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(54) Title: N-SUBSTITUTED-1H-1,2,4-TRIAZOLE-3-CARBOXAMIDES, ANALOGUES THEREOF, AND METHODS OF TREATMENT USING SAME

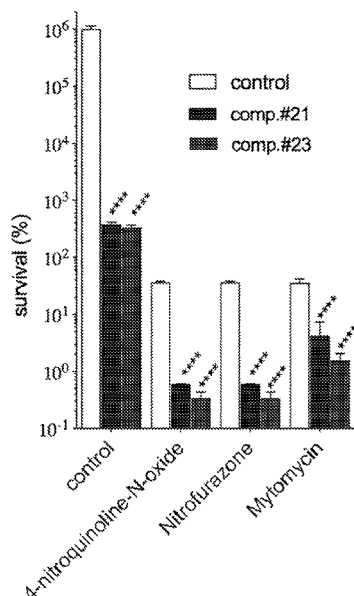


FIG. 9

(57) Abstract: The present invention relates to the discovery of novel N-(substituted)-1H-1,2,4-triazole-3-carboxamide compounds capable of inhibiting DksA activity in a bacteria cell. In certain embodiments, the compounds are capable of killing or weakening bacteria. In another aspect, the invention relates to methods of treating bacterial infection in a subject in need thereof, the method comprising administering to the subject a compound of the invention.

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N-Substituted-1H-1,2,4-Triazole-3-Carboxamides, Analogues Thereof, and
Methods of Treatment using Same

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application Serial
5 No. 62/721,392, filed August 22, 2018, which is incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under grant number
BX0002073 awarded by Veterans Affairs. The government has certain rights in the
10 invention.

BACKGROUND OF THE INVENTION

RNA polymerase-binding transcription factor DksA is a transcription factor protein
that acts by binding directly to RNA polymerase (RNAP). DksA is required for negative
regulation of rRNA expression and positive regulation of several amino acid biosynthesis
15 promoters. DksA is highly conserved among Gram-negative bacteria, making this protein
a potential target for small molecule antibiotics. At the tip of the coiled-coil domain, DksA
contains a pocket with Asp and Glu residues that serve as a potential ligand binding pocket
and drugable site. Inhibition of DksA by a small molecule could serve as a method of
weakening or killing a virulent bacteria.

20 There remains a need in the art for compounds and methods of treating bacterial
infection. In certain embodiments, the compounds and methods can target bacterial DksA
protein, inhibiting its activity, thereby weakening and/or killing the bacteria. The present
invention meets these needs.

BRIEF DESCRIPTION OF THE DRAWINGS

25 The following detailed description of specific embodiments of the invention will
be better understood when read in conjunction with the appended drawings. For the
purpose of illustrating the invention, there are shown in the drawings specific
embodiments. It should be understood, however, that the invention is not limited to the
precise arrangements and instrumentalities of the embodiments shown in the drawings.

30 FIGS. 1A-1D are graphs showing drug activity against DksA. FIG. 1A is a graph
showing the high conservation of several acidic residues at the tip of the coiled-coil
domain of DksA homologues from diverse Gram-negative bacteria. FIG. 1B is a graph
showing that Compound 916 (N-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamide,

prior art) has excellent activity against DksA-mediated repression of *rpsM* *in vitro* transcription. FIG. 1C is a graph showing that Compound 916 inhibits intracellular growth of *Salmonella* in J774 macrophage-like cells. The dash line represents intracellular growth of $\Delta dksA$ *Salmonella*. FIG. 1D is a graph showing that the addition (+) of 16 μ g/ml of

5 Compound 916 inhibits the intracellular expression of the SPI2 effector *sifA*

FIGS. 2A-2D are images showing the binding of drug to the pocket at the tip of DksA coiled-coil domain. FIG. 2A is an image of co-crystals of *Salmonella* recombinant DksA protein and compound 916. FIG. 2B is an *in silico* model of *E. coli* DksA protein (PDB 1TJL) with compound 916 docked into the putative compound-binding site at the tip
10 of the coiled-coil. The surface of DksA is depicted in shades of darker gray and lighter gray for most hydrophilic and hydrophobic, respectively. FIGS. 2C and 2D are *in silico* model images showing the predicted interactions of ATP (FIG. 2C) and compound 916 (FIG. 2D) with DksA pocket residues, depicted as dashed lines.

FIG. 3 is a graph showing the effects of increasing concentrations of compounds 1
15 and 2 on *livJ* *in vitro* transcription.

FIG. 4 is a graph showing the effects of increasing concentrations of compounds 3, 4, 5 and 6 on *livJ* *in vitro* transcription.

FIG. 5 is a graph showing the effects of increasing concentrations of compounds 7, 8, 9, 10 and 11 on *livJ* *in vitro* transcription.

20 FIG. 6 is a graph showing the effects of increasing concentrations of compounds 12, 13, 14, 15 and 16 on *livJ* *in vitro* transcription.

FIG. 7 is a graph showing the effects of increasing concentrations of compounds 17, 18, 19, 20, 21 and 22 on *livJ* *in vitro* transcription.

FIGS. 8A and 8B are graphs showing the effects of anti-DksA drugs on persistent
25 populations of intracellular *Salmonella*. FIG. 8A is a graph showing peritoneal mouse macrophages pretreated with interferon- γ and exposed to wild-type *Salmonella* or $\Delta dksA$. FIG. 8B is a graph showing survival percentage of infected cells following treatment with compounds #6 and #21 compared with wild-type *Salmonella* or $\Delta dksA$ cells.

FIG. 9 is a graph showing the synergistic effects of anti-DksA drugs used with
30 antibiotics that target DNA replication.

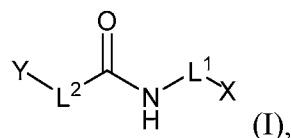
DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the discovery of novel *N*-(substituted)-1H-1,2,4-triazole-3-carboxamides and analogues thereof, which are capable of inhibiting DksA

activity in a bacterium. In certain embodiments, the compounds are capable of killing or weakening bacteria. In another aspect, the invention relates to methods of treating bacterial infection in a subject in need thereof, the method comprising administering to the subject a compound of the invention.

5 Compounds

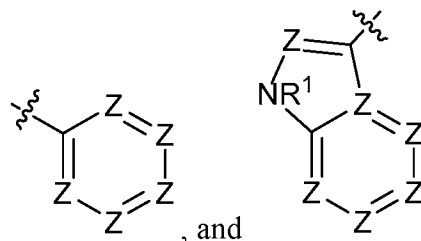
In one aspect, the invention provides a compound of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof:



wherein:

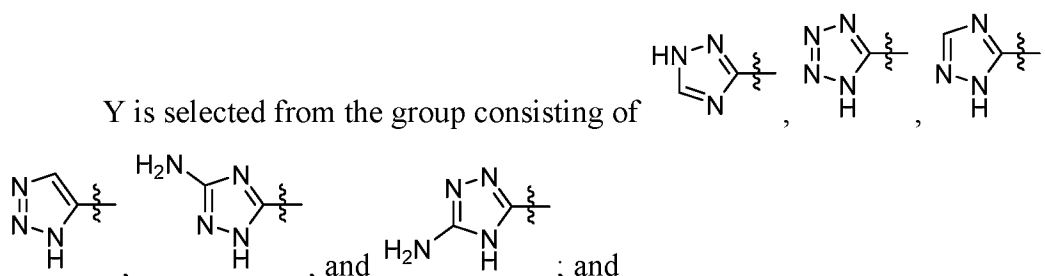
- 10 L^1 is selected from the group consisting of a bond, C_{1-6} alkylene optionally substituted with at least one selected from the group consisting of halogen and $\text{C}_1\text{-C}_6$ alkyl;

L^2 is selected from the group consisting of a bond and $-\text{CH}_2-$;



X is selected from the group consisting of H , , and ,

- 15 wherein each instance of Z is independently selected from the group consisting of N and CR^1 , provided that 0-2 occurrences of Z are N ;

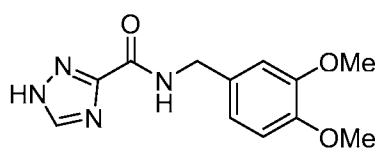


Y is selected from the group consisting of , , , , and

- 20 each instance of R^1 is independently selected from the group consisting of H , $-\text{OH}$, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{3-8} cycloalkyl, and C_{1-6} perhaloalkyl, or two adjacent R^1 groups are taken together to form a 4-6 membered homocyclic or heterocyclic fused ring (such as for example, cyclopentyl, cyclohexyl, 1,3-dioxolane, or 1,3-dioxane).

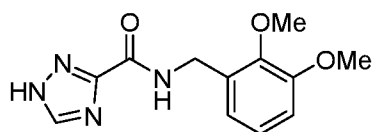
In certain embodiments, the compound of Formula (I) is at least one compound

selected from the group consisting of:



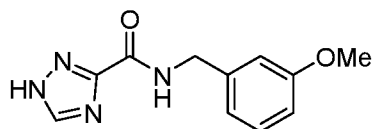
N-(3,4-dimethoxybenzyl)-1H-1,2,4-triazole-3-

carboxamide;



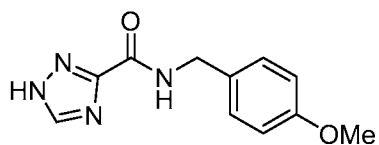
N-(2,3-dimethoxybenzyl)-1H-1,2,4-triazole-3-

5 carboxamide;



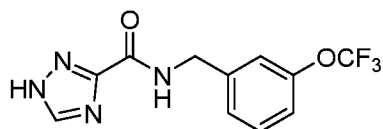
N-(3-methoxybenzyl)-1H-1,2,4-triazole-3-

carboxamide;



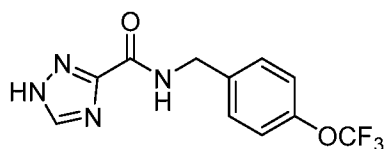
N-(4-methoxybenzyl)-1H-1,2,4-triazole-3-

carboxamide;



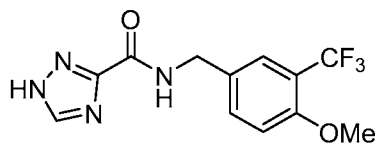
10 N-(3-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-

carboxamide;



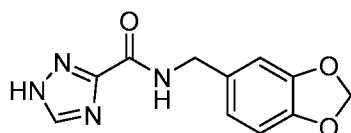
N-(4-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-

carboxamide;



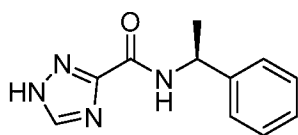
N-(4-methoxy-3-(trifluoromethyl)benzyl)-1H-1,2,4-

15 triazole-3-carboxamide;



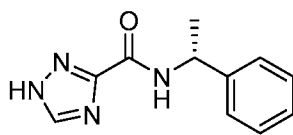
N-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-1,2,4-

triazole-3-carboxamide;



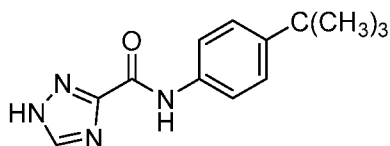
(*S*)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-

carboxamide;



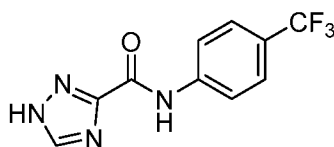
(*R*)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-

5 carboxamide;



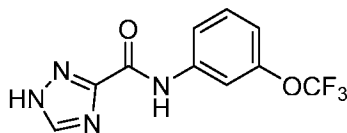
N-(4-(tert-butyl)phenyl)-1H-1,2,4-triazole-3-

carboxamide;



N-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-3-

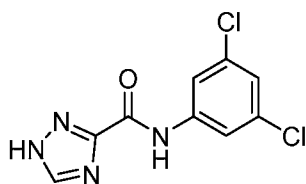
carboxamide;



10

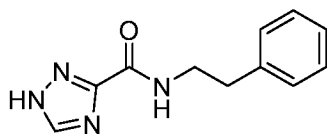
N-(3-(trifluoromethoxy)phenyl)-1H-1,2,4-triazole-3-

carboxamide;

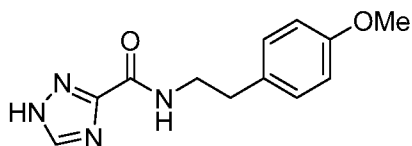


N-(3,5-dichlorophenyl)-1H-1,2,4-triazole-3-

carboxamide;



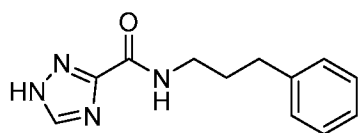
N-phenethyl-1H-1,2,4-triazole-3-carboxamide;



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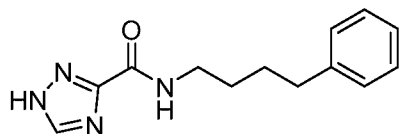
N-(4-methoxyphenethyl)-1H-1,2,4-triazole-3-

carboxamide;

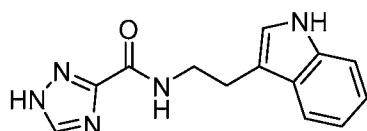


N-(3-phenylpropyl)-1H-1,2,4-triazole-3-

carboxamide;

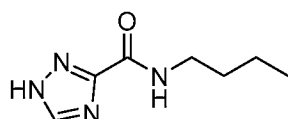


N-(4-phenylbutyl)-1H-1,2,4-triazole-3-carboxamide;



N-(2-(1H-indol-3-yl)ethyl)-1H-1,2,4-triazole-3-

5 carboxamide; and



N-butyl-1H-1,2,4-triazole-3-carboxamide.

In certain embodiments, the compound of Formula (I) is capable of binding DksA. In certain embodiments, the compound of Formula (I) inhibits DksA function. In other embodiments, the compound of Formula (I) inhibits the growth of gram-negative bacteria.

10 In yet other embodiments, the compound of Formula (I) kills gram-negative bacteria.

In certain embodiments, compounds of the invention include any compound of Formula (I) having an MIC value lower than about 32 $\mu\text{g/ml}$ capable of inhibiting *rpsM* *in vitro* transcription reactions with an IC_{50} value of less than about 100 nM.

In certain embodiments, the compound of Formula (I) is formulated as part of a pharmaceutical composition, further comprising at least one pharmaceutically acceptable carrier. In certain embodiments, the pharmaceutical composition further comprises at least one additional therapeutic agent. In other embodiments, the at least one additional therapeutic agent is at least one antimicrobial agent. In yet other embodiments, the at least one additional therapeutic agent is at least one antibiotic or antibacterial agent.

20 The compounds of the invention may possess one or more stereocenters, and each stereocenter may exist independently in either the (*R*) or (*S*) configuration. In certain embodiments, compounds described herein are present in optically active or racemic forms. The compounds described herein encompass racemic, optically-active, regioisomeric and stereoisomeric forms, or combinations thereof that possess the

25 therapeutically useful properties described herein. Preparation of optically active forms is

achieved in any suitable manner, including by way of non-limiting example, by resolution of the racemic form with recrystallization techniques, synthesis from optically-active starting materials, chiral synthesis, or chromatographic separation using a chiral stationary phase. In certain embodiments, a mixture of one or more isomer is utilized as the
5 therapeutic compound described herein. In other embodiments, compounds described herein contain one or more chiral centers. These compounds are prepared by any means, including stereoselective synthesis, enantioselective synthesis and/or separation of a mixture of enantiomers and/ or diastereoisomers. Resolution of compounds and isomers thereof is achieved by any means including, by way of non-limiting example, chemical
10 processes, enzymatic processes, fractional crystallization, distillation, and chromatography.

The methods and formulations described herein include the use of N-oxides (if appropriate), crystalline forms (also known as polymorphs), solvates, amorphous phases, and/or pharmaceutically acceptable salts of compounds having the structure of any
15 compound of the invention, as well as metabolites and active metabolites of these compounds having the same type of activity. Solvates include water, ether (*e.g.*, tetrahydrofuran, methyl *tert*-butyl ether) or alcohol (*e.g.*, ethanol) solvates, acetates and the like. In certain embodiments, the compounds described herein exist in solvated forms with pharmaceutically acceptable solvents such as water, and ethanol. In other
20 embodiments, the compounds described herein exist in unsolvated form.

In certain embodiments, the compounds of the invention exist as tautomers. All tautomers are included within the scope of the compounds recited herein.

In certain embodiments, compounds described herein are prepared as prodrugs. A "prodrug" is an agent converted into the parent drug *in vivo*. In certain embodiments, upon
25 *in vivo* administration, a prodrug is chemically converted to the biologically, pharmaceutically or therapeutically active form of the compound. In other embodiments, a prodrug is enzymatically metabolized by one or more steps or processes to the biologically, pharmaceutically or therapeutically active form of the compound.

In certain embodiments, sites on, for example, the aromatic ring portion of
30 compounds of the invention are susceptible to various metabolic reactions. Incorporation of appropriate substituents on the aromatic ring structures may reduce, minimize or eliminate this metabolic pathway. In certain embodiments, the appropriate substituent to decrease or eliminate the susceptibility of the aromatic ring to metabolic reactions is, by

way of example only, a deuterium, a halogen, or an alkyl group.

Compounds described herein also include isotopically-labeled compounds wherein one or more atoms is replaced by an atom having the same atomic number, but an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes suitable for inclusion in the compounds described herein include and are not limited to ^2H , ^3H , ^{11}C , ^{13}C , ^{14}C , ^{36}Cl , ^{18}F , ^{123}I , ^{125}I , ^{13}N , ^{15}N , ^{15}O , ^{17}O , ^{18}O , ^{32}P , and ^{35}S . In certain embodiments, isotopically-labeled compounds are useful in drug and/or substrate tissue distribution studies. In other embodiments, substitution with heavier isotopes such as deuterium affords greater metabolic stability (for example, increased in vivo half-life or reduced dosage requirements). In yet other embodiments, substitution with positron emitting isotopes, such as ^{11}C , ^{18}F , ^{15}O and ^{13}N , is useful in Positron Emission Topography (PET) studies for examining substrate receptor occupancy. Isotopically-labeled compounds are prepared by any suitable method or by processes using an appropriate isotopically-labeled reagent in place of the non-labeled reagent otherwise employed.

In certain embodiments, the compounds described herein are labeled by other means, including, but not limited to, the use of chromophores or fluorescent moieties, bioluminescent labels, or chemiluminescent labels.

The compounds described herein may form salts with acids and/or bases, and such salts are included in the present invention. In certain embodiments, the salts are pharmaceutically acceptable salts. The term "salts" embraces addition salts of free acids and/or bases that are useful within the methods of the invention. The term "pharmaceutically acceptable salt" refers to salts that possess toxicity profiles within a range that affords utility in pharmaceutical applications. Pharmaceutically unacceptable salts may nonetheless possess properties such as high crystallinity, which have utility in the practice of the present invention, such as for example utility in process of synthesis, purification or formulation of compounds useful within the methods of the invention.

Suitable pharmaceutically acceptable acid addition salts may be prepared from an inorganic acid or from an organic acid. Examples of inorganic acids include sulfate, hydrogen sulfate, hemisulfate, hydrochloric, hydrobromic, hydriodic, nitric, carbonic, sulfuric, and phosphoric acids (including hydrogen phosphate and dihydrogen phosphate). Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of

which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, trifluoromethanesulfonic, 2-hydroxyethanesulfonic, p-toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, alginic, β -hydroxybutyric, salicylic, galactaric, galacturonic acid, glycerophosphonic acids and saccharin (*e.g.*, saccharinate, saccharate). Salts may be comprised of a fraction of one, one or more than one molar equivalent of acid or base with respect to any compound of the invention.

Suitable pharmaceutically acceptable base addition salts of compounds of the invention include, for example, metallic salts including alkali metal, alkaline earth metal and transition metal salts such as, for example, calcium, magnesium, potassium, sodium and zinc salts. Pharmaceutically acceptable base addition salts also include organic salts made from basic amines such as, for example, N,N'-dibenzylethylene-diamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine. All of these salts may be prepared from the corresponding compound by reacting, for example, the appropriate acid or base with the compound.

Acid addition salts are generally formed by combining the target freebase with a salt former in a solvent, forming a solution, and collecting the salt as a solid. The molar ratio of salt former to free base may vary (*e.g.*, 1:1, 2:1, 1:2, etc.). A ratio of 1:1 may be preferred. Solvents may include, but are not limited to methyl ethyl ketone, methyl isobutyl ketone, ethyl acetate, isopropyl acetate, water, heptane, methyl *tert*-butyl ether, cyclohexane, toluene, methanol, ethanol, *n*-propanol, isopropanol, *n*-butanol, 2-butanol, isoamyl alcohol, tetrahydrofuran and acetonitrile, and mixtures thereof.

Synthesis

The compounds described herein, and other related compounds having different substituents are synthesized using techniques and materials described herein and as described, for example, in Fieser & Fieser's Reagents for Organic Synthesis, Vol. 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Vol. 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Vol. 1-40 (John Wiley and Sons, 1991), Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989), March, Advanced Organic Chemistry 4th Ed., (Wiley 1992); Carey & Sundberg, Advanced Organic Chemistry, 4th Ed., Vols. A and B (Plenum 2000,2001),

and Green & Wuts, Protective Groups in Organic Synthesis 3rd Ed., (Wiley 1999) (all of which are incorporated by reference for such disclosure). General methods for the preparation of compound as described herein are modified by the use of appropriate reagents and conditions, for the introduction of the various moieties found in the formula as provided herein.

Compounds described herein are synthesized using any suitable procedures starting from compounds that are available from commercial sources, or are prepared using procedures described herein. See, for example, Northen, *et al.*, 2002, J. Chem. Soc., Perkin Trans. 1, 108-115; DOI: 10.1039/B102224P.

Methods

In another aspect, the invention provides a method of inhibiting growth and/or killing bacteria. In certain embodiments, the method comprises contacting a bacterium with a compound or composition of the invention.

In certain embodiments, the bacterium is gram-negative. In other embodiments, the bacterium is at least one selected from the group consisting of, but not necessarily limited to, Enterobacteriaceae family members, including Klebsiella, E. coli, Proteus, Serratia, Salmonella, Yersinia, and Enterobacter, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Chlamydia trachomatis, Acinetobacter and Stenotrophomonas. In yet other embodiments, the bacterium has a DksA protein.

In certain embodiments, the invention provides a method of treating a bacterial infection in a subject in need thereof. In other embodiments, the method comprises administering to the subject a therapeutically effective amount of a pharmaceutical composition of the invention.

In certain embodiments, the bacterial infection is caused by a gram-negative bacterium. In other embodiments, the gram-negative bacterium is at least one selected from the group consisting of, but not necessarily limited to, Enterobacteriaceae family members, including Klebsiella, E. coli, Proteus, Serratia, Salmonella, Yersinia, and Enterobacter, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Chlamydia trachomatis, Acinetobacter and Stenotrophomonas. In yet other embodiments, the bacterium has a DksA protein.

This invention also encompasses the use of compound or composition of this disclosure in the manufacture of a medicament for the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection.

Similarly, this invention provides a compound or composition of this disclosure for use in the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection.

5 In these methods, the subject is a mammal. In these methods, the subject may be a human.

Combination and Concurrent Therapies

In one embodiment, the compositions of the invention are useful in the methods of present invention when used concurrently with at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein.

10 In one embodiment, the compositions of the invention are useful in the methods of present invention in combination with at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein. In certain embodiments, the compositions of the invention are useful in combination with one or more antimicrobial, antibacterial or antibiotic compounds or compositions.

15 These additional compounds may comprise compounds of the present invention or other compounds, such as commercially available compounds, known to treat, prevent, or reduce the symptoms of diseases and/or disorders contemplated herein. In certain embodiments, the combination of at least one compound of the invention or a salt thereof, and at least one additional compound useful for preventing and/or treating diseases and/or disorders contemplated herein, has additive, complementary or synergistic effects in the prevention and/or treatment of diseases and/or disorders contemplated herein.

20 As used herein, combinations of two or more compounds may refer to a composition wherein the individual compounds are physically mixed or wherein the individual compounds are physically separated. A combination therapy encompasses administering the components separately to produce the desired additive, complementary or synergistic effects.

A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid- $E_{\max}$ equation (Holford & Scheiner, 19981, Clin. Pharmacokinet. 6: 429-453), the equation of Loewe additivity (Loewe & Muischnek, 30 1926, Arch. Exp. Pathol Pharmacol. 114: 313-326), the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22: 27-55), and through the use of isobolograms (Tallarida & Raffa, 1996, Life Sci. 58: 23-28). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the

effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Administration/Dosing

5 The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations may be administered to the patient either prior to or after the onset of a bacterial infection. Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations
10 may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present invention to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a bacterial infection in the patient. An
15 effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior
20 medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound of the invention is
25 from about 0.01 mg/kg to 100 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

The compound may be administered to an animal as frequently as several times daily, or it may be administered less frequently, such as once a day, once a week, once
30 every two weeks, once a month, or even less frequently, such as once every several months or even once a year or less. It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day

administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on. The frequency of the dose will be readily apparent to the skilled artisan and will depend upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, the type and age of the animal, etc.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a bacterial infection in a patient.

In certain embodiments, the compositions of the invention are administered to the patient in dosages that range from one to five times per day or more. In other embodiments, the compositions of the invention are administered to the patient in range of dosages that include, but are not limited to, once every day, every two days, every three days to once a week, and once every two weeks. It will be readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the invention will vary from subject to subject depending on many factors including, but not limited to, age, disease or disorder to be treated, gender, overall health, and other

factors. Thus, the invention should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient will be determined by the attending physician taking all other factors about the patient into account.

5 Compounds of the invention for administration may be in the range of from about 1 μ g to about 7,500 mg, about 20 μ g to about 7,000 mg, about 40 μ g to about 6,500 mg, about 80 μ g to about 6,000 mg, about 100 μ g to about 5,500 mg, about 200 μ g to about 5,000 mg, about 400 μ g to about 4,000 mg, about 800 μ g to about 3,000 mg, about 1 mg to about 2,500 mg, about 2 mg to about 2,000 mg, about 5 mg to about 1,000 mg, about 10
10 mg to about 750 mg, about 20 mg to about 600 mg, about 30 mg to about 500 mg, about 40 mg to about 400 mg, about 50 mg to about 300 mg, about 60 mg to about 250 mg, about 70 mg to about 200 mg, about 80 mg to about 150 mg, and any and all whole or partial increments there-in-between.

 In some embodiments, the dose of a compound of the invention is from about 0.5
15 μ g and about 5,000 mg. In some embodiments, a dose of a compound of the invention used in compositions described herein is less than about 5,000 mg, or less than about 4,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments,
20 a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg,
25 or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

 In certain embodiments, the present invention is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the invention, alone or in combination with a second
30 pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of bacterial infection in a patient.

 The term “container” includes any receptacle for holding the pharmaceutical composition or for managing stability or water uptake. For example, in certain

embodiments, the container is the packaging that contains the pharmaceutical composition, such as liquid (solution and suspension), semisolid, lyophilized solid, solution and powder or lyophilized formulation present in dual chambers. In other embodiments, the container is not the packaging that contains the pharmaceutical composition, *i.e.*, the container is a receptacle, such as a box or vial that contains the packaged pharmaceutical composition or unpackaged pharmaceutical composition and the instructions for use of the pharmaceutical composition. Moreover, packaging techniques are well known in the art. It should be understood that the instructions for use of the pharmaceutical composition may be contained on the packaging containing the pharmaceutical composition, and as such the instructions form an increased functional relationship to the packaged product. However, it should be understood that the instructions may contain information pertaining to the compound's ability to perform its intended function, *e.g.*, treating, preventing, or reducing a bacterial infection in a patient.

Administration

Routes of administration of any of the compositions of the invention include inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal, and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, emulsions, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present invention are not limited to the particular formulations and compositions that are described herein.

Oral Administration

For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may

contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients that are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and lubricating agents such as magnesium stearate. The tablets may be uncoated or they may be coated by known techniques for elegance or to delay the release of the active ingredients. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert diluent.

Parenteral Administration

As used herein, "parenteral administration" of a pharmaceutical composition includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular, intrasternal injection, and kidney dialytic infusion techniques.

Formulations of a pharmaceutical composition suitable for parenteral administration comprise the active ingredient combined with a pharmaceutically acceptable carrier, such as sterile water or sterile isotonic saline. Such formulations may be prepared, packaged, or sold in a form suitable for bolus administration or for continuous administration. Injectable formulations may be prepared, packaged, or sold in unit dosage form, such as in ampules or in multidose containers containing a preservative. Formulations for parenteral administration include, but are not limited to, suspensions, solutions, emulsions in oily or aqueous vehicles, pastes, and implantable sustained-release or biodegradable formulations. Such formulations may further comprise one or more additional ingredients including, but not limited to, suspending, stabilizing, or dispersing agents. In one embodiment of a formulation for parenteral administration, the active ingredient is provided in dry (*i.e.*, powder or granular) form for reconstitution with a suitable vehicle (*e.g.*, sterile pyrogen-free water) prior to parenteral administration of the reconstituted composition.

Topical Administration

An obstacle for topical administration of pharmaceuticals is the stratum corneum layer of the epidermis. The stratum corneum is a highly resistant layer comprised of protein, cholesterol, sphingolipids, free fatty acids and various other lipids, and includes cornified and living cells. One of the factors that limit the penetration rate (flux) of a compound through the stratum corneum is the amount of the active substance that can be loaded or applied onto the skin surface. The greater the amount of active substance which is applied per unit of area of the skin, the greater the concentration gradient between the skin surface and the lower layers of the skin, and in turn the greater the diffusion force of the active substance through the skin. Therefore, a formulation containing a greater concentration of the active substance is more likely to result in penetration of the active substance through the skin, and more of it, and at a more consistent rate, than a formulation having a lesser concentration, all other things being equal.

Formulations suitable for topical administration include, but are not limited to, liquid or semi-liquid preparations such as liniments, lotions, oil-in-water or water-in-oil emulsions such as creams, ointments or pastes, and solutions or suspensions. Topically administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of the active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Enhancers of permeation may be used. These materials increase the rate of penetration of drugs across the skin. Typical enhancers in the art include ethanol, glycerol monolaurate, PGML (polyethylene glycol monolaurate), dimethylsulfoxide, and the like. Other enhancers include oleic acid, oleyl alcohol, ethoxydiglycol, laurocapram, alkanecarboxylic acids, dimethylsulfoxide, polar lipids, or N-methyl-2-pyrrolidone. One acceptable vehicle for topical delivery of some of the compositions of the invention may contain liposomes.

Buccal Administration

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets or lozenges made using conventional methods, and may contain, for example, 0.1 to 20% (w/w) of the active ingredient, the balance comprising an orally

dissolvable or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder or an aerosolized or atomized solution or suspension comprising the active ingredient. Such powdered, aerosolized, or aerosolized formulations, when
5 dispersed, preferably have an average particle or droplet size in the range from about 0.1 to about 200 nanometers, and may further comprise one or more of the additional ingredients described herein. The examples of formulations described herein are not exhaustive and it is understood that the invention includes additional modifications of these and other formulations not described herein, but known to those of skill in the art.

10 *Rectal Administration*

A pharmaceutical composition of the invention may be prepared, packaged, or sold in a formulation suitable for rectal administration. Such a composition may be in the form of, for example, a suppository, a retention enema preparation, and a solution for rectal or colonic irrigation.

15 Suppository formulations may be made by combining the active ingredient with a non-irritating pharmaceutically acceptable excipient which is solid at ordinary room temperature (*i.e.*, about 20°C) and which is liquid at the rectal temperature of the subject (*i.e.*, about 37°C in a healthy human). Suitable pharmaceutically acceptable excipients include, but are not limited to, cocoa butter, polyethylene glycols, and various glycerides.
20 Suppository formulations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Retention enema preparations or solutions for rectal or colonic irrigation may be made by combining the active ingredient with a pharmaceutically acceptable liquid carrier. As is well known in the art, enema preparations may be administered using, and may be
25 packaged within, a delivery device adapted to the rectal anatomy of the subject. Enema preparations may further comprise various additional ingredients including, but not limited to, antioxidants, and preservatives.

Additional Administration Forms

Additional dosage forms of this invention include dosage forms as described in
30 U.S. Patents Nos. 6,340,475, 6,488,962, 6,451,808, 5,972,389, 5,582,837, and 5,007,790. Additional dosage forms of this invention also include dosage forms as described in U.S. Patent Applications Nos. 20030147952, 20030104062, 20030104053, 20030044466, 20030039688, and 20020051820. Additional dosage forms of this invention also include

dosage forms as described in PCT Applications Nos. WO 03/35041, WO 03/35040, WO 03/35029, WO 03/35177, WO 03/35039, WO 02/96404, WO 02/32416, WO 01/97783, WO 01/56544, WO 01/32217, WO 98/55107, WO 98/11879, WO 97/47285, WO 93/18755, and WO 90/11757.

5 Definitions

As used herein, each of the following terms has the meaning associated with it in this section.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Generally, the nomenclature used herein and the laboratory procedures in animal pharmacology, pharmaceutical science, separation science and organic chemistry are those well-known and commonly employed in the art. It should be understood that the order of steps or order for performing certain actions is immaterial, so long as the present teachings remain operable. Moreover, two or more steps or actions can be conducted simultaneously or not.

As used herein, the articles “a” and “an” refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

As used herein, the term “alkoxy” employed alone or in combination with other terms means, unless otherwise stated, an alkyl group having the designated number of carbon atoms, as defined elsewhere herein, connected to the rest of the molecule via an oxygen atom, such as, for example, methoxy, ethoxy, 1-propoxy, 2-propoxy (or isopropoxy) and the higher homologs and isomers. A specific example is (C₁-C₃)alkoxy, such as, but not limited to, ethoxy and methoxy.

As used herein, the term “alkyl” by itself or as part of another substituent means, unless otherwise stated, a straight or branched chain hydrocarbon having the number of carbon atoms designated (*i.e.*, C₁-C₁₀ means one to ten carbon atoms) and includes straight, branched chain, or cyclic substituent groups. Examples include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *tert*-butyl, pentyl, neopentyl, hexyl, and cyclopropylmethyl. A specific embodiment is (C₁-C₆)alkyl, such as, but not limited to, ethyl, methyl, isopropyl, isobutyl, *n*-pentyl, *n*-hexyl and cyclopropylmethyl.

As used herein, the term “alkylene” by itself or as part of another substituent means, unless otherwise stated, a straight or branched hydrocarbon group having the

number of carbon atoms designated (*i.e.*, C₁-C₁₀ means one to ten carbon atoms) and includes straight, branched chain, or cyclic substituent groups, wherein the group has two open valencies. Examples include methylene, 1,2-ethylene, 1,1-ethylene, 1,1-propylene, 1,2-propylene and 1,3-propylene.

5 As used herein, the term “aromatic” refers to a carbocycle or heterocycle with one or more polyunsaturated rings and having aromatic character, *i.e.* having $(4n+2)$ delocalized π (pi) electrons, where n is an integer.

As used herein, the term “aryl,” employed alone or in combination with other terms, means, unless otherwise stated, a carbocyclic aromatic system containing one or
10 more rings (typically one, two or three rings) wherein such rings may be attached together in a pendent manner, such as a biphenyl, or may be fused, such as naphthalene. Examples include phenyl, anthracyl, and naphthyl.

As used herein, the term “aryl-(C₁-C₆)alkyl” refers to a functional group wherein a one to six carbon alkanediyl chain is attached to an aryl group, *e.g.*, -CH₂CH₂-phenyl or -
15 CH₂-phenyl (or benzyl). Specific examples are aryl-CH₂- and aryl-CH(CH₃)-. The term “substituted aryl-(C₁-C₆)alkyl” refers to an aryl-(C₁-C₆)alkyl functional group in which the aryl group is substituted. A specific example is substituted aryl(CH₂)-. Similarly, the term “heteroaryl-(C₁-C₆)alkyl” refers to a functional group wherein a one to three carbon alkanediyl chain is attached to a heteroaryl group, *e.g.*, -CH₂CH₂-pyridyl. A specific
20 example is heteroaryl-(CH₂)-. The term “substituted heteroaryl-(C₁-C₆)alkyl” refers to a heteroaryl-(C₁-C₆)alkyl functional group in which the heteroaryl group is substituted. A specific example is substituted heteroaryl-(CH₂)-.

As used herein, the term “cycloalkyl” by itself or as part of another substituent refers to, unless otherwise stated, a cyclic chain hydrocarbon having the number of carbon
25 atoms designated (*i.e.*, C₃-C₆ refers to a cyclic group comprising a ring group consisting of three to six carbon atoms) and includes straight, branched chain or cyclic substituent groups. Examples of (C₃-C₆)cycloalkyl groups are cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Cycloalkyl rings can be optionally substituted. Non-limiting examples of cycloalkyl groups include: cyclopropyl, 2-methyl-cyclopropyl, cyclopropenyl, cyclobutyl,
30 2,3-dihydroxycyclobutyl, cyclobutenyl, cyclopentyl, cyclopentenyl, cyclopentadienyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctanyl, decaliny, 2,5-dimethylcyclopentyl, 3,5-dichlorocyclohexyl, 4-hydroxycyclohexyl, 3,3,5-trimethylcyclohex-1-yl, octahdropentalenyl, octahydro-1*H*-indenyl, 3a,4,5,6,7,7a-hexahydro-3*H*-inden-4-yl,

decahydroazulenyl; bicyclo[6.2.0]decanyl, decahydronaphthalenyl, and dodecahydro-1*H*-fluorenyl. The term “cycloalkyl” also includes bicyclic hydrocarbon rings, non-limiting examples of which include, bicyclo-[2.1.1]hexanyl, bicyclo[2.2.1]heptanyl, bicyclo[3.1.1]heptanyl, 1,3-dimethyl[2.2.1] heptan-2-yl, bicyclo[2.2.2]octanyl, and
5 bicyclo[3.3.3]undecanyl.

As used herein, the term “halide” refers to a halogen atom bearing a negative charge. The halide anions are fluoride (F⁻), chloride (Cl⁻), bromide (Br⁻), and iodide (I⁻).

As used herein, the term “halo” or “halogen” alone or as part of another substituent refers to, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom.

10 As used herein, the language “pharmaceutically acceptable salt” refers to a salt of the administered compound prepared from pharmaceutically acceptable non-toxic acids and/or bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates (including hydrates) and clathrates thereof.

As used herein, the term “heteroalkyl” by itself or in combination with another
15 term refers to, unless otherwise stated, a stable straight or branched chain alkyl group consisting of the stated number of carbon atoms and one or two heteroatoms selected from the group consisting of O, N, and S, and wherein the nitrogen and sulfur atoms may be optionally oxidized and the nitrogen heteroatom may be optionally quaternized. The heteroatom(s) may be placed at any position of the heteroalkyl group, including between
20 the rest of the heteroalkyl group and the fragment to which it is attached, as well as attached to the most distal carbon atom in the heteroalkyl group. Examples include: -O-CH₂-CH₂-CH₃, -CH₂-CH₂-CH₂-OH, -CH₂-CH₂-NH-CH₃, -CH₂-S-CH₂-CH₃, and -CH₂CH₂-S(=O)-CH₃. Up to two heteroatoms may be consecutive, such as, for example, -CH₂-NH-OCH₃, or -CH₂-CH₂-S-S-CH₃.

25 As used herein, the term “heterocycle” or “heterocyclyl” or “heterocyclic” by itself or as part of another substituent refers to, unless otherwise stated, an unsubstituted or substituted, stable, mono- or multi-cyclic heterocyclic ring system that consists of carbon atoms and at least one heteroatom selected from the group consisting of N, O, and S, and wherein the nitrogen and sulfur heteroatoms may be optionally oxidized, and the nitrogen
30 atom may be optionally quaternized. The heterocyclic system may be attached, unless otherwise stated, at any heteroatom or carbon atom that affords a stable structure. A heterocycle may be aromatic or non-aromatic in nature. In certain embodiments, the heterocycle is a heteroaryl.

As used herein, the term “heteroaryl” or “heteroaromatic” refers to a heterocycle having aromatic character. A polycyclic heteroaryl may include one or more rings that are partially saturated. Examples include tetrahydroquinoline and 2,3-dihydrobenzofuryl.

Examples of non-aromatic heterocycles include monocyclic groups such as
5 aziridine, oxirane, thiirane, azetidine, oxetane, thietane, pyrrolidine, pyrroline, imidazoline, pyrazolidine, dioxolane, sulfolane, 2,3-dihydrofuran, 2,5-dihydrofuran, tetrahydrofuran, thiophane, piperidine, 1,2,3,6-tetrahydropyridine, 1,4-dihydropyridine, piperazine, morpholine, thiomorpholine, pyran, 2,3-dihydropyran, tetrahydropyran, 1,4-dioxane, 1,3-dioxane, homopiperazine, homopiperidine, 1,3-dioxepane, 4,7-dihydro-1,3-
10 dioxepin and hexamethyleneoxide.

Examples of heteroaryl groups include pyridyl, pyrazinyl, pyrimidinyl (such as, but not limited to, 2- and 4-pyrimidinyl), pyridazinyl, thienyl, furyl, pyrrolyl, imidazolyl, thiazolyl, oxazolyl, pyrazolyl, isothiazolyl, 1,2,3-triazolyl, 1,2,4-triazolyl, 1,3,4-triazolyl, tetrazolyl, 1,2,3-thiadiazolyl, 1,2,3-oxadiazolyl, 1,3,4-thiadiazolyl and 1,3,4-oxadiazolyl.

15 Examples of polycyclic heterocycles include indolyl (such as, but not limited to: 3-, 4-, 5-, 6-, and 7-indolyl), indolinyl, quinolyl, tetrahydroquinolyl, isoquinolyl (such as, but not limited to, 1- and 5-isoquinolyl), 1,2,3,4-tetrahydroisoquinolyl, cinnolinyl, quinoxaliny (such as, but not limited to, 2- and 5-quinoxaliny), quinazolinyl, phthalazinyl, 1,8-naphthyridinyl, 1,4-benzodioxanyl, coumarin, dihydrocoumarin, 1,5-
20 naphthyridinyl, benzofuryl (such as, but not limited to, 3-, 4-, 5-, 6- and 7-benzofuryl), 2,3-dihydrobenzofuryl, 1,2-benzisoxazolyl, benzothienyl (such as, but not limited to, 3-, 4-, 5-, 6-, and 7-benzothienyl), benzoxazolyl, benzothiazolyl (such as, but not limited to, 2-benzothiazolyl and 5-benzothiazolyl), purinyl, benzimidazolyl, benztriazolyl, thioxanthinyl, carbazolyl, carbolinyl, acridinyl, pyrrolizidinyl, and quinolizidinyl.

25 The aforementioned listing of heterocyclic and heteroaryl moieties is intended to be representative and not limiting.

As used herein, the term “substituted” refers to that an atom or group of atoms has replaced hydrogen as the substituent attached to another group.

As used herein, the term “substituted alkyl” or “substituted cycloalkyl” refers to
30 alkyl or cycloalkyl, as defined elsewhere herein, substituted by one, two or three substituents independently selected from the group consisting of halogen, -OH, alkoxy, tetrahydro-2-H-pyranyl, -NH₂, -NH(C₁-C₆ alkyl), -N(C₁-C₆ alkyl)₂, 1-methyl-imidazol-2-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, -C(=O)OH, -C(=O)O(C₁-C₆)alkyl,

trifluoromethyl, $-C\equiv N$, $-C(=O)NH_2$, $-C(=O)NH(C_1-C_6)alkyl$, $-C(=O)N((C_1-C_6)alkyl)_2$, $-SO_2NH_2$, $-SO_2NH(C_1-C_6 alkyl)$, $-SO_2N(C_1-C_6 alkyl)_2$, $-C(=NH)NH_2$, and $-NO_2$, in certain embodiments containing one or two substituents independently selected from halogen, $-OH$, alkoxy, $-NH_2$, trifluoromethyl, $-N(CH_3)_2$, and $-C(=O)OH$, in certain embodiments independently selected from halogen, alkoxy and $-OH$. Examples of substituted alkyls include, but are not limited to, 2,2-difluoropropyl, 2-carboxycyclopentyl and 3-chloropropyl.

For aryl, aryl-(C_1-C_3)alkyl and heterocyclyl groups, the term “substituted” as applied to the rings of these groups refers to any level of substitution, namely mono-, di-, tri-, tetra-, or penta-substitution, where such substitution is permitted. The substituents are independently selected, and substitution may be at any chemically accessible position. In certain embodiments, the substituents vary in number between one and four. In other embodiments, the substituents vary in number between one and three. In yet another embodiments, the substituents vary in number between one and two. In yet other embodiments, the substituents are independently selected from the group consisting of C_1-C_6 alkyl, $-OH$, C_1-C_6 alkoxy, halo, amino, acetamido and nitro. As used herein, where a substituent is an alkyl or alkoxy group, the carbon chain may be branched, straight or cyclic.

Unless otherwise noted, when two substituents are taken together to form a ring having a specified number of ring atoms (*e.g.*, R^2 and R^3 taken together with the nitrogen to which they are attached to form a ring having from 3 to 7 ring members), the ring can have carbon atoms and optionally one or more (*e.g.*, 1 to 3) additional heteroatoms independently selected from nitrogen, oxygen, or sulfur. The ring can be saturated or partially saturated, and can be optionally substituted.

Whenever a term or either of their prefix roots appear in a name of a substituent the name is to be interpreted as including those limitations provided herein. For example, whenever the term “alkyl” or “aryl” or either of their prefix roots appear in a name of a substituent (*e.g.*, arylalkyl, alkylamino) the name is to be interpreted as including those limitations given elsewhere herein for “alkyl” and “aryl” respectively.

In certain embodiments, substituents of compounds are disclosed in groups or in ranges. It is specifically intended that the description include each and every individual subcombination of the members of such groups and ranges. For example, the term “ C_{1-6} alkyl” is specifically intended to individually disclose C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_1-C_6 , C_1-C_5 ,

C1-C4, C1-C3, C1-C2, C2-C6, C2-C5, C2-C4, C2-C3, C3-C6, C3-C5, C3-C4, C4-C6, C4-C5, and C5-C6 alkyl.

As used herein, the term “bacteria” means a large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria have a wide range of shapes, ranging from spheres to rods and spirals. There are broadly speaking two different types of cell wall in bacteria, called Gram-positive and Gram-negative. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan and teichoic acids. In contrast, Gram-negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan surrounded by a second lipid membrane containing lipopolysaccharides and lipoproteins.

As used herein, the term “composition” or “pharmaceutical composition” refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient or subject. Multiple techniques of administering a compound exist in the art including, but not limited to, intravenous, oral, aerosol, parenteral, ophthalmic, nasal, pulmonary and topical administration.

A “disease” as used herein is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal’s health continues to deteriorate.

A “disorder” as used herein in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal’s state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal’s state of health.

The terms “patient,” “subject” or “individual” are used interchangeably herein, and refer to any animal, or cells thereof whether *in vitro* or *in situ*, amenable to the methods described herein. In a non-limiting embodiment, the patient, subject or individual is a human.

As used herein, the term “pharmaceutically acceptable” refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

As used herein, the term “pharmaceutically acceptable carrier” means a

pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function.

5 Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as
10 lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin,
15 sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer’s solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

20 As used herein, the term “pharmaceutically acceptable carrier” means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the invention within or to the patient such that it may perform its intended function.

25 Typically, such constructs are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be “acceptable” in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the invention, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as
30 lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive

oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; surface active agents; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the invention, and are physiologically acceptable to the patient. Supplementary active compounds may also be incorporated into the compositions. The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compound useful within the invention. Other additional ingredients that can be included in the pharmaceutical compositions used in the practice of the invention are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compounds prepared from pharmaceutically acceptable non-toxic acids, including inorganic acids, organic acids, solvates, hydrates, or clathrates thereof.

The term "prevent," "preventing" or "prevention," as used herein, means avoiding or delaying the onset of symptoms associated with a disease or condition in a subject that has not developed such symptoms at the time the administering of an agent or compound commences.

A "therapeutic" treatment is a treatment administered to a subject who exhibits signs of pathology, for the purpose of diminishing or eliminating those signs.

As used herein, the term "therapeutically effective amount" refers to an amount that is sufficient or effective to prevent or treat (delay or prevent the onset of, prevent the progression of, inhibit, decrease or reverse) a disease or condition described or contemplated herein, including alleviating symptoms of such disease or condition.

As used herein, the term "treatment" or "treating" is defined as the application or administration of a therapeutic agent, *i.e.*, a compound of the invention (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a condition contemplated herein, a

symptom of a condition contemplated herein or the potential to develop a condition contemplated herein, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect a condition contemplated herein, the symptoms of a condition contemplated herein or the potential to develop a condition contemplated herein.

5 Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation
10 on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual and
15 partial numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the
20 scope of this invention and covered by the claims appended hereto. For example, it should be understood, that modifications in reaction conditions, including but not limited to reaction times, reaction size/volume, and experimental reagents, such as solvents, catalysts, pressures, atmospheric conditions, *e.g.*, nitrogen atmosphere, and reducing/oxidizing agents, with art-recognized alternatives and using no more than routine
25 experimentation, are within the scope of the present application.

It is to be understood that, wherever values and ranges are provided herein, the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, all values and ranges encompassed by these values and ranges are meant to be encompassed within
30 the scope of the present invention. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application. The description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range and,

when appropriate, partial integers of the numerical values within ranges. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

The following examples further illustrate aspects of the invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

EXAMPLES

The invention is now described with reference to the following Examples. These Examples are provided for the purpose of illustration only, and the invention is not limited to these Examples, but rather encompasses all variations that are evident as a result of the teachings provided herein.

Materials and Methods

The ^1H and ^{13}C NMR spectra were recorded using a 400 MHz Bruker NMR, Avance III 400. The chemical shifts are reported in ppm. An Applied Biosystems Sciex 4000 (Applied Biosystems; Foster City, CA) was equipped with a Shimadzu HPLC (Shimadzu Scientific Instruments, Inc.; Columbia, MD) and Leap auto-sampler (LEAP Technologies; Carrboro, NC) was used to perform the LC/MS-MS. Nitrogen gas was procured from AirGas (Denver, CO). Reactions were monitored via silica gel IB2-F thin layer chromatography (TLC) plates from J.T. Baker (Phillipsburg, NJ). Silica Gel 60 Å 40-63 μm was purchased from Sorbent Technologies (Norcross, GA).

Anhydrous DMF (dimethylformamide), HPLC grade water, acetonitrile (ACN), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), hexanes, methanol (MeOH), methylene chloride (DCM), anhydrous sodium sulfate (Na_2SO_4), ammonium acetate (NH_4OAc) and formic acid were purchased from Fisher Scientific (Pittsburgh, PA). 1*H*-1,2,4-Triazole-3-carboxylic acid, DMSO- d_6 , 3,4-dimethoxyaniline, 1-hydroxybenzotriazole monohydrate, EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide), N-methylmorpholine, (3,4-dimethoxybenzyl) amine, (2,3-dimethoxybenzyl)amine, (4-methoxyphenyl)methanamine, 3-(trifluoromethoxy) aniline, piperonylamine, 4-(trifluoromethoxy)benzylamine, 3-methoxybenzylamine, 4-(trifluoromethoxy)benzylamine, 3-(trifluoromethoxy)benzylamine, 4-(trifluoromethoxy)benzylamine, 3-(trifluoromethoxy)benzylamine, 4-methoxy-3-

(trifluoromethyl)benzylamine, 4-methoxy-3-(trifluoromethyl)benzylamine, 4-(trifluoromethyl)aniline, butan-1-amine, 4-(tert-butyl)aniline, 4-(trifluoromethyl)aniline, 3,5-dichloroaniline, 3-phenylpropan-1-amine, 4-phenylbutan-1-amine, (*R*)-(+)- α -methyl benzyl amine, (*S*)-(α)-methyl benzyl amine, 2-(1*H*-indol-3-yl)ethanamine, 2-phenylethanamine, 2-(4-methoxyphenyl)ethanamine, (*R*)-(+)-4-Methoxy- α -methylbenzylamine were procured from Sigma-Aldrich Chemical Company (St. Louis, MO)

Procedures for *in vitro* transcription (IVT) assays

To measure the potency of a compound in inhibition of DksA biological function, *rpsM* transcripts *in vitro* was determined by non-radioactive *in vitro* transcription reactions with qPCR analysis with divergent compounds. Briefly, 5 nM pTIM-*rpsM* plasmid was mixed with 0.1 – 100 nM of compounds in 10 μ l reaction buffer (40 mM HEPES [pH 7.4], 2 mM MgCl₂, 60 mM potassium glutamate, 0.05% NP-40, 200 μ M ATP, 200 μ M GTP, 200 μ M CTP, 200 μ M UTP, and 5 nM *E.coli* RNA polymerase holoenzyme (NEB, Ipswich, MA) with 5 μ M DksA. The *in vitro* transcription was carried out at 37°C for 10 min, and then terminated at 70°C for 10 min. To remove template DNA after treated with DNaseI, DNA-free DNA Removal kit (ThermoFisher) was used according to manufacturer's direction. The resulting materials were used as templates to generate cDNA with 100 U MMLV reverse transcriptase (Promega), 0.45 μ M gene specific primer, and 20 U RNase inhibitor (Promega). The amount of cDNA synthesized for 1 h at 42°C was quantified by real-time PCR (qRT-PCR). The copy number of specific transcripts was calculated to the standard curve generated with known gene copy concentrations. The IC₅₀ is the concentration at which the curve passes through the 50% inhibition level. Standard deviation was calculated from 3 independent experiments.

Procedures for disk diffusion assays

Salmonella was grown overnight in LB broth at 37°C with shaking. The bacterial cultures were diluted by 1:1000 with PBS. 100 μ l of the diluted samples were transferred into E-salts glucose agar. The bacteria were spread on the surface of the agar. Two discs (BBL blank paper discs 6 mm) were placed per/plate, and 10 μ l of 10 mM or 100 mM stock compound were added to the discs. The plates were incubated at 37°C overnight. The diameter of inhibition was expressed in mm.

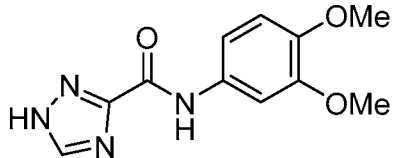
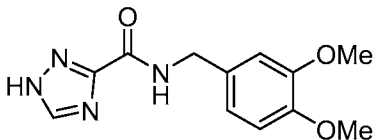
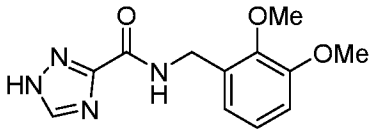
In Silico Modeling

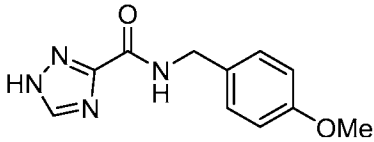
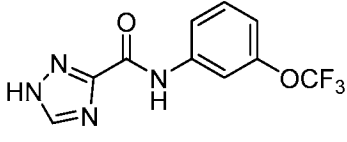
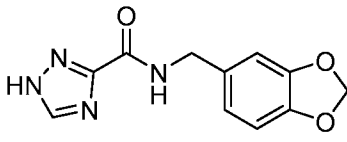
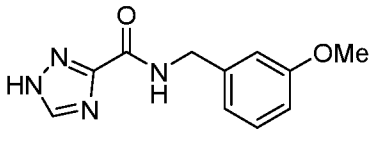
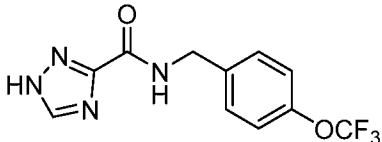
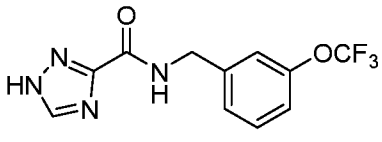
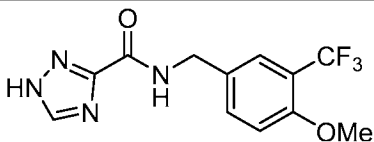
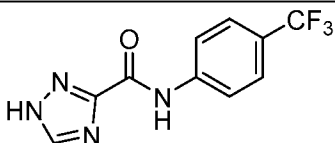
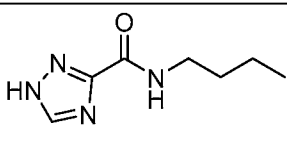
Structural investigation of DksA showed that there is an area of negatively charged

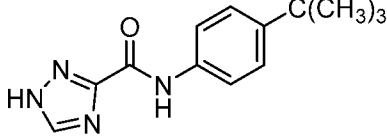
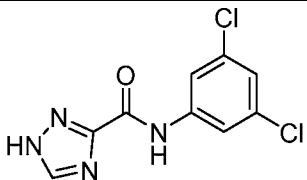
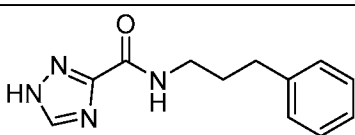
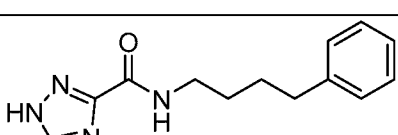
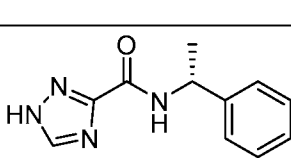
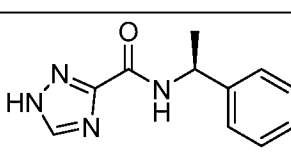
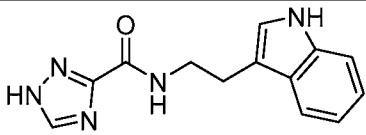
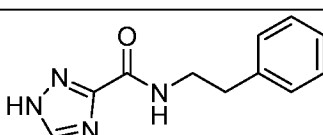
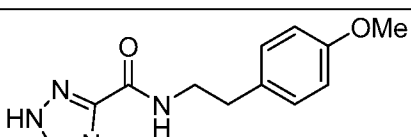
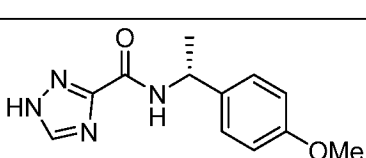
pocket in the coiled-coil domain, consisting of eight acidic residues: Asp⁶⁴, Asp⁶⁵, Asp⁷¹, Asp⁷⁴, Glu⁷⁹, Glu⁸⁰, Glu⁸¹, and Glu⁸⁵. DOCK (Kuntz, *et al.*, J. Mol. Biol. 161 (2), 269-288) was used to search several databases containing more than a hundred thousand small molecule drug candidates. Over 400 potential drug molecules matched the shapes and charges of acidic pocket at the tip of DksA. A panel of 40 compounds was selected from the top hits based on free energy binding scores. The surface complementarity between DksA and the compounds were inspected using a 3D modeling program. The interactions between these small molecules and DksA were further refined using energy minimization. These compounds were tested for their ability to interfere with DksA. The compound 916 (N-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamide, prior art) showed the best in vitro efficacy in preventing DksA-mediated repression of rpsM transcription. Modeling of compound 916 showed that the triazole ring was in van der Waals contact with Glu⁸⁰, Glu⁸¹, and also interacted through several hydrogen bonds. The phenol moiety was tucked into the cavity between Asp⁶⁵ and Glu⁷⁹. To improve the efficacy of candidate inhibitory drugs, compound 916 served as the basis for the development of additional compounds to screen. Based on free energy binding scores and molecular complementarity, several derivatives were identified with potentially greater binding affinity towards DksA.

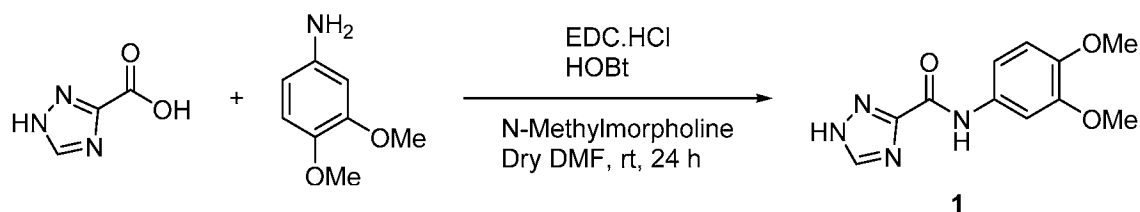
Example 1: Synthesis of Selected Compounds

Table 1. Compounds Synthesized

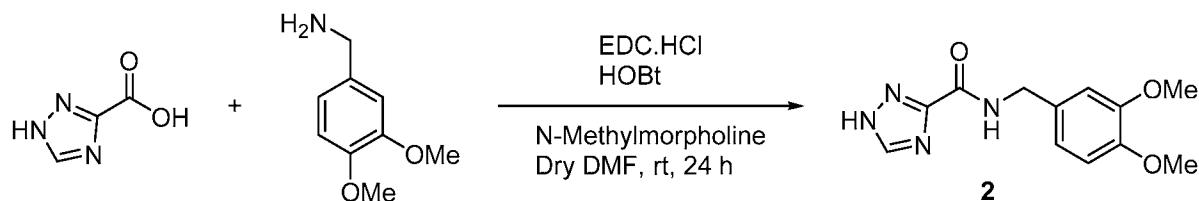
Cmpd. ID	Structure	Cmpd. Name	Molecular weight
1		N-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamide	248.24
2		N-(3,4-dimethoxybenzyl)-1H-1,2,4-triazole-3-carboxamide	262.26
3		N-(2,3-dimethoxybenzyl)-1H-1,2,4-triazole-3-carboxamide	262.26

4		N-(4-methoxybenzyl)-1H-1,2,4-triazole-3-carboxamide	232.24
5		N-(3-(trifluoromethoxy)phenyl)-1H-1,2,4-triazole-3-carboxamide	272.18
6		N-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-1,2,4-triazole-3-carboxamide	246.22
7		N-(3-methoxybenzyl)-1H-1,2,4-triazole-3-carboxamide	232.24
8		N-(4-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-carboxamide	286.21
9		N-(3-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-carboxamide	286.21
10		N-(4-methoxy-3-(trifluoromethyl)benzyl)-1H-1,2,4-triazole-3-carboxamide	300.24
11		N-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-3-carboxamide	256.18
12		N-butyl-1H-1,2,4-triazole-3-carboxamide	168.20

13		N-(4-(tert-butyl)phenyl)-1H-1,2,4-triazole-3-carboxamide	244.29
14		N-(3,5-dichlorophenyl)-1H-1,2,4-triazole-3-carboxamide	257.08
15		N-(3-phenylpropyl)-1H-1,2,4-triazole-3-carboxamide	230.27
16		N-(4-phenylbutyl)-1H-1,2,4-triazole-3-carboxamide	244.29
17		(R)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-carboxamide	216.24
18		(S)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-carboxamide	216.24
19		N-(2-(1H-indol-3-yl)ethyl)-1H-1,2,4-triazole-3-carboxamide	255.28
20		N-phenethyl-1H-1,2,4-triazole-3-carboxamide	216.24
21		N-(4-methoxyphenethyl)-1H-1,2,4-triazole-3-carboxamide	246.27
22		(R)-N-(1-(4-methoxyphenyl)ethyl)-1H-1,2,4-triazole-3-carboxamide	246.27

Synthesis of 1

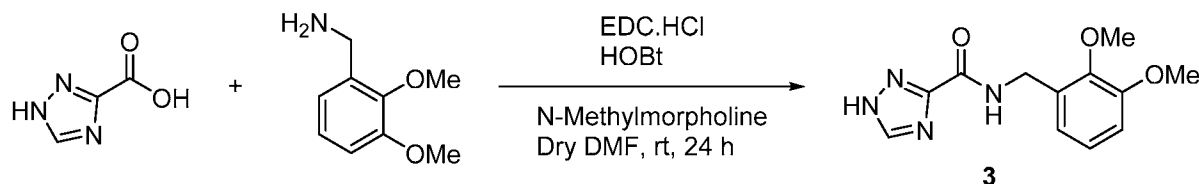
To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 3,4-dimethoxyaniline (322 mg, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into ice cold water and extracted with DCM (50 mL x 2). The DCM phase was washed with cold water (100 mL x 2). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and then chromatographed on silica gel using EtOAc as eluent to get the desired amide **1** (60 mg, 12% yield) as a light brown solid. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.79 (br s, 1H), 10.34 (br s, 1H), 8.58 (br s, 1H), 7.55-7.44 (m, 2H), 6.94-6.93 (m, 1H), 3.76-3.74 (m, 6H) ppm. MH⁺ = 263.2 m/z.

Synthesis of 2

To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (3,4-dimethoxybenzyl)amine (0.32 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24 h, and then slowly diluted into iced water and extracted with DCM (50 mL x 2). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (70:30) as

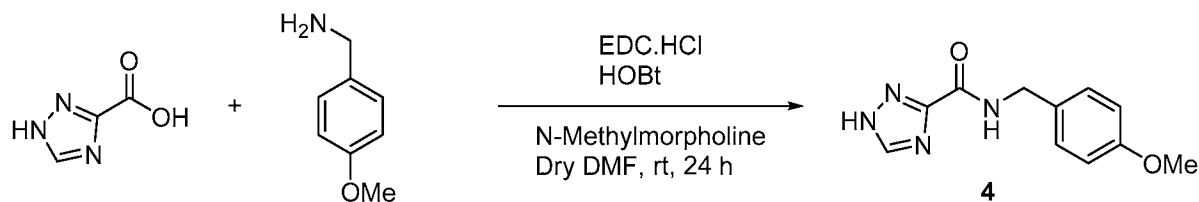
eluents to get the desired amide **2** (40.7 mg, 8% yield) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ14.74-14.50 (m, 1H), 9.06 (br s, 1H), 8.48-8.46 (m, 1H), 6.97 (m, 1H), 6.90-6.88 (m, 1H), 6.85-6.83 (m, 1H), 4.37 (d, *J* = 6.4 Hz, 2H), 3.73 (d, *J* = 3.6 Hz, 6H) ppm. MH⁺ = 263.2 m/z.

5 Synthesis of 3



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (2,3-dimethoxybenzyl)amine (0.31 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24 h, and then slowly diluted into ice cold water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (9:1) as eluents to get the desired amide **3** (104.9 mg, 20% yield) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ14.67 (br s, 1H), 8.94 (br s, 1H), 8.49 (br s, 1H), 7.03-6.99 (m, 1H), 6.99-6.94 (m, 1H), 6.85-6.82 (m, 1H), 4.48 (d, *J* = 6.4 Hz, 2H), 3.80 (s, 3H), 3.78 (s, 3H) ppm. MH⁺ = 233.2 m/z.

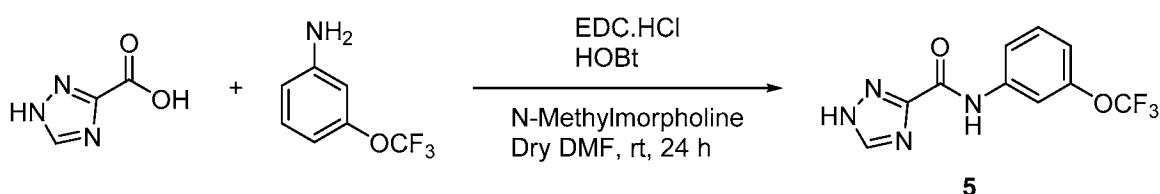
20 Synthesis of 4



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (4-methoxyphenyl)methanamine (0.27 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room

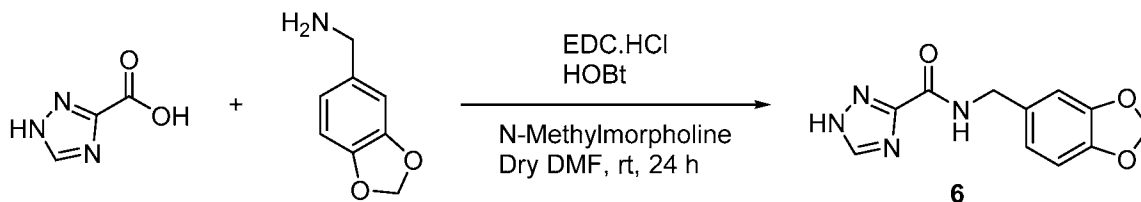
temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using MeOH/EtOAc/DCM (6:50:50, v/v/v) as eluents to get the desired amide **4** (137 mg, 29% yield) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.70-14.61 (m, 1H), 9.07 (br s, 1H), 8.45 (br s, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 4.36 (d, *J* = 6.4 Hz, 2H), 3.72 (s, 3H) ppm. MH⁺ = 233.2 m/z.

10 Synthesis of 5



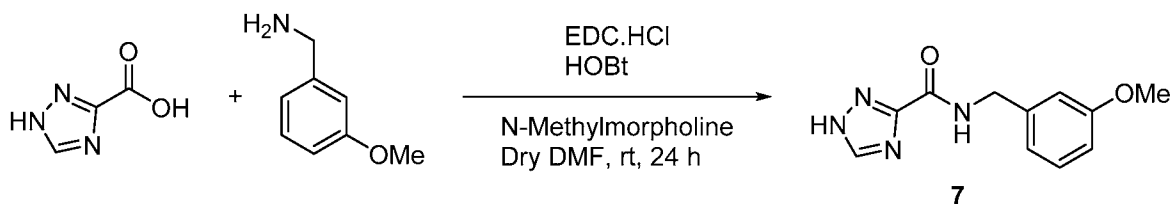
To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 3-(trifluoromethoxy)aniline (0.28 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (50:50) as eluents to get the desired amide **5** (123.8 mg, 23 % yield) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.89-14.74 (m, 1H), 10.82 (br s, 1H), 8.67 (br s, 1H), 8.00 (br s, 1H), 7.88 (d, *J* = 8 Hz, 1H), 7.49 (t, *J* = 8.2 Hz, 1H), 7.11 (d, *J* = 8 Hz, 1H); ¹⁹F-NMR (376 MHz, DMSO-d₆): δ -56.69 ppm. MH⁺ = 273.2 m/z.

25 Synthesis of 6



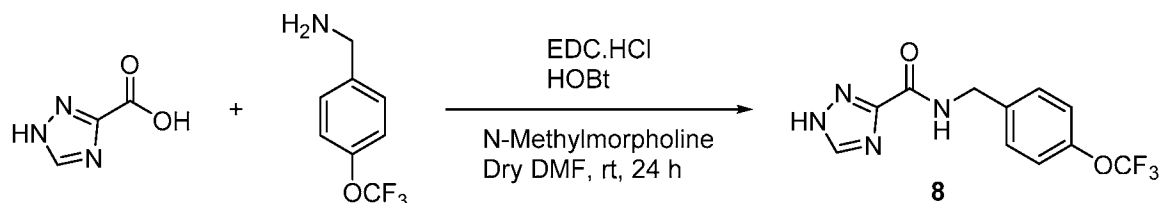
To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and Piperonylamine (0.26 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc as eluents to get the desired amide **6** (95 mg, 19 % yield) as a white solid. ¹H-NMR (400 MHz, DMSO-d₆): δ 15.58-13.83 (m, 1H), 9.10 (br s, 1H), 8.47 (br s, 1H), 6.90- 6.78 (m, 3H), 5.97 (s, 2H), 4.36 (d, *J* = 6.4 Hz, 2H) ppm. MH⁺ = 247.1 m/z.

Synthesis of 7



To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 3-methoxybenzylamine (0.28 mL, 2.2 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (2:98) as eluents to get the desired amide **7** (170.9 mg, 37 % yield) as a white solid compound. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.76-14.65 (m, 1H), 9.15 (br s, 1H), 8.48 (br s, 1H), 7.23 (t, *J* = 8 Hz, 1H), 6.90-6.88 (m, 2H), 6.82-6.80 (m, 1H), 4.43 (d, *J* = 6 Hz, 2H), 3.73 (s, 3H) ppm. MH⁺ = 233.2 m/z.

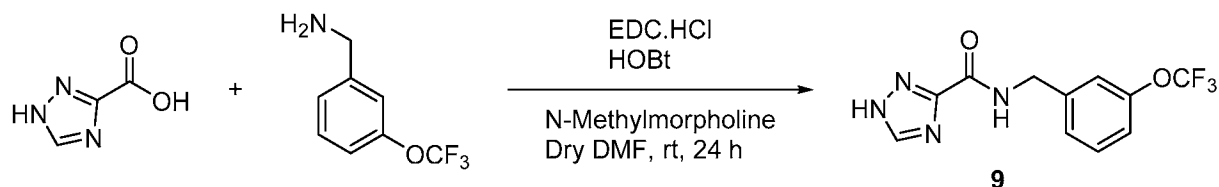
Synthesis of 8



To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (113 mg, 1.19 mmol) and 4-(trifluoromethoxy)benzylamine (0.20 mL, 1.31 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (200 mg, 1.31 mmol) and EDC (N-(3-

- 5 Dimethylaminopropyl)-N'-ethylcarbodiimide) (251 mg, 1.31 mmol) followed by N-methylmorpholine (0.52 mL, 4.76 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL).
 10 The DCM phase was dried over anhydrous Na₂SO₄, concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (8:92) as eluents to get the desired amide **8** (305.3 mg, 90 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-*d*₆): δ 14.80-14.01 (br s, 1H), 9.25 (br s, 1H), 8.50 (br s, 1H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8 Hz, 2H), 4.47 (d, *J* = 6 Hz, 2H); ¹⁹F -NMR (376 MHz, DMSO-*d*₆): δ -
 15 56.89 ppm. MH⁺ = 287.2 m/z.

Synthesis of 9



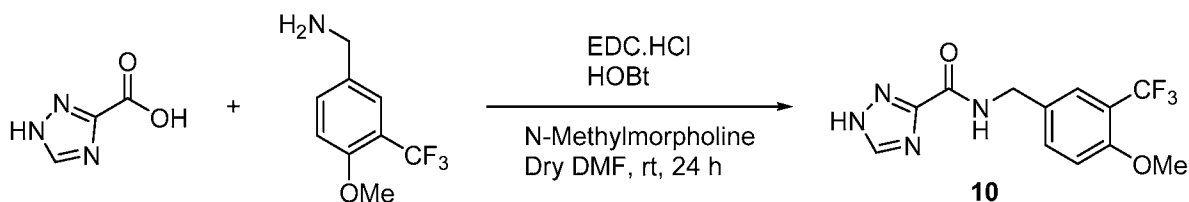
To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (113 mg, 1.19 mmol) and 3-(trifluoromethoxy)benzylamine (250 mg, 1.31 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (200 mg, 1.31 mmol) and EDC (N-(3-

20 Dimethylaminopropyl)-N'-ethylcarbodiimide) (251 mg, 1.31 mmol) followed by N-methylmorpholine (0.52 mL, 4.76 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and
 25 extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using DCM and EtOAc (10:90)

as eluents to get the desired amide **9** (108.7 mg, 32 % yield) as a white solid compound.

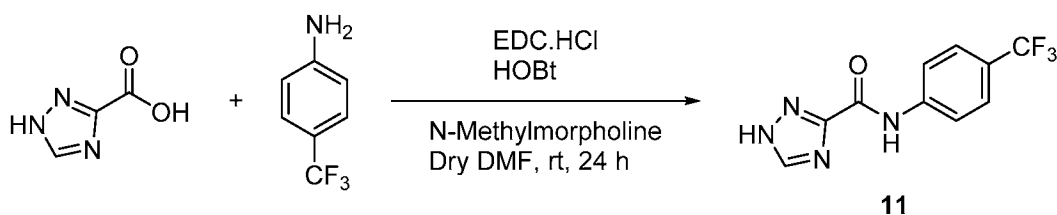
^1H -NMR (400 MHz, DMSO- d_6): δ 14.67 (br s, 1H), 9.28 (br s, 1H), 8.53 (br s, 1H), 7.48-7.44 (m, 1H), 7.36-7.34 (m, 1H), 7.30 (br s, 1H), 7.24-7.22 (m, 1H), 4.50 (d, J = 6.4 Hz, 2H); ^{19}F -NMR (376 MHz, DMSO- d_6): δ -56.70 ppm. MH^+ = 287.2 m/z.

5 Synthesis of 10



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (100 mg, 0.87 mmol) and 4-Methoxy-3- (trifluoromethyl)benzylamine (200 mg, 0.97 mmol) in dry DMF (10 mL) was added 1- hydroxybenzotriazole monohydrate (146 mg, 0.97 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (186 mg, 0.97 mmol) followed by N-methylmorpholine (0.38 mL, 3.48 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc as eluents to get the desired amide **10** (62.8 mg, 24 % yield) as a white solid compound. ^1H -NMR (400 MHz, DMSO- d_6): δ 14.66 (br s, 1H), 9.23 (br s, 1H), 8.49 (br s, 1H), 7.60-7.58 (m, 2H), 7.22 (d, J = 8.4 Hz, 1H), 4.43 (d, J = 6 Hz, 2H), 3.86 (s, 3H); ^{19}F -NMR (376 MHz, DMSO- d_6): δ -60.82 ppm. MH^+ = 301.4 m/z.

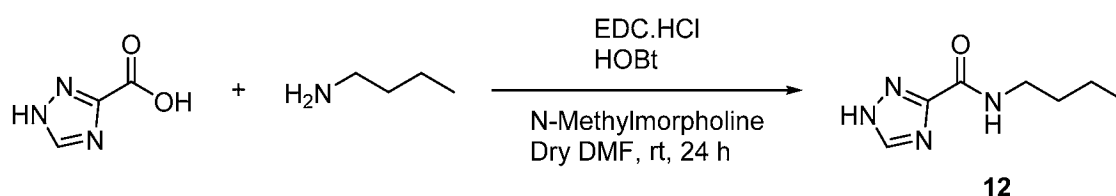
Synthesis of 11



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 4-(trifluoromethyl)aniline (0.28 mL, 2.2 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-

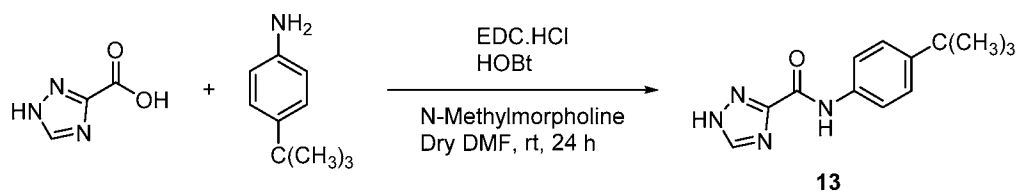
methymorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (80:20) as eluents to get the desired amide **11** (44.4 mg, 9 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-d₆): δ 14.86 (br s, 1H), 10.90 (br s, 1H), 8.70 (br s, 1H), 8.12 (d, *J* = 8 Hz, 2H), 7.75 (d, *J* = 8 Hz, 2H); ¹⁹F - NMR (376 MHz, DMSO-d₆): δ -60.46 ppm. MH⁺ = 257.0 m/z.

Synthesis of 12



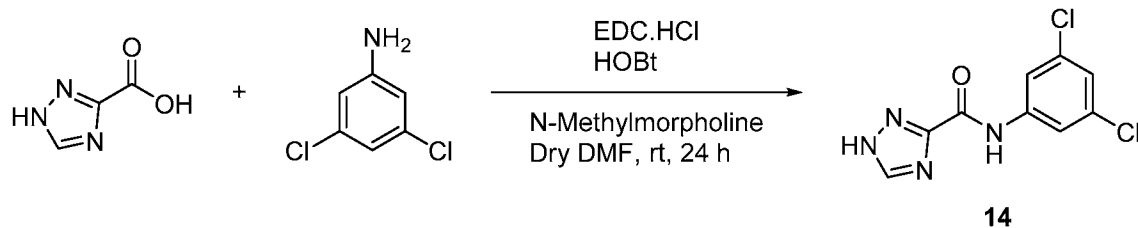
To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and butan-1-amine (0.21 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (80:20) as eluents to get the desired amide **12** (32 mg, 10 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-d₆): δ 14.75-14.44 (m, 1H), 8.58 (br s, 1H), 8.41 (br s, 1H), 3.26- 3.20 (m, 2H), 1.54-1.45 (m, 2H), 1.35-1.23 (m, 2H), 0.89 (t, *J* = 7.2 Hz, 3H) ppm. MH⁺ = 169.2 m/z.

Synthesis of 13



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 4-(tert-butyl)aniline (0.33 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (60:40) as eluents to get the desired amide **13** (123 mg, 25 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-d₆): δ 14.84-14.72 (m, 1H), 10.42 (br s, 1H), 8.58 (br s, 1H), 7.74 (d, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 8.4 Hz, 2H), 1.28 (s, 9H) ppm. MH⁺ = 245.2 m/z.

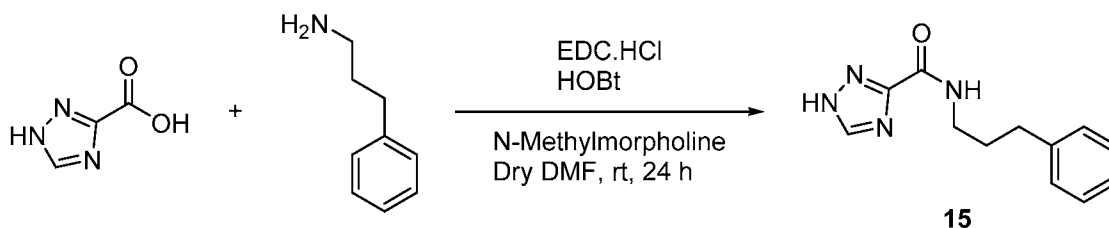
Synthesis of 14



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 3,5-dichloroaniline (340 mg, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated

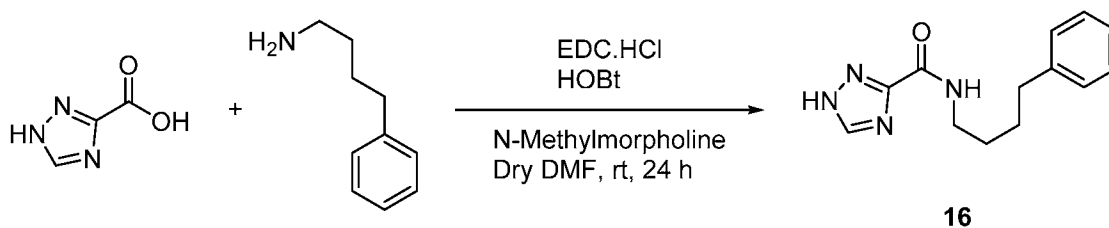
under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (60:40) as eluents to get the desired amide **14** (68.6 mg, 13 % yield) as a white solid compound. ^1H -NMR (400 MHz, DMSO- d_6): δ 14.85 (br s, 1H), 10.88 (br s, 1H), 8.69 (br s, 1H), 8.04-7.98 (m, 2H), 7.35-7.33 (m, 1H) ppm. $\text{MH}^+ = 257.3$ m/z.

5 Synthesis of 15



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 3-phenylpropan-1-amine (0.30 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc as eluents to get the desired amide **15** (69.7 mg, 15 % yield) as a white solid compound. ^1H -NMR (400 MHz, DMSO- d_6): δ 14.63 (br s, 1H), 8.68 (br s, 1H), 8.42 (br s, 1H), 7.30-7.18 (m, 5H), 3.40-3.24 (m, 2H), 2.61 (t, $J = 7.6$ Hz, 2H), 1.87-1.78 (m, 2H) ppm. $\text{MH}^+ = 231.3$ m/z.

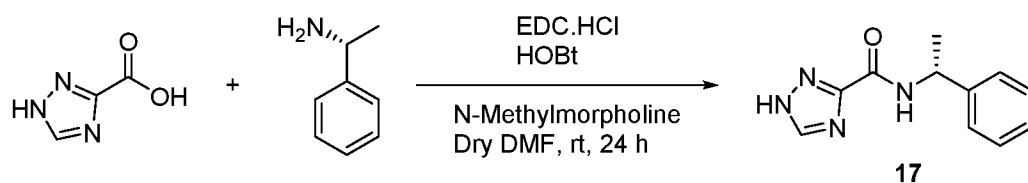
20 Synthesis of 16



To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 4-phenylbutan-1-amine (0.33 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room

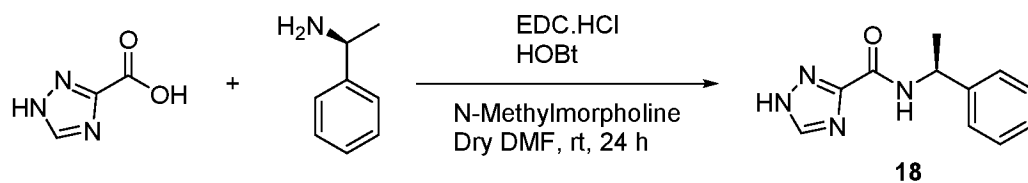
temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (75:25) as eluents to get the desired amide **16** (129.1 mg, 25 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-d₆): δ 14.64 (br s, 1H), 8.64 (br s, 1H), 8.42 (br s, 1H), 7.28-7.24 (m, 2H), 7.20-7.14 (m, 3H), 3.31-3.24 (m, 2H), 2.63-2.56 (m, 2H), 1.68-1.54 (m, 4H) ppm. MH⁺ = 245.3 m/z.

Synthesis of **17**



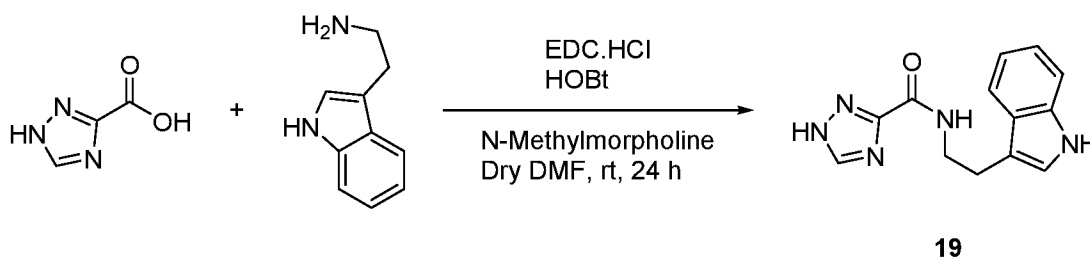
To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (R)-(+)-α-methyl benzyl amine (0.27 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (75:25) as eluents to get the desired amide **17** (88 mg, 20 % yield) as a white solid compound. ¹H -NMR (400 MHz, DMSO-d₆): δ 14.64 (br s, 1H), 8.97 (br s, 1H), 8.47 (br s, 1H), 7.42-7.40 (m, 2H), 7.34-7.30 (m, 2H), 7.25-7.21 (m, 1H), 5.19-5.12 (m, 1H), 1.50 (d, J = 6.8 Hz, 3H) ppm. MH⁺ = 217.1 m/z.

Synthesis of **18**



To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (S)- α -methyl benzyl amine (0.27 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using EtOAc and Hexanes (75:25) as eluents to get the desired amide **18** (211 mg, 49 % yield) as a white solid compound. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.67 (br s, 1H), 8.97 (br s, 1H), 8.47 (br s, 1H), 7.42-7.40 (m, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.25-7.21 (m, 1H), 5.20-5.12 (m, 1H), 1.50 (d, *J* = 6.8 Hz, 3H,) ppm. MH⁺ = 217.1 m/z.

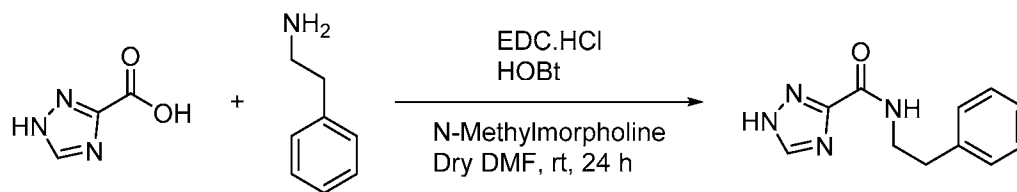
Synthesis of 19



To a reaction of 1*H*-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 2-(1*H*-indol-3-yl)ethanamine (0.34 mg, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (10:90) as eluents to get the desired amide **19** (193.3 mg, 38 % yield) as a white solid compound. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.90-14.48 (m, 1H), 10.82 (s, 1H), 8.80-8.13 (m, 2H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.35 (d, *J* = 8 Hz, 1H), 7.21-7.18 (m, 1H), 7.11-7.05 (m, 1H),

7.04-6.96 (m, 1H), 3.61-3.53 (m, 2H), 2.97 (t, $J = 7.6$ Hz, 2H) ppm. $MH^+ = 256.2$ m/z.

Synthesis of 20

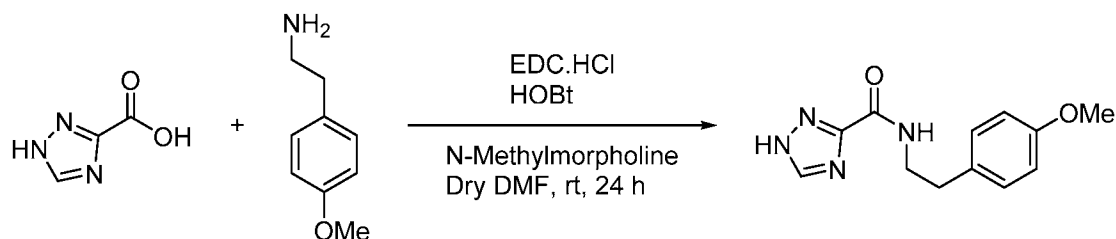


20

To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 2-phenylethanamine (0.27 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na_2SO_4 , filtered and concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (5:95) as eluents to get the desired amide **20** (189.4 mg, 44 % yield) as a white solid compound.

1H -NMR (400 MHz, $DMSO-d_6$): δ 14.63-14.52 (m, 1H), 8.65-8.25 (m, 2H), 7.33-7.17 (m, 5H), 3.55-3.44 (m, 2H), 2.85 (t, $J = 7.6$ Hz, 2H,) ppm. $MH^+ = 217.1$ m/z.

Synthesis of 21

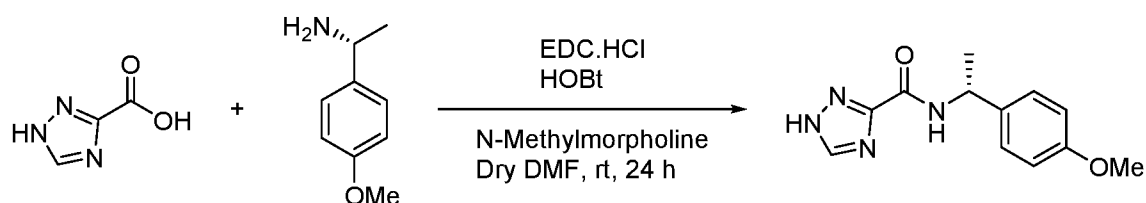


21

To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and 2-(4-methoxyphenyl)ethanamine (0.31 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24.0 h, and then slowly diluted into iced water and

extracted with DCM (2 x 50 mL). The DCM phase was washed with ice cold water (2 x 100 mL). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (6:94) as eluents to get the desired amide **21** (193 mg, 39 % yield) as a white solid compound. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.76-14.35 (br s, 1H), 8.80-8.30 (m, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 6.85 (d, *J* = 8.8 Hz, 2H), 3.72 (s, 3H), 3.48-3.40 (m, 2H), 2.78 (t, *J* = 7.6 Hz, 2H) ppm. MH⁺ = 247.1 m/z.

Synthesis of **22**



22

To a reaction of 1H-1,2,4-triazole-3-carboxylic acid (226 mg, 2.0 mmol) and (R)-1-(4-methoxyphenyl)ethan-1-amine (0.28 mL, 2.1 mmol) in dry DMF (10 mL) was added 1-hydroxybenzotriazole monohydrate (337 mg, 2.2 mmol) and EDC (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide) (422 mg, 2.2 mmol) followed by N-methylmorpholine (0.88 mL, 8.0 mmol via syringe). The mixture was stirred at room temperature under nitrogen and the solids were gradually dissolved. The contents were stirred at room temperature for 24 h, and then slowly diluted into iced water and extracted with DCM (50 mL x 2). The DCM phase was washed with ice cold water (100 mL x 2). The DCM phase was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and chromatographed on silica gel using MeOH and DCM (5:95) as eluents to get the desired amide **22** (287.1 mg, 58 % yield) as a white solid compound. ¹H-NMR (400 MHz, DMSO-d₆): δ 14.98-14.44 (m, 1H), 9.16-8.08 (m, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.18-5.04 (m, 1H), 3.73 (s, 3H), 1.48 (d, *J* = 7.2 Hz, 3H) ppm. MH⁺ = 247.1 m/z.

Table 2: Compound Solubility

Compound ID	16 mg/ml	10 mM	1 mM	100 μM	10 μM	1 μM
1	DMSO	water	water	water	water	water
2	DMSO	water	water	water	water	water

3	DMSO	water	water	water	water	water
4	DMSO	water	water	water	water	water
5	DMSO	DMSO	water	water	water	water
6	DMSO	water	water	water	water	water
7	DMSO	water	water	water	water	water
8	DMSO	DMSO	water	water	water	water
9	DMSO	DMSO	water	water	water	water
10	DMSO	water	water	water	water	water
11	DMSO	DMSO	DMSO	water	water	water
12	DMSO	water	water	water	water	water
13	DMSO	DMSO	water	water	water	water
14	DMSO	DMSO	DMSO	water	water	water
15	DMSO	water	water	water	water	water
16	DMSO	DMSO	water	water	water	water
17	DMSO	water	water	water	water	water
18	DMSO	water	water	water	water	water
19	DMSO	water	water	water	water	water
20	DMSO	water	water	water	water	water
21	DMSO	water	water	water	water	water
22	DMSO	water	water	water	water	water

Example 2: Identification of compounds that target DksA

The DksA protein is highly conserved among Gram-negative bacteria, making it a potential therapeutic target for a broad range of bacteria. Potential drugable sites in DksA zinc finger domain were assessed. Neither the highly conserved Cys¹¹⁴, which is buried deep in DksA globular domain, nor any of the other three cysteine residues in the zinc finger offered potential ligand-binding pockets. The Asp⁷⁴ residue is not only critical for DksA protein function but is also highly conserved among DksA orthologues. An area of negatively charged residues, including Asp⁶⁴, Asp⁷¹, Asp⁷⁴, Glu⁷⁹, Glu⁸⁰, Glu⁸¹, and Glu⁸⁵ (FIG. 1A), forms a conserved pocket at the tip of the coiled-coil domain of DksA. The region in the DksA crystal structure (PDB 1TJL) containing the pocket at the tip of DksA coiled-coil domain was selected as a small molecule binding target. The commercially-

available ZINC database were used for virtual screening of compounds.

The compounds obtained in this screen were further analyzed for binding to the acidic pocket at the tip of DksA coiled-coil domain using the computer search program Dock. The analysis identified over 400 compounds. A panel of 40 compounds was
5 selected from the top hits based on free energy binding scores. These compounds were investigated using the computer modeling program O. Two additional criteria were applied in order to narrow down the list of candidates: *First*, the compounds needed to fit deep into the pocket without any clashes; and *second*, the compounds bound to DksA should have minimal exposed moieties. The compounds selected were further refined
10 according to energy minimization as calculated by the CNS program. 23 compounds were obtained from MolPort and they were tested for their ability to interfere with DksA in a high throughput screen that combined *in vitro* transcription with highly specific and quantitative qRT-PCR. Five of these compounds, including N-(3,4-dimethoxyphenyl)-1H-1,2,4-triazole-3-carboxamide (**1**), prevented DksA-mediated repression of *rpsM in vitro*
15 transcription to some degree. The addition of 1 μ M **1** completely inhibited DksA-mediated repression of *rpsM in vitro* transcription (FIG. 1D). **1** inhibited DksA-mediated repression of *rpsM in vitro* transcription with an IC₅₀ value of 725 μ M, which is below the 1 μ M target for a screen of this type. As little as 0.1 μ M **1** completely inhibited DksA-dependent activation of *hisG in vitro* transcription. Without intending to be limited to any particular
20 theory, the effect that **1** has on DksA-dependent *rpsM* or *hisG* gene transcription is potentially due to differences in promoter activity. Addition of up to 10 μ M **1** did not affect *rpsM in vitro* transcription by the RNA polymerase itself.

Of all of the drugs that inhibited DksA function in the *in vitro* screens, **1** showed the greatest anti-Salmonella activity in disk diffusion assays. DksA controls transcription
25 of metabolic and virulence genes, allowing bacteria to both grow in minimum media and cause disease. Inhibition of bacterial growth in EG minimum media was used as an easy and reliable proxy of the DksA-dependent regulation of metabolism. The minimum inhibitory concentration of **1** against both *Salmonella Typhimurium* strain 14028s and *E. coli* strain 3110 was estimated to be 32 μ g/ml (i.e., 129 μ M). **1** had potent antimicrobial
30 activity against intracellular *Salmonella* growing in macrophages in complex cell culture media containing 10% fetal bovine serum (FIG. 1C), demonstrating excellent penetrability of **1** through cell host membranes. The poor intracellular growth of Δ *dksA* *Salmonella* was unaffected by **1**, suggesting this drug has minimal off-target effects. Compared to EG

media, lower concentrations of **1** resulted in the inhibition of *Salmonella* growth in macrophages, likely reflecting inhibition of DksA-dependent regulation of virulence programs. Accordingly, the addition of **1** to *Salmonella*-infected macrophages repressed transcription of a gene encoding a key effector of the SPI2 type III secretion system, whose expression is tightly regulated by DksA. The addition of 32 µg/ml of **1** did not change ($p = 0.3$) the low levels of lactate dehydrogenase (LDH) released from J774 cells 18 h after infection with wild-type or $\Delta dksA$ *Salmonella*, indicating this drug was well tolerated by host cells.

In silico modeling was used to inform SAR studies of **1** and DksA (FIG. 2B).

Many of the target compounds arrived at through *in silico* modeling which were found to dock in the pocket at the tip of the DksA coil-coiled region contained a purine-like ring, similar to ATP (FIG. 2C). Further analyses have shown excellent free energy binding scores for UTP, GTP and ATP. Oxygen atoms on the C2 and C4 positions in the pyrimidine ring of UTP make contact with Glu⁸¹ carboxylic group and Ala⁷⁷ backbone of DksA. Similarly, the amino group at the C6 position of ATP binds to Glu⁸⁰ carboxylic oxygen and Ala⁷⁷ backbone (FIG. 2C). In addition to interacting with the negatively charged residues at the rim of the pocket, phosphate groups of UTP and ATP establish hydrogen-bonds with Asn⁶⁸ and Asp⁷¹ that are located deep in the pocket. DksA Glu⁸⁰ and Glu⁸¹ at the rim of the pocket are also likely to help in the docking of **1** via hydrogen-bonding with N atoms in the carboxamide and the triazole group (FIG. 2D). In contrast to UTP and ATP, however, **1** does not interact with residues deep in the pocket.

Example 3: Inhibition assays using analogues of **1**

In a non-limiting example, wild-type *Salmonella* strain 14028s was grown overnight (18.5 h), and then diluted in EG minimal media to a density of about 10⁵ CFU/mL. The specimens were transferred to 3 ml to 14 ml tubes, and treated with the compound of interest to a final concentration of 32, 16, 8, 4, 2, or 1 mg/mL. Each test tube was incubated at 37 °C with shaking for 18 h and bacterial growth was evaluated. The MIC value was determined as the lowest concentration of compound that inhibited growth.

Table 3. Minimum inhibitory concentration

Cmpd. ID	Concentration tested						MIC (µg/ml)
	32 µg/ml	16 µg/ml	8 µg/ml	4 µg/ml	2 µg/ml	1 µg/ml	

1	--	+	+	+	+	+	32
2	+	+	+	+	+	+	ND
3	--	+/-	+	+	+	+	32
4	--	--	--	+	+	+	8
5	+	+	+	+	+	+	ND
6	--	--	--	+/-	+	+	8
7	--	--	--	+	+	+	8
8	+	+	+	+	+	+	ND
9	+	+	+	+	+	+	ND
10	+	+	+	+	+	+	ND
11	+	+	+	+	+	+	ND
12	--	--	--	+	+	+	8
13	+	+	+	+	+	+	ND
14	--	--	--	+	+	+	8
15	--	--	+	+	+	+	16
16	--	--	+/-	+	+	+	16
17	+	+	+	+	+	+	ND
18	+	+	+	+	+	+	ND
19	--	--	+	+	+	+	16
20	--	--	--	+	+	+	8
21	-	+	+	+	+	+	32
22	+	+	+	+	+	+	ND

--(No growth in EG) +(Growth in EG)

Example 4: *In vitro* transcription assays using analogues of 1

Compounds that showed MIC values of <16 µg/ml were evaluated for inhibition of DksA-dependent *in vitro* transcription reactions as described elsewhere herein (Materials and Methods section). The compounds were initially tested at 0.1 µM and 1 µM using as a target the promoter of the *livJ* gene that encodes a transporter of branch-chain amino acids (Table 4). Compounds that showed activity at 0.1 µM were selected for further study and IC₅₀ values were determined using the *rpsM* gene that encodes a ribosomal protein as the target. The compounds 12, 14, 15, 16, 19 and 20 inhibited *rpsM in vitro* transcription with IC₅₀ values of 73, 71, 51, 91, 75, and 90 nM, respectively.

Table 4. *In vitro* transcription assay results

Compound ID	0.1 μ M	1 μ M	IVT
1	no change	good repression	1 μ M
2	no change	no change	ND
3	no induction	no induction	0.1 μ M
4	no change	no change	ND
5	no induction	no induction	0.1 μ M
6	no change	no change	ND
7	no change	no change	ND
8	no induction	no induction	0.1 μ M
9	no induction	Repression	0.1 μ M
10	no change	no induction	1 μ M
11	good repression	good repression	0.1 μ M
12	no induction	no induction	0.1 μ M
13	no induction	no induction	0.1 μ M
14	no induction	no induction	0.1 μ M
15	no induction	no induction	0.1 μ M
16	no induction	good repression	0.1 μ M
17	no induction	no induction	0.1 μ M
18	no induction	no induction	0.1 μ M
19	no induction	no induction	0.1 μ M
20	no induction	no induction	0.1 μ M
21	no induction	no induction	0.1 μ M
22	no induction	no induction	0.1 μ M

Example 5: Macrophage killing assays

Salmonella was cleared after genetic or pharmacological inhibition of DksA. The stringent response is regulated by guanosine tetraphosphate and DksA. Bacterial strains unable to produce the stringent response regulator guanosine tetraphosphate cannot establish persistent infections. IFN γ -treated macrophages kill over 99% of wild-type Salmonella; however, the remaining 1% survive in a persistent state driven by the stringent response regulator guanosine tetraphosphate. This difference was used to develop

a persistent model of in vitro infection.

C57BL/6 mice were used in the assay following the IACUC guidelines. Mice (6-8 weeks) were inoculated intraperitoneally with 1 mg/mL of sodium periodate in PBS. The solution was sterilized by filtering through 0.22 μ M filter prior to injection. Peritoneal

macrophages were subsequently harvested from mice 4 days post inoculation (dpi). The exudate cells were resuspended in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% of heat-inactivated FBS, 15 mM HEPES, 2 mM L-glutamine, 1 mM of sodium pyruvate and 100 U/mL of Penicillin and Streptomycin (ThermoFisher Scientific). The peritoneal exudate cells were counted and adjusted to 3.5×10^6 cells/mL.

100 μ L were transferred onto a 96-well plate and incubated in 5% CO₂ at 37°C for 2 days. The cells in the treatment group were treated with interferon- γ (Peprotech Inc.) 24 h before infection with *Salmonella*.

Salmonella strains WT14028s and Δ dksA were grown in LB broth at 37°C with shaking for 20 h. Mouse peritoneal macrophages (with or without treatment of interferon- δ) were then challenged with *Salmonella* at an MOI of 2. Twenty-five minutes after *Salmonella* infection, some of the cells were lysed with 0.1% Triton to release intracellular bacteria. Cell lysates were then plated on LB agar and enumerated after 20 h incubation. The media of the remaining infected cells were replaced by pre-warmed RPMI medium containing 10 μ g/mL Gentamicin 25 min post infection.

The remaining infected cells were divided into different groups to be treated with compounds #6 and #21 at the final concentration of 32 μ g/mL and 8 μ g/mL, respectively. After additional 20 hrs incubation, cells were harvested and lysed. The lysates were plated onto LB agar plates, and subsequently counted after 20 h. Survival percentage is estimated by taking the number of bacteria at the later time point divided by the numbers of bacteria isolated 25 min after infection (FIG. 8B).

Data shown in FIG. 8A demonstrate that *Salmonella* deficient in dksA are not only more easily killed than wild-type controls in untreated macrophages, but are very susceptible to IFN γ -activated killing and are unable to establish a persistent infection. These findings show that DksA inhibitors prevent formation of persistent *Salmonella* infection and that drugs that inhibit DksA function can be used for treatment of persistent infections as monotherapy (FIG. 8B).

Example 6: Growth and survival assays

Salmonella strain 14028s was grown overnight at 37°C with shaking in LB broth.

Cell density was measured by spectrometer by following OD600. Cells were centrifuged and washed once with EG Minimal Salts Media (57.4 mM K₂HPO₄, 1.7 mM MgSO₄, 9.5 mM Citric acid, 16.7 mM H₅NNaPO₄, and 0.4% D-Glucose, pH 7.0). The specimens were then resuspended with EG media and adjusted to the final concentration of 1x10⁵ /ml.

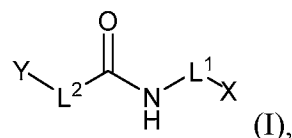
5 One hundred microliters of the cell dilution were plated onto LB agar plate to verify the starting concentration. Ten 5-ml cell culture tubes were prepared, and the diluted cells were transferred to the culture tubes at 1 ml/tube. The first culture tube was reserved as the control. The remaining nine tubes were divided into 3 groups (3 tubes each), which were treated with Nitrofurazone, 4-Nitroquinoline N-oxide, and Mitomycin
10 C at 8 µM, 8 µM, and 0.75 µM, respectively. Within each group, two of the tubes additionally received compounds #21 or #23 at 8 µg/ml and 16 µg/ml, respectively. All tubes were then incubated with shaking at 37°C for 20 hrs. Cells were collected, diluted, plated on LB agar plates, and subsequently counted. Survival percentages were calculated using bacteria counts divided by starting concentration.

15 Compounds #21 and #23 enhanced the anti-*Salmonella* activity of 4-nitroquinoline-N-oxide, nitrofurazone, and mytomycin, demonstrating that anti-DksA drugs sensitize persistent populations to antibiotic killing (FIG. 9). This data demonstrates that, in addition to serving as direct antibiotics, anti-DksA drugs are particularly effective in combination therapies.

20 The disclosures of each and every patent, patent application, and publication cited herein are hereby incorporated herein by reference in their entirety. While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art
25 without departing from the true spirit and scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.

What is claimed is:

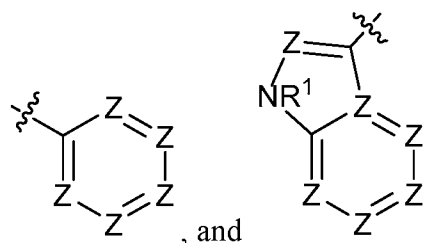
1. A compound of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof:



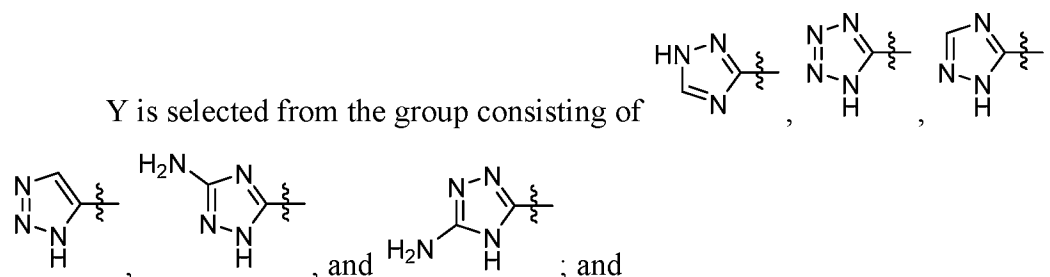
wherein:

L^1 is selected from the group consisting of a bond, C_{1-6} alkylene optionally substituted with at least one selected from the group consisting of halogen and C_{1-6} alkyl;

L^2 is selected from the group consisting of a bond and $-\text{CH}_2-$;

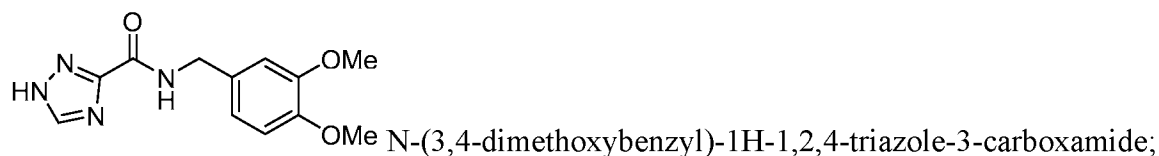


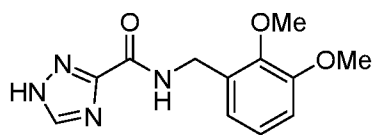
X is selected from the group consisting of H ,
 wherein each instance of Z is independently selected from the group consisting of N and CR^1 , provided that 0-2 occurrences of Z are N ;



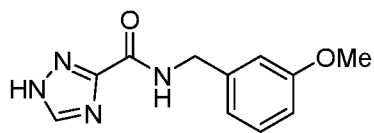
Y is selected from the group consisting of
 each instance of R^1 is independently selected from the group consisting of H , $-\text{OH}$, halogen, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} alkoxy, C_{1-6} haloalkoxy, C_{3-8} cycloalkyl, and C_{1-6} perhaloalkyl, or two adjacent R^1 groups are taken together to form a 4-6 membered homocyclic or heterocyclic fused ring.

2. The compound of claim 1, which is selected from the group consisting of:

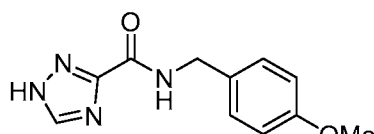




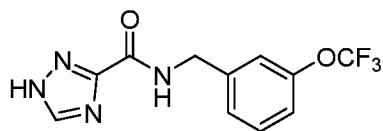
N-(2,3-dimethoxybenzyl)-1H-1,2,4-triazole-3-carboxamide;



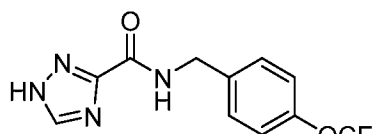
N-(3-methoxybenzyl)-1H-1,2,4-triazole-3-carboxamide;



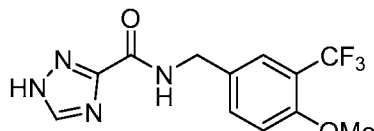
N-(4-methoxybenzyl)-1H-1,2,4-triazole-3-carboxamide;



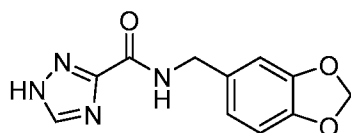
N-(3-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-carboxamide;



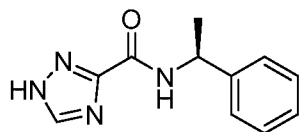
N-(4-(trifluoromethoxy)benzyl)-1H-1,2,4-triazole-3-carboxamide;



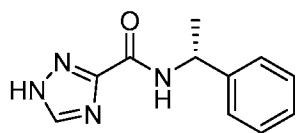
N-(4-methoxy-3-(trifluoromethyl)benzyl)-1H-1,2,4-triazole-3-carboxamide;



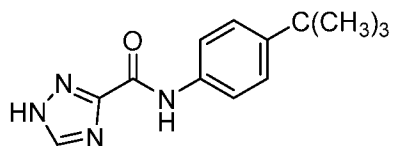
N-(benzo[d][1,3]dioxol-5-ylmethyl)-1H-1,2,4-triazole-3-carboxamide;



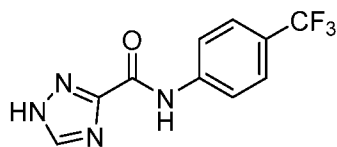
(S)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-carboxamide;



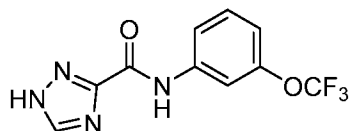
(R)-N-(1-phenylethyl)-1H-1,2,4-triazole-3-carboxamide;



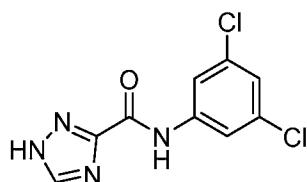
N-(4-(tert-butyl)phenyl)-1H-1,2,4-triazole-3-carboxamide;



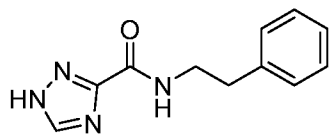
N-(4-(trifluoromethyl)phenyl)-1H-1,2,4-triazole-3-carboxamide;



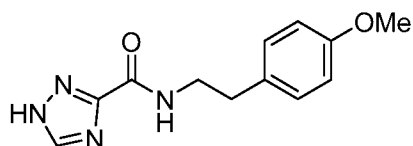
N-(3-(trifluoromethoxy)phenyl)-1H-1,2,4-triazole-3-carboxamide;



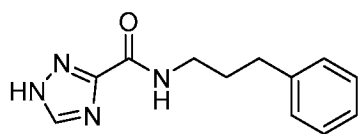
N-(3,5-dichlorophenyl)-1H-1,2,4-triazole-3-carboxamide;



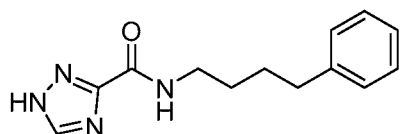
N-phenethyl-1H-1,2,4-triazole-3-carboxamide;



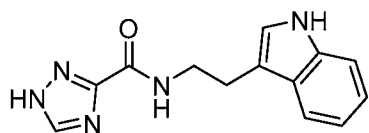
N-(4-methoxyphenethyl)-1H-1,2,4-triazole-3-carboxamide;

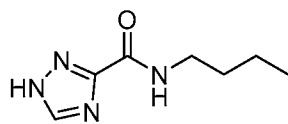


N-(3-phenylpropyl)-1H-1,2,4-triazole-3-carboxamide;



N-(4-phenylbutyl)-1H-1,2,4-triazole-3-carboxamide;

N-(2-(1H-indol-3-yl)ethyl)-1H-1,2,4-triazole-3-carboxamide;
and



N-butyl-1H-1,2,4-triazole-3-carboxamide.

3. A pharmaceutical composition comprising the compound of claim 1, further comprising at least one pharmaceutically acceptable carrier.
4. The pharmaceutical composition of claim 3, wherein the pharmaceutical composition is formulated for administration by at least one route selected from the group consisting of inhalational, oral, nasal, rectal, parenteral, sublingual, transdermal, transmucosal, intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, epidural, intrapleural, intraperitoneal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical.
5. A method of inhibiting growth of and/or killing a bacterium, the method comprising contacting the bacterium with the compound of claim 1 or 2.
6. The method of claim 5, wherein the bacterium is gram-negative.
7. The method of claim 6, wherein the bacterium is at least one selected from the group consisting of *Klebsiella*, *E. coli*, *Proteus*, *Serratia*, *Salmonella*, *Yersinia*, *Enterobacter*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Acinetobacter* and *Stenotrophomonas*.
8. The method of claim 5, wherein the bacterium has a DksA protein.
9. A method of treating a bacterial infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the compound of claim 1 or 2.
10. The method of claim 9, wherein the bacterium is gram-negative.

11. The method of claim 10, wherein the bacterium is at least one selected from the group consisting of *Klebsiella*, *E. coli*, *Proteus*, *Serratia*, *Salmonella*, *Yersinia*, *Enterobacter*, *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Acinetobacter* and *Stenotrophomonas*
12. The method of claim 9, wherein the bacterium has a DksA protein.
13. The method of claim 9, wherein the subject is a mammal.
14. The method of claim 13, wherein the subject is a human.
15. Use of compound or composition of claim 1 or claim 2 in the manufacture of a medicament for the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection.
16. A compound of claim 1 or claim 2, or a composition of claim 3, for use in the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection.

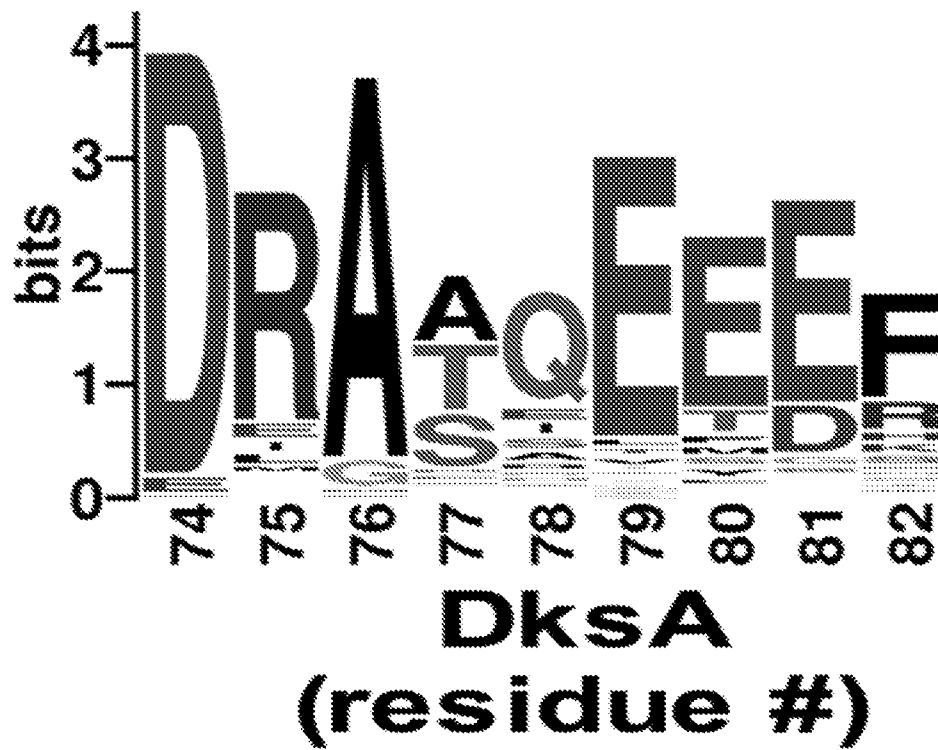


FIG. 1A

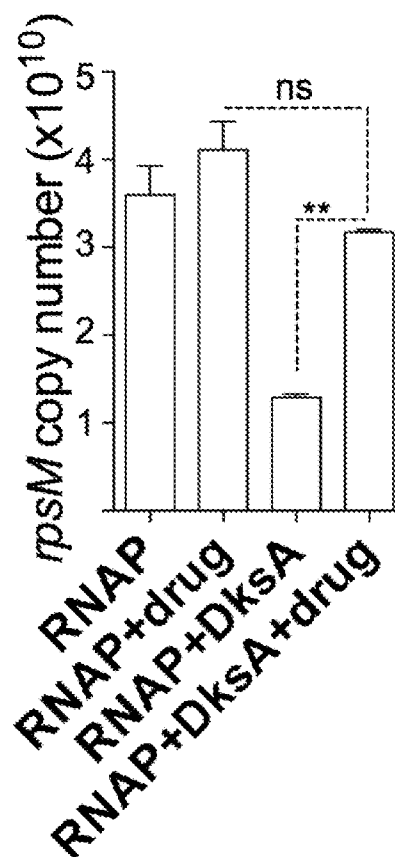


FIG. 1B

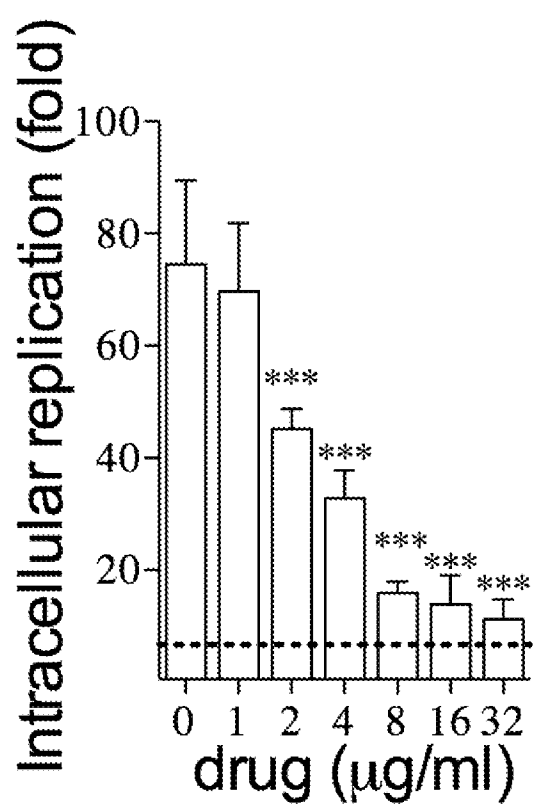


FIG. 1C

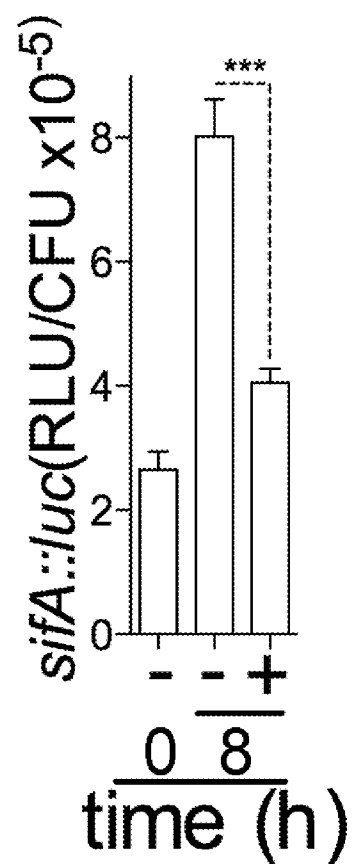


FIG. 1D

DksA &
Compound 916

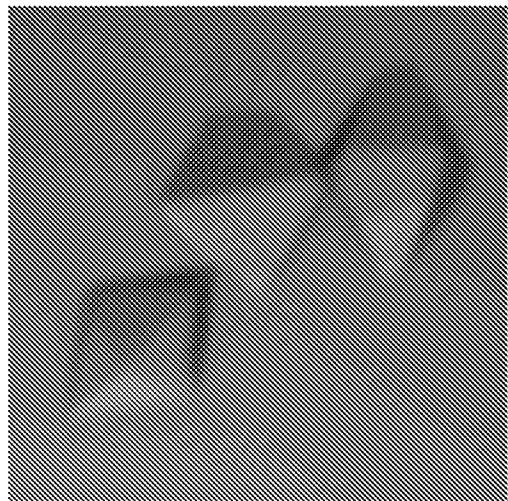


FIG. 2A

Compound 916

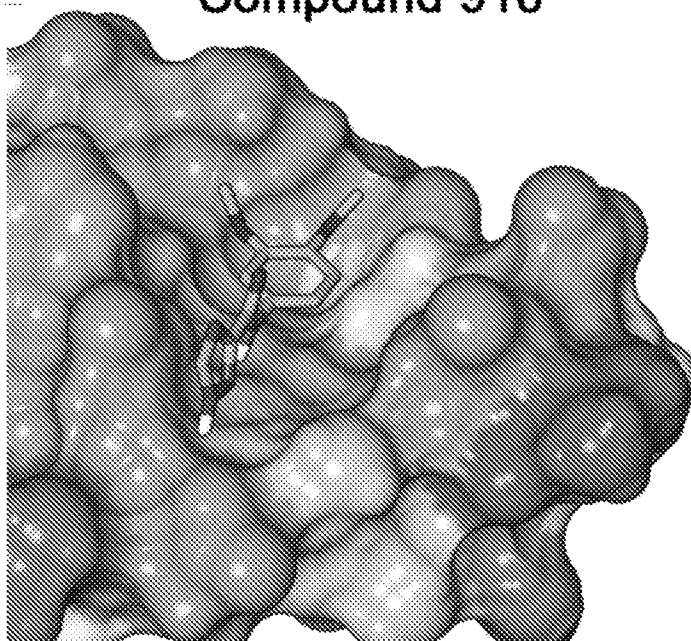


FIG. 2B

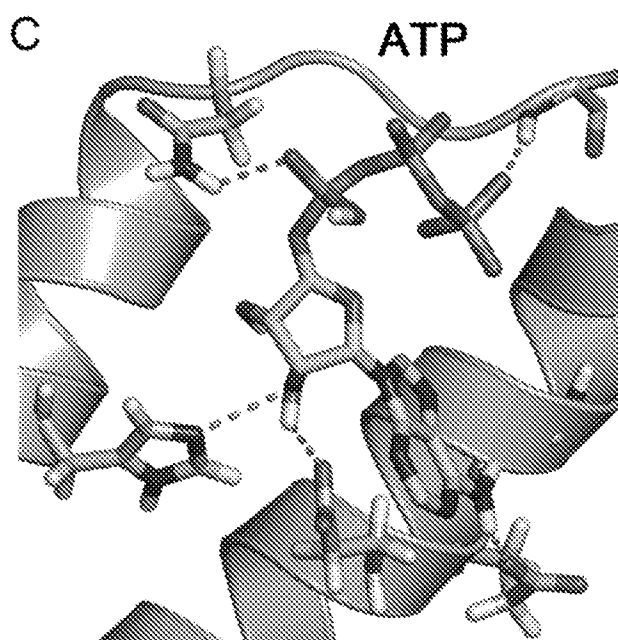


FIG. 2C

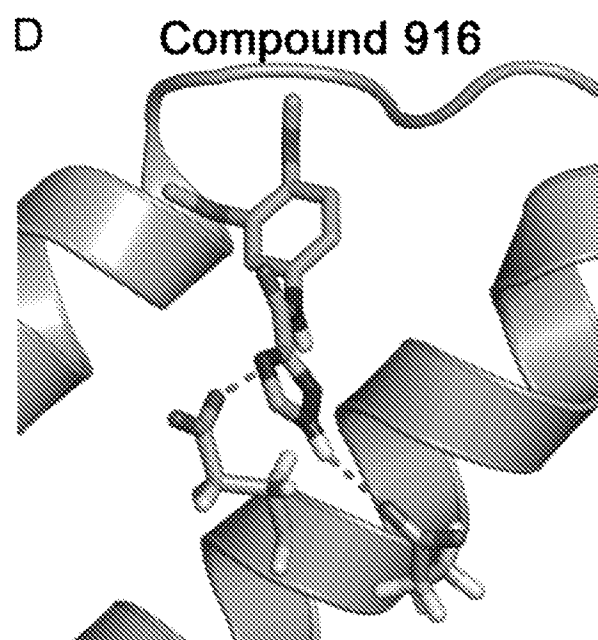


FIG. 2D

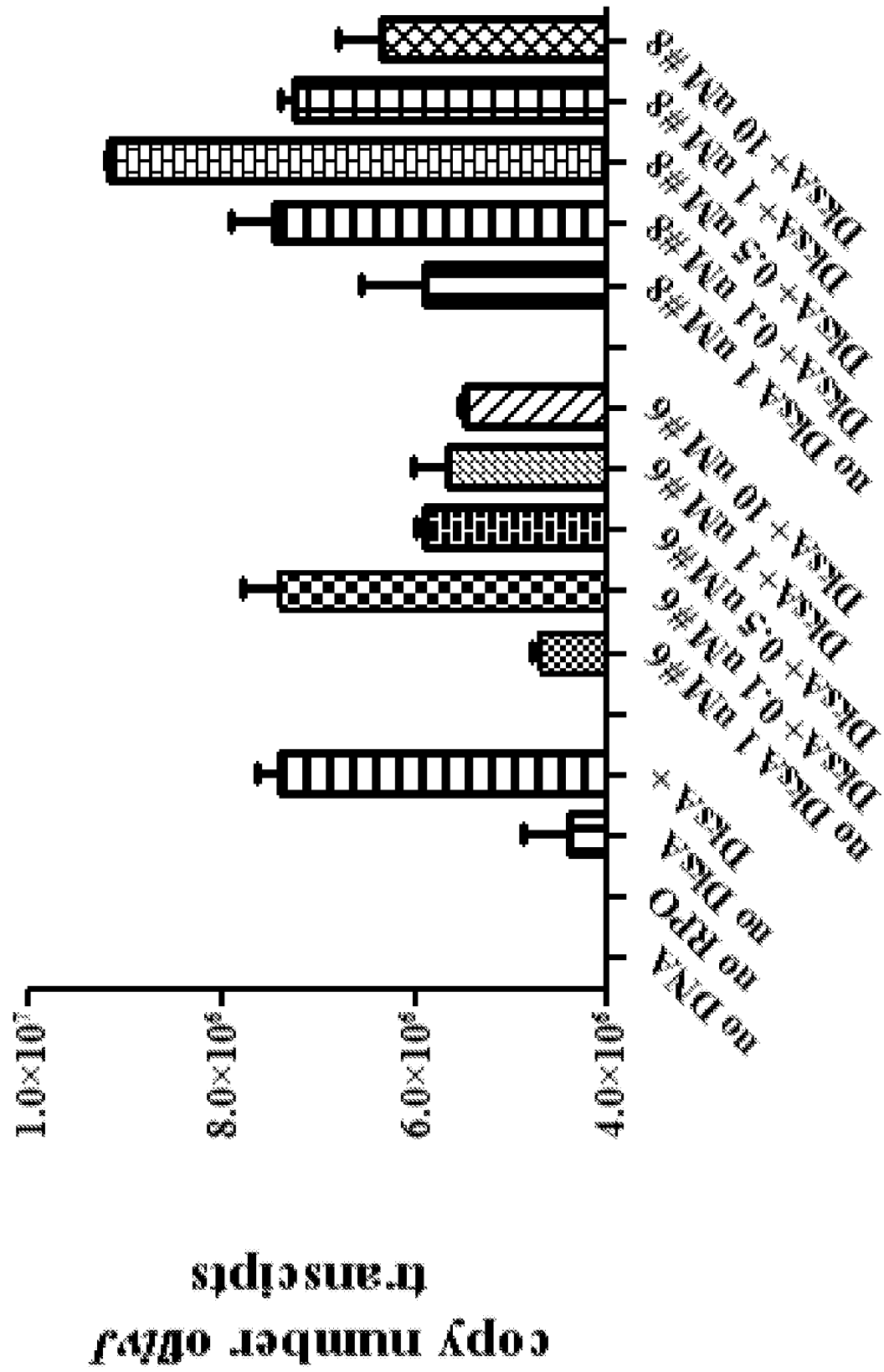


FIG. 3

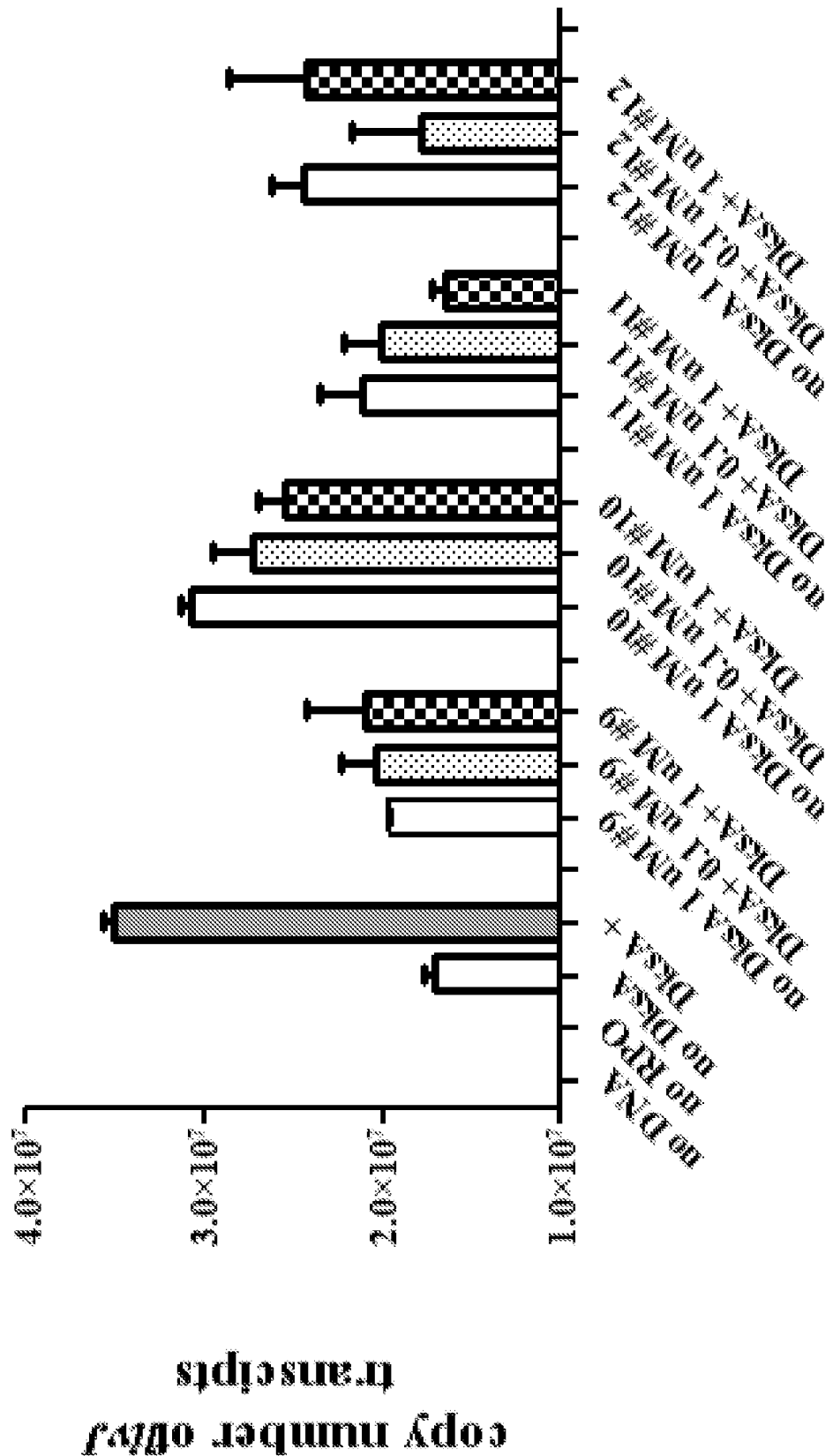


FIG. 4

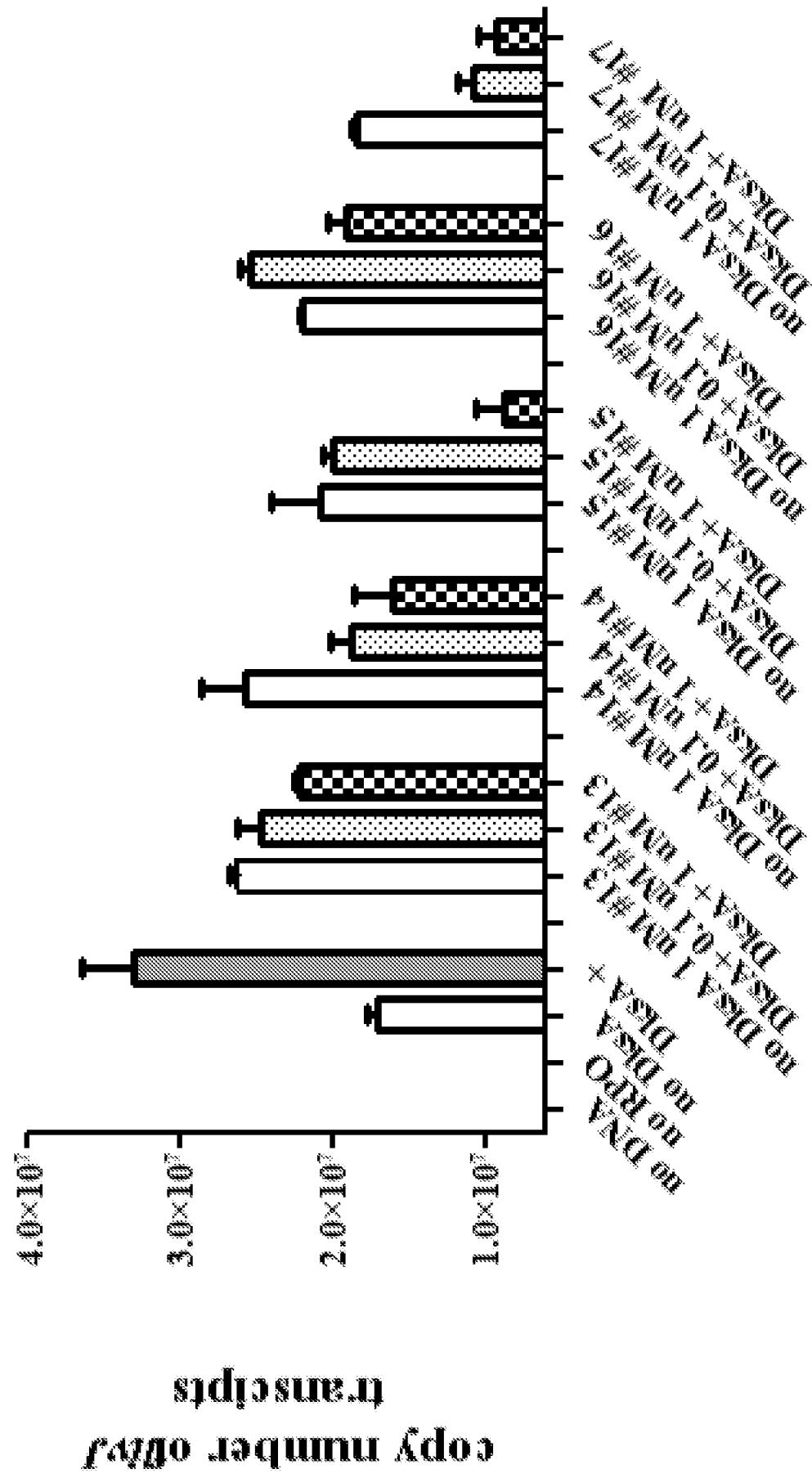


FIG. 5

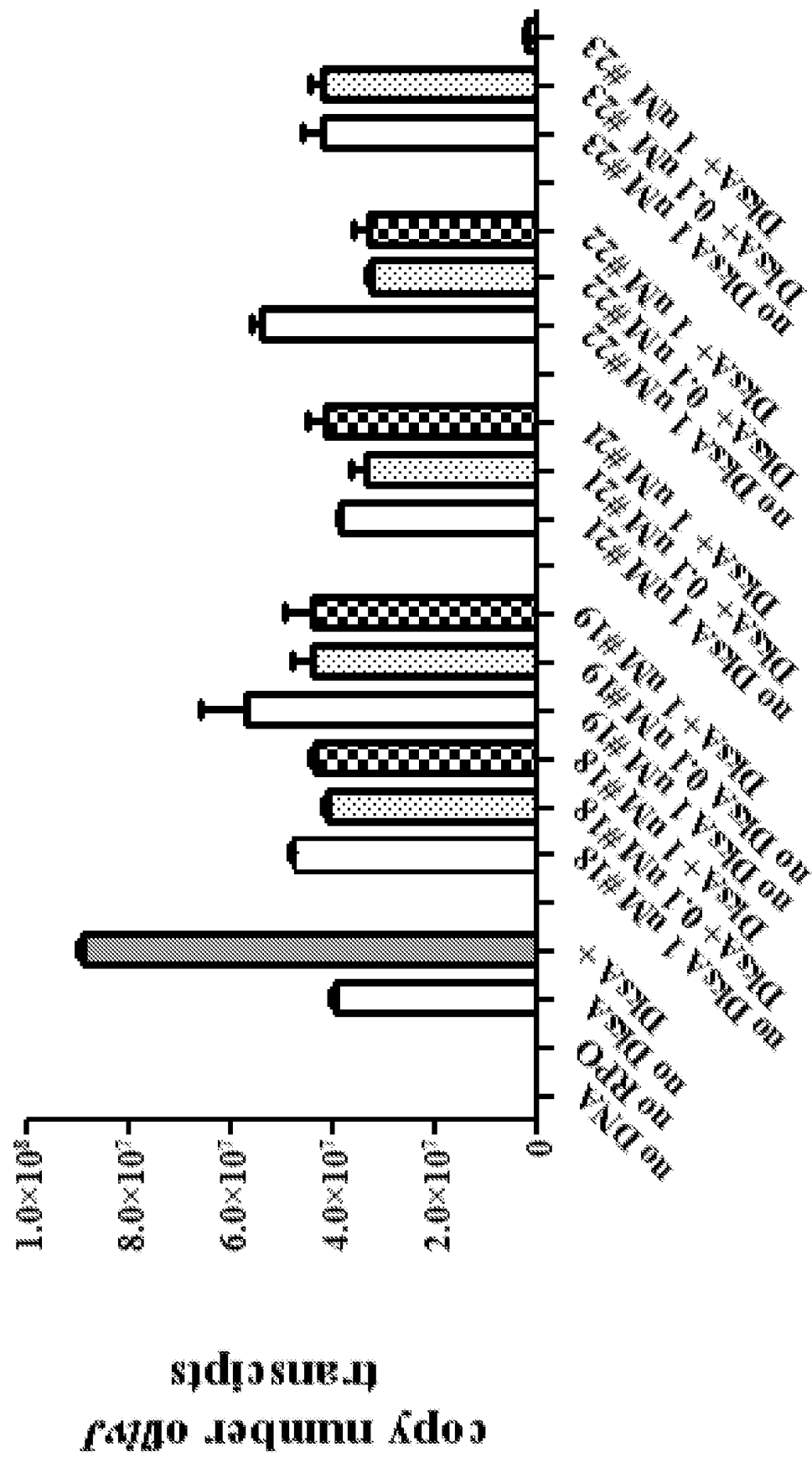


FIG. 6

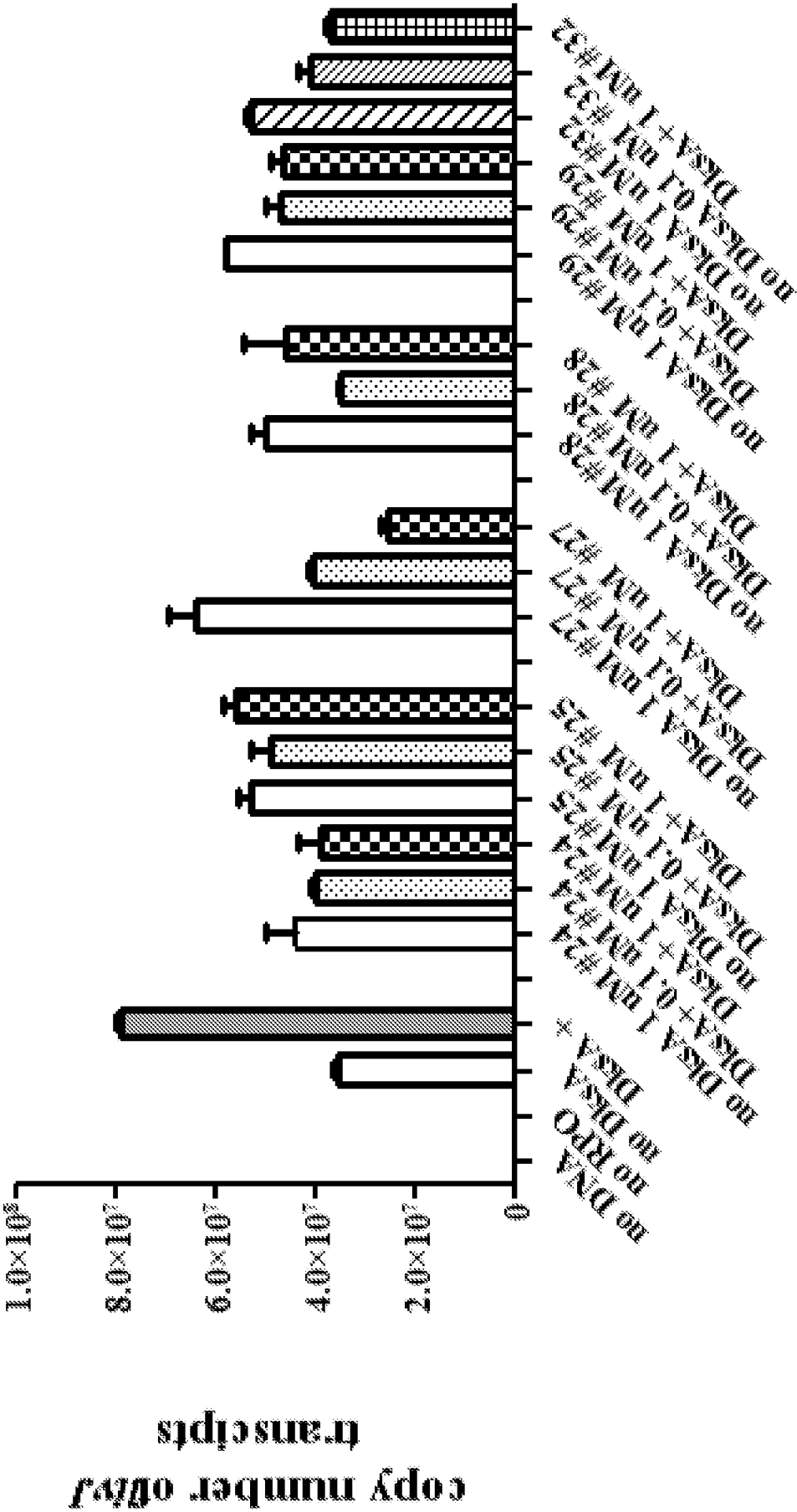


FIG. 7

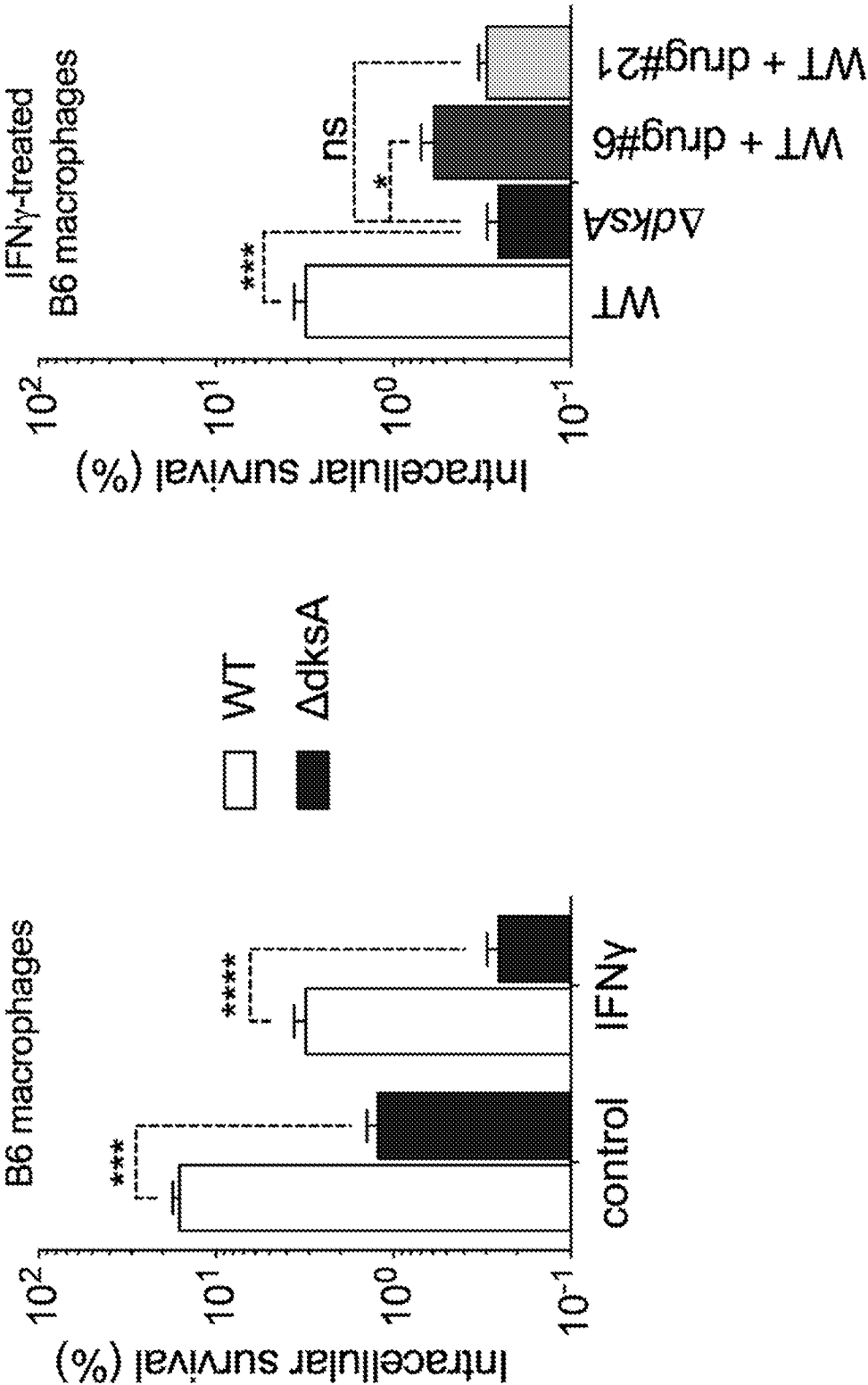


FIG. 8A

FIG. 8B

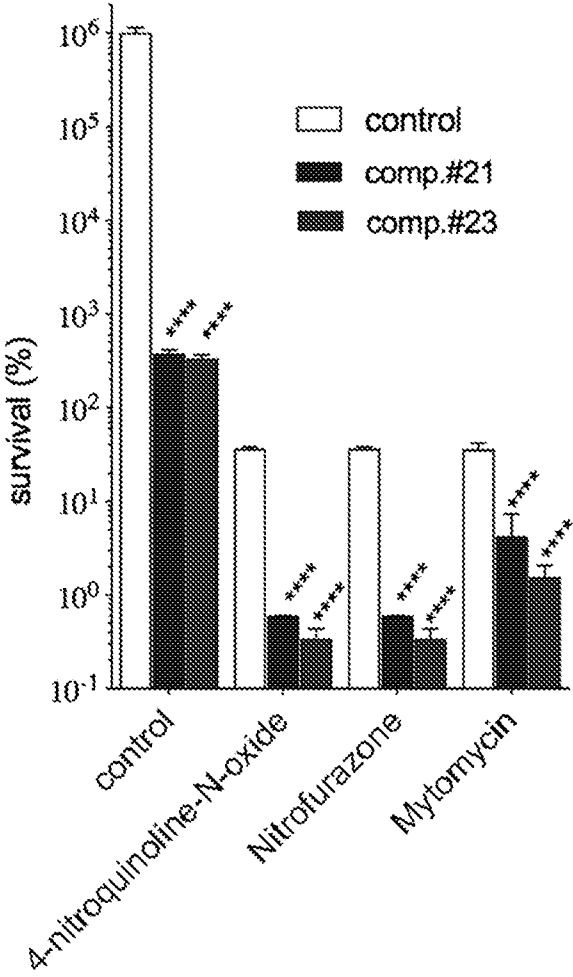


FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/047637

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 3-16

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/047637

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/4196; A61P 31/00; A61P 31/04; C07D 249/10 (2019.01)

CPC - A61K 31/4196; A61P 31/00; A61P 31/04; C07D 249/10 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

USPC - 514/383; 548/266.8 (keyword delimited)

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3,927,216 A (WITKOWSKI et al) 16 December 1975 (16.12.1975) entire document	1, 3, 4, 16
A	US 2006/0079485 A1 (YEDGAR et al) 13 April 2006 (13.04.2006) entire document	1, 3-16
A	US 4,006,159 A (NEWMAN) 01 February 1977 (01.02.1977) entire document	1, 3-16
A	US 2009/0264342 A1 (COTTAREL et al) 22 October 2009 (22.10.2009) entire document	1, 3-16

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

27 November 2019

Date of mailing of the international search report

27 DEC 2019

Name and mailing address of the ISA/US

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PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/047637

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

Claims 1 and 3-16 have been analyzed subject to the restriction that the claims read on a compound of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof: wherein: L1 is a bond; L2 is a bond; X is H; Y is the first shown structure.

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

Group I+: claims 1-16 are drawn to compounds of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof, pharmaceutical compositions thereof, methods of inhibiting growth of and/or killing a bacterium, methods of treating a bacterial infection in a subject in need thereof, and use of compounds.

The first invention of Group I+ is restricted to a compound of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof: wherein: L1 is a bond; L2 is a bond; X is H; Y is the first shown structure; pharmaceutical compositions thereof; methods of inhibiting growth of and/or killing a bacterium; methods of treating a bacterial infection in a subject in need thereof; and use of compounds. It is believed that claims 1 and 3-16 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a compound of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof: wherein: L1 is a bond; L2 is -CH₂-; X is H; Y is the first shown structure; pharmaceutical compositions thereof; methods of inhibiting growth of and/or killing a bacterium; methods of treating a bacterial infection in a subject in need thereof; and use of compounds. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

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

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables L1, L2, X, Y, and accordingly these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of a compound having the core structure of Formula (I), or a salt, solvate, enantiomer, diastereoisomer or tautomer thereof; a pharmaceutical composition comprising the compound, further comprising at least one pharmaceutically acceptable carrier; a method of inhibiting growth of and/or killing a bacterium, the method comprising contacting the bacterium with the compound; a method of treating a bacterial infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the compound; use of compound or composition in the manufacture of a medicament for the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection; and a compound or a composition, for use in the treatment of a bacterial infection in a mammal, particularly a gram-negative bacterial infection, these shared technical features do not represent a contribution over the prior art as disclosed by US 2006/0079485 A1 to Yedgar et al.

US 2006/0079485 A1 to Yedgar et al. teach a compound having the core structure of Formula (I) (Para. [0170],... ribavirin...); a pharmaceutical composition (Para. [0169],... a composition comprising a Lipid-conjugate. In another embodiment, compositions comprising Lipid-conjugates in admixture with conventional excipients...) comprising the compound (Para. [0169]), further comprising at least one pharmaceutically acceptable carrier (Para. [0169]); a method of inhibiting growth of and/or killing a bacterium (Para. [0008],... a method of Suppressing, inhibiting, preventing, or treating a pathogenic effect on a cell, comprising the step of contacting the cell with a compound...), the method comprising contacting the bacterium with the compound (Para. [0008]; Para. [0060],... the pathogen is a bacterium.); a method of treating a bacterial infection in a subject in need thereof (Para. [0009],... a method of Suppressing, inhibiting, preventing, or treating an infection in a subject, comprising the step of administering an effective amount of a lipid or phospholipid moiety bound to a physiologically acceptable monomer, dimer, oligomer, or polymer, and/or a pharmaceutically acceptable salt or a pharmaceutical product thereof to an infected Subject.; Para. [0060]), the method comprising administering to the subject a therapeutically effective amount of the compound (Paras. [0009] and [0060]); use of compound or composition in the manufacture of a medicament for the treatment of a bacterial infection in a mammal (Para. [0065],...the preparation of a pharmaceutical composition for treating a subject afflicted with a bacterial infection.); and a compound or a composition, for use in the treatment of a bacterial infection in a mammal (Para. [0065]; Para. [0071]. In another embodiment, the methods of the present invention may be used to treat a human infected with a pathogen.).

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