(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 August 2012 (16.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/109458 A1

(51) International Patent Classification:
A01N 43/60 (2006.01) *A61K 31/495* (2006.01)
A61K 31/50 (2006.01)

(21) International Application Number:
PCT/US2012/024507

(22) International Filing Date:
9 February 2012 (09.02.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/441,160 9 February 2011 (09.02.2011) US
61/516,651 6 April 2011 (06.04.2011) US
PCT/US2011/000617 6 April 2011 (06.04.2011) US

(71) Applicant *(for all designated States except US)*: **BIORELIX, INC.** [US/US]; 5 Science Park, 401 Winchester Avenue, New Haven, Connecticut 06511 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COISH, Philip D.G.** [CA/US]; 1365 Ridge Road, North Haven, Connecticut 06473 (US). **ARISTOFF, Paul Adrian** [US/US]; 8265 Trail Ridge, Dexter, Michigan 48130 (US). **DIXON, Brian R.** [US/US]; 1220 Johnson Road, Woodbridge, Connecticut 06525 (US).

(74) Agents: **LA, Brittany** et al.; Hoxie & Associates LLC, 75 Main Street, Suite 301, Millburn, New Jersey 07041 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

WO 2012/109458 A1

(54) Title: FLAVIN DERIVATIVES

(57) Abstract: The present invention relates novel flavin derivatives, composition comprising the same, their use and compositions for use as riboswitch ligands and/or anti-infectives.

FLAVIN DERIVATIVES

This application claims priority from U.S. Provisional Application Number 61/441,160 filed February 9, 2011, International Application Number PCT/US2011/000617 file April 6, 2011, and U.S. provisional application 61/516,651 filed April 6, 2011, the contents of each of which are incorporated by reference in their entirety.

TECHNICAL FIELD

[0001] The present invention relates to novel flavin derivatives, composition comprising the same, and their use and compositions for use as riboswitch ligands and/or anti-infectives.

BACKGROUND OF THE INVENTION

[0002] The fast growing rate of antibiotic resistance over the past decades has raised serious concerns that the antibiotic treatment options currently available will soon be ineffective. With the widespread usage of antibiotics in combination with the rapid growing rate of bacterial resistance in stark contrast with the decade-old chemical scaffolds available for their treatment, it is imperative that new drugs are developed in the battle against bacterial pathogens.

[0003] In many bacteria and fungi, RNA structures termed riboswitches regulate the expression of various genes crucial for survival or virulence. Typically located within the 5'-untranslated region (5'-UTR) of certain mRNAs, members of each known class of riboswitch can fold into a distinct, three-dimensionally structured receptor that recognizes a specific organic metabolite. When the cognate metabolite is present at sufficiently high concentrations during transcription of the mRNA, the riboswitch receptor binds to the metabolite and induces a structural change in the nascent mRNA that prevents expression of the open reading frame (ORF), thereby altering gene expression. In the absence of the cognate metabolite, the riboswitch folds into a structure that does not interfere with the expression of the ORF.

[0004] Sixteen different classes of riboswitches have been reported. Members of each class of riboswitch bind to the same metabolite and share a highly conserved sequence and secondary structure. Riboswitch motifs have been identified that bind to thiamine pyrophosphate (TPP), flavin mononucleotide (FMN), glycine, guanine, 3'-5'-cyclic eiguanylic acid (c-di-GMP), molybdenum cofactor, glucosamine-6-phosphate (GlcN6P),

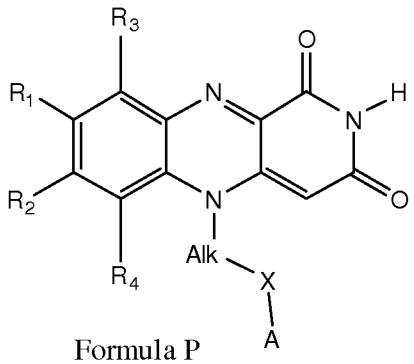
lysine, adenine, and adocobalamin (AdoCbl) riboswitches. Additionally, four dinstinct riboswitch motifs have been identified that recognize S-adenosylmethionine (SAM) I, II and III, IV and two distinct motifs that recognize pre-queosine-1 (PreQ1). Several antimetabolite ligands have also been identified that bind to known riboswitch classes, including pyrithiamine pyrophosphate (PTPP) which binds TPP riboswitches, *L*-aminoethylcysteine (AEC) and DL-4-oxalysine which bind to lysine riboswitches and roseoflavin and FMN which bind to FMN riboswitches.The riboswitch-receptors bind to their respective ligands in an interface that approaches the level of complexity and selectivity of proteins. This highly specific interaction allows riboswitches to discriminate against most intimately related analogs of ligands. For instance, the receptor of a guanine-binding riboswitch from *Bacillus subtilis* forms a three-dimensional structure such that the ligand is almost completely enveloped. The guanine is positioned between two aromatic bases and each polar functional group of the guanine hydrogen bonds with four additional riboswitch nucleotides surrounding it. This level of specificity allows the riboswitch to discriminate against most closely related purine analogs. Similarly, studies of the SAM-binding riboswitches reveal that nearly every functional group of SAM is critical in binding the ligands, allowing it to discriminate highly similar compounds such as S-adenosylhomocysteine (SAH) and S-adenosylmethionine (SAM), which only differ by a single methyl group. Likewise, TPP riboswitches comprise one subdomain that recognizes every polar functional group of the 4-amino-5-hydroxymethyl-2-methylpyrimidine (HMP) moiety, albeit not the thiazole moiety, and another subdomain that coordinates two metal ions and several water molecules to bind the negatively charged pyrophosphate moiety of the ligand. Similar to TPP, guanine and SAM riboswitches, FMN riboswitches form receptor structures that are highly specific for the natural metabolite FMN. It is by this highly specific interaction that allows for the design of small molecules for the regulation of specific genes.

[0005] FMN riboswitches are of particular interest of this invention because it is believed that the riboswitch binds to flavin mono-nucleotide (FMN) and represses the expression of enzymes responsible for riboflavin and FMN biosynthesis. Riboflavin is a water-soluble vitamin that is converted by flavokinases and FAD synthases to co-factors FMN and FAD, which are indispensable cofactors involved in energy metabolism and metabolism of fats, ketones, carbohydrates and proteins crucial for all living organisms. Although vertebrates rely on uptake of vitamin from their gut for riboflavin sources, most

prokaryotes, fungi and plants synthesize the necessary riboflavin for survival. It is therefore suggested that compounds that are selective for FMN riboswitches may be useful against bacterial pathogens by causing disregulation of biosynthesis of riboflavin crucial for survival or virulence. In addition, no examples of the FMN, TPP, nor any other riboswitch class have presently been identified in humans. Therefore, riboswitches appear to offer the potential for the discovery of selective antipathogenic drugs. It is therefore the objective of this invention to provide novel flavin derivatives for targeting FMN riboswitches and methods of treating infections comprising administering flavin derivatives. Various flavin derivatives that target FMN riboswitch are disclosed in PCT/US2009/004576, PCT/US2010/001876 and PCT/US2011/000617, the contents of which are incorporated by reference in their entirety. The current application provides further flavin derivatives that target the FMN and/or the CD3299 riboswitch and/or are active against various bacterial strains.

SUMMARY OF THE INVENTION

[0006] The invention relates to a compound of Formula P:



Formula P

wherein:

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one hydroxy or C_{1-4}alkoxy (e.g., ethoxy) group;

(ii) X is a single bond, -S- or –O-;

(iii) A is aryl (e.g., phenyl) or heteroaryl (e.g. pyridinyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

(iv) R_1 is H, C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl), or C_{1-4}alkoxy (e.g., methoxy);

(v) R_2 is H, C_{1-4}alkyl (e.g., methyl or n-propyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

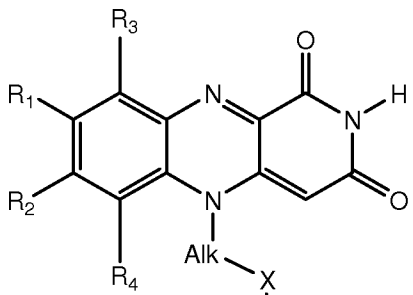
(vi) R_3 is H or C_{1-4}alkyl (e.g., methyl);

(vii) R_4 is H or C_{1-4}alkyl (e.g., methyl);

(viii) R_a and R_b are independently H, C_{1-4}alkyl (e.g., methyl) or C_{3-8}cycloalkyl (e.g., cyclopropyl, cyclopentyl);

in free or salt form.

[0007] In a further embodiment, the invention provides a compound of Formula II'':



Formula II"

wherein:

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one hydroxy or C_{1-4}alkoxy group;

(ii) X is a single bond, -S- or –O-;

(iii) A is aryl (e.g., phenyl) or heteroaryl (e.g. pyridinyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

(iv) R_1 is H or C_{1-4}alkyl (e.g., methyl), or C_{1-4}alkoxy (e.g., methoxy);

(v) R_2 is H or C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

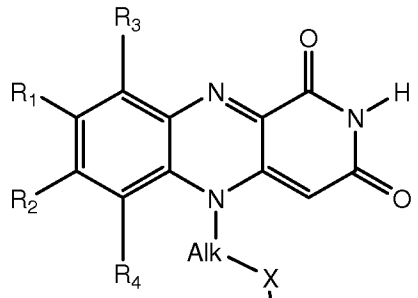
(vi) R_3 is H or C_{1-4}alkyl (e.g., methyl);

(vii) R_4 is H or C_{1-4}alkyl (e.g., methyl);

(viii) R_a and R_b are independently H, C_{1-4}alkyl (e.g., methyl) or C_{3-8}cycloalkyl (e.g., cyclopropyl, cyclopentyl);

in free or salt form.

[0008] In still a further embodiment, the invention provides a compound of Formula II:



Formula II

wherein:

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one hydroxy or C_{1-4}alkoxy group;

(ii) X is a single bond, -S- or –O-;

(iii) A is aryl (e.g., phenyl) or heteroaryl (e.g. pyridinyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

(iv) R_1 is H, C_{1-4}alkyl (e.g., methyl) or C_{1-4}alkoxy (e.g., methoxy);

(v) R_2 is H, C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

(vi) R_3 is H or C_{1-4}alkyl (e.g., methyl);

(vii) R_4 is H or C_{1-4}alkyl (e.g., methyl);

(viii) R_a and R_b are independently H, C_{1-4}alkyl (e.g., methyl) or C_{3-8}cycloalkyl (e.g., cyclopropyl, cyclopentyl);

in free or salt form.

[0009] In yet a further embodiment, the invention provides a compound of the following formulae:

2.1 a compound of Formula P, wherein Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one hydroxy or C_{1-4}alkoxy (e.g., ethoxy) group;

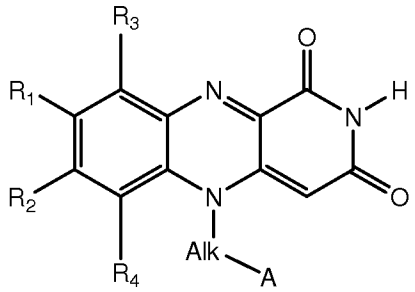
2.2 a compound of Formula P or 2.1, wherein Alk is n-propylene substituted with one or more C_{1-6}alkyl (e.g., methyl), for example Alk is $-CH_2CH_2CH(CH_3)-$;

2.3 a compound of Formula P, II'' or II or 2.1, wherein Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one hydroxy or C_{1-4}alkoxy group;

2.4 a compound of Formula P, II'' or II or 2.1, wherein Alk is n-propylene;

2.5 a compound of Formula P, II'' or II or any of 2.1-2.4, wherein X is a single bond, -S- or –O-;

2.6 a compound of Formula P, II'' or II or any of 2.1-2.5, wherein X is a single bond, wherein said compound is represented by a compound of formula II';



Formula II'

2.7 a compound of Formula P, II'' or II or any of 2.1-2.6, wherein A is aryl (e.g., phenyl) or heteroaryl (e.g. pyridinyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., $-OCF_3$);

2.8 a compound of Formula P, II'' or II or any of 2.1-2.7, wherein A is aryl (e.g., phenyl);

2.9 a compound of Formula P, II'' or II or any of 2.1-2.8, wherein A is phenyl;

2.10 a compound of Formula P, II'' or II or any of 2.1-2.7, wherein A is aryl (e.g., phenyl) substituted with one or more C_{1-4}alkyl (e.g., methyl) or halo (e.g., Cl, F);

2.11 a compound of Formula P or any of 2.1-2.10, wherein R_1 is H, C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl) or C_{1-4}alkoxy (e.g., methoxy);

2.12 a compound of Formula P or any of 2.1-2.11, wherein R_1 is C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl);

2.13 a compound of Formula P, II'' or II or any of 2.1-2.10, wherein R_1 is H, C_{1-4}alkyl (e.g., methyl), or C_{1-4}alkoxy (e.g., methoxy);

2.14 a compound of Formula P, II'' or II or any of 2.1-2.13, wherein R_1 is methyl;

2.15 a compound of Formula P or any of 2.1-2.14, wherein R_2 is H, C_{1-4}alkyl (e.g., methyl or n-propyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

2.16 a compound of Formula P, II'' or II or any of 2.1-2.14, wherein R_2 is H, C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

2.17 a compound of Formula P, II'' or II or any of 2.1-2.14, wherein R_2 is H, C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_1alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

2.18 a compound of Formula P, II'' or II or any of 2.1-2.16, wherein R_2 is methyl;

2.19 a compound of Formula P or any of 2.1-2.15, wherein R_2 is n-propyl;

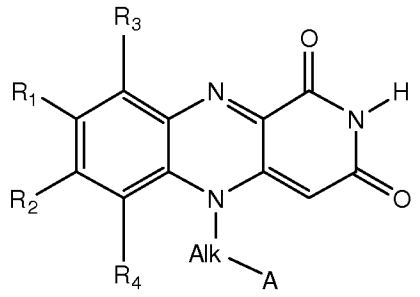
2.20 a compound of Formula P, II'' or II or any of 2.1-2.19, wherein R_3 is H or C_{1-4}alkyl (e.g., methyl);

2.21 a compound of Formula P, II'' or II or any of 2.1-2.20, wherein R_3 is H;

2.22 a compound of Formula P, II'' or II or any of 2.1-2.21, wherein R_4 is H or C_{1-4}alkyl (e.g., methyl);

2.23 a compound of Formula P, II'' or II or any of 2.1-2.22, wherein R_4 is H;

2.24 a compound of Formula P or any of 2.1-2.23, wherein X is a single bond, wherein said compound is represented by a compound of formula II':



Formula II'

and

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one C_{1-4}alkoxy (e.g., ethoxy) group;

(ii) A is aryl (e.g., phenyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl) or halo (e.g., Cl, F);

(iii) R_1 is H or C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl);

(iv) R_2 is H or C_{1-4}alkyl (e.g., methyl or n-propyl);

(v) R_3 is H;

(vi) R_4 is H;

2.25 a compound of Formula P, II'' or II or or any of 2.1-2.23, wherein:

Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one hydroxy or C_{1-4}alkoxy group;

X is a single bond, -S- or –O-;

A is aryl (e.g., phenyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

R_1 is C_{1-4}alkyl (e.g., methyl);

R_2 is C_{1-4}alkyl (e.g., methyl);

R_3 is H;

R_4 is H;

2.26 a compound of Formula P, II'' or II or any of 2.1-2.23, wherein:

Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one hydroxy or C_{1-4}alkoxy group;

X is a single bond;

A is aryl (e.g., phenyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

R_1 is C_{1-4}alkyl (e.g., methyl);

R_2 is C_{1-4}alkyl (e.g., methyl);

R_3 is H;

R_4 is H;

2.27 a compound of Formula P, II'' or II or any of 2.1-2.23, wherein:

Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene);

X is a single bond;

A is aryl (e.g., phenyl);

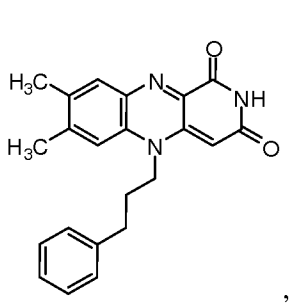
R_1 is C_{1-4}alkyl (e.g., methyl);

R_2 is C_{1-4}alkyl (e.g., methyl);

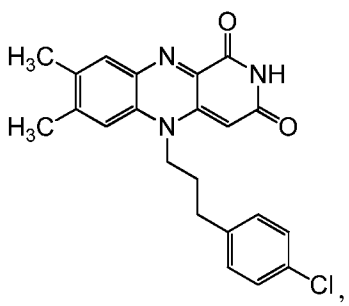
R_3 is H;

R_4 is H;

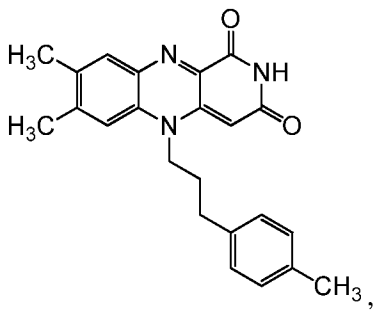
2.28 any of the preceding formulae, wherein the compound of Formula P, II'' or II is selected from any of the following:



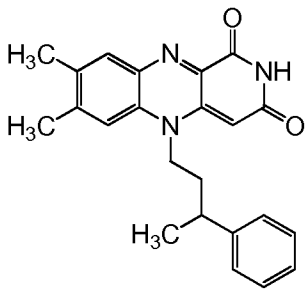
,



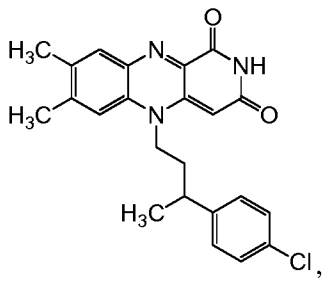
,



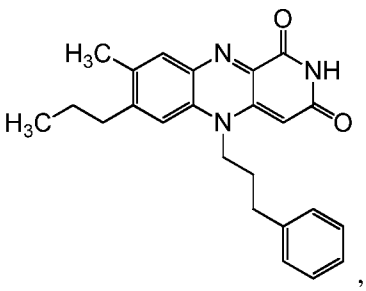
,



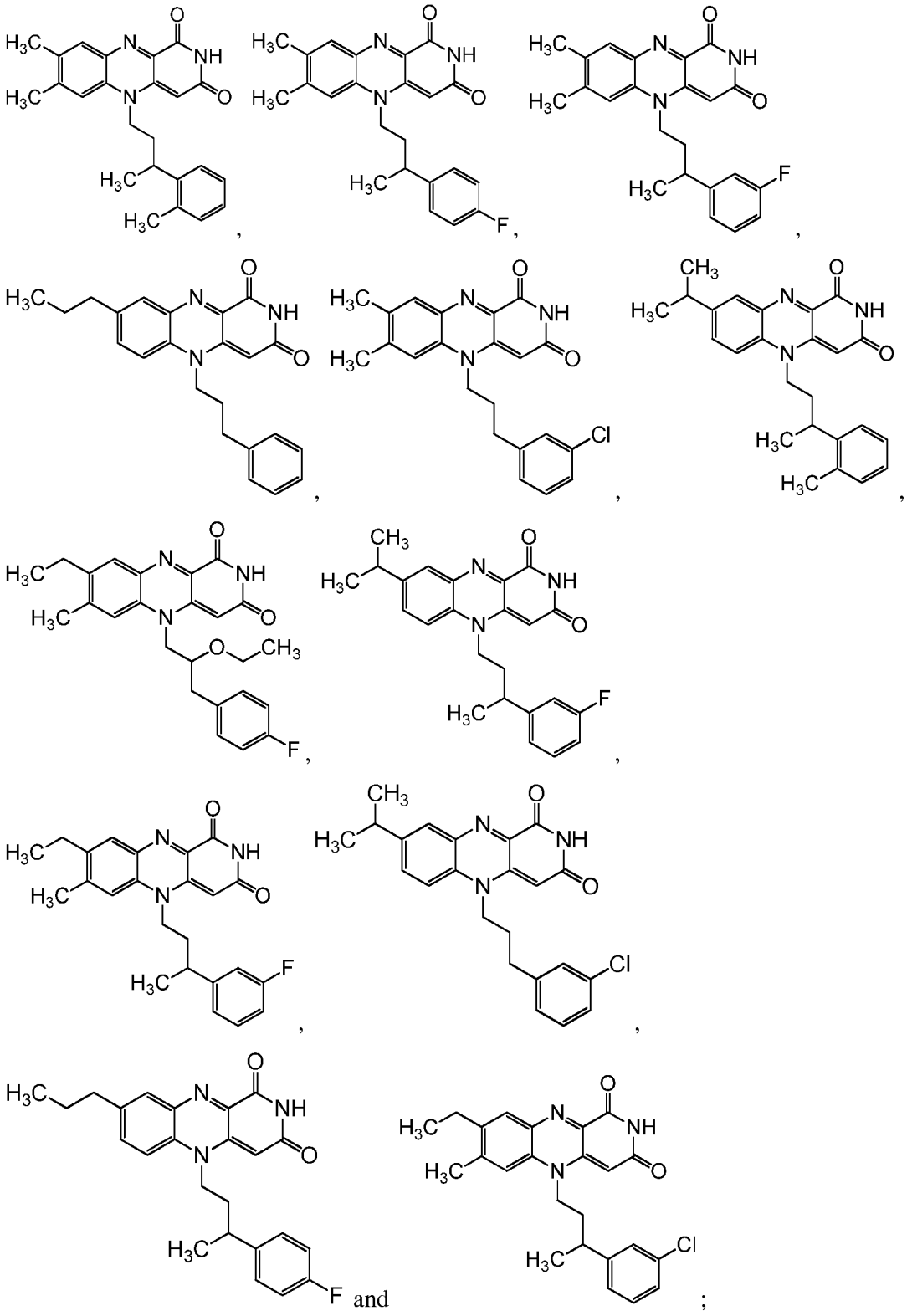
,



,

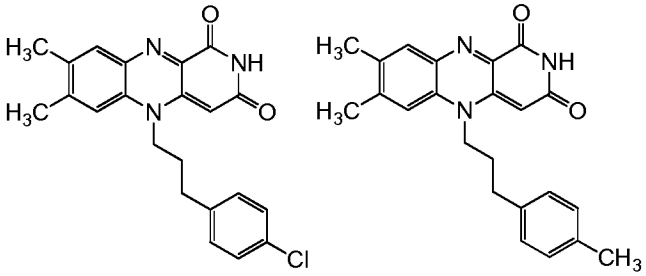


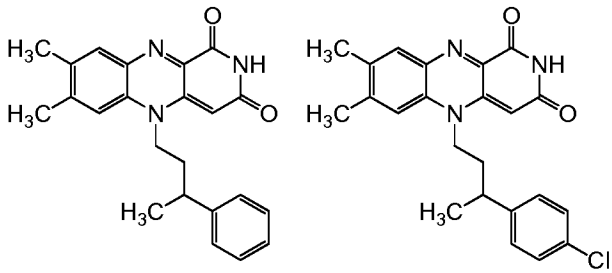
,



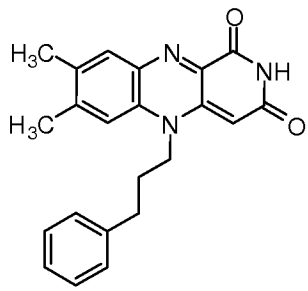
and ;

2.29 any of the preceding formulae, wherein the compound of Formula P, II'' or II is selected from any of the following:





2.30 any of the preceding formulae, wherein the compound of Formula P, II'' or II is



2.31 any of the preceding formulae, wherein the compound of Formula P, II'' or II binds to FMN and/or CD3299 riboswitch, e.g., with an Imax of greater than 20%, preferably greater than 30%, more preferably greater than 40%, still more preferably greater than 50% in an assay, for example, as described in Example A, and/or has a Minimum Inhibitory Concentration (MIC) of less than or equal to 64μg/mL, more preferably less than or equal to 32μg/mL, still more preferably less than or equal to 16μg/mL, most preferably less than or equal to 2μg/mL, for example, in an assay as described in Example B;

in free or salt form.

[0010] In a particular embodiment, the compound of the invention is a compound as hereinbefore described (e.g., Formula P, II'', II', II, or any of 2.1-2.31), wherein X is a

single bond and the remaining substituent is as defined in any of the formula described therein (e.g., Formula P, II'', II', II, or any of 2.1-2.31), in free or salt form.

[0011] In the second aspect, the invention provides a pharmaceutical composition comprising a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form in admixture with a pharmaceutically acceptable diluent or carrier.

[0012] In the third aspect, the invention provides a method for the treatment or prophylaxis of a bacterial infection (Method P) comprising administering to a subject in need thereof an effective amount of a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form.

[0013] In a further embodiment of the third aspect, Methods P as hereinbefore described are useful for the treatment or prophylaxis of a Gram-positive or Gram-negative bacterial infection (Method P-A). In another specific embodiment, Method P, is useful for treating a bacterial infection including, but not limited to an infection by one or more of the following bacteria: *Clostridium difficile* (or *C. difficile*), *Staphylococcus epidermidis, Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli*, *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio cholerae*, *Brucella melitensis*, *Bacillus anthracis*, *Francisella tularensis*, *Moraxella catarrhalis, Klebsiella pneumoniae, Yersinia pestis*, *Streptococcus viridans, Enterococcus faecium,* and/or *Borrelia burgdorferi* bacteria (Method P-B). Patients taking antibiotics, particularly those with a broad spectrum activity, are particularly vulnerable to *C. difficile* infection as a result of the use of antibiotics which disrupts the normal intestinal flora, leading to an overgrowth of *C. difficile*, causing an infection ranging from asymptomatic to severe and life-threatening condition. The exemplified Compounds of the Invention, e.g., compounds of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, particularly compounds of Examples 1-16 selectively inhibit *C. difficile* bacteria in a minimum inhibitory concentration assay as described in Example B below. Further, various compounds of the invention, e.g., various compounds of Formula P, e.g., various compounds of Examples 1-16 are also active against the CD3299 riboswitch. Therefore, in a particular embodiment, Method P is particularly useful for treating an infection caused by *Clostridium difficile*. In addition to having MIC activity against *C. Difficile* and some having CD3299 riboswitch activity, the

Compounds of the Invention, particularly Examples 1-16 are also active against the FMN riboswitch. Compounds which are active against FMN riboswitch are generally also active against *Staphylococcus aureus* and/or *Clostridium difficile* infections. Therefore, in particular embodiment, the compounds of the invention, particular compounds of Examples 1-16 may also be useful for the treatment of a *Staphylococcus aureus* infection.

[0014] In still another embodiment of the third aspect, Method P as hereinbefore described is useful for the treatment or prophylaxis of a disease, infection or condition selected from a group consisting of anthrax, staphylococcal scalded skin syndrome (staph infections), pneumonia, impetigo, boils, cellulitis, folliculitis, furuncles, carbuncles, scalded skin syndrome, abscesses, meningitis, osteomyelitis endocarditis, Toxic Shock Syndrome (TSS), septicemia, acute sinusitis, otitis media, septic arthritis, endocarditis, peritonitis, pericarditis, brain abscess, tularemia, urinary tract infection, empyema, food poisoning, diarrhea, conjunctivitis and clostridium difficile associated disease (CDAD), comprising administering to a subject in need thereof an effective amount of a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form (Method P-D).

[0015] Without being bound to any particular theory, it is believed that the current invention provides methods of treating a bacterial infection via a novel mechanism, e.g., by utilizing riboswitch-ligand binding to alter gene expression. Therefore in one aspect, the compounds of the invention, particularly compounds of Examples 1-16 bind to FMN riboswitches, thereby affecting downstream riboflavin biosynthesis. In another aspect, various compounds of the invention are also active against the CD3299 riboswitch, thereby affecting expression of the adjacent coding region. Compounds that are active against CD3299 and/or FMN riboswitch are particularly selective against *C. difficile*. As such, the Compounds of the Invention, e.g., the compounds of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, particularly Examples 1-16, in free or pharmaceutically acceptable salt form, are effective in treating an infection wherein traditional antibiotics are rendered ineffective due to drug resistance. Therefore, in a particular embodiment, the invention provides Method P as hereinbefore described wherein the infection is by an infectious agent which is resistant to a drug that is not a riboswitch ligand (Method P-E). In a further embodiment, the infection to be treated in Method P is a *C. difficile* infection, wherein the infection is by an infectious agent which is resistant to any drug that is not a

riboswitch ligand, e.g., fluoroquinolone (e.g., ciprofloxacin- and/or levofloxacin-resistant infection), metronidazole and/or vancomycin. In another embodiment, the compounds of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form may be useful for a *Staphylococcus aureus* infection which is resistant to one or more drugs selected from a group consisting of a penicillin, vancomycin, cephalosporin and methicillin. In a particular embodiment, the infection is a methicillin-resistant *Staphylococcus aureus* infection.

[0016] It will be noted that various compounds of the Invention have a low CC_{50} value in an assay as disclosed in Example C and therefore, may have anti-metabolite activities which may interfere with DNA biosynthesis. Therefore, in one embodiment, these compounds may be useful as an anti-cancer or anti-viral agent. In another embodiment, the compounds that have a low MIC and/or a high I_{max} value in an assay as disclosed in Example B and A respectively, and a low CC_{50} value in an assay as disclosed in Example C are used as an antibacterial, for topical administration.

[0017] In the fourth aspect, the invention provides a method for the treatment of an infection in a plant (Method P-F) comprising administering to such plant an effective amount of a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form.

[0018] In the fifth aspect, the invention provides use of a compound, or use of a pharmaceutical composition comprising a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form, (in the manufacture of a medicament) for the treatment or prophylaxis of an infection, e.g., a bacterial infection as described in Methods P, or any of Methods P-A through P-F.

[0019] In the sixth aspect, the invention provides a pharmaceutical composition comprising a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form, for use in the treatment of any disease or condition as hereinbefore described, e.g., in any of Methods P or Methods P-A through P-F.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The term "riboswitch" or "riboswitches" is an art recognized term and refers to an mRNA which comprises a natural aptamer that binds target metabolite and an expression platform which changes in the RNA structure to regulate genes. The term

"riboswitch ligand" refers to any compound such as a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31,, in free or salt form, that binds to that particular riboswitch. For example, "FMN riboswitch" refers to a riboswitch that binds a metabolite such as flavin mono-nucleotide (FMN) or other ligands such as the compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or salt form, and which affects downstream FMN biosynthesis and transport proteins. Without intended to be bound by any particular theory, it is believed the binding of the ligand to its riboswitch induces a conformational change in the bacterial mRNA such that the expression of the ORF is altered, for example, such that the expression of enzymes responsible for, e.g., riboflavin and FMN biosynthesis is repressed or overexpressed.

[0021] "CD3299 riboswitch" refers to a riboswitch found in *C. difficile*, controlling the gene designated CD3299. The 5'UTR and beginning of ORF from CD3299 gene of C. difficile 630, accession number AM180355 is as follows:

SEQ ID NO: 1:

TTACAGCTTTCTGATTTTGATAAATTTAAAACTTACCATCTAATACTAAT
AACAGGTTAATTTTATCTAATTATTATAGATTCTCATACTGTGCCTTATT
CTATCTATAAATACAATTTAAGTGTCCATATTGAAATATTTGTATTGTA
ATACAGCTGGATATTACTTAAATCCAATTGTTTCCATTATAATTTTATGT
TAAAATAATATTACAAAATACATCT**GTTTTTCTTCATAAACGGGTGAA**
ATTCCCTATCGGCGGTAAAAGCCCGCGAGCCTTATGGCATAATTTG
GTCATATTCCAAAGCCAACAGTAAAATCTGGATGGTAGAAGAAAAT
AGTATATGAGTACCTTTATGTAATTTTACATGAGTAATCTATACAAATC
CTTCAACTACCGTATTTATTCATGAAATTAGACACATTCAAG***GTACCTA***
ATATACAGGTGCTTTTTTTGTTGTTTATTTTACAATTATATCGTACTTATA
AAATCTATTAAGATTGGAGTGTTATC***ATGAAACAAA***AATGGATAGTATT
GATTATCATCTGTATTGGTGTATTTATGTCTACTCTTGATGGAAGTATAC
TAAATATCGCAAA

In the above depiction of the sequence, the riboswitch is highlighted in **bold,** and is

SEQ ID N0: 2

GTTTTTCTTCATAAACGGGTGAAATTCCCTATCGGCGGTAAAAGCC
CGCGAGCCTTATGGCATAATTTGGTCATATTCCAAAGCCAACAGTA
AAATCTGGATGGTAGAAGAAAATA

The ORF start site in the above sequence is downstream from the riboswitch and is depicted in *italics* and is:

SEQ ID NO: 3

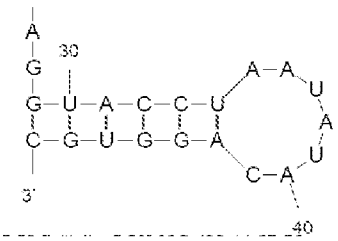
ATGAAACAAA

The putative terminator hairpin is in *bold italics* and is:

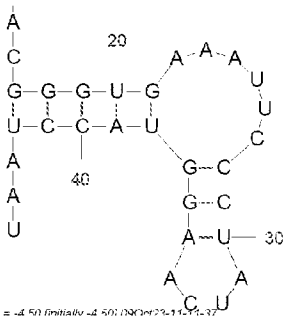
SEQ ID NO: 4

GTACCTAATATACAGGTGC

The hairpin can form a loop having a structure as depicted in Formula 1:



A possible antiterminator has a structure as depicted in Formula 2:



Various Compounds of the Invention, e.g., Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or salt form, bind well to the CD3299 riboswitch and have antibacterial activity against *C. difficile*, provided these compounds possess physicochemical characteristics amenable to uptake into the bacteria.

[0022] The term "infection" encompasses an infection by a Gram-positive or Gram-negative bacteria. In one embodiment, the infection is by a Gram-positive bacteria. In another embodiment, the infection is by a Gram-negative bacteria. In still another embodiment, the infection is an infection by one or more bacteria selected from a group consisting of *Clostridium difficile, Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli, Haemophilus influenzae*, *Enterococcus faecalis, Streptococcus pyogenes, Listeria monocytogenes*, *Salmonella enterica*, *Vibrio cholerae*, *Brucella melitensis*, *Bacillus anthracis*, *Francisella tularensis, Moraxella catarrhalis, Klebsiella pneumoniae,*

Yersinia pestis, *Streptococcus viridans, Enterococcus faecium,* and/or *Borrelia burgdorferi*. In a further embodiment, the infection is a *Clostridium difficile* and/or *Staphylococcus aureus* infection. In a particular embodiment, the infection is an infection which is resistant to a drug which is not a riboswitch ligand. In a further aspect of this particular embodiment, the infection is an infection which is resistant to one or more drugs selected from a group consisting of penicillin, vancomycin, cephalosporin, methicillin and fluoroquinolone (e.g., ciprofloxacin- and/or levofloxacin). In a particular embodiment, the infection is a methicillin-resistant *Staphylococcus aureus* (MRSA) infection. In another particular embodiment, the infection is a fluoroquinolone-resistant (e.g., ciprofloxacin- and/or levofloxacin-resistant), metronidazole and/or vancomycin-resistant *C. difficile* infection.

[0023] The term "bacteria" or "bacterial" include, but are not limited to *Clostridium difficile, Staphylococcus epidermidis, Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli*, *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio cholerae*, *Brucella melitensis*, *Bacillus anthracis*, *Francisella tularensis*, *Moraxella catarrhalis, Klebsiella pneumoniae, Yersinia pestis*, *Streptococcus viridans, Enterococcus faecium,* and/or *Borrelia burgdorferi* .

[0024] If not otherwise specified or clear from context, the following terms as used herein have the following meetings:

a. "Alkyl" as used herein is a saturated or unsaturated hydrocarbon moiety, preferably saturated, e.g., one to eight, e.g., one to six, e.g., one to four carbon atoms in length, which may be linear or branched (e.g., n-butyl or tert-butyl) unless otherwise specified, and may be optionally substituted, e.g., mono-, di-, or tri-substituted on any one of the carbon atoms, e.g., with C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy, halogen (e.g., chloro or fluoro), haloC_{1-4}alkyl (e.g., trifluoromethyl), hydroxy, and carboxy. For example, "C_1-C_8 alkyl" denotes alkyl having 1 to 8 carbon atoms. Examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, 3-methylpentyl, 4-methylpentyl, n-pentyl, n-hexyl and n-heptyl. Wherein the alkyl group is unsaturated or partially saturated, it is denoted as "alkenyl" or "alkynyl". Therefore, n-prop-2-en-1-yl is intended to be $-CH_2$-CH=CH_2.

b. For the avoidance of doubt, the term "alkylene" is intended to denote an alkyl group bridging between two substituents (e.g., between the flavin core structure and another substituent, for example –X-A). Therefore C_{1-4}alkylene, e.g., methylene, ethylene, n-propylene and n-butylene are intended to represent $–CH_2–$, $–CH_2CH_2–$, $–CH_2CH_2CH_2–$ and $–CH_2CH_2CH_2CH_2–$ respectively. Wherein the alkylene group is unsaturated or partially saturated, it is denoted as "alkenylene" or "alkynylene". Therefore, n-but-2-enylene is intended to be $–CH_2-CH=CHCH_2-$.

c. "Aryl" as used herein is a monocyclic or polycyclic aromatic hydrocarbon, preferably phenyl, optionally substituted, e.g., with C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy, halogen (e.g., chloro or fluoro), haloC_{1-4}alkyl (e.g., trifluoromethyl), hydroxy, carboxy, or an additional aryl or heteroaryl.

d. "Cycloalkyl" refers to a saturated or unsaturated nonaromatic hydrocarbon moiety, preferably saturated, preferably comprising three to eight carbon atoms, at least some of which form a nonaromatic mono- or bicyclic, or bridged cyclic structure.

e. "Heterocycloalkyl" refers to a cycloalkyl as defined above wherein at least one of the carbon atoms is replaced with a heteroatom selected from N, O, S. Therefore, "C_{3-8}heterocycloalkyl" or "heteroC_{3-8}cycloalkyl" refers to a 3- to 8-membered non-aromatic ring system containing at least one heteroatom selected from N, O and S.

f. Wherein the substituent is connected via an alkyl group, e.g., -C_{0-4}alkyl-C_{3-8}cycloalkyl or aryl-C_{1-4}alkyl, it is understood that the alkyl group may be saturated or unsaturated or linear or branched. Wherein the substituent is connected via the C_0-alkyl, it is understood that the alkyl is not present and the connectivity is directly to the next substituent. For example, wherein the substituent is -C_0alkyl-C_{3-8}cycloalkyl, it is understood that the alkyl group is not present and the cycloalkyl (e.g., cyclopropyl) is directly connected.

[0025] The Compounds of the Invention or any of the compounds disclosed herein (e.g. a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31), may exist in free or salt, e.g., as acid addition salts, or prodrug form. An acid-addition salt of a compound of the invention which is sufficiently basic, for example, an acid-addition salt with, for

example, an inorganic or organic acid, for example hydrochloric, hydrobromic, sulphuric, phosphoric, acid acetic, trifluoroacetic, citric, maleic acid, toluene sulfonic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic acid, and the like. In addition a salt of a compound of the invention which is sufficiently acidic is an alkali metal salt, for example a sodium or potassium salt, an alkaline earth metal salt, for example a calcium or magnesium salt, an ammonium salt or a salt with an organic base which affords a physiologically-acceptable cation, for example a salt with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine. In a particular embodiment, the salt of the compound of the invention is a trifluoroacetic or hydrochloric acid addition salt. In another embodiment, the salt of the compound of the invention is an acetic acid addition salt.

[0026] In this specification, unless otherwise indicated, language such as Compounds of the Invention is to be understood as embracing the compounds disclosed herein, such as a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in any form, for example free or acid addition salt or prodrug form, or where the compounds contain acidic substituents, in base addition salt form. The Compounds of the Invention are intended for use as pharmaceuticals, therefore pharmaceutically acceptable salts are preferred. Salts which are unsuitable for pharmaceutical uses may be useful, for example, for the isolation or purification of free Compounds of the Invention, and are therefore also included.

[0027] The Compounds of the Invention may comprise one or more chiral carbon atoms. The compounds thus exist in individual isomeric, e.g., enantiomeric or diasteriomeric form or as mixtures of individual forms, e.g., racemic/diastereomeric mixtures. Any isomer may be present in which the asymmetric center is in the (*R*)-, (*S*)-, or (*R,S*)- configuration. The invention is to be understood as embracing both individual optically active isomers as well as mixtures (e.g., racemic/diasteromeric mixtures) thereof. Accordingly, the Compound of the Invention may be a racemic mixture or it may be predominantly, e.g., in pure, or substantially pure, isomeric form, e.g., greater than 70% enantiomeric excess ("ee"), preferably greater than 80% ee, more preferably greater than 90% ee, most preferably greater than 95% ee. The purification of said isomers and the separation of said isomeric mixtures may be accomplished by standard

techniques known in the art (e.g., column chromatography, preparative TLC, preparative HPLC, simulated moving bed and the like).

[0028] Geometric isomers by nature of substituents about a double bond or a ring may be present in cis (=*Z*-) or trans (=*E*-) form, and both isomeric forms are encompassed within the scope of this invention.

[0029] As will be appreciated by those skilled in the art, the Compounds of the Invention may exhibit keto-enol tautomerization. Therefore, the invention as defined in the present invention is to be understood as embracing both the structures as setforth herewith and their tautomeric forms.

[0030] It is also intended that the Compounds of the Invention encompass their stable isotopes. For example, the hydrogen atom at a certain position on the Compounds of the Invention may be replaced with deuterium. It is expected that the activity of compounds comprising such isotopes would be retained and/or it may have altered pharmacokinetic or pharmacodynamic properties. In addition to therapeutic use, compounds comprising such isotopes and having altered pharmacokinetic or pharmacodynamic properties would also have utility for measuring pharmacokinetics of the non-isotopic analogs.

[0031] Compounds of the Invention may in some cases also exist in prodrug form. The term "prodrug" is an art recognized term and refers to a drug precursors prior to administration, but generate or release the active metabolite *in vivo* following administration, via some chemical or physiological process. For example, when the Compounds of the Invention (e.g., a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31) contain a hydroxy group, these substituents may be esterified to form physiologically hydrolysable and acceptable esters (e.g., acyl esters, e.g., $CH_3C(O)$-O-Compound). As used herein, "physiologically hydrolysable and acceptable esters" means esters of Compounds of the Invention which are hydrolysable under physiological conditions to yield hydroxy on the one hand and acid, e.g., carboxylic acid on the other (e.g., Drug-O-$C(O)$-CH_3 → Drug-OH + CH_3COOH), which are themselves physiologically tolerable at doses to be administered. Similarly, wherein the compounds of the invention contain an amine group, prodrug of such amine, e.g., amino acid, carbamic acid ester, amide prodrugs may also exist wherein the prodrug is cleaved to release the active amine metabolite *in vivo* following administration. Further details of amine prodrugs may may be found in Jeffrey P. Krise and Reza Oliyai, Biotechnology:

Pharmaceutical Aspects, Prodrugs, Volume 5, Part 3, pages 801-831, the contents of which are herein incorporated by reference in their entirety. As will be appreciated, the term thus embraces conventional pharmaceutical prodrug forms.

Methods of using Compounds of the Invention

[0032] The Compounds of the Invention are useful for the treatment of an infection, particularly an infection by bacteria including but not limited to *Clostridium difficile, Staphylococcus epidermidis, Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli*, *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio cholerae*, *Brucella melitensis*, *Bacillus anthracis*, *Francisella tularensis*, *Moraxella catarrhalis, Klebsiella pneumoniae, Yersinia pestis*, *Streptococcus viridans, Enterococcus faecium,* and/or *Borrelia burgdorferi* bacteria. In one particular embodiment, the bacteria is *Clostridium difficile*. In another particular embodiment, the bacteria is*Staphylococcus aureus*.

[0033] The invention therefore provides methods of treatment of any one or more of the following conditions: anthrax infection, staphylococcal scalded skin syndrome (staph infections), pneumonia, impetigo, boils, cellulitis, folliculitis, furuncles, carbuncles, scalded skin syndrome, abscesses, meningitis, osteomyelitis endocarditis, Toxic Shock Syndrome (TSS), septicemia, acute sinusitis, otitis media, septic arthritis, endocarditis, peritonitis, pericarditis, brain abscess, tularemia, urinary tract infection, empyema, food poisoning, diarrhea, conjunctivitis and clostridium difficile associated disease (CDAD); comprising administering an effective amount of a compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or pharmaceutically acceptable salt form, to a subject in need thereof.

[0034] The words "treatment" and "treating" are to be understood accordingly as embracing prophylaxis and treatment or amelioration of symptoms of disease as well as treatment of the cause of the disease. In one particular embodiment, the invention encompasses prophylaxis of symptoms of disease or cause of the disease. In another particular embodiment, the invention encompasses treatment or amelioration of symptoms of disease or cause of the disease.

[0035] The term "subject" as used herein encompasses human and/or non-human (e.g., animal).

[031] Dosages employed in practicing the present invention will of course vary depending, e.g. on the particular disease or condition to be treated, the particular Compound of the Invention used, the mode of administration, and the therapy desired. Administration of a therapeutically active amount of the therapeutic compositions is defined as an amount effective, at dosages and for periods of time necessary to achieve the desired result. For example, a therapeutically effective amount of a Compound of the Invention reactive with at least a portion of the FMN or the CD3299 riboswitch may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. Dosage regiment 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. In general, satisfactory results, e.g. for the treatment of diseases as hereinbefore set forth are indicated to be obtained on oral administration at dosages of the order from about 0.01 to 2.0 mg/kg. In larger mammals, for example humans, an indicated daily dosage for oral administration will accordingly be in the range of from about 0.75 to 1000 mg, conveniently administered once, or in divided doses 2 to 4 times, daily or in sustained release form. Unit dosage forms for oral administration thus for example may comprise from about 0.2 to 75 mg, 250 mg,1000 mg, e.g. from about 0.2 or 2.0 to 50, 75, 100, 250, 500, 750 or 1000 mg of a Compound of the Invention, together with a pharmaceutically acceptable diluent or carrier therefor.

[0036] Pharmaceutical compositions comprising the Compounds of the Invention may be prepared using conventional diluents or excipients and techniques known in the galenic art. Thus oral dosage forms may include tablets, capsules, solutions, suspensions, spray-dried dispersions [e.g. Eudragit L100] and the like. The term "pharmaceutically acceptable carrier" as used herein is intended to include diluents such as saline and aqueous buffer solutions. The Compounds of the Invention may be administered in a convenient manner such as by injection such as subcutaneous, intravenous, by oral administration, inhalation, transdermal application, intravaginal application, topical application, intranasal, sublingual or rectal administration. Depending on the route of administration, the active compound may be coated in a material to protect the compound from the degradation by enzymes, acids and other natural conditions that may inactivate

the compound. In one embodiment, the compound may be orally administered. In another embodiment, the compound is administered via topical application.

[0037] In certain embodiment, the Compounds of the Invention may be administered alone or in conjunction, e.g., at or about the same time or simultaneously and separately or simultaneously in an admixture, with another agent, e.g., an agent to facilitate entry or permeability of the Compounds of the Invention into the cell, e.g., an antimicrobial cationic peptide. Antimicrobial cationic peptides include peptides which contain (1) a disulfide-bonded β-sheet peptides; (2) amphipathic α-helical peptides; (3) extended peptides; or (4) loop-structured peptides. Examples of cationic peptide include but are not limited to defensins, cecropins, melittins, magainins, indolicidins, bactenecin and protegrins. Other examples of antimicrobial cationic peptides include but are not limited to human neutrophil defensin-1 (HNP-1), platelet microbicidal protein-1 (tPMP), inhibitors of DNA gyrase or protein synthesis, CP26, CP29, CP11CN, CP10A, Bac2A-NH_2 as disclosed in Friedrich et al., *Antimicrob. Agents Chemother.* (2000) 44(8):2086, the contents of which are hereby incorporated by reference in its entirety. Further examples of antibacterial cationic peptides include but are not limited to polymyxin e.g., polymixin B, polymyxin E or polymyxin nonapeptide. Therefore, in another embodiment, the Compounds of the Invention may be administered in conjunction with polymyxin, e.g., polymyxin B, polymyxin E or polymyxin nonapeptide, preferably polymyxin B.

[0038] In still another embodiment, the Compounds of the Invention may be administered alone or in conjunction, e.g., at or about the same time, simultaneously and separately, or simultaneously in an admixture, with other antimicrobial agents, e.g., other antifungal or other systemic antibacterial (bactericidal or bacteriostatic) agents. Examples of bacterial agents include agents which inhibit bacterial cell wall synthesis (e.g., penicillins, cephalosporins, carbapenems, vancomycin), agents which damage cytoplasmic membrane (e.g., polymixins as discussed above), agents which modify the synthesis or metabolism of nucleic acids (e.g., quinolones, rifampin, nitrofurantoin), agents which inhibit protein synthesis (aminoglycosides, tetracyclines, chloramphenicol, erythomycin, clindamycin), agents which interfer with the folate synthesis (e.g., folate-inhibitors), agents which modify energy metabolism (e.g., sulfonamides, trimethoprim) and/or other antibiotics (beta-lactam antibiotic, beta-lactamase inhibitors). Specific anti-infective agents, particularly antibacterial and antifungal agents, are discussed in Remington: The

Science and Practice of Pharmacy, Chapter 90, pp. 1626-1684 (21st Ed., Lippincott Williams & Wilkins 2005), the contents of which are hereby incorporated by reference.

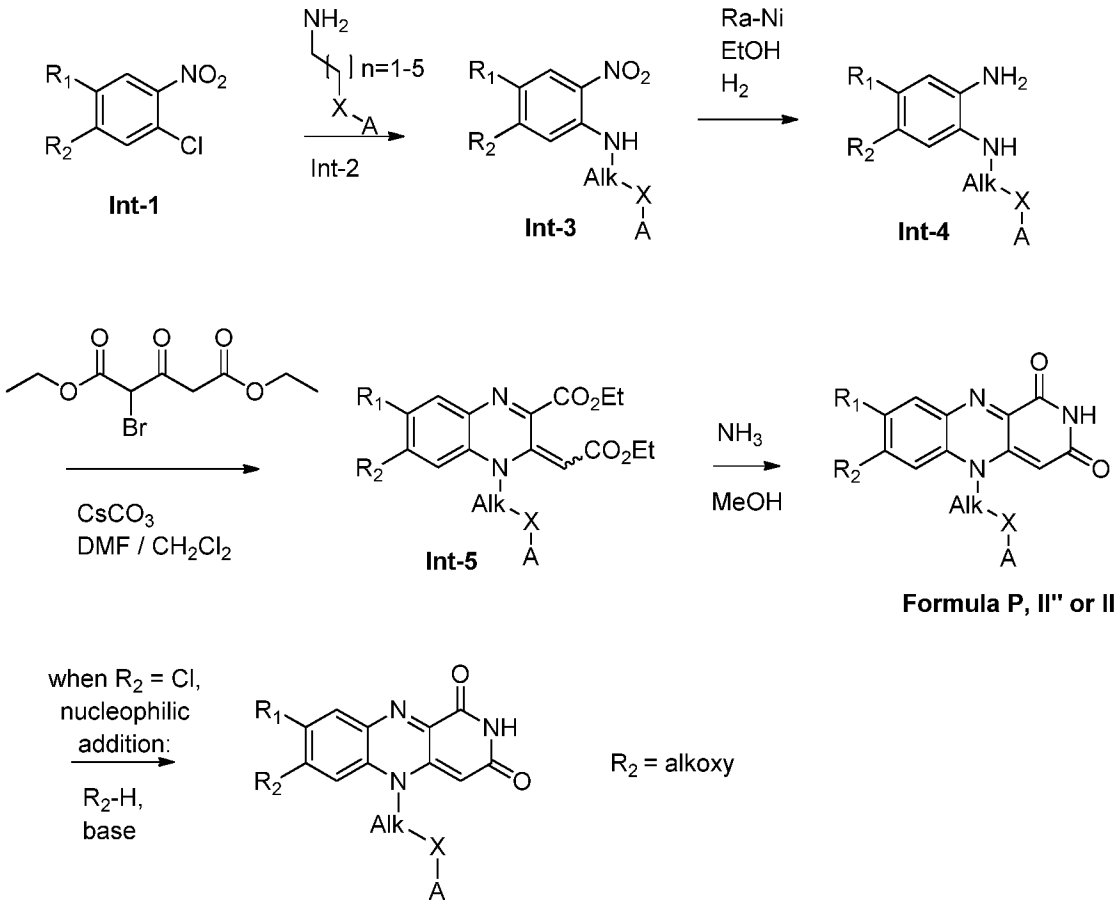
Methods of making the Compounds of the Invention:

[0039] The compounds of the Invention, e.g., compound of Formula P, II'' or II, e.g., any of formulae 2.1-2.31, in free or salt form may be made using the methods as described and exemplified herein and by methods similar thereto and by methods known in the chemical art. Such methods include, but not limited to, those described below. In the description of the synthetic methods described herein, it is to be understood that all proposed reaction conditions, including choice of solvent, reaction atmosphere, reaction temperature, duration of the experiment and workup procedures, are chosen to be the conditions standard for that reaction, which should be readily recognized by one skilled in the art. Therefore, at times, the reaction may require to be run at elevated temperature or for a longer or shorter period of time. It is understood by one skilled in the art of organic synthesis that functionality present on various portions of the molecule must be compatible with the reagents and reactions proposed. If not commercially available, starting materials for these processes may be made by procedures, which are selected from the chemical art using techniques which are similar or analogous to the synthesis of known compounds. All references cited herein are hereby incorporated by reference in their entirety.

[0040] The synthetic methods for the Compounds of the Invention are illustrated below either in the generic synthetic scheme and/or in the specific Examples, which methods are claimed individually and/or collectively. The significances for the substituents are as set forth above in Formula P, II'' or II, e.g., any of formulae 2.1-2.31, unless otherwise indicated.

[0041] Generally, the compounds of Formula P, II'' or II, e.g., any of formulae 2.1-2.31 may be prepared by reacting **Intermediate-5** (**Int-5**) with ammonia in a pressure tube. **Int-5** may be prepared by reacting **Intermediate-4** (**Int-4**) with diethyl 2-bromo-3-oxopentanedioate in the presence of a base, e.g., cesium carbonate, in a solvent, for example, a mixture of dimethylformamide (DMF) and methylene chloride (CH_2Cl_2). **Int-4** may be may be prepared by converting **Intermediate-3** (**Int-3**) to **Int-4**, for example, by catalytic hydrogenation, e.g., by reacting **Int-3** with a metal, e.g., Raney-Nickel, and hydrogen gas in a solvent such as ethanol. In turn, **Int-3** may be prepared by reacting

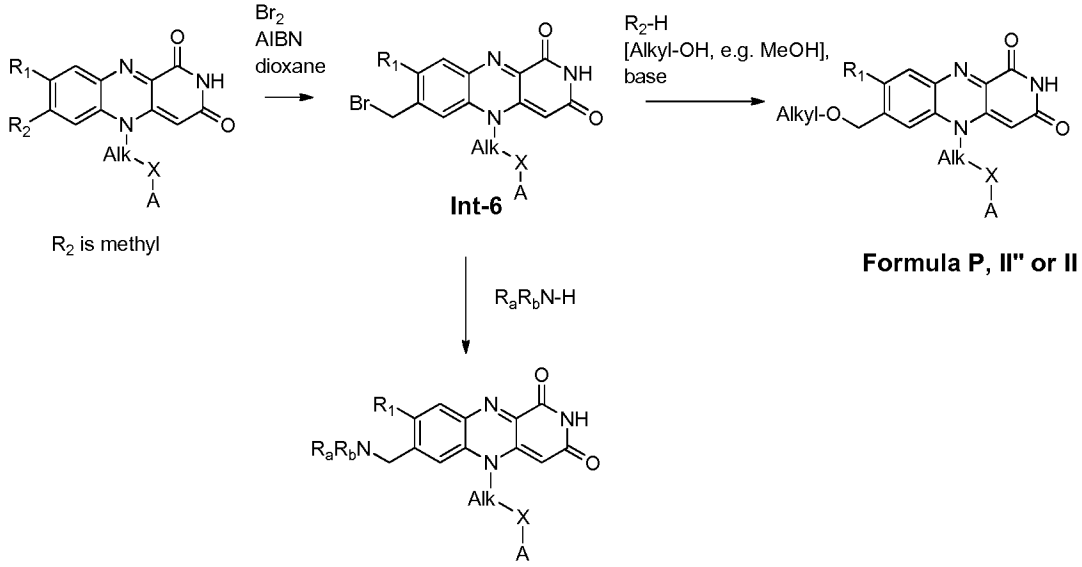
Intermediate-1 (**Int-1**) with NH_2-Alk-X-A (**Int-2**), wherein Alk, X and A are defined in Formula P, II'' or II or any of 2.1-2.31 to yield **Int-3**. **Int-1** is either commercially available or may be prepared by methods known in the art. Wherein R_2 of compounds of Formula P, II'' or II is alkoxy, this compound may be prepared by reacting a compound of Formula P, II'' or II, wherein R_2 is halo, e.g., chloro, with R_2-H, e.g., methanol, in the presence of a base. The methods for preparing a compound of Formula P, II'' or II may be described in the reaction scheme below, wherein all substituents are defined in Formula P, II'' or II or any of 2.1-2.31:



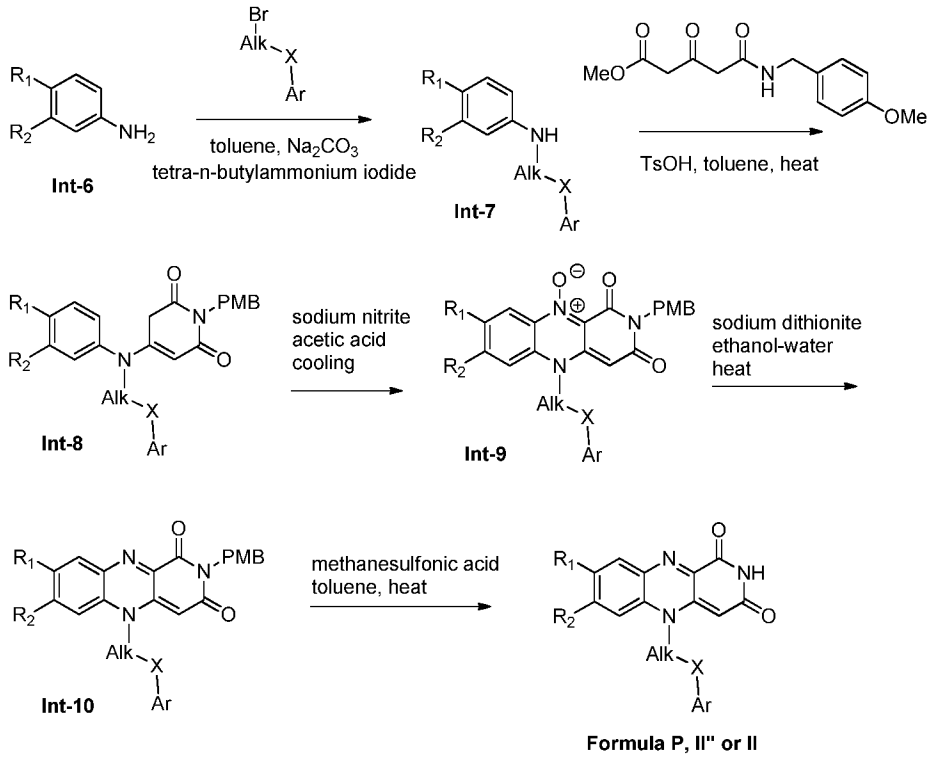
[0042] Wherein R_2 of the compounds of Formula P, II'' or II is (C_{1-4}alkoxy)-methyl, these compounds may be prepared by first halogenating the compound of Formula P, II'' or II, wherein R_2 is methyl, for example by reacting such compound with a halogen, e.g., bromine, e.g., optionally in the presence of a catalyst such as azobisisobutyronitrile (AIBN). The resulting intermediate, **Int-6**, may then react with a

R_2-H, wherein R_2-H is e.g. methanol, in the presence of a base to provide the corresponding alkoxy-methyl product.

[0043] Wherein R_2 of the compounds of Formula P, II'' or II is -methyl-N(R_a)(R_b), e.g., -CH_2-N(CH_3)$_2$, this compound may be prepared by halogenating the compounds of Formula P, II'' or II, wherein R_2 is e.g., a methyl group, for example by reacting bromine with the compounds of Formula P, II'' or II, wherein R_2 is methyl, optionally in the presence of a catalyst such as azobisisobutyronitrile (AIBN). The resulting intermediate, **Int-6**, may then react with an amine, HN(R_a)(R_b), e.g. HN(CH_3)$_2$, to provide a Compound of Formula P, II'' or II wherein R_2 is -methyl-N(R_a)(R_b), e.g., -CH_2-N(CH_3)$_2$. This preparation may be summarized in the following reaction scheme:



[0044] Wherein R_2 of the compounds of Formula P, II" or II is hydrogen, these compounds may be prepared by heating **Intermediate-10 (Int-10)** with acid [e.g. methanesulfonic acid] in toluene. **Int-10** may be prepared by heating **Intermediate-9 (Int-9)** with sodium dithionite in a mixture of ethanol and water. **Intermediate-9 (Int-9)** may be prepared by treating **Intermediate-8 (Int-8)** with sodium nitrite in acetic acid with cooling. **Int-8** may be prepared by condensing **Intermediate-7 (Int-7)** with methyl 5-[(4-methoxybenzyl)amino]-3,5-dioxopentanoate in the presence of acid [e.g. *p*-toluenesulfonic acid monohydrate] in toluene. In turn, **Intermediate-7 (Int-7)** may be prepared with Br-Alk-X-Ar , wherein Alk, X, and A are defined in any of Formulae P, II", or II. This preparation may be summarized in the following reaction scheme:



Examples:

Binding of ligand to riboswitch:

Example A:

[0045] An in-line probing assay, as described in Regulski and Breaker, "In-line probing analysis of riboswitches", (2008), *Methods in Molecular Biology,* Vol 419, pp 53-67, the contents of which are incorporated by reference in their entirety, is used to estimate the dissociation binding constants for the interaction of each of the ligands described herein with either an FMN riboswitch amplified from the genome of *Bacillus subtilis* or a CD3299 riboswitch amplified from *Clostridium difficile*. Precursor mRNA leader molecules are prepared by in vitro transcription from templates generated by PCR and [5'-^{32}P]-labeling using methods described previously (Regulski and Breaker, In-line probing analysis of riboswitches (2008), *Methods in Molecular Biology* Vol 419, pp 53-67). Approximately 5 nM of labeled RNA precursor is incubated for 41 hours at 25°C in 20 mM $MgCl_2$, 50 mM Tris/HCl (pH 8.3 at 25°C) in the presence or absence of a fixed concentration of each ligand. Binding to the FMN and CD3299 riboswitches are measured at 20 μM and 100 μM, respectively. In-line cleavage products are separated on 10%

polyacrylamide gel electrophoresis (PAGE), and the resulting gel is visualized using a Molecular Dynamics Phosphorimager. The location of products bands corresponding to cleavage are identified by comparison to a partial digest of the RNA with RNase T1 (G-specific cleavage) or alkali (nonspecific cleavage).

[0046] In-line probing exploits the natural ability of RNA to self-cleave at elevated pH and metal ion concentrations (pH ≈ 8.3, 25 mM $MgCl_2$) in a conformation-dependent manner. For self-cleavage to occur, the 2'-hydroxyl of the ribose must be "in-line" with the phosphate-oxygen bond of the internucleotide linkage, facilitating a S_N2P nucleophilic transesterification and strand cleavage. Typically, single-stranded regions of the riboswitch are dynamic in the absence of an active ligand, and the internucleotide linkages in these regions can frequently access the required in-line conformation. Binding of an active ligand to the riboswitch generally reduces the dynamics of these regions, thereby reducing the accessibility to the in-line conformation, resulting in fewer in-line cleavage events within those regions. These ligand-dependent changes in RNA cleavage can be readily detected by denaturing gel electrophoresis. The relative binding affinity of each ligand is expressed as I_{max}, wherein I_{max} represents the percent inhibition of in-line cleavage at selected internucleotide ligands in the presence of a fixed ligand concentration (20 μM for the FMN riboswitch and 100 μM for the CD3299 riboswitch) normalized to the percent inhibition in the absence of ligand and the percent inhibition in the presence of a saturation concentration of a control ligand. 100 μM FMN is used as a control ligand for estimating binding to the FMN riboswitch and 100 μM of 7,8-dimethyl-10-(3-phenylpropyl)benzo[g]pteridine-2,4(3H,10H)-dione (which is a compound which has a high affinity against the CD3299 riboswitch) is used as a control ligand for estimating binding to the CD3299 riboswitch.

[0047] The experiments show that the exemplified Compounds of the Invention, e.g., compounds of Examples 1-16 have a binding affinity to the FMN riboswitch with an I_{max} value of greater than 20%, some up to80% compared to the control at 20 μM. In other instances, various compounds of the invention have a binding affinity to the CD3299 switch with an I_{max} value of greater than 20% compared to the control at 100μM.

MIC Assay

Example B:

[0048] The MIC assays are carried out in a final volume of 100 µL in 96-well clear round-bottom plates according to methods established by the Clinical Laboratory Standards Institute (CLSI). Briefly, test compound suspended in 100 % DMSO (or another suitable solubilizing buffer) is added to an aliquot of media appropriate for a given pathogen to a total volume of 50 µL. This solution is serially diluted by 2-fold into successive tubes of the same media to give a range of test compound concentrations appropriate to the assay. To each dilution of test compound in media is added 50 µl of a bacterial suspension from an overnight culture growth in media appropriate to a given pathogen. Final bacterial inoculum is approximately 10^5-10^6 CFU/well. After growth for 18-24 hours at 37° C, the MIC is defined as the lowest concentration of antimicrobial agent that completely inhibits growth of the organism as detected by the unaided eye, relative to control for bacterial growth in the absence of added antibiotic. Ciprofloxacin is used as an antibiotic-positive control in each screening assay. Each of the bacterial cultures that are available from the American Type Culture Collection (ATCC, www.atcc.org) is identified by its ATCC number.

[0049] The experiments show that the exemplified compounds of the invention, e.g., the compounds of Examples 1-16 have a minimum inhibitory concentration (MIC) of less than 2 µg/mL against at least one of the bacteria selected from *C. difficile MMX3581* (clinical), *C. difficile* ATCC *43596, C. difficile* ATCC 700057 (MMX 4381), *C. difficile* ATCC BAA-1805 (NAP1), *C. difficile* ATCC BAA-1382 (MMX4820), *C. difficile* 43255 (MMX4821), *C. difficile* ATCC BAA-1803 (NAP1) and *C. difficile* ATCC BAA-1870 (NAP1).

[0050] All of the exemplified compounds of the invention have either an I_{max} value of greater than 20% in an assay as described in Example A (compared to at least one of the two controls) and/or a MIC of less than or equal to 2µg/mL against at least one of the bacterial strains as decribed in Example B. In certain embodiment, certain compounds of the invention have either an I_{max} value of greater than 50% in an assay as described in Example A (compared to at least one of the two controls) and/or a MIC of less than or equal to 2µg/mL against at least one of the bacterial strains as decribed in Example B.

Cytotoxic Assay

Example C:

[0051] The cytotoxic effects of test compounds on HepG2 are measured with a commercially available cell viability assay kit from Promega. On day 1, HepG2 cells (~1 x 10^4 cells) are seeded into each well in 96-well plate and cultured for approximately 24 h at 37°C in a 5% CO_2 atmosphere under saturating humidity. On day 2, test compounds and DMSO controls are added to appropriate wells to give a range of test compound concentrations appropriate to the assay. Terfenadine is also added to each plate as a positive cytotoxic control. Control wells containing medium without cell are prepared to obtain a value for background luminescence. Assay plates are then cultured for approximately 24 h at 37°C in a 5% CO2 atmosphere under saturating humidity. On day 3, assay plates are removed from 37°C incubator and equilibrated to 22°C. Once equilibrated, CellTiter-Glo® reagent is added to each well containing cell culture medium, followed by mixing to allow cell lysis. The CellTiter-Glo® Assay measures the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells. This assay generates a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. After the assay plate is incubated at room temperature for approximately 10 min to stabilize luminescent signal, luminescence is recorded on PerkinElmer luminometer. CC_{50} is defined as the concentration of test compounds in μM to result in 50% reduction in luminescence signal relative to the signal for untreated cells.

[0052] The experiments show that the exemplified compounds of the invention have a CC_{50} value of greater than or equal to 19 μM. The exemplified compounds of the invention generally have a MIC to cytotox ratio of at least 1:20.

Synthesis of the Compounds of the Invention:

[0053] Temperatures are given in degrees Celsius (°C); unless otherwise stated, operations are carried out at room or ambient temperature, that is, at a temperature in the range of 18-25 °C. Chromatography means flash chromatography on silica gel; thin layer chromatography (TLC) is carried out on silica gel plates. NMR data is in the delta values of major diagnostic protons, given in parts per million (ppm) relative to the deuterium lock signal of the deuterated solvent utilized. Conventional abbreviations for signal shape are used. For mass spectra (MS), the lowest mass major ion is reported for molecules where isotope splitting results in multiple mass spectral peaks. Solvent mixture compositions are

given as volume percentages or volume ratios. In cases where the NMR spectra are complex, only diagnostic signals are reported.

HPLC Methods:

[0054] **Method D**: Agilent 1100 HPLC, Agilent XDB C18 50 x 4.6 mm 1.8 micron column, 1.5 mL/min., Solvent A-Water (0.1% TFA), Solvent B -Acetonitrile (0.07% TFA), Gradient -5 min. 95%A to 95%B; 1min. hold; then recycle, UV Detection @ 210 and 254nm.

[0055] **Method G**: Agilent 1100 HPLC, Agilent XDB C18 50 x 4.6 mm 5 micron column, 1.5 mL/min., Solvent A-Water (0.1% TFA), Solvent B-Acetonitrile (0.07% TFA), Gradient -6 min. 95%A to 95%B; 1 min. hold; then recycle, UV Detection @ 210 and 250nm.

Terms and abbreviations:

br = broad,
CH_3CN = acetonitrile,
d = doublet,
CH_2Cl_2 or DCM = dichloromethane,
DMF = *N,N*-dimethylforamide,
DCM = dichloromethane
DMSO = dimethyl sulfoxide,
Et_2O = diethyl ether,
EtOAc = ethyl acetate,
h = hour(s),
hep = heptet,
Hex = hexane,
HPLC = high performance liquid chromatography,
m = multiplet,
min. = minute(s)
MeOH = methanol,
$NaHCO_3$ = sodium bicarbonate,
Na_2SO_4 = sodium sulfate,

NH_3 = ammonia gas,

NMR = nuclear magnetic resonance,

p = pentet,

PMB = *para*-methoxybenzyl

rt = room temperature,

RNA = ribonucleic acid,

RNase T1 = an endoribonuclease that specifically degrades single-stranded RNA at G residues,

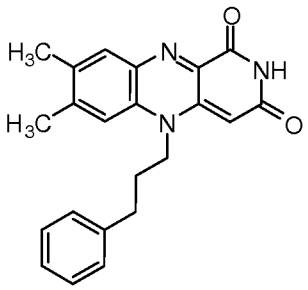
s = singlet,

t = triplet,

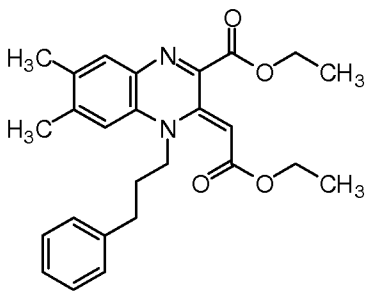
THF = tetrahydrofuran,

Example 1

7,8-Dimethyl-5-(3-phenylpropyl)pyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione



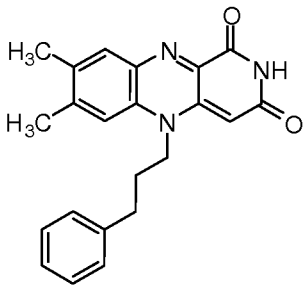
Step 1 Preparation of ethyl (3Z)-3-(2-ethoxy-2-oxoethylidene)-6,7-dimethyl-4-(3-phenylpropyl)-3,4-dihydroquinoxaline-2-carboxylate



[0056] Cesium carbonate (5.38 g, 16.5 mmol) is added to a solution of 4,5-dimethyl-N-(3-phenylpropyl)benzene-1,2-diamine (0.600 g, 2.36 mmol) in 40 mL of 1:1 DMF / CH_2Cl_2 followed by diethyl 2-bromo-3-oxopentanedioate (4.64 g, 16.5 mmol) and the mixture is stirred at rt under N_2 overnight. The mixture is evaporated to dryness and the residue is partitioned between 50 mL of CH_2Cl_2 and 50 mL of water. The layers are

separated and the aqueous phase is extracted with 2 x 50 mL of CH_2Cl_2. The combined organic layers are extracted with 3 x 50 mL of water. Drying over Na_2SO_4 and evaporation gives 3.5 g of a red oil. Chromatography on 150 g of silica gel in 30% EtOAc / hexane gives desired product (0.57 g, 56%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.53 (s, 1 H), 7.38 (d, 1 H), 7.41 (d, 1 H), 7.27 - 7.34 (m, 3 H), 6.49 (s, 1 H), 5.06 (s, 1 H), 4.40 (q, 2 H), 4.15 (q, 2 H), 3.80 (q, 2 H), 2.84 (q, 2 H), 2.24 (m, 6 H), 2.15 (m, 2 H), 1.43 (t, 3 H), 1.31 (t, 3 H); MS (ESI$^+$) for $C_{26}H_{30}N_2O_4$ *m/z* 435 $(M+H)^+$.

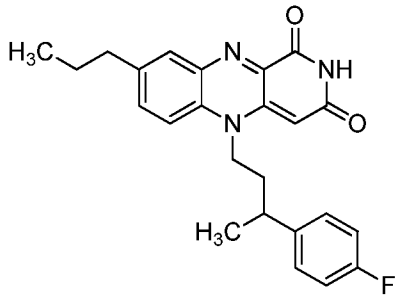
Step 2 Preparation of 7,8-dimethyl-5-(3-phenylpropyl)pyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione



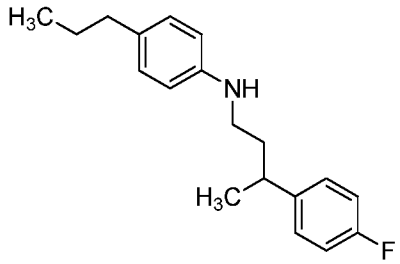
[0057] Ethyl (3Z)-3-(2-ethoxy-2-oxoethylidene)-6,7-dimethyl-4-(3-phenylpropyl)-3,4-dihydroquinoxaline-2-carboxylate (0.300 g, 0.690 mmol) is taken up in 20 mL of MeOH and the solution is cooled in an ice water bath. Ammonia gas is bubbled through the solution for 5 minutes in a pressure tube. The solution is stirred at rt overnight in the capped pressure tube. The pressure tube is opened slowly to allow NH_3 to evolve. The remaining solution is evaporated to give 0.2 g of a dark solid. The solid is adsorbed onto silica gel and chromatographed on 50 g of silica gel. The column is eluted with 1% MeOH /DCM (1L) followed by 1.5 % MeOH / CH_2Cl_2 (1.5L). The product elutes in the 1.5% MeOH / DCM. Evaporation of the fractions containing product gives 0.07 g of a purple solid. Crystallization from CH_3CN (25 mL) gives 12 mg of the product as a purple solid. 1H NMR (400 MHz, DMSO-d_6) δ ppm 11.17 (s, 1 H), 7.63 (s, 1 H), 7.20 - 7.36 (m, 6 H), 5.27 (d, 1 H), 4.01 (br s, 2 H), 2.82 (t, 2 H), 2.35 (s, 3 H), 2.29 (s, 3 H), 1.93 (br s, 2 H); MS (ESI$^+$) for $C_{22}H_{21}N_3O_2$ *m/z* 360 $(M+H)^+$, HPLC retention time: 3.84 min. (Method D).

Example 2

5-[3-(4-fluorophenyl)butyl]-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione

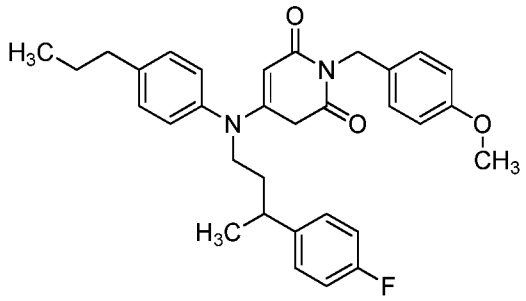


Step 1 Preparation of *N*-[3-(4-Fluorophenyl)butyl]-4-propylaniline



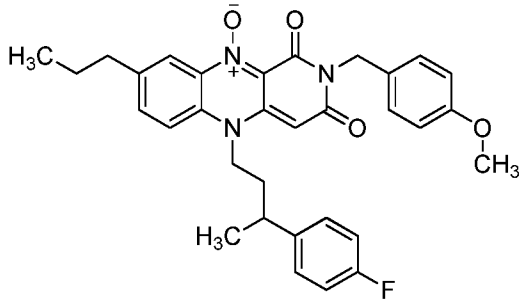
[0058] A solution of 4-propylaniline (0.18 mL, 1.2 mmol) and 1-(3-bromo-1-methylpropyl)-4-fluorobenzene (0.25 g, 1.1 mmol) in toluene (4.8 mL) is treated with sodium bicarbonate (0.18 g, 2.1 mmol) and tetra-*n*-butylammonium iodide (0.040 g, 0.11 mmol). The pale yellow reaction mixture is heated at 80 °C for 24.5 h and at reflux (113 °C) for 4 h. The reaction mixture is cooled to rt and partitioned between water (10 mL) and EtOAc (10 mL). The separated aqueous layer is extracted with EtOAc (3 x 10 mL) and the combined organics are washed with brine (1 x 25 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford a tan oil. Purification by column chromatography (2 x 22 cm silica; Hex, 1:1:98 Et_2O/CH_2Cl_2/Hex, 2:2:96 Et_2O/CH_2Cl_2/Hex) afforded the title compound as a yellow oil (0.21 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22-7.15 (m, 2H), 7.05-6.94 (m, 4H), 6.51-6.45 (m, 2H), 3.40 (br s, 1H), 3.08-2.94 (m, 2H), 2.93-2.81 (m, 1H), 2.52-2.44 (m, 2H), 1.97-1.80 (m, 2H), 1.66-1.51 (m, 2H), 1.29 (d, 3H), 0.93 (t, 3H); MS (ESI+) for $C_{19}H_{24}FN$ *m/z* 286.2 $(M+H)^+$; HPLC retention time: 3.79 min (Method D).

Step 2 Preparation of 4-{[3-(4-Fluorophenyl)butyl](4-propylphenyl)amino}-1-(4-methoxybenzyl)pyridine-2,6(1*H*,3*H*)-dione



[0059] A slurry of *N*-[3-(4-fluorophenyl)butyl]-4-propylaniline (0.16 g, 0.56 mmol) and methyl 5-[(4-methoxybenzyl)amino]-3,5-dioxopentanoate (0.19 g, 0.67 mmol) in toluene (60 mL) is treated with *p*-toluenesulfonic acid monohydrate (9.3 mg, 0.049 mmol). The reaction mixture is heated at reflux (131 °C) for 18 h and allowed to cool to rt. The reaction mixture is concentrated under reduced pressure and purified by column chromatography (2 x 22 cm silica; Hex, 5%, 10%, 20%, 30% EtOAc/Hex) to afford the title compound as a light tan oil (0.21 g, 73%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41-7.35 (m, 2H), 7.23-7.17 (m, 2H), 7.07-7.00 (m, 2H), 6.99-6.90 (m, 4H), 6.83-6.77 (m, 2H), 5.05 (s, 1H), 4.92 (s, 2H), 3.76 (s, 3H), 3.47-3.36 (m, 1H), 3.34-3.23 (m, 1H), 3.19-3.05 (m, 2H), 2.70-2.56 (series of m, 3H), 1.95-1.79 (m, 2H), 1.71-1.59 (m, 2H), 1.21 (d, 3H), 0.97 (t, 3H); MS (ESI+) for $C_{32}H_{35}FN_2O_3$ *m/z* 515.2 $(M+H)^+$; MS (ESI–) for $C_{32}H_{35}FN_2O_3$ *m/z* 513.3 $(M-H)^-$; HPLC retention time: 5.50 min. (Method D).

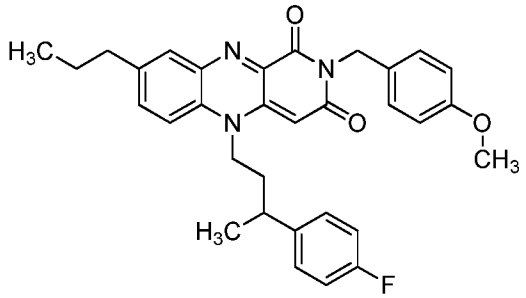
Step 3 Preparation of 5-[3-(4-Fluorophenyl)butyl]-2-(4-methoxybenzyl)-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione 10-oxide



[0060] A solution of 4-{[3-(4-fluorophenyl)butyl](4-propylphenyl)amino}-1-(4-methoxybenzyl)pyridine-2,6(1*H*,3*H*)-dione (0.21 g, 0.41 mmol) in acetic acid (2.4 mL) is cooled to 15 °C (ice water bath). Sodium nitrite (34 mg, 0.49 mmol) is added in one portion and the cold bath is removed. The bright red-orange reaction mixture is stirred for 20 min. to afford a dark purple mixture. At 30 min., the reaction mixture is poured into ice water (20 mL) to afford a finely dispersed purple solid. The solid remaining in the reaction flask is taken up in CH_2Cl_2 (30 mL) and added to the aqueous mixture. The separated

aqueous layer is extracted with CH_2Cl_2 (1 x 20 mL), neutralized with $NaHCO_3$ (solid), and extracted with CH_2Cl_2 (3 x 10 mL). The combined organic layers are washed with saturated aqueous $NaHCO_3$ (1 x 30 mL), brine (1 x 30 mL), dried (Na_2SO_4) and concentrated under reduced pressure to afford a dark purple, glassy residue. Purification by column chromatography (5 x 17 cm silica; Hex, 10%, 20%, 40%, 50%, 60% EtOAc/Hex) afforded the title compound as a deep violet oil (60 mg, 30%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18-8.13 (m, 1H), 7.55-7.49 (m, 2H), 7.36-7.30 (m, 1H), 7.30-7.23 (m, 3H), 7.14-7.06 (m, 2H), 6.84-6.78 (m, 2H), 6.74-6.60 (m, 1H), 5.27 (s, 1H), 5.14 (s, 2H), 3.88-3.73 (m, 1H), 3.76 (s, 3H), 3.70-3.58 (m, 1H), 2.95-2.84 (m, 1H), 2.64 (t, 2H), 2.16-1.87 (series of m, 2H), 1.70-1.57 (m, 2H), 1.39 (d, 3H), 0.93 (t, 3H); MS (ESI+) for $C_{32}H_{32}FN_3O_4$ *m/z* 542.3 $(M+H)^+$; MS (ESI–) for $C_{32}H_{32}FN_3O_4$ *m/z* 586.3 $(M+HCO_2)^-$; HPLC retention time: 5.08 min. (Method D).

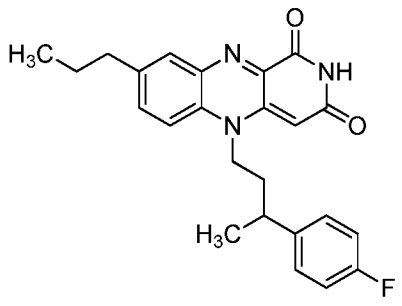
Step 4 Preparation of 5-[3-(4-fluorophenyl)butyl]-2-(4-methoxybenzyl)-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione



[0061] A solution of 5-[3-(4-fluorophenyl)butyl]-2-(4-methoxybenzyl)-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione 10-oxide (60 mg, 0.11 mmol) in ethanol (2 mL)/THF (4 mL)/water (2 mL) is heated to 50 °C to afford a homogeneous solution. Sodium dithionite (0.038 g, 0.22 mmol) is added to afford a red-purple reaction mixture, which is heated at 55 °C for 1 h 10 min. and allowed to cool to rt. The reaction mixture is partitioned between water (20 mL) and CH_2Cl_2 (25 mL). The separated aqueous layer is extracted with CH_2Cl_2 (2 x 10 mL) and the combined organic layers are washed with brine (10 mL), dried (Na_2SO_4), and concentrated under reduced pressure to afford the title compound as a red-purple foam, which is carried on without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85-7.80 (m, 1H), 7.55-7.48 (m, 2H), 7.36-7.30 (m, 1H), 7.28-7.22 (m, 2H), 7.14-7.06 (m, 2H), 6.85-6.78 (m, 2H), 6.78-6.70 (m, 1H), 5.30 (s, 1H), 5.16 (s, 2H), 3.84-3.58 (series of m, 2H), 3.76 (s, 3H), 2.95-2.83 (m, 1H), 2.63 (t, 2H),

2.11-1.88 (series of m, 2H), 1.72-1.60 (m, 2H), 1.36 (d, 3H), 0.93 (t, 3H); MS (ESI+) for $C_{32}H_{32}FN_3O_3$ *m/z* 526.2 $(M+H)^+$; HPLC retention time: 5.21 min. (Method D).

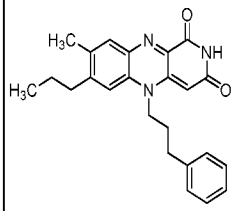
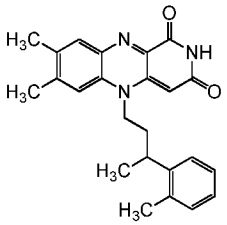
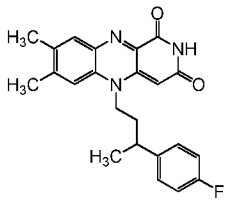
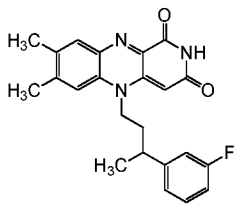
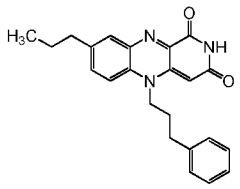
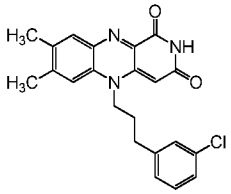
Step 5 Preparation of 5-[3-(4-fluorophenyl)butyl]-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione

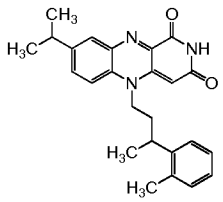
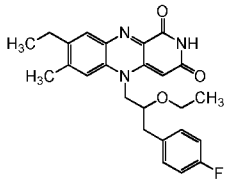
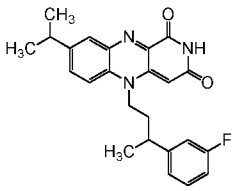
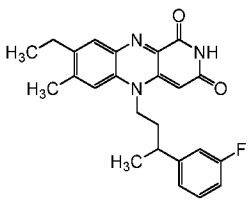
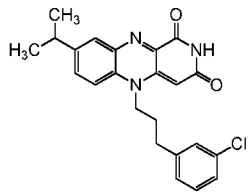


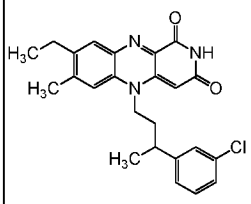
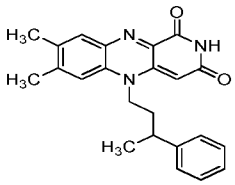
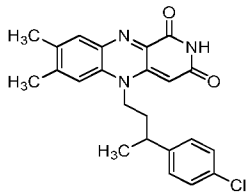
[0062] A solution of the 5-[3-(4-fluorophenyl)butyl]-2-(4-methoxybenzyl)-8-propylpyrido[3,4-*b*]quinoxaline-1,3(2*H*,5*H*)-dione in methanesulfonic acid (2.2 mL, 33 mmol) is diluted with toluene (5.7 mL) and the biphasic mixture is heated at 50 °C for 5 h. The reaction mixture is diluted with water (30 mL) and CH_2Cl_2 (70 mL) and the mixture is poured over ice and adjusted to pH 8 with solid $NaHCO_3$. The separated aqueous layer is extracted with CH_2Cl_2 (3 x 20 mL) and the combined organics are dried (Na_2SO_4) and concentrated under reduced pressure to afford a red-purple solid. Purification by column chromatography (4 x 15 cm silica; Hex, 20%, 40%, 50%, 60%, 70%, 80%, 90% EtOAc/Hex doped with 0.1% i-PrOH) afforded the title compound as a dark purple amorphous solid (44 mg, 98%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.22 (s, 1H), 7.68-7.63 (m, 1H), 7.54-7.48 (m, 1H), 7.41-7.32 (m, 2H), 7.31-7.20 (m, 1H), 7.20-7.11 (m, 2H), 5.05 (br s, 1H), 4.12-3.98 (m, 1H), 3.67-3.54 (m, 1H), 3.09-2.99 (m, 1H), 2.64 (t, 2H), 1.96-1.80 (m, 2H), 1.68-1.56 (m, 2H), 1.29 (d, 3H), 0.90 (t, 3H); MS (ESI+) for $C_{24}H_{24}FN_3O_2$ *m/z* 406.1 $(M+H)^+$; MS (ESI–) for $C_{24}H_{24}FN_3O_2$ *m/z* 404.2 $(M-H)^-$, 450.1 $(M+HCO_2)^-$; HPLC retention time: 4.29 min. (Method D).

[0063] The compounds of the invention particularly those compounds as set forth in Table 1 below which are disclosed and claimed either individually and/or collectively may generally be prepared using similar procedures as set forth in Examples 1-2 above. It is to be understood that the appropriate reagents, solvents and reaction condition for those reactions are used as apparent to one skilled in the art.

Table 1

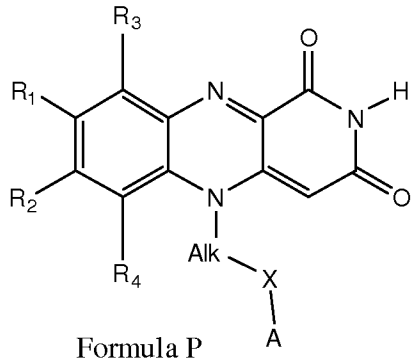
Ex.	Structure	LC-MS MH+ (m/z)	HPLC retention time (min.)	HPLC method	Preparation	Name
3		388.3	4.70	G	Prepared as described for the synthesis of Example 1 starting from 4-methyl-2-nitro-5-propyl-phenylamine and (3-bromo-propyl)-benzene	8-methyl-5-(3-phenylpropyl)-7-propylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
4		388.3	4.54	G	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-2-methyl-benzene	7,8-dimethyl-5-(3-(o-tolyl)butyl)pyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
5		392.2	4.317	G	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-4-fluorobenzene	5-(3-(4-fluorophenyl)butyl)-7,8-dimethylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
6		392.3	4.31	G	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-3-fluoro-benzene	5-(3-(3-fluorophenyl)butyl)-7,8-dimethylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
7		374.3	4.47	G	Prepared as described for the synthesis of Example 2 starting from 4-propyl-phenylamine and (3-bromo-propyl)-benzene	5-(3-phenylpropyl)-8-propylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
8		394.2	4.004	D	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-propyl)-3-chloro-benzene	5-(3-(3-chlorophenyl)propyl)-7,8-dimethylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione

9		402.2	4.8	G	Prepared as described for the synthesis of Example 2 starting from 4-isopropyl-phenylamine and 1-(3-bromo-1-methyl-propyl)-2-methyl-benzene	8-isopropyl-5-(3-(o-tolyl)butyl)pyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
10		436.5	4.53	G	Prepared as described for the synthesis of Example 1 from [2-ethoxy-3-(4-fluoro-phenyl)-propyl]-(4-ethyl-5-methyl-2-nitro-phenyl)-amine which was prepared using the methodology described for Example 17 in WO2011126567A1 and 2-ethoxy-3-(4-fluoro-phenyl)-propylamine (prepared as described for intermediate W in WO2011126567A1) and 1-bromo-4-ethyl-5-methyl-2-nitro-benzene	5-(2-ethoxy-3-(4-fluorophenyl) propyl)-8-ethyl-7-methylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
11		406.1	4.6	G	Prepared as described for the synthesis of Example 2 starting from 4-isopropyl-phenylamine and 1-(3-bromo-1-methyl-propyl)-3-fluoro-benzene	5-(3-(3-fluorophenyl) butyl)-8-isopropylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
12		406.4	4.2	D	Prepared as described for the synthesis of Example 1 starting from 4-ethyl-5-methyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-3-fluoro-benzene	8-ethyl-5-(3-(3-fluorophenyl) butyl)-7-methylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione
13		408.2	4.64	G	Prepared as described for the synthesis of Example 2 starting from 4-isopropyl-phenylamine and 1-(3-bromo-propyl)-3-chloro-benzene	5-(3-(3-chlorophenyl) propyl)-8-isopropylpyrido[3,4-b]quinoxaline-1,3(2H,5H)-dione

14		422.2	4.4	G	Prepared as described for the synthesis of Example 1 starting from 4-ethyl-5-methyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-3-chloro-benzene	5-(3-(3-chlorophenyl) butyl)-8-ethyl-7-methylpyrido [3,4-b]quinoxaline -1,3(2H,5H)-dione
15		374.3	4.36	G	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)benzene	7,8-dimethyl-5-(3-phenylbutyl)p yrido[3,4-b]quinoxaline -1,3(2H,5H)-dione
16		408.1	4.59	G	Prepared as described for the synthesis of Example 1 starting from 4,5-dimethyl-2-nitro-phenylamine and 1-(3-bromo-1-methyl-propyl)-4-chloro-benzene	5-[3-(4-chlorophenyl) butyl]-7,8-dimethylpyri do[3,4-b]quinoxaline -1,3(2H,5H)-dione

CLAIMS

1. A compound of Formula P:



Formula P

wherein:

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one hydroxy or C_{1-4}alkoxy (e.g., ethoxy) group;

(ii) X is a single bond, -S- or –O-;

(iii) A is aryl (e.g., phenyl) or heteroaryl (e.g. pyridinyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl), C_{1-4}alkoxy (e.g., methoxy), hydroxy, halo (e.g., Cl, F), haloC_{1-4}alkyl (e.g., CF_3) or -O-haloC_{1-4}alkyl (e.g., -OCF_3);

(iv) R_1 is H, C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl), or C_{1-4}alkoxy (e.g., methoxy);

(v) R_2 is H, C_{1-4}alkyl (e.g., methyl or n-propyl), C_{1-4}alkoxy (e.g., methoxy), halo (e.g., Cl), C_{3-8}cycloalkyl-C_{1-4}alkyl, -C_{1-4}alkyl-N(R_a)(R_b), (C_{1-4}alkoxy)-C_{1-4}alkyl or (2-C_{1-4}alkoxyethoxy)-C_{1-4}alkyl;

(vi) R_3 is H or C_{1-4}alkyl (e.g., methyl);

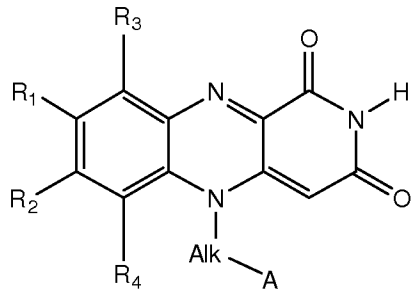
(vii) R_4 is H or C_{1-4}alkyl (e.g., methyl);

(viii) R_a and R_b are independently H, C_{1-4}alkyl (e.g., methyl) or C_{3-8}cycloalkyl (e.g., cyclopropyl, cyclopentyl);

in free or salt form.

2. The compound according to claim 1, wherein X is a single bond which compound

is represented by a compound of formula II':



Formula II'

wherein:

(i) Alk is C_{1-6}alkylene (e.g., n-propylene, n-butylene, n-pentylene) optionally substituted with one or more C_{1-6}alkyl (e.g., methyl) or one C_{1-4}alkoxy (e.g., ethoxy) group;

(ii) A is aryl (e.g., phenyl) optionally substituted with one or more C_{1-4}alkyl (e.g., methyl) or halo (e.g., Cl, F);

(iii) R_1 is H or C_{1-4}alkyl (e.g., methyl, ethyl, n-propyl or isopropyl);

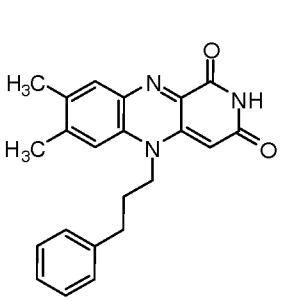
(iv) R_2 is H or C_{1-4}alkyl (e.g., methyl or n-propyl);

(v) R_3 is H;

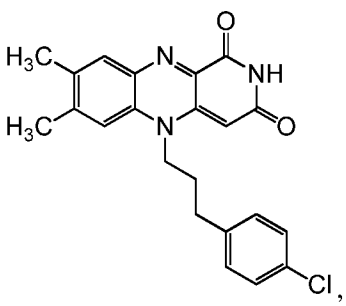
(vi) R_4 is H;

in free or salt form.

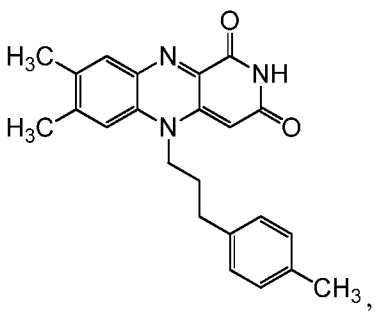
3. The compound according to claim 1 or 2 selected from any one of the following:



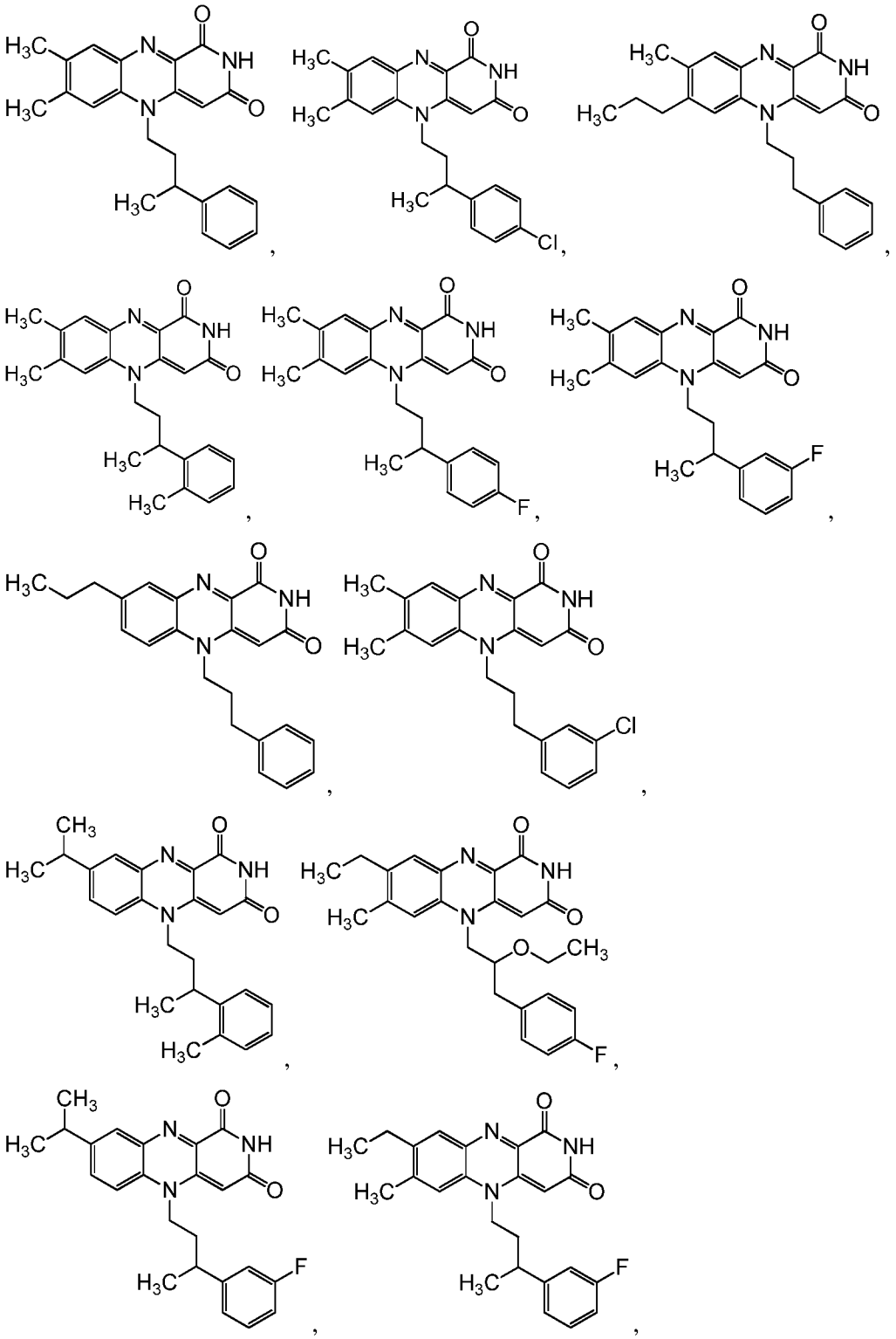
,

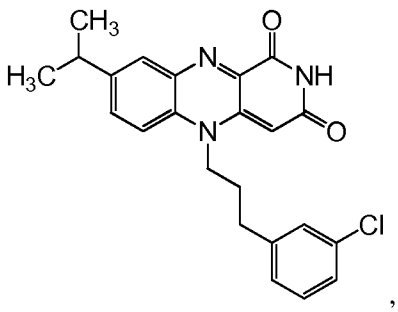


,

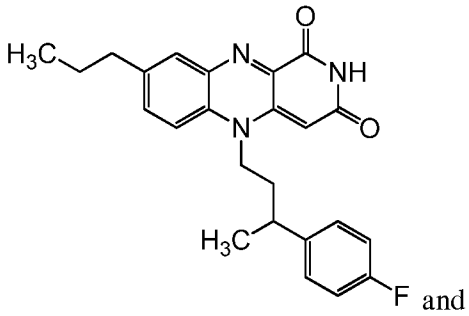


,

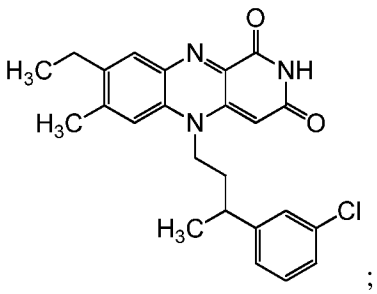




,



and



;

in free or salt form.

4. A pharmaceutical composition comprising the compound according to any one of claims 1-3, in free or pharmaceutically acceptable salt form, in admixture with a pharmaceutically acceptable diluent or carrier.

5. A method for the treatment or prophylaxis of a bacterial infection comprising administering to a patient in need of such treatment an effective amount of a compound according to any one of claims 1-3, in free or pharmaceutically acceptable salt form.

6. The method according to any one of claims 5, wherein the infection is a Gram-positive or Gram-negative bacterial infection.

7. The method according to any one of claims 5-6, wherein the bacterial infection is selected from a group consisting of *Clostridium difficile, Staphylococcus epidermidis, Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Escherichia coli*, *Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Listeria monocytogenes*, *Salmonella enterica*, *Vibrio cholerae*, *Brucella melitensis*, *Bacillus anthracis*, *Francisella tularensis*, *Moraxella catarrhalis, Klebsiella pneumoniae, Yersinia*

pestis, *Streptococcus viridans, Enterococcus faecium,* and *Borrelia burgdorferi* .

8. The method according to any one of claims 5-7, wherein the bacterial infection is a *C. difficile* infection.

9. The method according to any one of claims 5-7, wherein the bacterial infection is a *Staphylococcus aureus* infection.

10. The method according to any of claims 5-9, wherein said infection is by an infectious agent which is resistant to a drug that is not a riboswitch ligand.

11. The method according to claim 9 or 10, wherein the infection is an infection which is resistant to one or more drugs selected from a group consisting of a penicillin, vancomycin, cephalosporin and methicillin.

12. The method according to claim 11, wherein the infection is a methicillin-resistant *Staphylococcus aureus* infection.

13. The method according to claim 8 or 10, wherein the infection is by an infectious agent which is resistant to fluoroquinolone (e.g., ciprofloxacin- and/or levofloxacin-resistant infection), metronidazole and/or vancomycin.

14. The method according to any one of claims 5-13, wherein such method is effective for the treatment or prophylaxis of a disease, condition or infection selected from a group consisting of anthrax, staphylococcal scalded skin syndrome (staph infections), pneumonia, impetigo, boils, cellulitis, folliculitis, furuncles, carbuncles, scalded skin syndrome, abscesses, meningitis, osteomyelitis endocarditis, Toxic Shock Syndrome (TSS), septicemia, acute sinusitis, otitis media, septic arthritis, endocarditis, peritonitis, pericarditis, brain abscess, tularemia, urinary tract infection, empyema, food poisoning, diarrhea, conjunctivitis and clostridium difficile associated disease (CDAD).

15. A method for the treatment or prophylaxis of a bacterial infection in a plant comprising administering to said plant an effective amount of a compound of any one of claims 1-3, in free or pharmaceutically acceptable salt form.

16. Use of a compound according to any one of claims 1-3, in free or pharmaceutically acceptable salt form in the manufacture of a medicament for the treatment or prophylaxis of a bacterial infection as described in any one of claims 5-15.

17. A pharmaceutical composition according to any one of claims 1-3, in free or pharmaceutically acceptable salt form for use in the manufacture of a medicament for the treatment or prophylaxis of a bacterial infection as described in any one of claims 5-15.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24507

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/60; A61K 31/50, 31/495 (2012.01)
USPC - 514/250
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)
USPC: 514/250

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/249; 544/344, 345 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,EPAB,JPAB), Google Scholar
$flavin, riboflavin analog, derivative, mimic, mimetic, $isoalloxazine, $phenylpropyl$isoalloxazine, riboswitch

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/019208 A1 (DIXON et al.) 18 February 2010 (18.02.2010) para [0005]-[0007], pg 95 This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP	1-3
Y	CARLSON et al. Improved Chemical Syntheses of 1- and 5-Deazariboflavin, J. Org. Chem., 2004, Vol 69, pp 2614-2617; Abstract; pg 2614, col 1, para 1 to col 2, para 1; Fig 1 Downloaded from: http://www.biochem.wisc.edu	1-3
Y	US 3,920,650 A (SPENCER) 18 November 1975 (18.11.1975) col 1, ln 3-21; col 7, ln 57 to col 8, ln 22; claim 5	1-3
A	KITTLEMAN et al. Characterization and Mechanistic Studies of Type II Isopentenyl Diphosphate: Dimethylallyl Diphosphate Isomerase from Staphylococcus aureus. Biochemistry, 2007, Vol 46(28): pp 8401-8413; entire document Downloaded from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2515275/pdf/nihms60389.pdf	1-3
A	Ott et al. The RFN riboswitch of Bacillus subtilis is a target for the antibiotic roseoflavin produced by Streptomyces davawensis. RNA Biology, 2009, Vol 6:3, pp 276-280; Fig 1 Downloaded from: http://www.landesbioscience.com	1-3

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 May 2012 (05.05.2012)	31 MAY 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/24507

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

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

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

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

3. ☒ Claims Nos.: 4-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ 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.:

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.