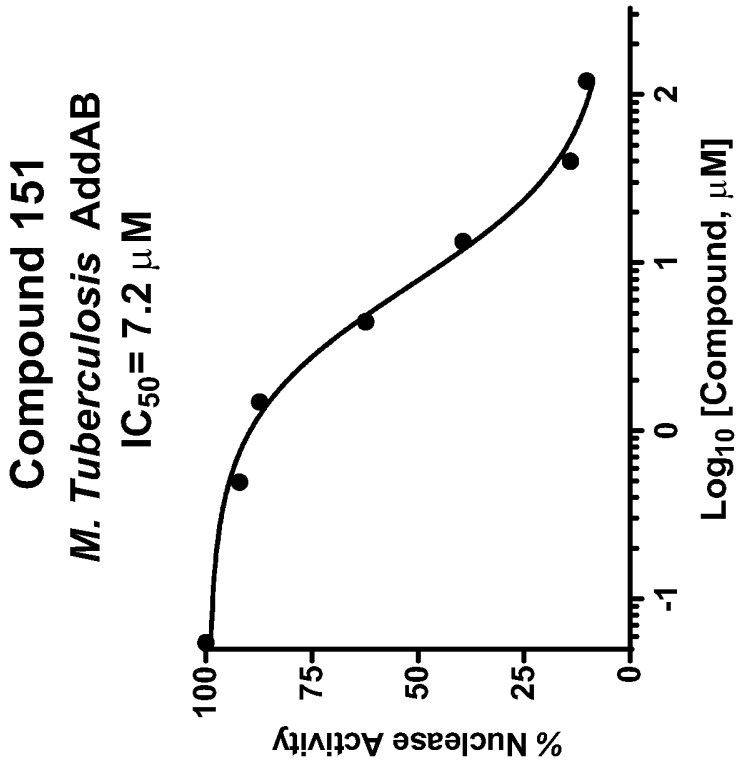

Compound 151
M. Tuberculosis AddAB
IC50= 7.2 μM
% Nuclease Activity
100
75
50
25
0
-1
0
1
2
Log10 [Compound, μM]

Fig. 23

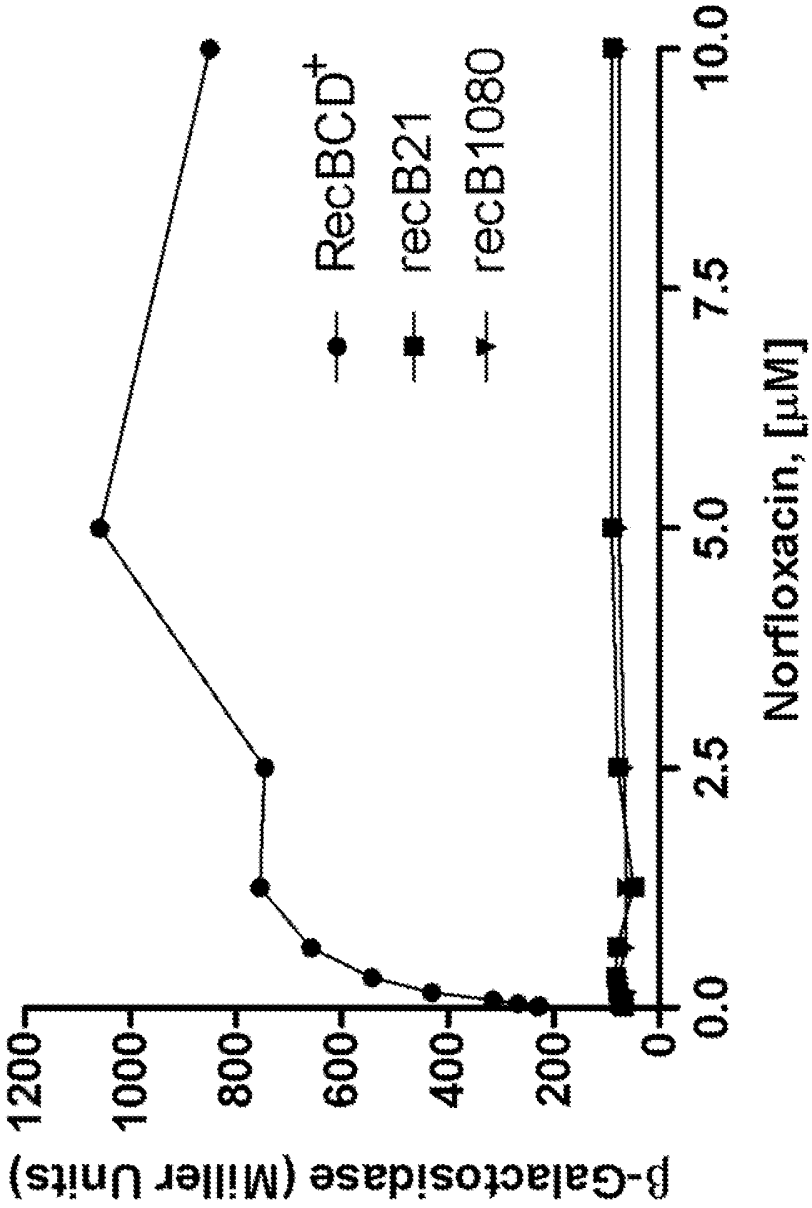

β-Galactosidase (Miller Units)
1200
1000
800
600
400
200
0
0.0
2.5
5.0
7.5
10.0
Norfloxacin, [μM]
RecBCD+
recB21
recB1080

Fig. 24

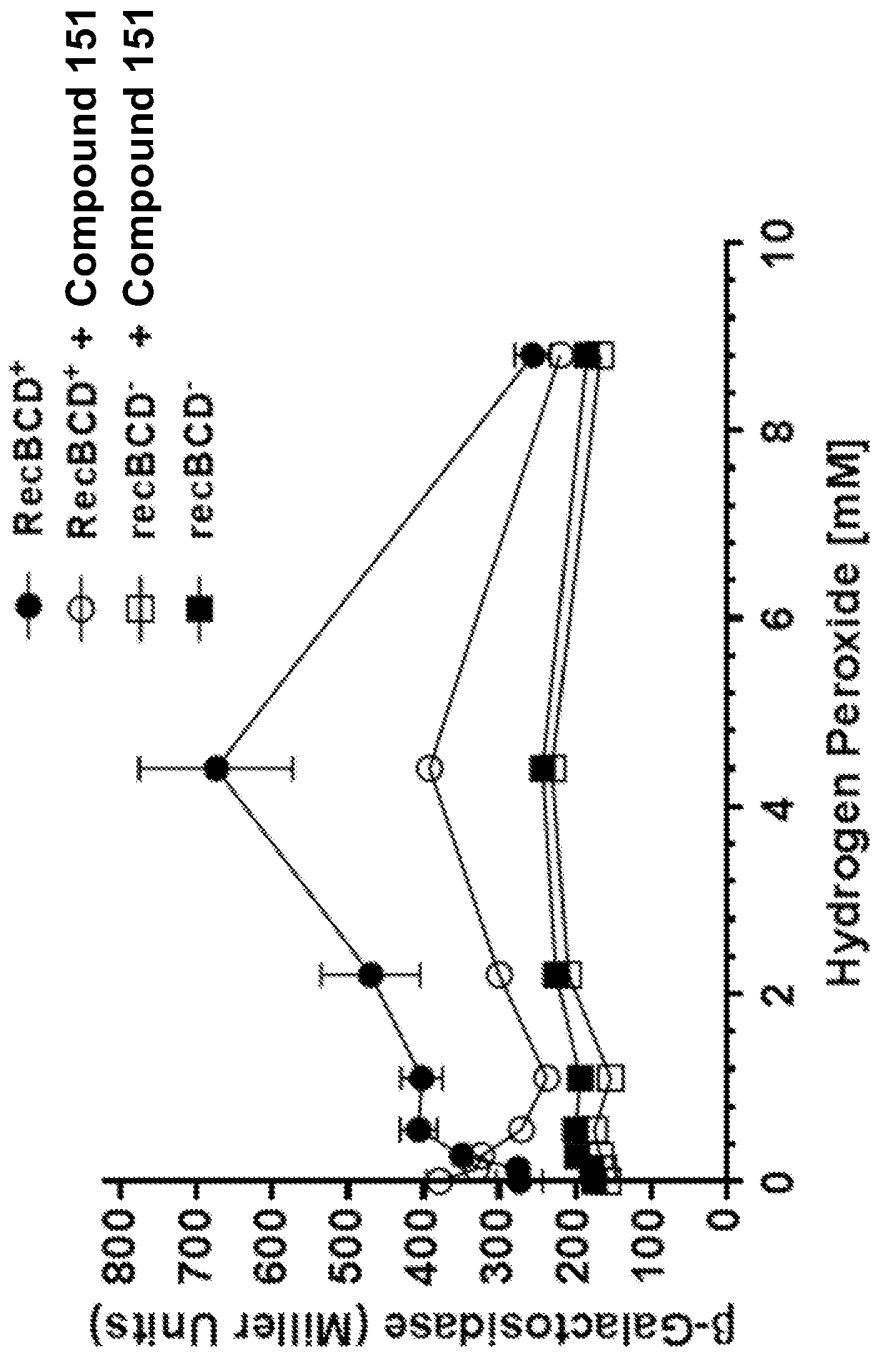

RecBCD+
RecBCD+ + Compound 151
recBCD- + Compound 151
recBCD-
β-Galactosidase (Miller Units)
800
700
600
500
400
300
200
100
0
0
2
4
6
8
10
Hydrogen Peroxide [mM]

Fig. 25

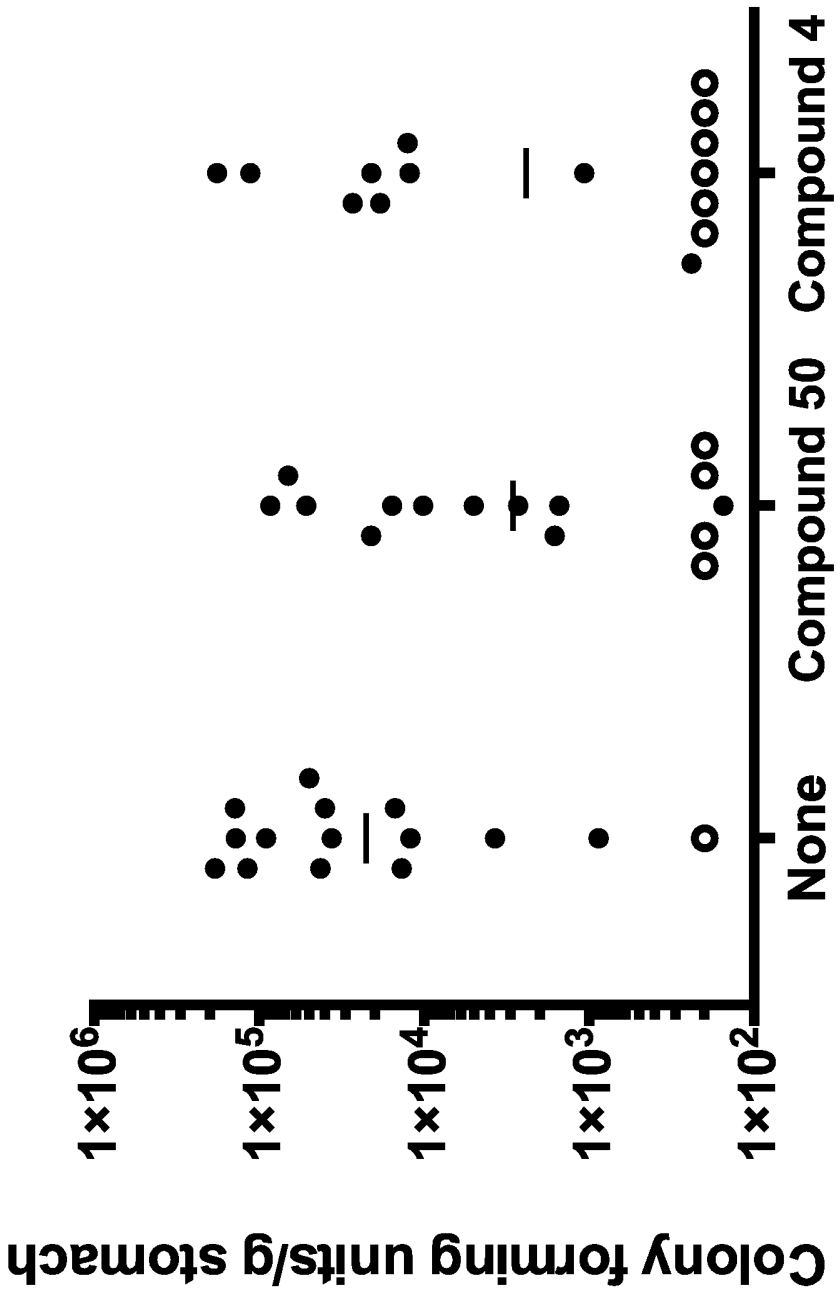

Colony forming units/g stomach
1×10^6
1×10^5
1×10^4
1×10^3
1×10^2
None
Compound 50
Compound 4
Compound (20 uM)

Fig. 26

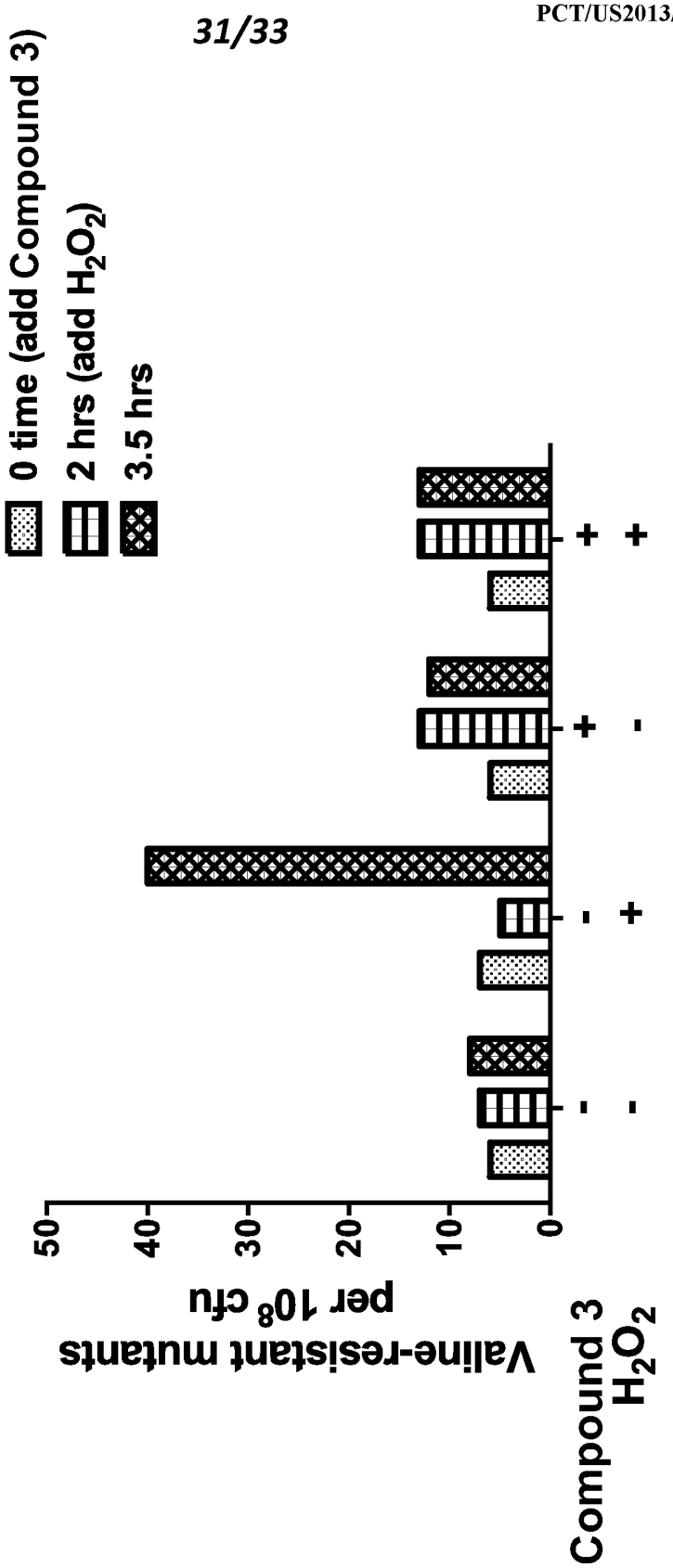

0 time (add Compound 3)
2 hrs (add H_2O_2)
3.5 hrs
Valine-resistant mutants per 10^8 cfu
0
10
20
30
40
50
Compound 3
H_2O_2
- -
- +
+ -
+ +

Fig. 27

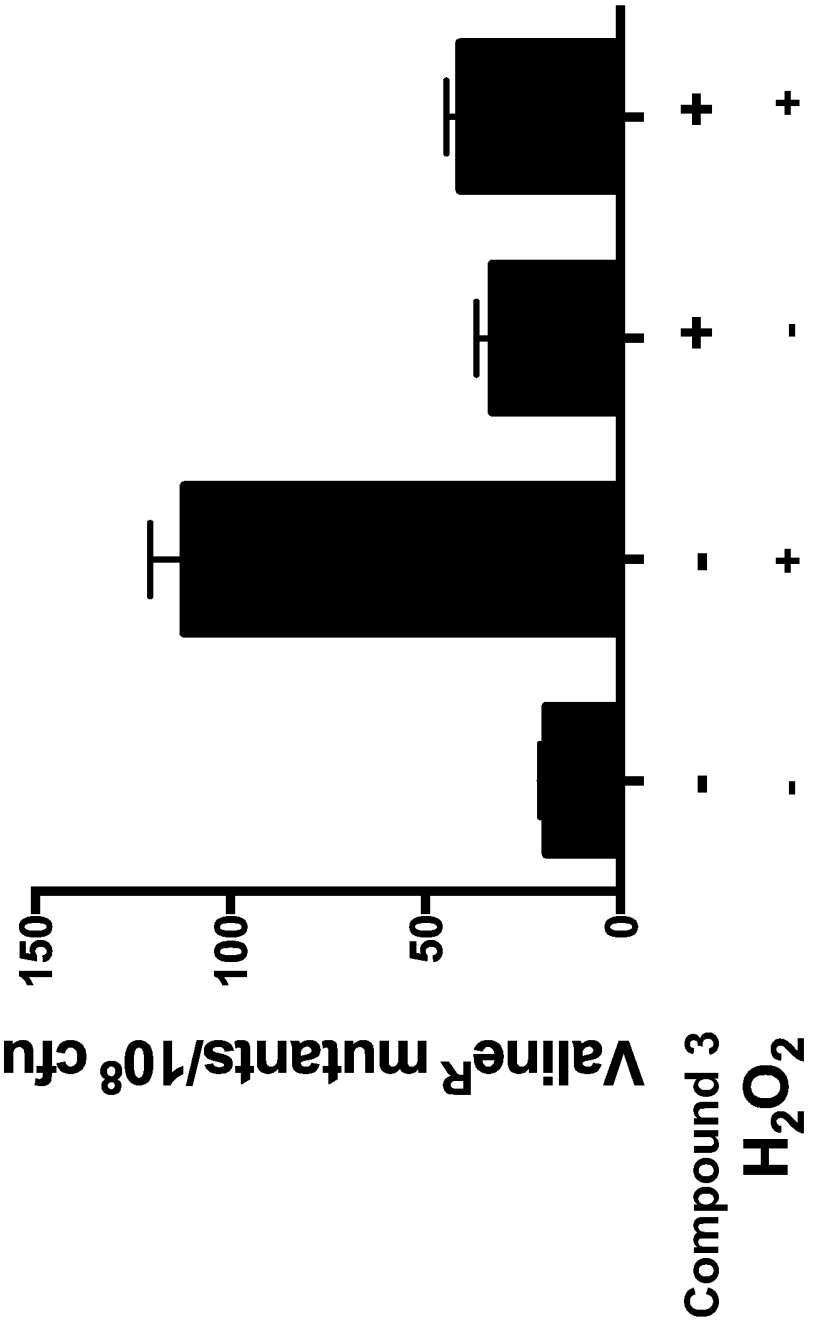


Fig. 28

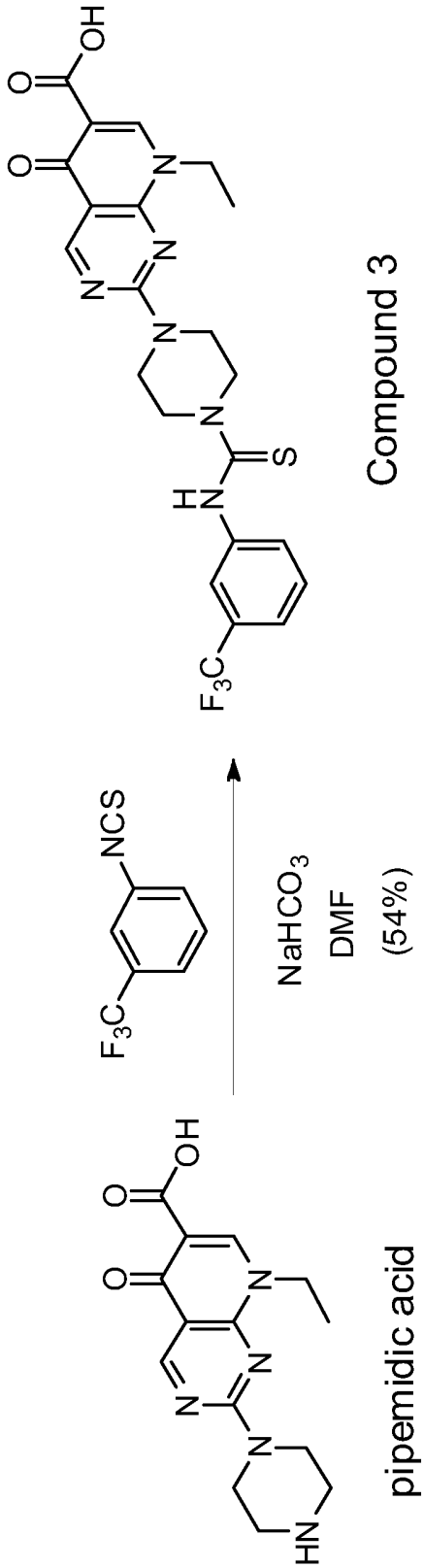


Fig. 29