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[Continued on next page]

(54) Title: PYRROLOMYCINS AND METHODS OF USING THE SAME

(57) Abstract: Provided herein are pyrrolomycin derivatives, which can be used to modulate Mcl-1, inhibit proliferation of bacteria and pathogens, as well as to treat infectious diseases and cancers.

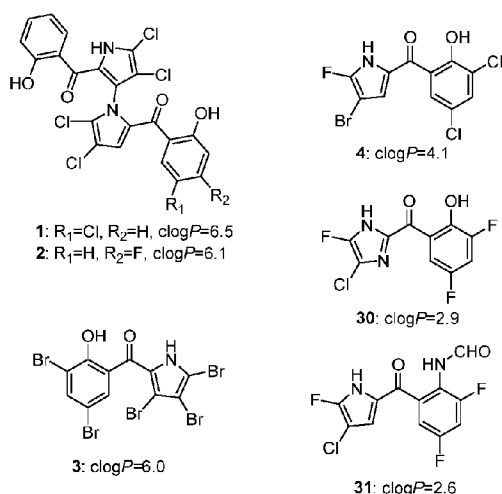


Figure 1

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PYRROLOMYCINS AND METHODS OF USING THE SAME

BACKGROUND

[0001] Bacterial resistance to antibiotics is a widespread problem with grave implications for public health. Most bacteria have a genome that consists of a single chromosome and, consequently, replicate using a process that is simpler than mitosis or meiosis. Because bacteria can grow and divide at a rate that is much faster than that of eukaryotic cells, they can undergo evolution, i.e., natural selection, to produce a significant number of multidrug-resistant strains on a time scale that is short enough to pose a serious threat to human health worldwide. Much of the rise in multidrug-resistant bacterial strains can be attributed to the overuse and misuse of antibiotics, by patients and physicians alike. Multidrug-resistant bacterial strains may also be engineered by persons with nefarious goals, e.g., bioterrorists. Therefore, the development of new antibiotics must be an ongoing endeavor.

[0002] *Bacillus anthracis* (BA) and *Staphylococcus aureus* (SA) are important human pathogens, but for very different reasons. BA is the causative agent of anthrax, which is a serious and fatal disease, and is considered an agent of biological warfare or terrorism.^{1,2} Although it holds great potential as a biological weapon of mass destruction (WMD), the intentional use of this organism to infect our population has, to date, been limited to the letter attacks in 2001. While efforts to develop better-defined vaccines to the prophylaxis of anthrax are actively ongoing, their use for administration to humans is limited and their availability is very restricted.³ Although doxycycline and ciprofloxacin can be used to treat anthrax upon immediate administration to infected patients,^{4,5} BA resistance to ciprofloxacin, doxycycline and macrolides have appeared in the literature.⁶⁻⁸ Furthermore, capable terrorists can engineer the resistance to these antibiotics.

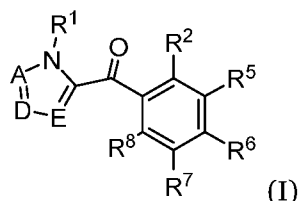
[0003] In contrast to BA, SA is one of the most common causes of infection in the U.S., attributable in large part to its propensity to become multidrug-resistant.^{9,10} The Infectious Disease Society of America has classified it as one of the "ESKAPE" pathogens that can readily develop resistance to the biocidal action of antibiotics.^{11,12} Currently, vancomycin is the most common first-line treatment for antibiotic-resistant SA infections.¹³ However, its overutilization has resulted in an increase in vancomycin-resistant *Staphylococcus aureus* strains.^{9,14-16} Further, except for the addition of the oxazolidinone linezolid¹⁷ in 2000, the lipopeptide daptomycin¹⁸ in 2003, and the FDA's recent approval of ceftaroline,¹⁹ tedizolid,²⁰ and dalbavancin,²¹ the options to treat infections caused by SA are limited. Additionally, although these recently approved and late-stage antibiotics in development may prove invaluable in combating SA resistance, these bacteria will almost inevitably develop resistance to new antibacterial agents introduced to the clinic.²² For instance, reports of resistance to linezolid and daptomycin have quickly emerged upon their introduction. In

essence, antibiotic resistance is occurring faster than new compounds can be introduced into clinical practice.

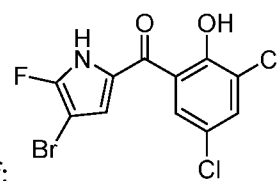
[0004] Compounding this problem is the propensity of SA to form biofilms, which are a leading cause of chronic infections in medical devices and are tolerant to most currently available antibiotics. Biofilm-growing bacteria are known to mutate at a higher frequency compared to planktonic, isogenic bacteria, and thus can more quickly undergo natural selection and gain resistance to antibiotics. Additionally, biofilms thrive under hypoxic conditions, which retards growth, metabolic activity, and protein synthesis. Thus, when bacteria are producing biofilms, they are said to be in a non-replicative, “stationary” phase. Most drugs are ineffective against bacteria in such a phase, even when their planktonic progenitors remain susceptible to the same drugs. To date, no antibiofilm agents have been made available for clinical use. Thus, there is a need for new drug for combating biofilm-related infections.

SUMMARY

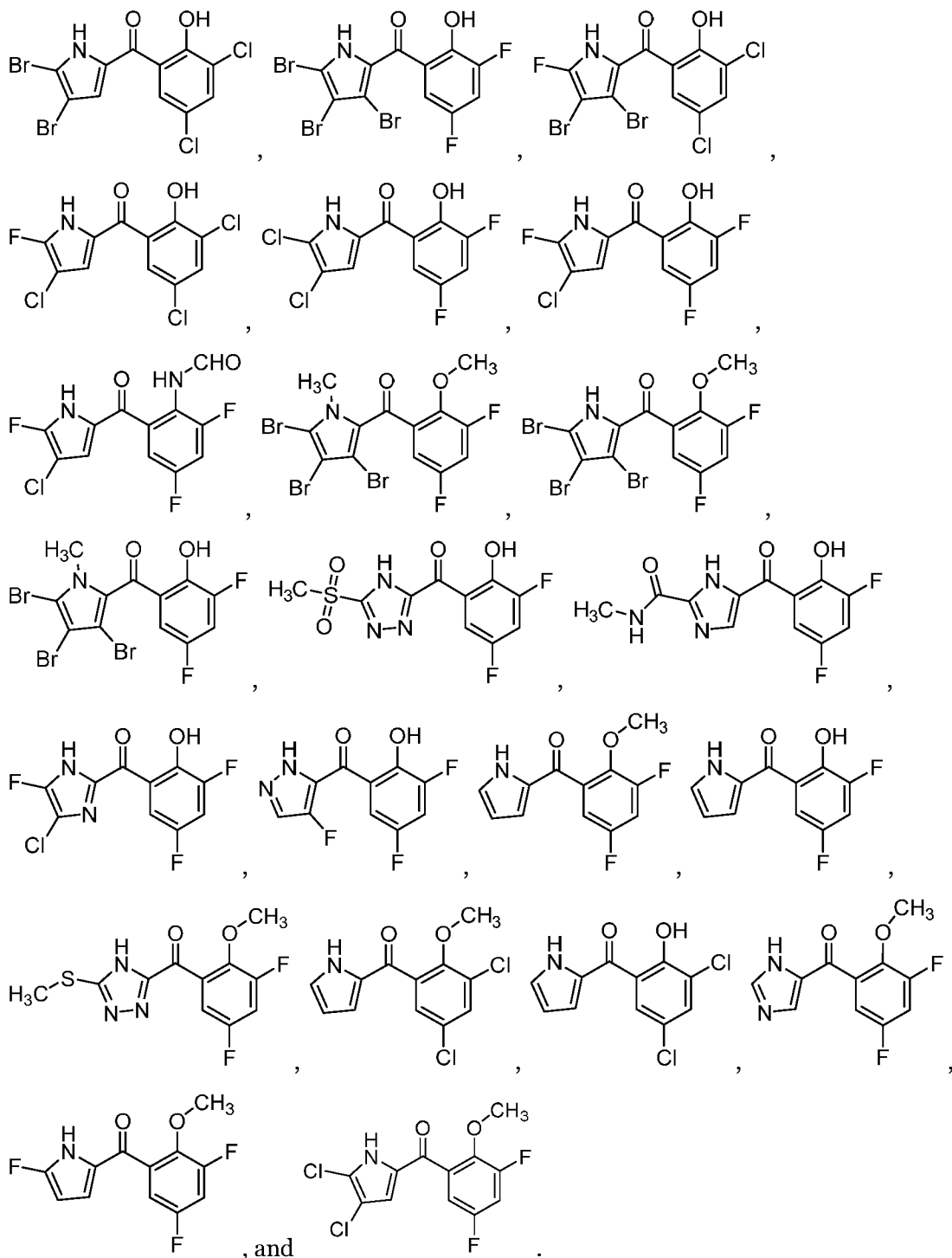
[0005] Provided herein are pyrrolomycin compounds, compositions of pyrrolomycin compounds, and methods of using pyrrolomycins. Provided herein are compounds, or pharmaceutically acceptable salts thereof, of formula (I):



wherein R¹ is H, C₁₋₁₀alkyl, or COR⁹; R² is OH, C₁₋₁₀alkoxy, NHC(O)R⁹, or NHSO₂R¹⁰; A and D are each CR³ or N; E is CR⁴ or N; each R³ is H, halogen, C₁₋₁₀haloalkyl, C₁₋₁₀haloalkoxy, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹; R⁴ is H, halogen, C₁₋₁₀haloalkyl, or C₁₋₁₀haloalkoxy; R⁵, R⁶, R⁷, and R⁸ are each H, F, Cl, CF₃, or OCF₃; R⁹ is H, C₁₋₁₀alkyl, or C₆₋₁₀aryl; and R¹⁰ is C₁₋₁₀alkyl or C₆₋₁₀aryl, with the proviso that (a) at least one of A, D, and E is other than N, (b) at least one R³ is other than H, and (c) at least one of R⁵, R⁶, R⁷, and R⁸ is other than H. In some cases, R¹ is H or C₁₋₁₀alkyl. In some cases, R¹ is H. In various cases, R² is NHC(O)R⁹ or NHSO₂R¹⁰. In various cases, R² is OH or OCH₃. In some cases, R² is OH. In various cases, R³ is halogen, CF₃, OCF₃, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹. In various cases, R³ is CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹. In various cases, R³ is F, Cl, or Br. In some cases, R³ is F or Cl. In various cases, R⁴ is H, halogen, CF₃, or OCF₃. In various cases, R⁴ is F, Br, or Cl. In various cases, R⁵ and R⁷ are each H. In various cases, R⁶ and R⁸ are each independently F or Cl. In some cases,



the compound has a structure selected from the group consisting of:



[0006] Further provided are pharmaceutical formulations comprising a compound as disclosed herein and a pharmaceutically acceptable excipient.

[0007] Also provided are methods of inhibiting the growth or proliferation of bacteria in a subject, comprising contacting the bacteria with a compound or a composition as disclosed herein in an amount sufficient to inhibit the growth or proliferation of the bacteria. In some cases, the bacteria are Gram-positive bacteria. In some cases, the bacteria are Gram-negative bacteria. In various cases, the bacteria are of a genus selected from *Staphylococcus*, *Enterococcus*, *Bacillus*, *Streptococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Clostridium*, *Citrobacter*, *Serratia*, *Neisseria*, *Corynebacterium*, *Cyanobacterium*, *Salmonella*, *Shigella*, *Helicobacter*, *Brucella*, *Borrelia*, *Bordetella*, *Bartonella*, *Bacteroides*, *Burkholderia*, *Mycobacterium*, *Mycoplasma*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Francisella*, *Haemophilus*, *Legionella*, *Leptospira*, *Listeria*, *Rickettsia*, *Vibrio*, *Ureaplasma*, *Yersinia*, *Treponema*, *Proteus*, *Stenotrophomonas*, *Plesiomonas*, *Nocardia*, *Actinomyces*, *Moraxella*, *Erysipelothrix*, *Actinobacillus*, *Anaplasma*, *Pasteurella*, *Alcaligenes*, *Achromobacter*, and *Candida*.

[0008] Further provided are methods of killing a pathogen in a subject, comprising contacting the pathogen with a compound or a composition as disclosed herein in an amount sufficient to kill the pathogen. In some cases, the pathogen can be Gram-positive bacteria. In some cases, the pathogen can be Gram-negative bacteria. In various cases, the pathogen can be bacteria of a genus selected from *Staphylococcus*, *Enterococcus*, *Bacillus*, *Streptococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Clostridium*, *Citrobacter*, *Serratia*, *Neisseria*, *Corynebacterium*, *Cyanobacterium*, *Salmonella*, *Shigella*, *Helicobacter*, *Brucella*, *Borrelia*, *Bordetella*, *Bartonella*, *Bacteroides*, *Burkholderia*, *Mycobacterium*, *Mycoplasma*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Francisella*, *Haemophilus*, *Legionella*, *Leptospira*, *Listeria*, *Rickettsia*, *Vibrio*, *Ureaplasma*, *Yersinia*, *Treponema*, *Proteus*, *Stenotrophomonas*, *Plesiomonas*, *Nocardia*, *Actinomyces*, *Moraxella*, *Erysipelothrix*, *Actinobacillus*, *Anaplasma*, *Pasteurella*, *Alcaligenes*, *Achromobacter*, or *Candida*.

[0009] Also provided are methods of inhibiting the growth of biofilms, comprising contacting the biofilm with a compound or a composition as disclosed herein in an amount sufficient to inhibit the growth of biofilms. In some cases, the biofilm is due to a pathogen. In some cases, the pathogen can be Gram-positive bacteria. In some cases, the pathogen can be Gram-negative bacteria. In various cases, the pathogen can be bacteria of a genus selected from *Staphylococcus*, *Enterococcus*, *Bacillus*, *Streptococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Clostridium*, *Citrobacter*, *Serratia*, *Neisseria*, *Corynebacterium*, *Cyanobacterium*, *Salmonella*, *Shigella*, *Helicobacter*, *Brucella*, *Borrelia*, *Bordetella*, *Bartonella*, *Bacteroides*, *Burkholderia*, *Mycobacterium*, *Mycoplasma*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Francisella*, *Haemophilus*, *Legionella*, *Leptospira*, *Listeria*, *Rickettsia*, *Vibrio*, *Ureaplasma*, *Yersinia*,

Treponema, Proteus, Stenotrophomonas, Plesiomonas, Nocardia, Actinomyces, Moraxella, Erysipelothrix, Actinobacillus, Anaplasma, Pasteurella, Alcaligenes, Achromobacter, or Candida.

[0010] The foregoing summary is not intended to define every aspect of the disclosure, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document.

[0011] In addition to the foregoing, the disclosure includes, as an additional aspect, all embodiments of the disclosure narrower in scope in any way than the variations specifically mentioned above. With respect to aspects described or claimed with “a” or “an,” it should be understood that these terms mean “one or more” unless context unambiguously requires a more restricted meaning. With respect to elements described as one or more within a set, it should be understood that all combinations within the set are contemplated. If aspects of the invention are described as “comprising” a feature, embodiments also are contemplated “consisting of” or “consisting essentially of” the feature.

[0012] Although the applicant(s) invented the full scope of the claims appended hereto, the claims appended hereto are not intended to encompass within their scope the prior art work of others. Therefore, in the event that statutory or judicially-recognized prior art within the scope of a claim is brought to the attention of the applicants by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention. Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, and all such features are intended as aspects of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 shows known pyrrolomycins, their parent marinopyrroles, and bioisosteres of pyrrolomycins.

[0014] Figure 2 shows (A) the bactericidal activity of pyrrolomycins **4** and **18** against *Bacillus anthracis*, compared with that of ciprofloxacin, after 1 h, 3 h, 6 h, and 24 h periods; and (B) the bactericidal activity of pyrrolomycins **4** and **18** against *Staphylococcus aureus* compared with that of vancomycin.

[0015] Figure 3 shows the minimum bactericidal concentration of pyrrolomycin **4** against *Staphylococcus aureus* after 24 h compared with that of vancomycin.

[0016] Figure 4 shows the anti-biofilm activity of pyrrolomycin **4** against *Staphylococcus aureus* compared with that of vancomycin after 24 h, 48 h, and 120 h.

[0017] Figure 5 shows the plasma concentration of pyrrolomycin **4** in mice after oral administration as a function of time.

DETAILED DESCRIPTION

[0018] Provided herein are compounds of formula (I) which are pyrrolomycin derivatives. The compounds disclosed herein are potent antibiofilm agents.

Compounds of formula (I)

[0019] Marinopyrrole derivatives **1** and **2** (Figure 1) have recently been reported as potent anti-methicillin resistant *Staphylococcus aureus* (MRSA) agents with MIC (minimum inhibitory concentration) values in the submicrogram/mL range.²³⁻²⁵ A series of compounds called 'pyrrolomycins' were reported to have anti- Gram-positive activity, and pyrrolomycin **3** also showed antibiofilm activity with MIC in the submicrogram/mL range. Like pyrrolomycin **3**, marrinopyrroles **1** and **2** have clogP values that are beyond the desired range of therapeutic drugs as stipulated by Lipinski's rule of five (RO5).²⁶ In order to improve their physicochemical and drug-like properties, the bis-pyrrole marinopyrroles were truncated into monomers and fluorine atoms to both aromatic rings were introduced, resulting in a series of novel pyrrolomycins and their bioisosteres (**4**, **30**, and **31** in Figure 1) with the clogP values of up to four log units lower than those of pyrrolomycin **3** and marinopyrrole **1**. These results demonstrated that novel fluorinated pyrrolomycin not only exhibited potent activity against SA with an MIC of 73 ng/mL but also showed a lack of mammalian cell cytotoxicity. Most importantly, these compounds demonstrated potent antibiofilm activity at a concentration of 8.0 µg/mL without the occurrence of bacterial resistance. In addition, novel A, B, C, D, and E Series compounds (Chart 1) have been designed to further improve the physicochemical and drug-like properties of these compounds by bioisosteric replacement of the pyrrole and dihalophenol moieties.

[0020] These results suggest that a network of hydrogen bond donor-acceptor-donor (present by the pyrrole NH-carbonyl-phenol OH) is important for anti-SA activity. Therefore, seven more series of novel pyrrolomycins and their bioisosteric replacements of both pyrrole and dihalophenol moieties were designed (Chart 1). Besides introduction of additional functional groups to both the pyrrole and benzene rings in the A1 Series, the bioisosteric replacement of the pyrrole with imidazole (B Series and C Series), pyrazole (D Series) and triazole (E Series) is investigated. The electron-withdrawing substituents on the pyrrole in A Series can stabilize the pyrrole system to avoid potential metabolic liability.^{27,28}

Toxicity associated with the pyrroles bearing electron-donating groups was documented. The amide (A2 series) and sulfonamide (A3 Series) are designed as surrogates for the dihalophenol moiety of the pyrrolomycins. The NH from amide or sulfonamide groups is expected to serve as a hydrogen bond donor as that of the phenol OH group. This replacement is designed to avoid the potential metabolic liability of the dihalophenol group. Fluorine atoms are incorporated since fluorinated pyrrolomycins have improved physicochemical, drug-like properties and antibacterial activities, and as fluorinated drugs often have improved bioactivities and pharmacokinetic and pharmacodynamic profiles.²⁹⁻³¹ These bioisosteric replacements generate new chemical entities. In turn, bioisosteric replacements are expected to alter the metabolism and improve the drug-like properties. Electron-withdrawing groups F, Cl, CF₃, CN, carboxamide, sulfonamide, and sulfone in the A-E Series compounds reduces the electron density and/or mask sites of the five-membered heteroaromatic rings to avoid generation of reactive metabolites by cytochrome P-450.

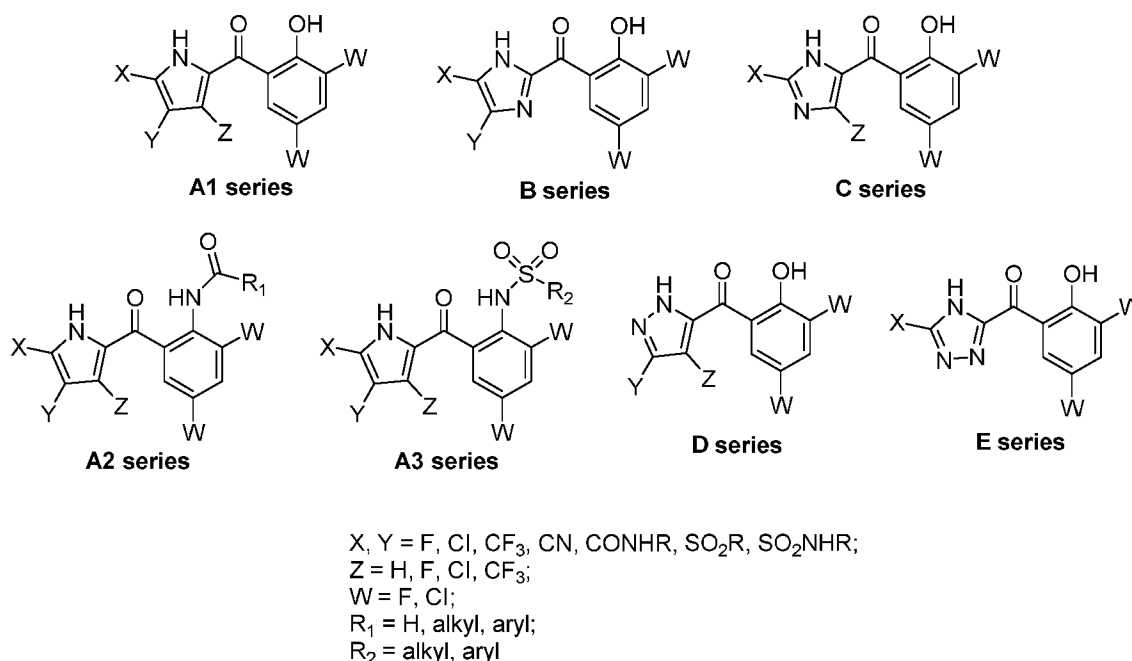
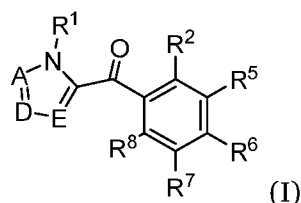


Chart 1. Novel pyrrolomycins and their bioisosteres

[0021] Thus, provided herein are compounds, or pharmaceutically acceptable salts thereof, having a structure of formula (I):



wherein R¹ is H, C₁₋₁₀alkyl, or COR⁹; R² is OH, C₁₋₁₀alkoxy, NHC(O)R⁹, or NHSO₂R¹⁰; A and D are each CR³ or N; E is CR⁴ or N; each R³ is H, halogen, C₁₋₁₀haloalkyl, C₁₋₁₀haloalkoxy, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹; R⁴ is H, halogen, C₁₋₁₀haloalkyl, or C₁₋₁₀haloalkoxy; R⁵, R⁶, R⁷,

and R⁸ are each H, halogen, CF₃, or OCF₃; R⁹ is H, C₁₋₁₀alkyl, or C₆₋₁₀aryl; and R¹⁰ is C₁₋₁₀alkyl or C₆₋₁₀aryl, with the proviso that (a) at least one of A, D, and E is other than N, (b) at least one R³ is other than H, and (c) at least one of R⁵, R⁶, R⁷, and R⁸ is other than H.

[0022] As used herein, the term “C₁₋₁₀alkyl” refers to substituted or unsubstituted saturated hydrocarbon groups, including straight-chain alkyl and branched-chain alkyl groups that contain from 1 to 10 carbons in the chain, including haloalkyl groups such as trifluoromethyl and 2,2,2-trifluoroethyl, etc. C₀alkyl indicates a hydrogen where the group is in a terminal position, a bond if internal.

[0023] As used herein, the term “C₆₋₁₀aryl” refers to a monocyclic or polycyclic aromatic group that contains from 6 to 10 carbon atoms, preferably a monocyclic or bicyclic aromatic group, e.g., phenyl or naphthyl. Unless otherwise indicated, an aryl group can be unsubstituted or substituted with one or more, and in particular one to four groups independently selected from, for example, halogen, alkyl, alkenyl, OCF₃, NO₂, CN, NC, OH, alkoxy, amino, CO₂H, CO₂alkyl, aryl, and heteroaryl. Exemplary aryl groups include, but are not limited to, phenyl, naphthyl, tetrahydronaphthyl, chlorophenyl, methylphenyl, methoxyphenyl, trifluoromethylphenyl, nitrophenyl, 2,4-methoxychlorophenyl, and the like. In some specific cases, the aryl can be substituted with one or more of halogen, alkyl and alkoxy.

[0024] As used herein, the term “alkoxy” refers to an alkyl group having an oxygen attached thereto. Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

[0025] As used herein, the term “haloalkyl” refers to an alkyl substituted by one or more fluorine, chlorine, bromine and/or iodine atoms. In some embodiments, the alkyl group is substituted by one, two, or three fluorine and/or chlorine atoms. In some embodiments, the haloalkyl group is a C₁₋₁₀ haloalkyl group. Non-limiting exemplary haloalkyl groups include fluoromethyl, 2-fluoroethyl, difluoromethyl, trifluoromethyl, pentafluoroethyl, 1,1-difluoroethyl, 2,2-difluoroethyl, 2,2,2-trifluoroethyl, 3,3,3-trifluoropropyl, 4,4,4-trifluorobutyl, and trichloromethyl groups.

[0026] As used herein, the term “haloalkoxy” refers to a haloalkyl attached to a terminal oxygen atom. Non-limiting exemplary haloalkoxy groups include fluoromethoxy, difluoromethoxy, trifluoromethoxy, and 2,2,2-trifluoroethoxy.

[0027] In some embodiments, A and D are each CR³ and E is CR⁴. In some embodiments, A and D are each CR³, and E is N. In some embodiments, A is CR³, D is N, and E is CR⁴. In some embodiments, A is CR³, and D and E are N. In some embodiments, A is N, D is CR³, and E is CR⁴. In some cases, the compound has a structure of Series A1, A2, or A3. In some cases, the compound has a structure of Series B, C, D, or E.

[0028] In some embodiments, R³ is halogen, CF₃, OCF₃, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹. In some embodiments, R³ is CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹. In some embodiments, R³ is F, Cl, or Br. In some embodiments, R³ is F or Cl.

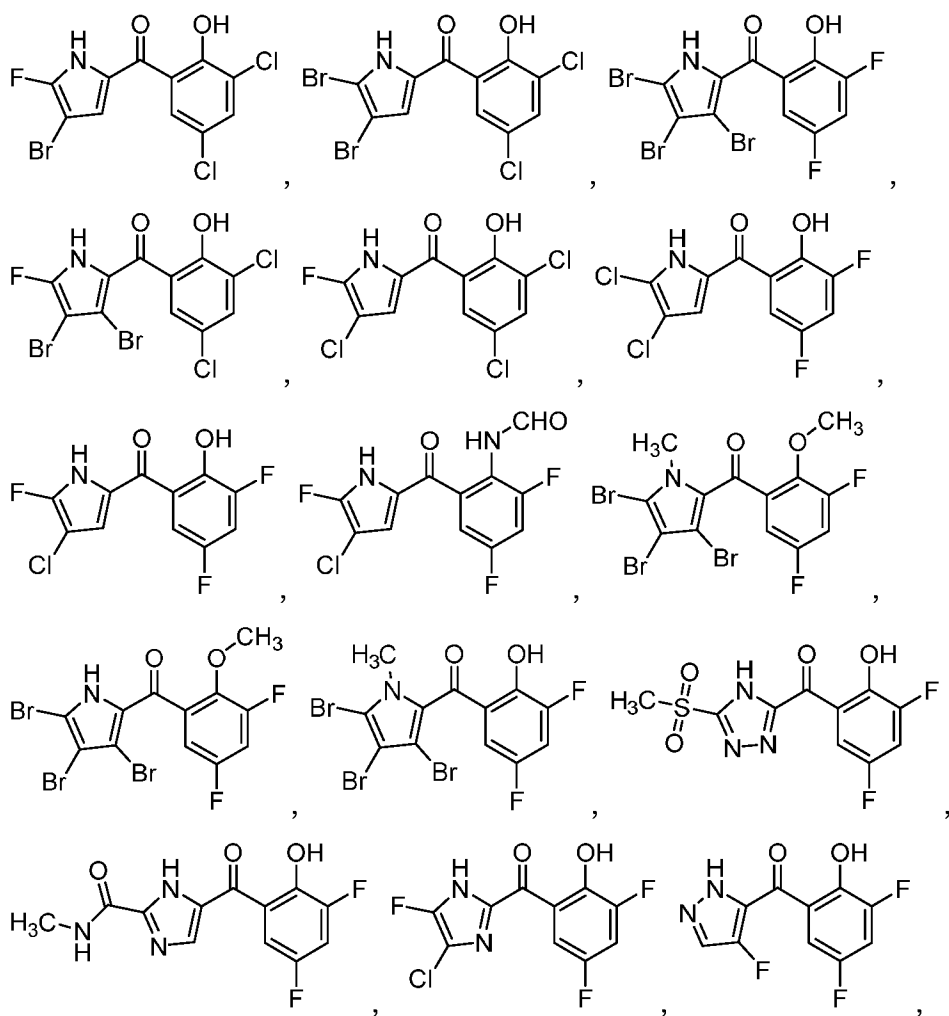
[0029] In some embodiments, R⁴ is H, halogen, CF₃, or OCF₃. In some cases, R⁴ is F or Cl.

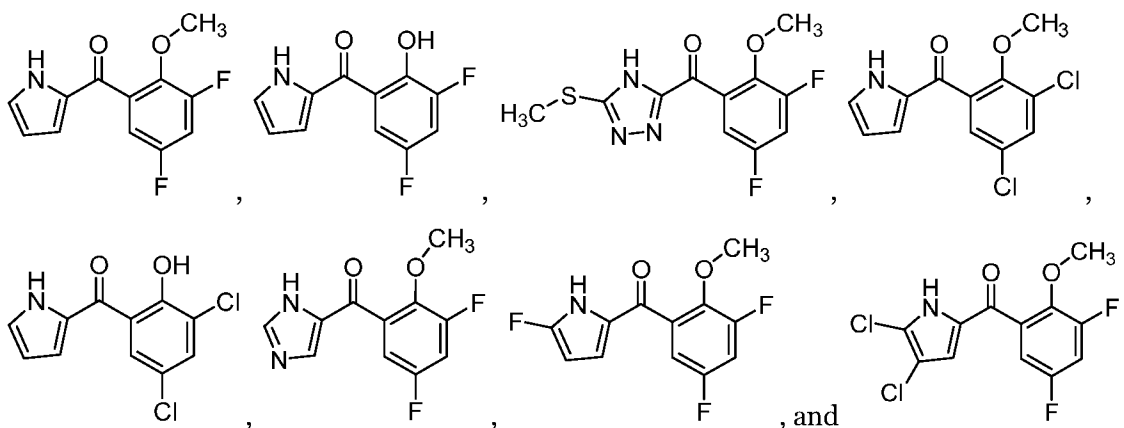
[0030] In some embodiments, R² is NHC(O)R⁹ or NHSO₂R¹⁰. In some embodiments, R² is OH or OCH₃. In some embodiments, R² is OH.

[0031] In some embodiments, R¹ is H or C₁₋₁₀alkyl. In some embodiments, R¹ is H.

[0032] In some embodiments, R⁶ and R⁸ are each H. In some embodiments, R⁷ and R⁹ are each independently F or Cl.

[0033] Specifically contemplated compounds include:





[0034] The compounds described herein also include pharmaceutically acceptable salts of the compounds disclosed herein. As used herein, the term “pharmaceutically acceptable salt” refers to a salt formed by the addition of a pharmaceutically acceptable acid or base to a compound disclosed herein. As used herein, the phrase “pharmaceutically acceptable” refers to a substance that is acceptable for use in pharmaceutical applications from a toxicological perspective and does not adversely interact with the active ingredient. Pharmaceutically acceptable salts, including mono- and bi- salts, include, but are not limited to, those derived from organic and inorganic acids such as, but not limited to, acetic, lactic, citric, cinnamic, tartaric, succinic, fumaric, maleic, malonic, mandelic, malic, oxalic, propionic, hydrochloric, hydrobromic, phosphoric, nitric, sulfuric, glycolic, pyruvic, methanesulfonic, ethanesulfonic, toluenesulfonic, salicylic, benzoic, and similarly known acceptable acids. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418; Journal of Pharmaceutical Science, 66, 2 (1977); and "Pharmaceutical Salts: Properties, Selection, and Use A Handbook; Wermuth, C. G. and Stahl, P. H. (eds.) Verlag Helvetica Chimica Acta, Zurich, 2002 [ISBN 3-906390-26-8.

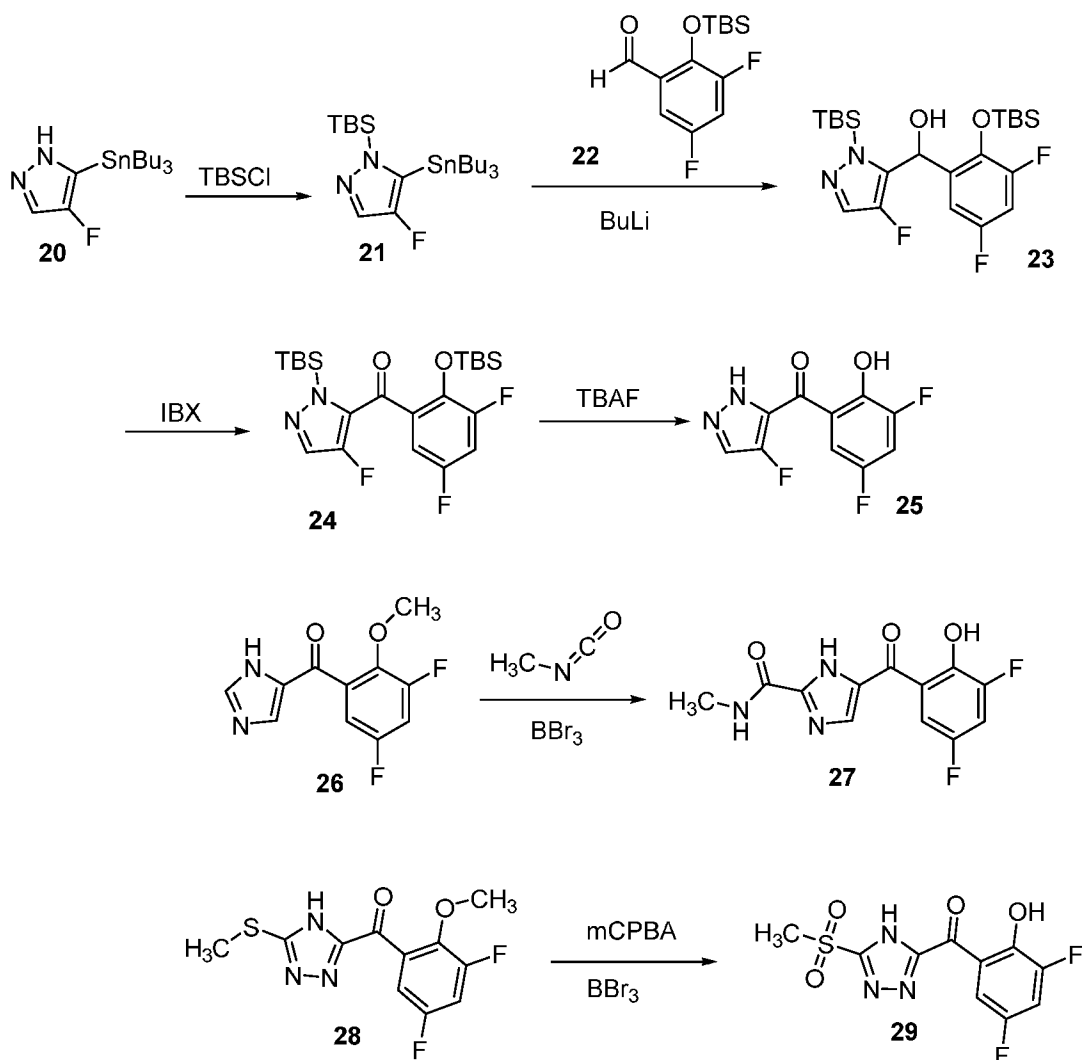
Synthesis of Compounds of formula (I)

[0035] Compounds disclosed herein can be prepared in a variety of ways using commercially available starting materials, compounds known in the literature, or from readily prepared intermediates, by employing standard synthetic methods and procedures either known to those skilled in the art, or in light of the teachings herein. Standard synthetic methods and procedures for the preparation of organic molecules and functional group transformations and manipulations can be obtained from the relevant scientific literature or from standard textbooks in the field. Although not limited to any one or several sources, classic texts such as Smith, M. B., March, J., March' s Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition, John Wiley & Sons: New York, 2001 ; and Greene, T.W., Wuts, P.G. M., Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons: New York, 1999, are useful and recognized reference textbooks of organic synthesis known to those in the art. The following descriptions of synthetic methods are designed to

illustrate, but not to limit, general procedures for the preparation of compounds of the present invention.

[0036] The synthetic processes disclosed herein can tolerate a wide variety of functional groups; therefore various substituted starting materials can be used. The processes generally provide the desired final compound at or near the end of the overall process, although it may be desirable in certain instances to further convert the compound to a pharmaceutically acceptable salt, ester or prodrug thereof.

[0037] Scheme 1 provides representative syntheses of azole-pyrrolomycin bioisosteres. Protection of a commercially available pyrazole **20** with t-butyldimethylsilyl (TBS) furnishes **21**. Transmetalation of tributylstannyl pyrazole **21** with butyllithium (BuLi) at -78°C provides a corresponding lithiated species that reacts with aldehyde **22** to yield alcohol **23**. Oxidation of **23** using 2-iodoxybenzoic acid (IBX) followed by removal of TBS-protecting groups furnishes **25**. Similarly, the corresponding imidazole and triazole precursors, **26** and **28**, yield final compounds **27** and **29**, respectively.



Scheme 1

Pharmaceutical formulations, dosing, and routes of administration

[0038] Compounds disclosed herein can be formulated into a pharmaceutical formulation using a pharmaceutically acceptable excipient. The formulation can include a therapeutically effective amount of the compound.

[0039] The terms “therapeutically effective amount” and “prophylactically effective amount,” as used herein, refer to an amount of a compound sufficient to treat, ameliorate, or prevent the identified disease or condition, or to exhibit a detectable therapeutic, prophylactic, or inhibitory effect. The effect can be detected by, for example, an improvement in clinical condition, reduction in symptoms, or by any of the assays or clinical diagnostic tests described herein. The precise effective amount for a subject will depend upon the subject's body weight, size, and health; the nature and extent of the condition; and the therapeutic or combination of therapeutics selected for administration. Therapeutically and prophylactically effective amounts for a given situation can be determined by routine experimentation that is within the skill and judgment of the clinician.

[0040] Dosages of the therapeutic can alternately be administered as a dose measured in mg/kg. Contemplated mg/kg doses of the disclosed therapeutics include about 0.001 mg/kg to about 1000 mg/kg. Specific ranges of doses in mg/kg include about 0.1 mg/kg to about 500 mg/kg, about 0.5 mg/kg to about 200 mg/kg, about 1 mg/kg to about 100 mg/kg, about 2 mg/kg to about 50 mg/kg, and about 5 mg/kg to about 30 mg/kg.

[0041] As herein, the compounds described herein may be formulated in pharmaceutical compositions with a pharmaceutically acceptable excipient, carrier, or diluent. The compound or composition comprising the compound is administered by any route that permits treatment of the disease or condition. One route of administration is oral administration. Additionally, the compound or composition comprising the compound may be delivered to a patient using any standard route of administration, including parenterally, such as intravenously, intraperitoneally, intrapulmonary, subcutaneously or intramuscularly, intrathecally, topically, transdermally, rectally, orally, nasally or by inhalation. Slow release formulations may also be prepared from the agents described herein in order to achieve a controlled release of the active agent in contact with the body fluids in the gastro intestinal tract, and to provide a substantial constant and effective level of the active agent in the blood plasma. The compound may be embedded for this purpose in a polymer matrix of a biological degradable polymer, a water-soluble polymer or a mixture of both, and optionally suitable surfactants. Embedding can mean in this context the incorporation of micro-particles in a matrix of polymers. Controlled release formulations are also obtained through encapsulation of dispersed micro-particles or emulsified micro-droplets via known dispersion or emulsion coating technologies.

[0042] Administration may take the form of single dose administration, or a compound as disclosed herein can be administered over a period of time, either in divided doses or in a continuous-release formulation or administration method (*e.g.*, a pump). However the compounds of the embodiments are administered to the subject, the amounts of compound administered and the route of administration chosen should be selected to permit efficacious treatment of the disease condition.

[0043] In an embodiment, the pharmaceutical compositions are formulated with one or more pharmaceutically acceptable excipient, such as carriers, solvents, stabilizers, adjuvants, diluents, etc., depending upon the particular mode of administration and dosage form. The pharmaceutical compositions should generally be formulated to achieve a physiologically compatible pH, and may range from a pH of about 3 to a pH of about 11, preferably about pH 3 to about pH 7, depending on the formulation and route of administration. In alternative embodiments, the pH is adjusted to a range from about pH 5.0 to about pH 8. More particularly, the pharmaceutical compositions may comprise a therapeutically or prophylactically effective amount of at least one compound as described herein, together with one or more pharmaceutically acceptable excipients. Optionally, the pharmaceutical compositions may comprise a combination of the compounds described herein, or may include a second active ingredient useful in the treatment or prevention of bacterial infection (*e.g.*, anti-bacterial or anti-microbial agents).

[0044] Formulations, *e.g.*, for parenteral or oral administration, are most typically solids, liquid solutions, emulsions or suspensions, while inhalable formulations for pulmonary administration are generally liquids or powders. A pharmaceutical composition can also be formulated as a lyophilized solid that is reconstituted with a physiologically compatible solvent prior to administration. Alternative pharmaceutical compositions may be formulated as syrups, creams, ointments, tablets, and the like.

[0045] The term “pharmaceutically acceptable excipient” refers to an excipient for administration of a pharmaceutical agent, such as the compounds described herein. The term refers to any pharmaceutical excipient that may be administered without undue toxicity.

[0046] Pharmaceutically acceptable excipients are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there exists a wide variety of suitable formulations of pharmaceutical compositions (see, *e.g.*, Remington's Pharmaceutical Sciences).

[0047] Suitable excipients may be carrier molecules that include large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Other exemplary

excipients include antioxidants (*e.g.*, ascorbic acid), chelating agents (*e.g.*, EDTA), carbohydrates (*e.g.*, dextrin, hydroxyalkylcellulose, and/or hydroxyalkylmethylcellulose), stearic acid, liquids (*e.g.*, oils, water, saline, glycerol and/or ethanol) wetting or emulsifying agents, pH buffering substances, and the like. Liposomes are also included within the definition of pharmaceutically acceptable excipients.

[0048] The pharmaceutical compositions described herein are formulated in any form suitable for an intended method of administration. When intended for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, non-aqueous solutions, dispersible powders or granules (including micronized particles or nanoparticles), emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions, and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation.

[0049] Pharmaceutically acceptable excipients particularly suitable for use in conjunction with tablets include, for example, inert diluents, such as celluloses, calcium or sodium carbonate, lactose, calcium or sodium phosphate; disintegrating agents, such as cross-linked povidone, maize starch, or alginic acid; binding agents, such as povidone, starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc.

[0050] Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

[0051] Formulations for oral use may be also presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example celluloses, lactose, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with non-aqueous or oil medium, such as glycerin, propylene glycol, polyethylene glycol, peanut oil, liquid paraffin or olive oil.

[0052] In another embodiment, pharmaceutical compositions may be formulated as suspensions comprising a compound of the embodiments in admixture with at least one pharmaceutically acceptable excipient suitable for the manufacture of a suspension.

[0053] In yet another embodiment, pharmaceutical compositions may be formulated as dispersible powders and granules suitable for preparation of a suspension by the addition of suitable excipients.

[0054] Excipients suitable for use in connection with suspensions include suspending agents (*e.g.*, sodium carboxymethylcellulose, methylcellulose, hydroxypropyl

methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth, gum acacia); dispersing or wetting agents (e.g., a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycethanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate)); and thickening agents (e.g., carbomer, beeswax, hard paraffin or cetyl alcohol). The suspensions may also contain one or more preservatives (e.g., acetic acid, methyl or n-propyl p-hydroxy-benzoate); one or more coloring agents; one or more flavoring agents; and one or more sweetening agents such as sucrose or saccharin.

[0055] The pharmaceutical compositions may also be in the form of oil-in water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth; naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids; hexitol anhydrides, such as sorbitan monooleate; and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

[0056] Additionally, the pharmaceutical compositions may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous emulsion or oleaginous suspension. This emulsion or suspension may be formulated by a person of ordinary skill in the art using those suitable dispersing or wetting agents and suspending agents, including those mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,2-propane-diol.

[0057] The sterile injectable preparation may also be prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile fixed oils may be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids (e.g., oleic acid) may likewise be used in the preparation of injectables.

[0058] To obtain a stable water-soluble dose form of a pharmaceutical composition, a pharmaceutically acceptable salt of a compound described herein may be dissolved in an aqueous solution of an organic or inorganic acid, such as 0.3 M solution of succinic acid, or more preferably, citric acid. If a soluble salt form is not available, the compound may be

dissolved in a suitable co-solvent or combination of co-solvents. Examples of suitable co-solvents include alcohol, propylene glycol, polyethylene glycol 300, polysorbate 80, glycerin and the like in concentrations ranging from about 0 to about 60% of the total volume. In one embodiment, the active compound is dissolved in DMSO and diluted with water.

[0059] The pharmaceutical composition may also be in the form of a solution of a salt form of the active ingredient in an appropriate aqueous vehicle, such as water or isotonic saline or dextrose solution. Also contemplated are compounds which have been modified by substitutions or additions of chemical or biochemical moieties which make them more suitable for delivery (*e.g.*, increase solubility, bioactivity, palatability, decrease adverse reactions, etc.), for example by esterification, glycosylation, PEGylation, etc.

[0060] In some embodiments, the compounds described herein may be formulated for oral administration in a lipid-based formulation suitable for low solubility compounds. Lipid-based formulations can generally enhance the oral bioavailability of such compounds.

[0061] As such, pharmaceutical compositions comprise a therapeutically or prophylactically effective amount of a compound described herein, together with at least one pharmaceutically acceptable excipient selected from the group consisting of medium chain fatty acids and propylene glycol esters thereof (*e.g.*, propylene glycol esters of edible fatty acids, such as caprylic and capric fatty acids) and pharmaceutically acceptable surfactants, such as polyoxyl 40 hydrogenated castor oil.

[0062] In some embodiments, cyclodextrins may be added as aqueous solubility enhancers. Exemplary cyclodextrins include hydroxypropyl, hydroxyethyl, glucosyl, maltosyl and maltotriosyl derivatives of α -, β -, and γ -cyclodextrin. A specific cyclodextrin solubility enhancer is hydroxypropyl- α -cyclodextrin (BPBC), which may be added to any of the above-described compositions to further improve the aqueous solubility characteristics of the compounds of the embodiments. In one embodiment, the composition comprises about 0.1% to about 20% hydroxypropyl- α -cyclodextrin, more preferably about 1% to about 15% hydroxypropyl- α -cyclodextrin, and even more preferably from about 2.5% to about 10% hydroxypropyl- α -cyclodextrin. The amount of solubility enhancer employed will depend on the amount of the compound of the invention in the composition.

Methods of use

[0063] The compounds disclosed herein can modulate Mcl-1 activity. The compounds disclosed herein are capable of inhibiting growth or proliferation of a pathogen, or bacteria. Thus, provided herein are methods of killing a pathogen in a subject by contacting the pathogen or bacteria with a compound or formulation as disclosed herein. In some cases, the pathogen can be Gram-positive bacteria or Gram-negative bacteria. Some specifically contemplated bacteria include *Staphylococcus*, *Enterococcus*, *Bacillus*, *Streptococcus*,

Klebsiella, Acinetobacter, Pseudomonas, Enterobacter, Escherichia, Clostridium, Citrobacter, Serratia, Neisseria, Corynebacterium, Cyanobacterium, Salmonella, Shigella, Helicobacter, Brucella, Borrelia, Bordetella, Bartonella, Bacteroides, Burkholderia, Mycobacterium, Mycoplasma, Campylobacter, Chlamydia, Chlamydophila, Francisella, Haemophilus, Legionella, Leptospira, Listeria, Rickettsia, Vibrio, Ureaplasma, Yersinia, Treponema, Proteus, Stenotrophomonas, Plesiomonas, Nocardia, Actinomyces, Moraxella, Erysipelothrix, Actinobacillus, Anaplasma, Pasteurella, Alcaligenes, Achromobacter, and Candida. In various embodiments, the pathogen is methicillin-resistant, carbapenem-resistant, fluoroquinone-resistant, vancomycin-resistant, or multidrug-resistant.

[0064] The compounds disclosed herein can inhibit the growth of biofilms. The compounds disclosed herein can treat an infectious disease, and/or be used as an antibiotic.

[0065] As used herein, the term "infectious disease" refer to a condition in which an infectious organism or agent is present in a detectable amount in the blood or in a normally sterile tissue or normally sterile compartment of a subject. Infectious organisms and agents include viruses, bacteria, fungi, and parasites. The terms encompass both acute and chronic infections, as well as sepsis.

[0066] The methods disclosed herein can be for a cell, or for an organism. In some cases, the method is practiced on a mammal, e.g., a human.

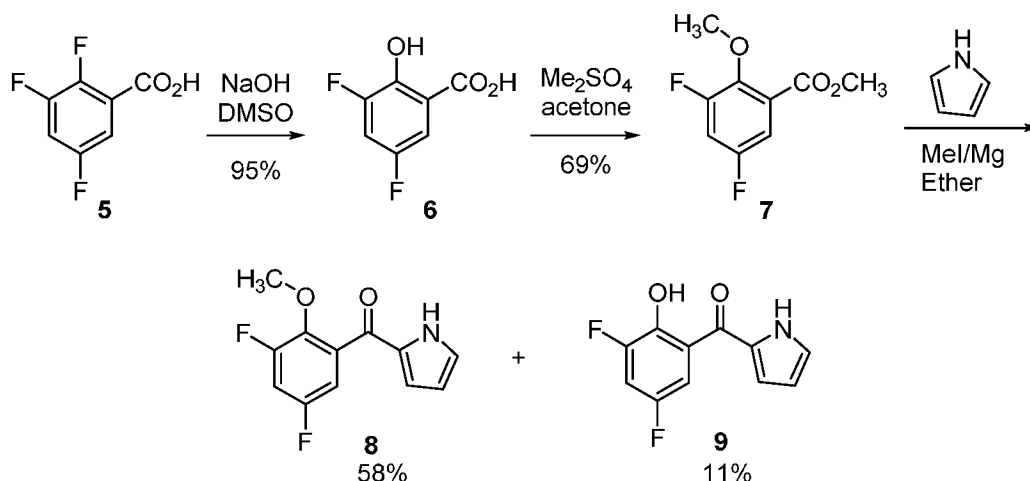
[0067] Aspects described as methods of treatment should also be understood to include first or subsequent "medical use" aspects of the invention or "Swiss use" of compositions for the manufacture of a medicament for treatment of the same disease or condition.

EXAMPLES

[0068] **General Experimental Procedures.** All chemicals and solvents were purchased from commercial suppliers and used without further purification. Melting points were determined on a Stanford Research Systems EZ-Melt apparatus and are uncorrected. Flash column chromatography was performed on silica gel 60, with a mesh size of 0.040-0.063 mm (EMD chemicals, Billerica, MA, USA). ¹H NMR (500 MHz) and ¹³C NMR (125 MHz) spectra were recorded on a Bruker advance III at ambient temperature. High-resolution mass spectra were obtained by electrospray ionization. Analytical HPLC was performed on an Agilent 1260 Infinity with diode array detectors and auto samplers. Preparative HPLC was performed on a Shimadzu LC-20A series. All target compounds after HPLC purification (preparative C18 column, 5 μm, 150 x 21.2 mm and analytical C18 column, 5 μm, 150 x 4.6 mm, UV 214 nm and 254 nm) possessed purities of 95%.

Example 1

[0069] Synthesis of Novel Pyrrolomycin Derivatives.

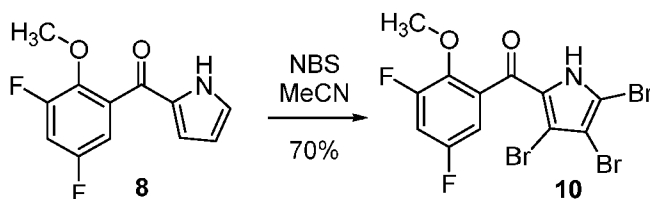
Synthesis of Compounds **8** and **9**

[0070] Compound 8. Step A: After a mixture of 2,3,5-trifluorobenzoic acid (5 g, 28.4 mmol), NaOH (4.5 g, 112.5 mmol) and anhydrous DMSO (60 mL) was stirred at 130 °C for 3 h, the reaction mixture was poured into ice water (200 mL), acidified to ~pH 1, and extracted with ether (3 x 100 mL). The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered and evaporated to give a crude product, 3,5-difluorosalicic acid **6** as a white solid (4.7 g, 95%). ¹H NMR (500 MHz, CDCl₃) δ 10.22 (s, 1H), 7.41 (ddd, *J* = 8.0, 3.0, 2.0 Hz, 1H), 7.15 (dd, *J* = 9.0, 3.0 Hz, 1H). Step B: To a solution of **6** (6.6 g, 37.9 mmol) in acetone (80 mL) were added K₂CO₃ (15 g, 108.7 mmol) and dimethyl sulfate (10 mL, 105.6 mmol). The resulting mixture was refluxed for 18 h, cooled to room temperature, and solvent was evaporated. The residue was partitioned between ether and water. The organic layer was washed with 1N NaOH solution and brine, dried over anhydrous MgSO₄, filtered and evaporated to dryness. The crude product was purified by silica gel column chromatography (4% ethyl acetate in hexane) to afford the desired product as a white solid **7** (5.3 g, 69%). ¹H NMR (500 MHz, CDCl₃) δ 7.28 (ddd, *J* = 8.5, 3.0, 2.0 Hz, 1H), 7.02 (dd, *J* = 9.0, 3.5 Hz, 1H), 3.94 (d, *J* = 0.9 Hz, 3H), 3.92 (s, 3H). Step C: A solution of methyl iodide (CH₃I, 1.24 mL, 19.9 mmol) in ether (10 mL) was added dropwise to a stirred magnesium turning (0.45 g, 18.8 mmol) in ether (5 mL) to maintain a gentle reflux. After the mixture was stirred for 0.5 h at room temperature, pyrrole (1.50 mL, 20.0 mmol) was added dropwise to maintain a gentle reflux. The resulting mixture was refluxed for 0.5 h and cooled to room temperature. To the pyrrolylmagnesium iodide was added dropwise a solution of **7** (2.0 g, 9.9 mmol) in ether (40 mL). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated NH₄Cl solution and extracted with ether (3 x 100 mL). The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered and evaporated to dryness. The residue was purified by silica gel column chromatography (9% ethyl acetate in hexane) to afford the desired product **8** as a brown solid (1.36 g, 58%). mp 85.7 – 87.1 °C. ¹H NMR (500

MHz, CDCl₃) δ 10.65 (s, 1H), 7.22–7.16 (m, 1H), 7.02–6.91 (m, 2H), 6.71–6.66 (m, 1H), 6.32–6.25 (m, 1H), 3.87 (d, $J = 1.3$ Hz, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 182.05 (dd, $J = 3.2, 2.0$ Hz), 157.34 (dd, $J = 245.6, 11.0$ Hz), 155.71 (dd, $J = 250.8, 11.9$ Hz), 142.39 (dd, $J = 12.0, 3.9$ Hz), 135.36 (dd, $J = 7.8, 2.5$ Hz), 131.69 (s), 127.49 (s), 121.57 (s), 111.59 (s), 111.27 (dd, $J = 23.8, 3.7$ Hz), 106.95 (dd, $J = 26.6, 23.2$ Hz), 62.89 (dd, $J = 4.6, 0.6$ Hz); HRMS (EI-TOF) M^+ calcd for C₁₂H₉F₂NO₂ 237.0601, found 237.0603; HPLC purity, 95.6%.

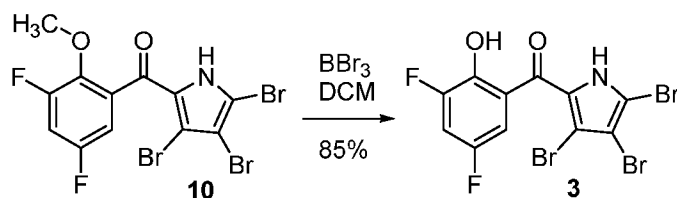
[0071] **Compound 9**. After silica gel column purification of the reaction mixture above and purification by preparative HPLC, a demethylated byproduct **9** was obtained as a yellow solid (0.25 g, 11%). mp 161.7 – 162.5 °C. ¹H NMR (500 MHz, CDCl₃) δ 11.50 (s, 1H), 9.56 (s, 1H), 7.59–7.51 (m, 1H), 7.25–7.18 (m, 1H), 7.14–7.06 (m, 2H), 6.47–6.41 (m, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 184.92 (t, $J = 2.9$ Hz), 153.88 (dd, $J = 241.3, 10.4$ Hz), 151.80 (dd, $J = 251.4, 11.6$ Hz), 147.46 (dd, $J = 12.6, 3.1$ Hz), 129.74 (s), 126.85 (s), 120.59 (dd, $J = 7.8, 4.0$ Hz), 120.42 (s), 112.49 (s), 111.49 (dd, $J = 23.7, 4.0$ Hz), 110.03 (dd, $J = 27.1, 21.3$ Hz); HRMS (EI-TOF) M^+ calcd for C₁₁H₇F₂NO₂ 223.0445, found 223.0444; HPLC purity, 100%.

Synthesis of Compound 10



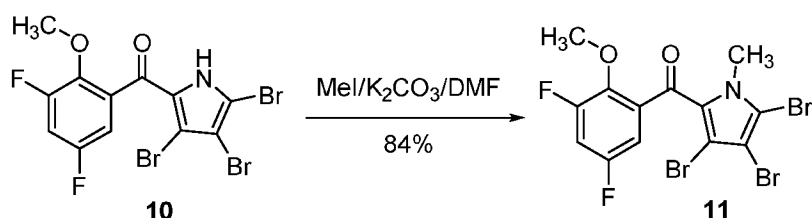
[0072] To a solution of **8** (100 mg, 0.42 mmol) in acetonitrile (2 mL) was added *N*-bromosuccinimide (NBS, 225 mg, 1.27 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 20 h and evaporated to dryness. The residue was partitioned between ether and water. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered and evaporated. The residue was purified by silica gel column chromatography (5% ethyl acetate in hexane) to afford **10** as a white solid (140 mg, 70%). mp 178.9 – 179.7 °C. ¹H NMR (500 MHz, Acetone-*d*₆) δ 12.37 (s, 1H), 7.26 (ddd, $J = 11.6, 8.5, 3.0$ Hz, 1H), 7.06 (ddd, $J = 7.9, 2.8, 1.8$ Hz, 1H), 3.83 (d, $J = 1.5$ Hz, 3H). ¹³C NMR (125 MHz, Acetone-*d*₆) δ 180.48 (dd, $J = 3.5, 2.1$ Hz), 158.53 (dd, $J = 244.8, 11.1$ Hz), 156.00 (dd, $J = 250.7, 12.2$ Hz), 142.65 (dd, $J = 12.0, 3.9$ Hz), 135.48 (dd, $J = 8.4, 2.9$ Hz), 131.22 (s), 111.35 (s), 111.24 (dd, $J = 24.3, 3.7$ Hz), 108.18 (s), 107.68 (dd, $J = 27.0, 23.4$ Hz), 106.19 (s), 62.58 (d, $J = 5.1$ Hz); HRMS (EI-TOF) M^+ calcd for C₁₂H₆Br₃F₂NO₂ 470.7917, found 470.7919; HPLC purity, 100%.

Synthesis of Compound **3**



[0073] To a solution of **10** (60 mg, 0.13 mmol) in CH_2Cl_2 (3 mL) was added a solution of boron tribromide (BBr_3 , 0.38 mL, 1M solution in CH_2Cl_2) dropwise at -78°C . The resulting mixture was stirred at this temperature for 2 h, quenched with water and extracted with CH_2Cl_2 . The organic layer was washed with brine, dried over anhydrous MgSO_4 , filtered and evaporated to dryness. The residue was purified by silica gel column chromatography (6% ethyl acetate in hexane) to afford the desired product as a yellow solid. This compound was purified by preparative HPLC to yield 50 mg (85%): mp $151.8 - 152.7^\circ\text{C}$. ^1H NMR (500 MHz, Acetone- d_6) δ 12.35 (s, 1H), 9.47 (s, 1H), 7.38–7.23 (m, 1H), 7.19–7.06 (m, 1H); ^{13}C NMR (125 MHz, Acetone- d_6) δ 182.50 (dd, $J = 3.3, 2.3$ Hz), 155.58 (dd, $J = 240.1, 10.5$ Hz), 152.17 (dd, $J = 245.2, 11.8$ Hz), 142.44 (dd, $J = 14.9, 3.1$ Hz), 131.19 (s), 127.47 (dd, $J = 8.1, 3.0$ Hz), 112.18 (dd, $J = 24.2, 3.9$ Hz), 110.47 (s), 108.26 (dd, $J = 27.3, 22.5$ Hz), 107.64 (s), 106.11 (s); HRMS $[\text{M}+\text{H}]^+$ calcd for $\text{C}_{11}\text{H}_5\text{Br}_3\text{F}_2\text{NO}_2$ 457.7838, found 457.7852; HPLC purity, 98.6%.

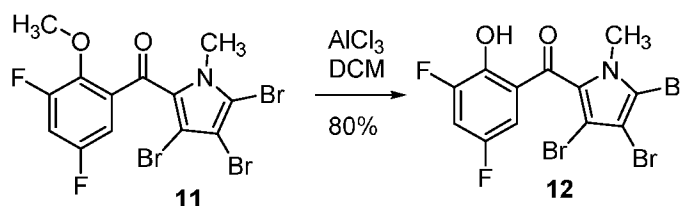
Synthesis of Compound **11**



[0074] To a solution of **10** (63 mg, 0.133 mmol) in DMF (1.0 mL) at room temperature were added K_2CO_3 (55 mg, 0.40 mmol) and iodomethane (0.083 mL, 1.33 mol). The resulting mixture was stirred at room temperature for 3 h, and evaporated to dryness. The residue was partitioned between ether and water. The organic layer was washed with brine, dried over anhydrous MgSO_4 , filtered and evaporated to afford **11** as a white solid (55 mg, 84%). This compound was purified by preparative HPLC. mp $107.4 - 108.1^\circ\text{C}$. ^1H NMR (500 MHz, CDCl_3) δ 6.99 (ddd, $J = 11.2, 8.1, 3.0$ Hz, 1H), 6.83 (ddd, $J = 7.6, 2.9, 1.7$ Hz, 1H), 4.01 (s, 3H), 3.85 (d, $J = 1.8$ Hz, 3H). ^{13}C NMR (125 MHz, CDCl_3) δ 182.12 (dd, $J = 3.5, 2.2$ Hz), 157.71 (dd, $J = 246.7, 10.9$ Hz), 155.24 (dd, $J = 252.2, 11.7$ Hz), 142.39 (dd, $J = 11.8, 3.9$ Hz), 135.17 (dd, $J = 8.0, 3.1$ Hz), 130.56 (s), 117.01 (s), 110.97 (dd, $J = 23.9, 3.7$ Hz), 109.32

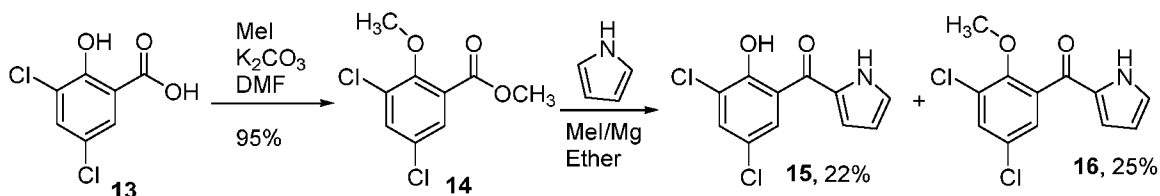
(s), 107.69 (dd, $J = 26.5, 23.1$ Hz), 104.94 (s), 62.23 (d, $J = 5.7$ Hz), 37.63 (s); HRMS (EI-TOF) M^+ calcd for $C_{13}H_8Br_3F_2NO_2$ 484.8073, found 484.8072; HPLC purity, 100%.

Synthesis of Compound **12**



[0075] A mixture of **11** (45 mg, 0.092 mmol) and $AlCl_3$ (74 mg, 0.55 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 2 h to give compound **12** as a yellow solid (35 mg, 80%). This compound was purified by preparative HPLC. mp 156.2 – 157.2 °C. 1H NMR (500 MHz, $CDCl_3$) δ 11.01 (s, 1H), 7.25 – 7.21 (m, 1H), 7.16 (ddd, $J = 10.6, 8.0, 2.9$ Hz, 1H), 3.79 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 188.45 (t, $J = 3.0$ Hz), 153.56 (dd, $J = 241.7, 10.1$ Hz), 151.31 (dd, $J = 251.4, 11.2$ Hz), 147.50 (dd, $J = 12.7, 2.9$ Hz), 129.55 (s), 120.20 (dd, $J = 7.8, 3.8$ Hz), 114.46 (s), 114.24 (dd, $J = 23.8, 4.1$ Hz), 111.57 (dd, $J = 27.2, 21.2$ Hz), 106.09 (s), 104.33 (s), 36.53 (s); HRMS (EI-TOF) M^+ calcd for $C_{12}H_6Br_3F_2NO_2$ 470.7917, found 470.7914; HPLC purity, 100%.

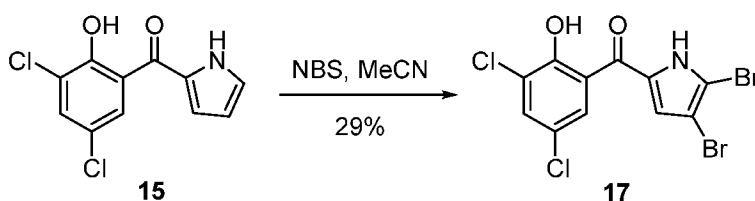
Synthesis of Compounds **15** and **16**



[0076] **Compound 16.** Step A: To a solution of 3,5-dichlorosalicylic acid (**13**, 20.0 g, 0.097 mol) in DMF (150 mL) on an ice water bath were added K_2CO_3 (32.0 g, 0.232 mol) and iodomethane (24.0 mL, 0.384 mol). The resulting mixture was stirred at room temperature for 24 h, and evaporated to dryness. The residue was partitioned between ether and water. The organic layer was washed with 1N NaOH solution and brine, dried over anhydrous $MgSO_4$, filtered and evaporated to afford the desired product **14** as a white solid (21.6 g, 95%). 1H NMR (500 MHz, $CDCl_3$) δ 7.68 (d, $J = 2.7$ Hz, 1H), 7.54 (d, $J = 2.7$ Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H). Step B: A solution of CH_3I (1.33 mL, 21.3 mmol) in ether (10 mL) was added dropwise to a stirred magnesium turning (0.51 g, 21.2 mmol) in ether (5 mL) to maintain a gentle reflux. After stirred for 0.5 h at room temperature, pyrrole (1.50 mL, 21.6 mmol) was added dropwise to maintain a gentle reflux. The resulting mixture was refluxed for 0.5 h and cooled to room temperature. To the pyrrolylmagnesium iodide solution was

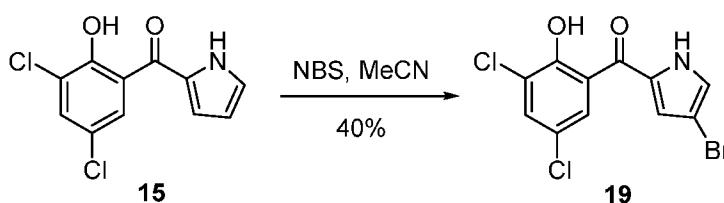
added dropwise the solution of **14** (2 g, 8.5 mmol) in ether (40 mL). After stirred at room temperature for 2 h, the reaction mixture was quenched with saturated NH₄Cl solution and extracted with ether. The organic layer was washed with brine, dried over anhydrous MgSO₄, filtered and evaporated. The residue was purified by silica gel column chromatography (9% ethyl acetate in hexane) to afford the desired product **16** as a brown solid (0.58 g, 25%). ¹H NMR (500 MHz, CDCl₃) δ 9.45 (br s, 1H), 7.51 (d, *J* = 2.6 Hz, 1H), 7.33 (d, *J* = 2.5 Hz, 1H), 7.19–7.15 (m, 1H), 6.71–6.67 (m, 1H), 6.37–6.29 (m, 1H), 3.85 (s, 3H).

[0077] Compound 15. After silica gel column purification of the reaction mixture above, a demethylated byproduct **7** was obtained as a yellow solid (0.5 g, 22%). mp 158.0 – 159.0 °C. ¹H NMR (500 MHz, CDCl₃) δ 12.27 (s, 1H), 9.56 (br s, 1H), 7.96 (d, *J* = 2.5 Hz, 1H), 7.58 (d, *J* = 2.4 Hz, 1H), 7.26–7.21 (m, 1H), 7.13–7.08 (m, 1H), 6.48–6.42 (m, 1H); HRMS (EI-TOF) M⁺ calcd for C₁₁H₇Cl₂NO₂ 254.9854, found 254.9862; HPLC purity, 98.5%. Synthesis of Compound **17**



[0078] To a stirred solution of **15** (30 mg, 0.12 mmol) in CH₃CN (1.0 mL) was added a solution of NBS (63 mg, 0.35 mmol) in CH₃CN (0.5 mL) dropwise on an ice water bath under argon atmosphere. The resulting mixture was stirred at room temperature for 40 h. The precipitate was collected by filtration and washed with ice-cold CH₃CN to give **17** as a yellow solid (15 mg, 29%). This compound was purified by preparative HPLC. ¹H NMR (500 MHz, CDCl₃) δ 11.88 (s, 1H), 9.64 (s, 1H), 7.82 (d, *J* = 2.5 Hz, 1H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.05 (d, *J* = 2.9 Hz, 1H); HRMS (EI-TOF) M⁺ calcd for C₁₁H₅Br₂Cl₂NO₂ 410.8064, found 410.8065; HPLC purity, 100%.

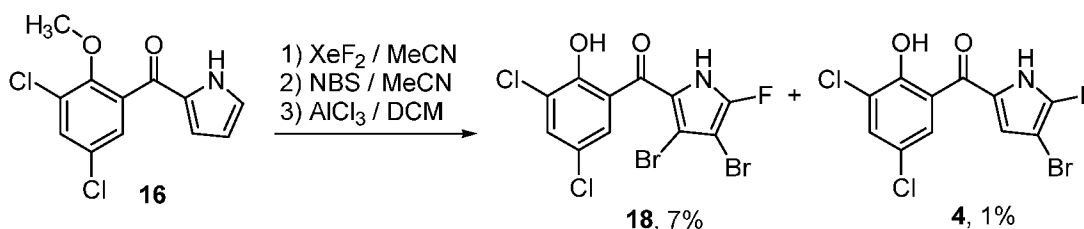
Synthesis of Compound **17**



[0079] To a stirred solution of **15** (100 mg, 0.4 mmol) in CH₃CN (5.0 mL) was added a solution of NBS (71.2 mg, 0.4 mmol) in CH₃CN (1 mL) dropwise on an ice water bath under argon atmosphere. The resulting mixture was stirred at room temperature for 4 h. The

precipitate was collected by filtration and washed with ice-cold CH₃CN to give **19** as a yellow solid (52.8mg, 40%). This compound was purified by preparative HPLC. mp 222.2–223.1 °C. ¹H NMR (500 MHz, Acetone-*d*₆) δ 11.89 (s, 1H), 11.70 (s, 1H), 7.98 (d, *J* = 2.5 Hz, 1H), 7.75 (d, *J* = 2.5 Hz, 1H), 7.49 (dd, *J* = 3.1, 1.3 Hz, 1H), 7.25 (s, 1H). ¹³C NMR (125 MHz, Acetone-*d*₆) δ 184.90 (s), 156.82 (s), 135.12 (s), 130.49 (s), 129.81 (s), 128.31 (s), 124.29 (s), 124.06 (s), 122.47 (s), 122.08 (s), 99.20 (s). HRMS (EI-TOF) M⁺ calcd for C₁₁H₆BrCl₂NO₂ 332.8959, found 332.8957; HPLC purity: 100%.

Synthesis of Compounds **4** and **18**



[0080] Compound 18. To a stirred solution of **16** (1.0 g, 3.7 mmol) in CH₃CN (20 mL) on an ice water bath were added XeF₂ (1.0 g, 5.9 mmol) under argon atmosphere. The resulting mixture was heated to 30 °C for 48 h, and then evaporated to dryness. The residue was treated with NBS (1.3 g, 7.4 mmol) followed by AlCl₃ (1.98 g, 14.8 mmol). After flash chromatography (silica gel/ 2%–9% ethyl acetate in hexane and purification by preparative HPLC, 111 mg of **18** (7%) was obtained. mp 171.9–172.7 °C. ¹H NMR (500 MHz, CDCl₃) δ 10.51 (s, 1H), 9.31 (s, 1H), 7.73 (d, *J* = 2.5 Hz, 1H), 7.59 (d, *J* = 2.4 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃) δ 184.84 (s), 154.83 (s), 148.22 (d, *J* = 274.6 Hz), 135.30 (s), 130.93 (s), 124.06 (s), 123.47 (s), 120.57 (s), 119.79 (s), 108.98 (s), 84.63 (d, *J* = 14.0 Hz); HRMS (EI-TOF) M⁺ calcd for C₁₁H₄Br₂Cl₂FNO₂ 428.7970, found 428.7962; HPLC purity, 97.8%.

[0081] Compound 4. Same as above, after flash chromatography (silica gel/ 2%–9% ethyl acetate in hexane and purification by preparative HPLC, 13 mg of **4** (1%) was obtained. mp 207.7 – 208.9 °C. ¹H NMR (500 MHz, Acetone-*d*₆) δ 12.23 (s, 1H), 11.69 (s, 1H), 7.92 (d, *J* = 2.5 Hz, 1H), 7.71 (d, *J* = 2.5 Hz, 1H), 7.23 (d, *J* = 4.3 Hz, 1H); ¹³C NMR (125 MHz, Acetone-*d*₆) δ 184.24 (d, *J* = 3.0 Hz), 156.45 (s), 150.90 (d, *J* = 269.4 Hz), 135.06 (s), 129.78 (s), 124.49 (s), 124.17 (s), 122.69 (s), 122.63 (s), 122.09 (s), 78.48 (d, *J* = 14.9 Hz); HRMS (EI-TOF) M⁺ calcd for C₁₁H₅BrCl₂FNO₂ 350.8865, found 350.8853; HPLC purity, 100%.

Example 2

General Procedures

[0082] Mammalian Cytotoxicity Assays. HeLa (ATCC, Manassas, VA, USA) cells were cultured in 96-well plates at a density of 2 x 10⁴ cells/well in Dulbecco's modified Eagles

medium (DMEM, GIBCO Invitrogen Corp, Carlsbad, CA) with 10% heat inactivated fetal bovine serum (FBS) (GIBCO) and an antibiotic mixture containing penicillin and streptomycin (Life Technologies, Grand Island, NY). Cells were treated with various concentrations of the PL compounds for 24 h in growth medium. Cell viability was determined by a colorimetric CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI) based on the manufacture's instruction. Data were recorded at the absorbance of 490 nm. Each condition was tested in triplicate. The results are shown in Table 1.

[0083] Minimum Inhibitory Concentration Assays. Broth microdilution assays were performed as described in the third edition of the ASM Clinical Microbiology Procedures Handbook. Briefly, serial 2-fold dilutions of pyrrolomycins were made in Muller Hinton II broth (cation-adjusted)(CAMHB) (Becton, Dickinson and Company, Sparks, MD), containing 1%-5% DMSO in Costar 96-well microtiter plates (Corning, Kennebunk, ME). Bacterial cultures were prepared using the direct colony suspension method to 0.5 McFarland units and each well was inoculated with 10 μ L of this suspension. Plates were incubated statically at 37 °C for 20-24 h. MIC values were reported as the lowest concentration of antibiotic in which no growth was seen.

[0084] Minimum Bactericidal Concentration Assays. MBC assays were performed according to a published protocol.³³ Overnight cultures grown in CAMHB were adjusted to an OD₆₀₀ of 0.05 in 25 mL of CAMHB in a 250-mL flask. They were then allowed to grow at 37°C with agitation at 250 rpm to exponential phase (OD₆₀₀=0.5) at which point the cultures were diluted to achieve a concentration of 5x10⁶ cfu/mL. One hundred microliters of the diluted, exponential phase bacteria were added to 1mL of the pyrrolomycins, and control antibiotics, vancomycin and ciprofloxacin for SA and BA, respectively, in a 10-mL tube. Bacteria were harvested at 0, 1, 3 and 24 h and plated for viable cell counts and reported as cfu/mL.

[0085] Anti-staphylococcal biofilm activity. Static biofilms were generated using a published protocol. Briefly, overnight cultures of *S. aureus* were diluted to an OD₆₀₀ of 0.05 in TSB-NaCl/Glc, and 200 μ L was inoculated into wells of a Costar 3596 plate (Corning life Sciences, Acton, MA), that had been coated overnight at 4 °C with 20% human plasma (Sigma). After 24 h of static growth, at 37 °C the biofilms were washed two times with PBS and the remaining biofilm was treated with pyrrolomycin derivatives in Muller Hinton II broth (cation-adjusted) (Becton, Dickinson and Company, Sparks, MD) supplemented with 5% DMSO (Sigma). After 24, 48 or 120 h of treatment, the remaining biofilm was washed two times with PBS and then re-suspended in 100 μ L PBS, serial diluted and plated for cfu.

[0086] Pharmacokinetics of pyrrolomycin 4. A Waters ACQUITY ultra-performance liquid chromatography (UPLC) system (Waters, Milford, MA) coupled to a 4000 Q TRAP[®] quadrupole linear ion trap hybrid mass spectrometer with an electrospray ionization (ESI) source (Applied Biosystems, MDS Sciex, Foster City, CA) was used throughout. All chromatographic separations were performed with an ACQUITY UPLC[®] BEH Shield RP18 column (2.1×100mm, 1.7µm; Waters) equipped with an ACQUITY UPLC C₁₈ guard column (Waters, Milford, MA). Mobile phase A consisted of 0.1% acetic acid and mobile phase B was methanol (MeOH). The initial mobile phase composition was 80% B for the first 3 min and was gradually increased to 95%B in 4.25 min and held constant for 1.25 min. Mobile phase B was then reset to 80% in 0.25 min and the column was equilibrated for 1 min before the next injection. A flow rate of 0.25 mL/min was used and the injection volume of all samples was 10 µL. MS/MS analyses were performed with negative ESI mode and using the following parameters: ion spray voltage, -4500 V; source temperature, 500 °C; curtain gas (nitrogen), 10 arbitrary units; and collision gas (nitrogen), "High". Specific detection was performed by monitoring the transition 349.67→160.8 m/z for **4**. In addition, for the detection of potential **4** metabolites, multiple reaction monitoring–information–dependent acquisition–enhanced product ion, neutral loss, precursor ion and enhanced MS scans were used as described previously.³⁷⁻⁴⁰

[0087] Plasma samples were prepared by protein precipitation using ice-cold methanol. Sixty-five µL of methanol was added to 25 µL plasma samples pre-spiked with 10 µL of internal standard (500 ng/ml). Samples were then vortexed, and centrifuged at 16,000 × g for 10 min. After centrifugation at 16,000 × g for 10 min, 80 µL supernatant were mixed with 40 mL of 10% methanol in H₂O. Further, 10 µL of each sample was used for LC-MS/MS analysis.

[0088] Eight-week-old healthy male Balb/c mice were purchased from Charles River Laboratories. Sterilized 7012 Teklad diets (Harlan) were used for mice, and water was provided *ad libitum*. A 10 mg/kg oral dose was administered as 250 µL of solution (**4**, 1 mg/mL) in a mixture of DMSO-PEG400-PG-EtOH-Cremophore-PBS (2/20/10/10/5/53 % v/v) to a 25 g mouse. A 1.0 mg/kg intravenous dose was administered as a 100 µL of solution (**4**, 0.25 mg/mL) in the same DMSO-PEG400-PG-EtOH-Cremophore-PBS mixture to a 25 g mouse. Oral doses were administered via oral gavage, whereas intravenous doses were administered via the tail vein. Blood samples were collected at 0, 5, 15, 30, 45 min, and 1, 2, 4, 8, 24, 48, and 72 h post oral administration, and at 0, 5, 30 min, and 1, 3, 8, 24, 48, and 72 h post intravenous dosing. Plasma was separated by centrifugation of blood samples at 2000 × g for 5 min at 4 °C within 1 h of sample collection. The pharmacokinetic parameters were determined using the noncompartmental analysis module of WinNonlin (version 5.1, Pharsight, Mountain View, CA). The absolute bioavailability (F) was calculated

as the ratio between the AUC_{0-infinity} from oral and intravenous routes, after dose normalization using the following equation:

$$\%F_{Absolute} = \frac{AUC_{Oral} \times Dose_{i.v.}}{AUC_{i.v.} \times Dose_{oral}} \times 100$$

Results and Discussion

[0089] Mammalian cytotoxicity was determined for all active pyrrolomycins with MIC values <0.20 µg/mL using human Hela cells (Table 1). Selectivity Index (SI) was defined as a ratio of IC₅₀ of human Hela cytotoxicity/MIC against either BA or SA (both in [temL]). Pyrrolomycins **3** and **4** had SI values of >1,484 and >751 against BA and >275 and >484 against SA, respectively, demonstrating that they are selective antibacterial agents without toxicity at concentrations several hundred to a thousand-fold higher than their MICs. The Ligand Efficiency (LE) of each compound was calculated to determine the binding energy per non-hydrogen atom of a ligand to its binding partner.^{34,35} LE is used as a parameter for optimization of both physicochemical properties and pharmacological properties.³⁶

[0090] The bactericidal activities of pyrrolomycins **4** and **18** were compared with that of ciprofloxacin against BA for a period of 24 hours (Figure 2A). Compound **4** reduces BA culture viability by 60% in 60 minutes and compound **18** reduces viability by 92%, while ciprofloxacin fails to kill BA at the same concentration of 0.063 µg/mL. In 24 h, compound **4** reduces BA culture viability by 99.9% but ciprofloxacin reduces viability by only 30%. For compound **4** against SA at a concentration of 4 µg/mL, 100% killing was observed with no growth of colonies after incubation for 48 hours (Figure 2B).

[0091] The results show that the MBC value of pyrrolomycin **4** is 4 µg/mL in 24 hours while 100% killing of SA requires 8 µg/mL of vancomycin (Figure 3, n = 4). As shown in Figure 4 (n = 4), compound **4** kills 84% biofilms in 24 hours at a concentration of 8.0 µg/mL, while vancomycin kills only 11% at 32 µg/mL. Compound **4** continues to achieve 97% biofilm killing at the corresponding concentration in 48 hours. In 120 hours, pyrrolomycin **4** kills >99.9% biofilms at 8.0 µg/mL, while vancomycin kills only 39% at 32 µg/mL. Compound **4** disrupts pre-formed biofilms at the concentration of 8.0 µg/mL in five days.

[0092] Compounds **1-4**, **8-12**, **15**, **17**, and **18** were evaluated for their ability to inhibit growth of BA, SA (Table 1), and other Gram-positive pathogens including *E. faecium*, vancomycin-resistant *Enterococcus* and methicillin-resistant *S. epidermidis*. Gram-negative ESKAPE pathogens, including *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp were also evaluated. Although some derivatives were found to have a broad spectrum of activity against Gram-negative pathogens, the compounds reported here exhibited the most pronounced inhibitory activity against Gram-positive bacteria.

[0093] Structure-activity relationship (SAR)—*Bacillus anthracis*.

Marinopyrrole derivative **1** is as potent as ciprofloxacin against BA with an MIC value of 0.063 µg/mL. Marinopyrrole **2** is two-fold less potent than ciprofloxacin with an MIC of 0.125 µg/mL. Importantly, it was determined that both pyrrolomycins **3** and **4**, which are fragments of marinopyrrole, exhibited higher potency than the marinopyrroles with MIC values of 0.031 µg/mL and 0.047 µg/mL, respectively. More significantly, these novel fluorinated pyrrolomycins **3** and **4** are the most efficient binders that have ever been reported as anti-BA agents with LEs of 0.60 and 0.59, respectively (Table 1). A four-fold increase in anti-BA activity was observed upon replacing bromine with fluorine, as exhibited by **4** and **17** with MIC values of 0.047 µg/mL and 0.188 µg/mL, respectively (Table 1). Dibromo congener **18**, with an extra bromine substituted in the pyrrole ring, is not only three-fold less potent (MIC = 0.125 µg/mL) than **4**, but also has a clogP value that is almost a full log unit higher (Table 1). Compound **4** has a clogP of 4.1 and the clogP values of **17** and **18** are 4.7 and 4.9, respectively. In general, more lipophilic compounds possess more potent antibacterial activity. This finding demonstrated that the least lipophilic compound **4** exhibited the most potent antibacterial activity. The most potent pyrrolomycin is **3** with fluorine substitutions on both para- and ortho positions of the benzene ring. This further reinforced the idea that introducing fluorine atoms into pyrrolomycin not only increased the potency but also improved the physicochemical and drug-like properties of this compound.

[0094] SAR—*Staphylococcus aureus*. Pyrrolomycins **4** and **17** are equally potent against SA, with MIC values of 0.073 µg/mL similar to that of marinopyrrole **1** although the LE values of the former almost twice that of the latter (see Table 2). Compounds **4** and **5** have the same anti-staphylococcal activity while their structures differ by only one atom (fluorine vs. bromine) at the A position. Compound **19** (A = CH) is at least 54-fold (MIC > 4.0 µg/mL) less active than its fluorine or bromine congeners **4** and **17** (Table 1). Although there is no obvious fluorine effect on anti-staphylococcal activity (MIC) observed between compound **4** and **17**, they do have different bactericidal activities. Compounds **15** and **9** are 21–57-fold less potent than compounds **4**, **17**, and **18**, suggesting that A and D should be either CF or CBr. Compound **18** is less active than **4**. Compound **10** with methyl substituted at W is 18-fold less potent than **3**. Both **11** and **12** are 24-fold less active than **3**. These results suggest that neither the pyrrole nitrogen nor the hydroxyl group in the benzene ring can be substituted to achieve potent anti-SA activity.

[0095] Pharmacokinetics of pyrrolomycin 4. A sensitive and specific LC-MS/MS method has been developed for determination of **4** in mouse plasma.³⁷⁻⁴⁰ Following intravenous (IV) and oral dosing in mice, the oral bioavailability of **4** was found to be 35%. The compound was well absorbed with maximum plasma concentration (C_{max}) observed after 0.25 h of oral administration. The elimination half-life was 6.04 hours and 6.75 hours

following IV and oral administration, respectively. The AUC for the IV dose at 1 mg/kg was 3.8 $\mu\text{g}\cdot\text{h}/\text{mL}$ and 13.2 $\mu\text{g}\cdot\text{h}/\text{mL}$ for the oral dose at 10 mg/kg, respectively. The plasma concentrations vs. time profiles after IV and oral administration of **4** in mice are shown in Figure 5. After both IV and oral administration, parent compound **4** was detectable in plasma up to 72 hours with the detection limit of 0.8 ng/mL.

Table 1

Compound ID	clogP ^a	MIC ^b		LE ^c		MIC ^d		LE		IC ₅₀ ^e		SI	
		BA	SA	BA	SA	BA	SA	SA	BA	BA	SA	BA	SA
1	6.5	0.063	0.078	0.30	0.29	0.078	0.29	>40.9	>649	>524	>40.9	>649	>524
2	6.1	0.125	0.19	0.29	0.28	0.19	0.28	>52.8	>422	>280	>52.8	>422	>280
3	5.5	0.031	0.17	0.60	0.54	0.17	0.54	>46.0	>1484	>275	>46.0	>1484	>275
4	4.1	0.047	0.073	0.59	0.57	0.073	0.57	>35.3	>751	>484	>35.3	>751	>484
8	2.1	> 4.0	>4.0	ND	ND	>4.0	ND	ND	ND	ND	ND	ND	ND
9	2.7	> 4.0	>4.0	ND	ND	>4.0	ND	ND	ND	ND	ND	ND	ND
10	4.1	1.5	3.0	ND	ND	3.0	ND	ND	ND	ND	ND	ND	ND
11	4.3	> 4.0	>4.0	ND	ND	>4.0	ND	ND	ND	ND	ND	ND	ND
12	5.0	> 4.0	>4.0	ND	ND	>4.0	ND	ND	ND	ND	ND	ND	ND
15	3.4	> 4.0 ^g	>4.0 ^e	ND	ND ^f	>4.0 ^e	ND ^f	ND	ND	ND	ND	ND	ND
17	4.7	0.188	0.073	0.50	0.54	0.073	0.54	>41.4	>220	>567	>41.4	>220	>567
18	4.9	0.125	0.19	0.51	0.51	0.19	0.51	>43.2	>346	>229	>43.2	>346	>229
19	4.2	ND ^h	>4.0	ND	ND	>4.0	ND	ND	ND	ND	ND	ND	ND

^aCalculated using ChemAxon MarvinSketch version 15.7.6.0, Hungary; ^bMIC: Minimum Inhibitory Concentration of pyrrolomycin in µg/mL against *Bacillus*

anthracis V770-NP1-R (n ≥ 3) with ciprofloxacin as a positive control (MIC = 0.063 µg/mL); ^cLE: Ligand Efficiency ≈ -ΔG/HAC, defined as the free energy of binding divided by the number of non-hydrogen atoms (Heavy Atom Count); ^dMIC: Minimum Inhibitory Concentration of pyrrolomycin in µg/mL against

Staphylococcus aureus USA300 (n ≥ 3) with vancomycin as a positive control (MIC = 2.0 µg/mL); ^eIC₅₀: Concentration of the pyrrolomycin in µg/mL at which

the human Hela cell proliferation was inhibited by 50% with respect to the untreated growth control, the highest concentration tested was 100 µM; ^fSI: Selectivi

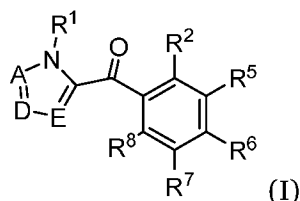
Index was defined as a ratio of IC₅₀ of human Hela cell cytotoxicity to MIC; ^gHighest concentration tested was 4 µg/mL; ^hND: not determined.

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What is Claimed is:

1. A compound, or pharmaceutically acceptable salt thereof, having a structure of formula (I):



wherein

R¹ is H, C₁₋₁₀alkyl, or COR⁹;

R² is OH, C₁₋₁₀alkoxy, NHC(O)R⁹, or NHSO₂R¹⁰;

A and D are each independently CR³ or N;

E is CR⁴ or N;

each R³ is H, halogen, C₁₋₁₀haloalkyl, C₁₋₁₀haloalkoxy, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹;

R⁴ is H, halogen, C₁₋₁₀haloalkyl, or C₁₋₁₀haloalkoxy;

R⁵, R⁶, R⁷, and R⁸ are each H, halogen, CF₃, or OCF₃;

R⁹ is H, C₁₋₁₀alkyl, or C₆₋₁₀aryl; and

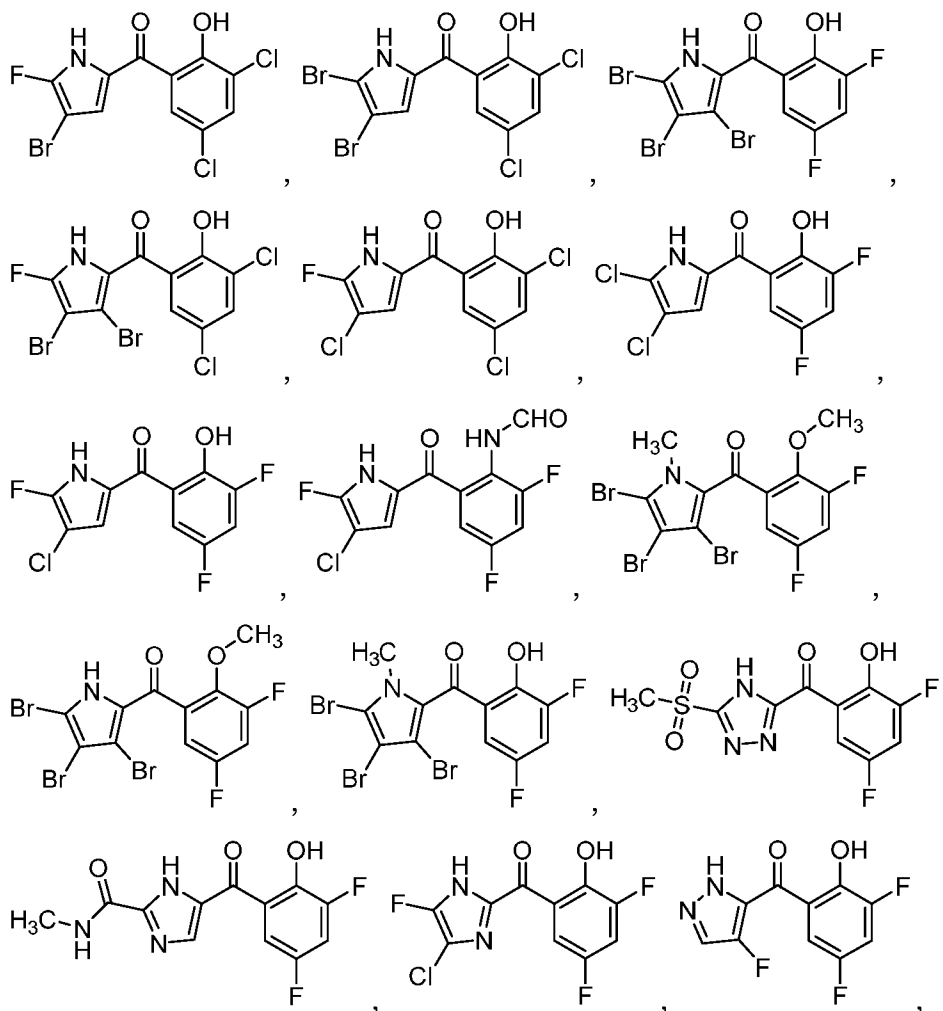
R¹⁰ is C₁₋₁₀alkyl or C₆₋₁₀aryl,

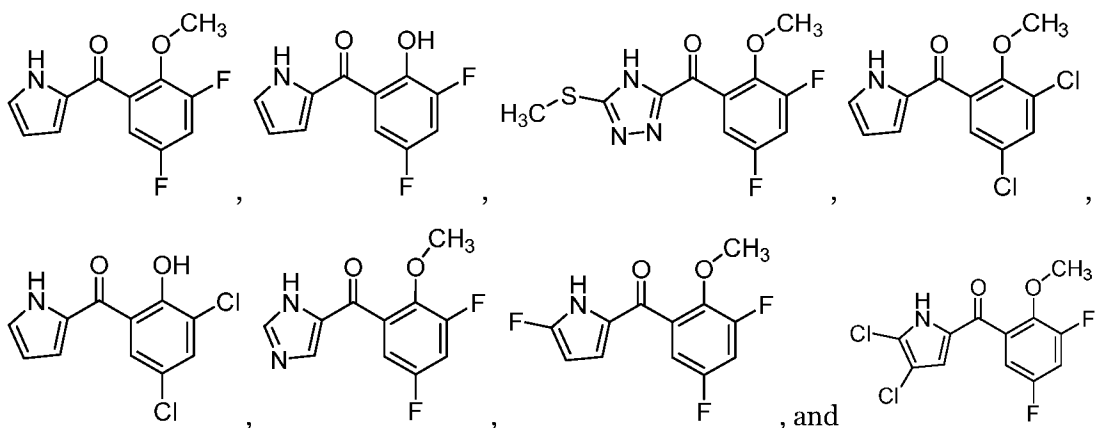
with the proviso that (a) at least one of A, D, and E is other than N, (b) at least one R³ is other than H, and (c) at least one of R⁵, R⁶, R⁷, and R⁸ is other than H.

2. The compound of claim 1, wherein A and D are each CR³ and E is CR⁴.
3. The compound of claim 1, wherein A and D are each CR³, and E is N.
4. The compound of claim 1, wherein A is CR³, D is N, and E is CR⁴.
5. The compound of claim 1, wherein A is CR³, and D and E are N.
6. The compound of claim 1, wherein A is N, D is CR³, and E is CR⁴.
7. The compound of any one of claims 1 to 6, wherein R³ is halogen, CF₃, OCF₃, CN, CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹.
8. The compound of claim 7, wherein R³ is CONHR⁹, SO₂R¹⁰, or SO₂NHR⁹.
9. The compound of claim 7, wherein R³ is F, Cl, or Br.
10. The compound of claim 9, wherein R³ is F or Cl.
11. The compound of any one of claims 1 to 10, wherein R⁴ is H, halogen, CF₃, or OCF₃.
12. The compound of claim 11, wherein R⁴ is F or Cl.

13. The compound of any one of claims 1 to 12, wherein R^2 is NHC(O)R^9 , or $\text{NHSO}_2\text{R}^{10}$.
14. The compound of any one of claims 1 to 12, wherein R^2 is OH or OCH_3 .
15. The compound of claim 14, wherein R^2 is OH.
16. The compound of any one of claims 1 to 15, wherein R^1 is H or C_{1-10} alkyl.
17. The compound of claim 16, wherein R^1 is H.
18. The compound of any one of claims 1 to 17, wherein R^5 and R^7 are each H
19. The compound of any one of claims 1 to 18, wherein R^6 and R^8 are each independently F or Cl.

20. The compound of claim 1 having a structure selected from the group consisting of:





21. The compound of any one of claims 1 to 20 in the form of a pharmaceutically acceptable salt.
22. A pharmaceutical formulation comprising the compound of any one of claims 1 to 21 and a pharmaceutically acceptable excipient.
23. A method of modulating Mcl-1 in a cell, comprising contacting the cell with the compound of any one of claims 1 to 21 or the formulation of claim 22.
24. The method of claim 23, wherein the cell is a cancerous cell.
25. A method of inhibiting the growth or proliferation of bacteria in a subject, comprising contacting the bacteria with the compound of any one of claims 1 to 21 or the composition of claim 22.
26. A method of killing a pathogen in a subject, comprising contacting the pathogen with the compound of any one of claims 1 to 21 or the composition of claim 22.
27. A method of inhibiting the growth of biofilms, comprising contacting the biofilm with the compound of any one of claims 1 to 21 or the composition of claim 22.
28. The method of claim 27, wherein the biofilm is due to a pathogen.
29. The method of any one of claims 25, 26, and 28, wherein the pathogen or bacteria is Gram-positive bacteria.
30. The method of any one of claims 25, 26, and 28, wherein the pathogen or bacteria is Gram-negative bacteria.
31. The method of any one of claims 25, 26, and 28, wherein the pathogen or bacteria is *Staphylococcus*, *Enterococcus*, *Bacillus*, *Streptococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Escherichia*, *Clostridium*, *Citrobacter*, *Serratia*, *Neisseria*, *Corynebacterium*, *Cyanobacterium*, *Salmonella*, *Shigella*, *Helicobacter*, *Brucella*, *Borrelia*, *Bordetella*, *Bartonella*, *Bacteroides*, *Burkholderia*, *Mycobacterium*, *Mycoplasma*, *Campylobacter*, *Chlamydia*, *Chlamydomphila*, *Francisella*, *Haemophilus*, *Legionella*,

Leptospira, Listeria, Rickettsia, Vibrio, Ureaplasma, Yersinia, Treponema, Proteus, Stenotrophomonas, Plesiomonas, Nocardia, Actinomyces, Moraxella, Erysipelothrix, Actinobacillus, Anaplasma, Pasteurella, Alcaligenes, Achromobacter, or Candida.

32. The method of any one of claims 25, 26, and 28 to 31, wherein the pathogen or bacteria is methicillin-resistant, carbapenem-resistant, fluoroquinone-resistant, vancomycin-resistant, or multidrug-resistant.

33. A method of treating infectious disease in a subject comprising administering to the subject a therapeutically effective amount of a compound of any one of claims 1 to 21 or the pharmaceutical formulation of claim 22.

34. The compound of any one of claims 1 to 21 for use in the preparation of a medicament.

35. The compound of any one of claims 1 to 21 for use as an antibiotic.

36. Use of a compound of any one of claims of 1 to 21 as an antibiotic.

37. The method of any one of claims 25 to 33, wherein the subject is a mammal.

38. The method of claim 37, wherein the mammal is a human.

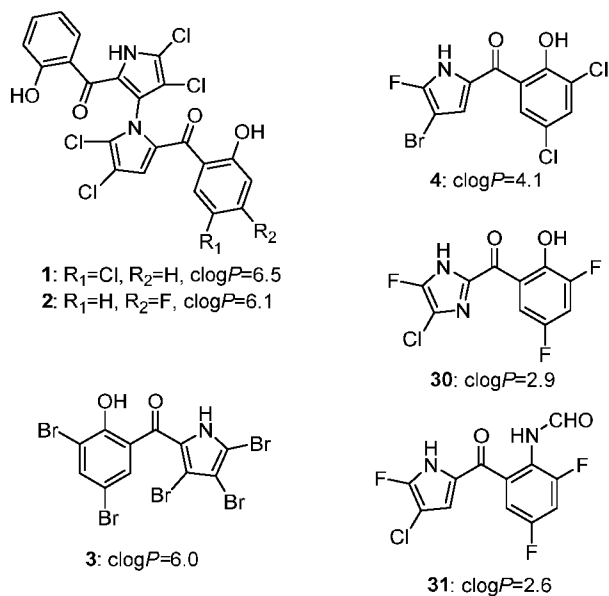


Figure 1

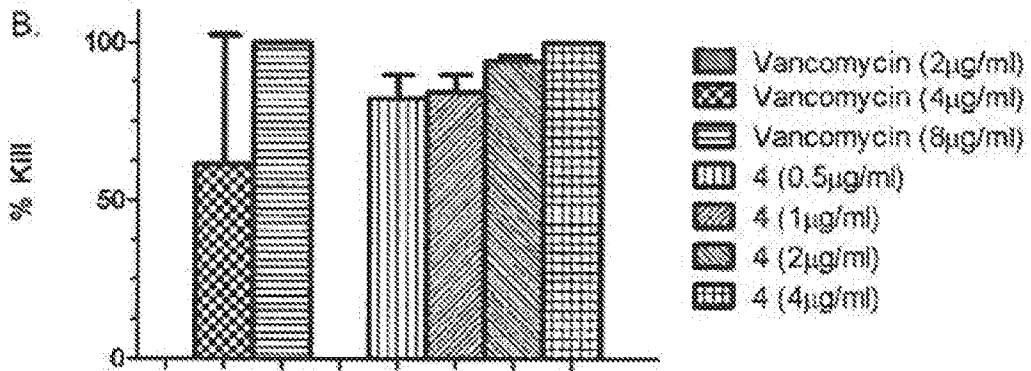
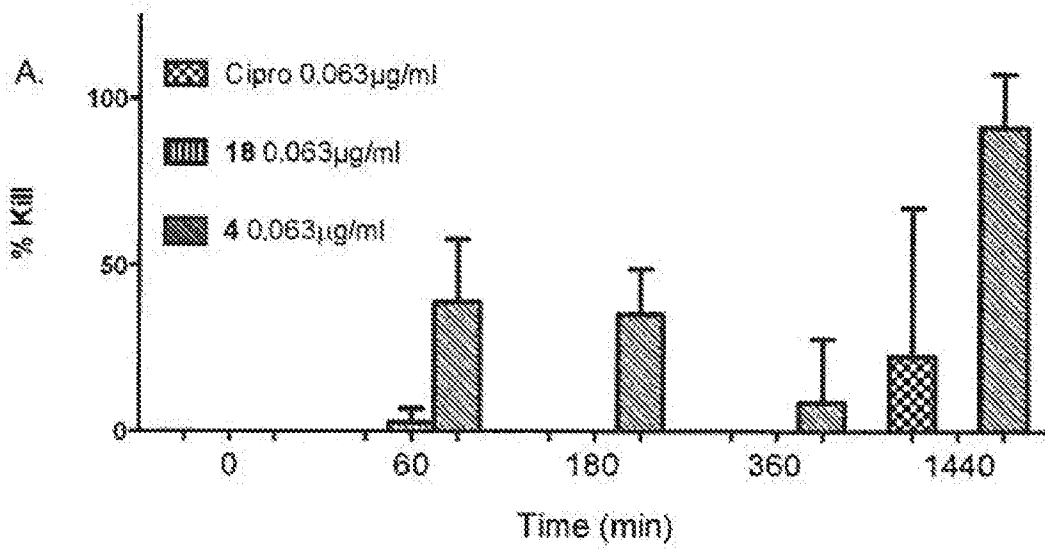


Figure 2

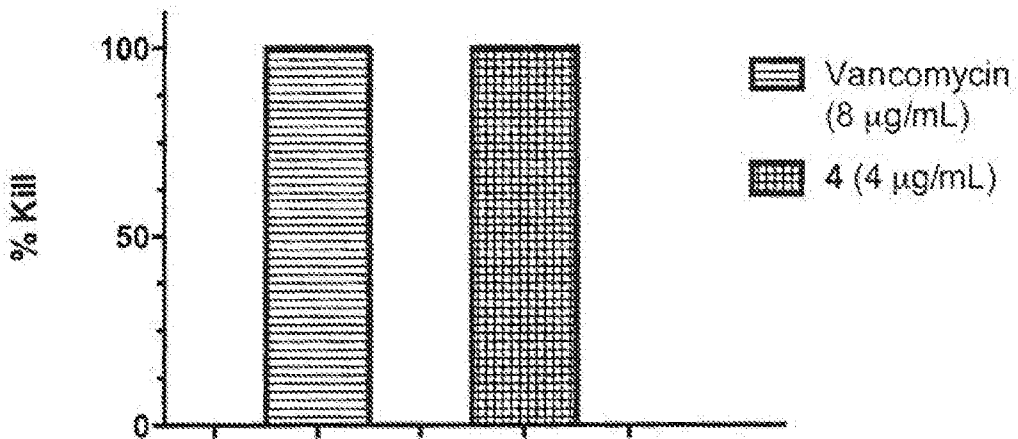


Figure 3

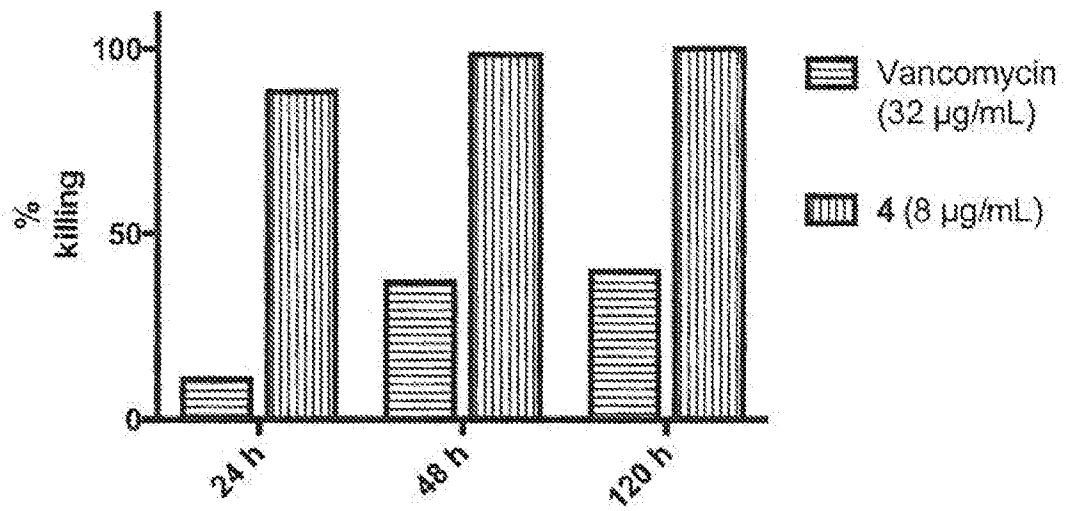


Figure 4

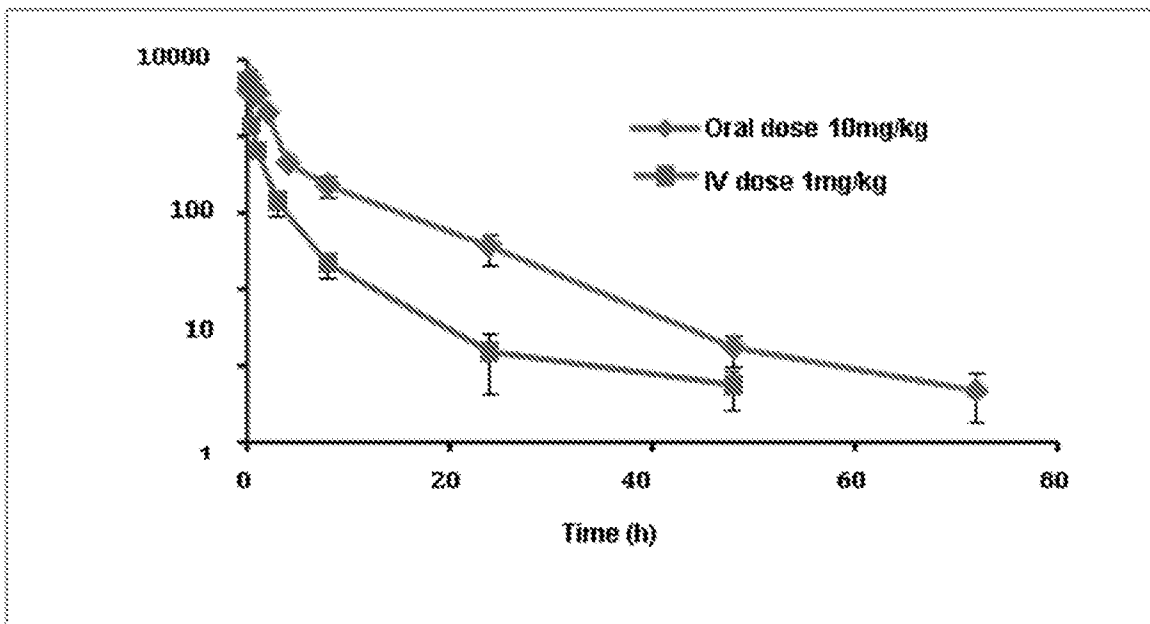


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/42423

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.: 11-19, 21-38
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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/42423

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07D 409/12, 333/28, 409/10 (2016.01)

CPC - C07D 409/12, 333/28, 409/10

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)

IPC(8): C07D 409/12, 333/28, 409/10 (2016.01)

CPC: C07D 409/12, 333/28, 409/10

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO; PubMed; Google/Google Scholar; pyrrolomycin, mcl-1, bacteria, pathogen, infection, cancer, pyrrole, pyrazole, imidazole, triazole, myeloid, cell, leukemia

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUBCHEM. CID 23634462. 07 January 2008, pp. 1-9 [online], [retrieved on 2016-08-29]. Retrieved from the Internet <URL: https://pubchem.ncbi.nlm.nih.gov/compound/23634462#section=Top >; page 3, formula	1-2, 7/1-2, 9/7/1-2, 10/9/7/1-2
X	WO 2015/101670 A2 (ELEXOPHARM GMBH) 09 July 2015; pages 22-24, 30	1-6, 7/1-6, 8/7/1-6, 9/7/1-6, 10/9/7/1-6, 20
A	US 4,443,466 A (KARJALAINEN, AJ et al.) 17 April 1984; entire document	1-6, 7/1-6, 8/7/1-6, 9/7/1-6, 10/9/7/1-6, 20

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

29 August 2016 (29.08.2016)

Date of mailing of the international search report

28 SEP 2016

Name and mailing address of the ISA/

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