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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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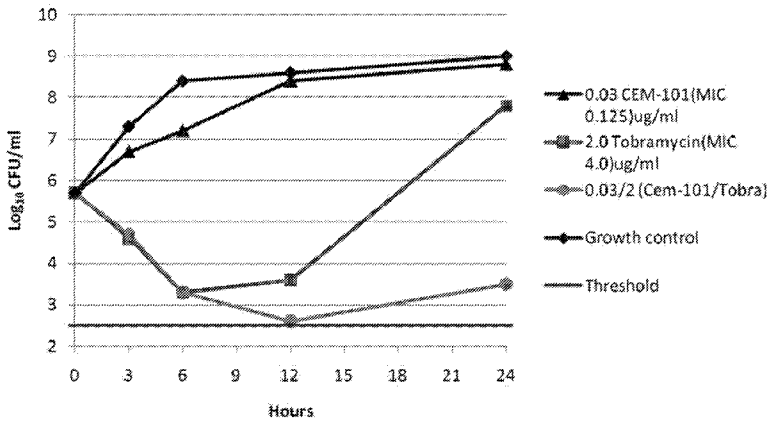

FIG. 1

(57) Abstract: Compounds, compositions, methods, and uses are described herein for the treatment of respiratory diseases, including cystic fibrosis. Inhalation formulations of macrolide antibiotics are also described herein. The treatment of bacterial infections continues to be an important endeavor of pharmaceutical research and development. The specter of bacterial resistance to currently available antibiotics is ever-present, and accordingly, new and improved compounds, pharmaceutical formulations, treatment methods, and treatment protocols are needed.

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METHODS FOR TREATING RESPIRATORY DISEASES AND FORMULATIONS THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims, under 35 U.S.C. § 119(e), the benefit of and priority to U.S. Provisional Application No. 61/781,197 filed March 14, 2013, which is hereby incorporated herein by reference.

TECHNICAL FIELD

The invention described herein pertains to the treatment of respiratory diseases, including cystic fibrosis. The invention described herein also pertains to inhalation formulations of macrolide antibiotics.

BACKGROUND AND SUMMARY OF THE INVENTION

The treatment of bacterial infections continues to be an important endeavor of pharmaceutical research and development. The specter of bacterial resistance to currently available antibiotics is ever-present, and accordingly, new and improved compounds, pharmaceutical formulations, treatment methods, and treatment protocols are needed. In addition, bacterial infections present themselves in a wide range of tissues, and in many cases, those tissues pose particular challenges for successful treatment. For example, new treatments of bacterial infections of the respiratory system, including acute and chronic pulmonary and endobronchial infections, are needed.

Many antibiotics do not achieve sufficiently high lung concentrations to be effectively used in the treatment and/or prophylaxis of acute and chronic pulmonary and endobronchial diseases. For example, aminoglycoside penetration into the bronchial secretions has been reported to be poor, at approximately only about 12% of the peak serum concentration (Rev. Infect. Dis., 3:67 (1981)). In addition, it has been reported that sputum itself is inhibitory to the bioactivity of aminoglycosides because of its high ionic strength and the presence of divalent cations (Advances in Pediatric Infections Diseases, 8:53 (1993)). Sputum also contains mucin glycoproteins and DNA, which bind aminoglycosides. It has also been reported that to overcome the inhibitory activity, the concentration of aminoglycosides in the sputum would need to be increased to about ten times the minimum inhibitory concentration of the particular target pathogen, such as Pseudomonas aeruginosa isolates (J. Infect. Dis., 148:1069 (1983)).

It has also been reported that it is particularly difficult to treat cystic fibrosis (CF), a common genetic disease that is characterized by the inflammation and progressive destruction of lung tissue. The debilitation of the lungs in CF patients is associated with accumulation of purulent sputum produced as a result of chronic endobronchial infections

caused by pathogenic bacteria, such as H. influenzae, Staphylococcus aureaus, and Pseudomonas aeruginosa, and the like. Nearly all individuals suffering from CF eventually die of respiratory failure.

Because certain antibiotics, like aminoglycosides, penetrate poorly into the sputum, to achieve therapeutic concentrations in sputum, high dose parenteral administration is required. Such dosing regimens increase the risk of systemic toxicity including ototoxicity and nephrotoxicity because the serum contains high aminoglycoside concentrations. Intravenous therapy may also increase hardship on the patient, and require hospitalization, which increases treatment costs and exposes the patient to potential other infections. It is appreciated that during infection, the bacteria may predominantly reside in the smaller airways, such as the terminal and respiratory bronchioles, and that the bacteria may predominantly colonize in the larger airways. It has also been reported that when azithromycin is administered by inhalation and other intrabronchial routes, the half-life in the pharynx and lungs is undesirably long, leading to a higher potential for resistance.

Tobramycin inhalation solution is currently the only aerosol antibiotic approved for use for the treatment of bacterial infections in patients with CF. It has been reported that the aerosol administration of tobramycin reduces the potential for systemic toxicity. However, it has also been reported that long term use has been associated with multiple-antibiotic-resistant P. aeruginosa strains. Thus, there is a need for the development of different treatments, including classes of aerosol antibiotics for the treatment, of chronic lung infections in patients with CF.

It has been unexpectedly discovered that unlike azithromycin, triazole-containing macrolides described herein have an optimal half-life in the pharynx and lungs, which allows for efficacy in treating disease in the lungs with a lower potential for resistance development. It has also been surprisingly discovered that the triazole-containing macrolides described herein may be administered by inhalation, including intranasal and oral inhalation, and other nasal, sinus, respiratory tract, pulmonary, and intrabronchial routes. It has also been unexpectedly discovered that the triazole-containing macrolides described herein exhibit a large volume of distribution.

It has also been discovered that the macrolides described herein are useful in treating respiratory tract infections (RTIs). It has been surprisingly discovered that the compounds described herein also achieve sufficiently high lung levels upon oral administration. Accordingly, methods are described herein for the treatment and/or prophylaxis of acute and chronic pulmonary and endobronchial diseases, where the methods include the step of administering or co-administering one or more macrolides described herein to a host animal.

The macrolides may be administered by a variety of routes, including but not limited to oral, parenteral, inhalation, and like routes of administration. Without being bound by theory, it is believed herein that the utility of the macrolides described herein is due at least in part to the unexpectedly high lung tissue levels of the compounds following administration, including oral and parenteral administration. It has also been surprisingly discovered that the compounds do not have to be administered by inhalation to achieve efficacious lung levels.

It has also been discovered that the macrolide compounds described herein are useful in the treatment and/or prophylaxis of acute and chronic pulmonary and endobronchial diseases, such as diseases caused by or exacerbated by bacteria, including Pseudomonas aeruginosa seen in CF patients, chronic bronchitis, and bronchiectasis. It has been discovered that the macrolides described herein have potent anti-inflammatory activities, and therefore are useful in treating the inflammatory component of various pulmonary and endobronchial diseases, such as CF.

It has also been discovered that the macrolides described herein may be co-administered with other antibiotics, such as aminoglycosides, fluoroquinolones, aztreonam, fosfomycin, and the like, and that such co-administration give unexpectedly high efficacy. Without being bound by theory, it is believed herein that the unexpectedly high efficacy may be due to one or more properties of the macrolides. One such property may be that the macrolides have been shown to not antagonize the activity of other antibiotics, such as aminoglycoside antibiotics, which has been reported for other antibacterial agents during co-administration. Another such property may be that the macrolides surprisingly synergize the activity of other antibiotics, such as aminoglycoside antibiotics.

It has also been discovered that the macrolides described herein are useful in treating diseases that are caused at least in part by Escherichia coli, Enterobacteria species, Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Serratia marcescens, Haemophilus influenzae, Burkholderia cepacia, Stenotrophomonas maltophilia, Alcaligenes xylosoxidans, multidrug resistant Pseudomonas aeruginosa. The macrolides may be administered alone or in combination with other antibiotics, such as aminoglycosides, fluoroquinolones, aztreonam, fosfomycin, and the like.

Described herein are compounds, compositions, formulations, uses in the manufacture of medicaments, and methods for treating respiratory infections, and related diseases, including cystic fibrosis (CF), diseases caused at least in part by Mycobacterium avium complex (MAC) or Mycobacterium hominus (MAH), patients suffering from infection co-morbid with HIV, AIDS and/or AIDS related diseases, and other immunocompromised patients suffering from infection. Without being bound by theory, it is believed herein that

efficacy in treating disease such as CF is due at least in part to the combination of antibacterial and anti-inflammatory activity of the compounds administered.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows that synergy against MRSA SA 2230 is observed with achievable levels of CEM-101 co-administered with tobramycin.

FIG. 2 shows the effect of various macrolides on PMA-induced MMP9 production. Effects of macrolides on phorbol 12-myristate 13-acetate (PMA)-induced MMP9 activation in U937 cells. Cells were pretreated with CEM-101 (10 to 100 μM) or erythromycin, clarithromycin, azithromycin, or telithromycin (33 to 333 μM) for 1 h, followed by PMA (50 ng/mL) treatment for 48 h. After 48 h supernatants were collected for zymography. MMP9 enzyme activity was measured by gelatin zymography. Data are expressed relative to standard. Values represent means of four experiments for CEM-101 and three experiments for each of erythromycin, clarithromycin, azithromycin, and telithromycin, ± SEM. $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. treatment with PMA only).

FIG. 3 shows the effect of various macrolides on LPS-induced TNFα production. Effects of macrolides on lipopolysaccharide (LPS)-induced TNFα release in PMA-differentiated U937 cells. Cells were pretreated with CEM-101 (10 to 100 μM) or erythromycin, clarithromycin, azithromycin, or telithromycin (33 to 333 μM) for 1 h, followed by LPS (100 ng/mL) stimulation for 4 h. LPS-induced TNFα release was evaluated by ELISA. Values represent means of three experiments ± SEM. $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. treatment with LPS only).

FIG. 4 shows the effect of various macrolides on LPS-induced IL-8 production. Effects of macrolides on lipopolysaccharide (LPS)-induced CXCL8 release in PMA-differentiated U937 cells. Cells were pretreated with CEM-101 (10 to 100 μM) or erythromycin, clarithromycin, azithromycin, or telithromycin (33 to 333 μM) for 1 h, followed by LPS (100 ng/mL) stimulation for 4 h. LPS-induced CXCL8 release was evaluated by ELISA. Values represent means of three experiments ± SEM. $^{\#\#}$ $p < 0.01$ (vs. non-treatment control), * $p < 0.05$, ** $p < 0.01$ (vs. treatment with LPS only).

DETAILED DESCRIPTION

In one embodiment, the compounds, compositions, formulations, and methods include one or more macrolides described herein. In another embodiment, the compounds, compositions, and formulations are adapted for oral administration. In another embodiment, the compounds, compositions, and formulations are adapted for parenteral administration. In

another embodiment, the compounds, compositions, and formulations are adapted for administration by inhalation. In another embodiment, the methods include oral administration. In another embodiment, the methods include parenteral administration. In another embodiment, the methods include administration by inhalation.

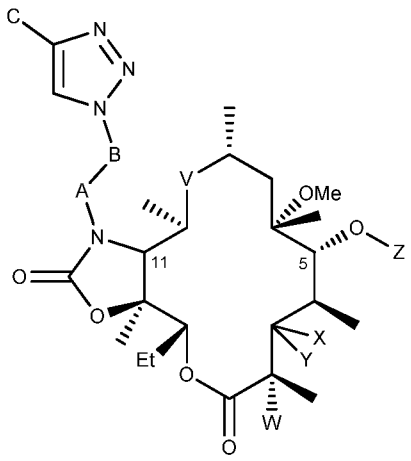
It has been unexpectedly discovered herein that the triazole-containing ketolide antibiotics and fluoro derivatives thereof, such as CEM-101 and related compounds, are effective anti-inflammatory agents and as such are effective in treating CF. In particular, triazole-containing ketolide antibiotics and fluoro derivatives thereof described herein are effective in treating the bacterial and inflammatory aspects of CF.

It is also discovered herein that the compounds described herein exhibit high solution stability even during long term storage.

In another embodiment, compounds, compositions, and methods are described herein for treating CF that includes both a bacterial and inflammatory component.

The invention described herein is further illustrated by the following enumerated and non-limiting clauses:

1. A method for treating a pulmonary or endobronchial disease in a host animal, the method comprising the step of administering to the host animal a therapeutically effective amount of one or more compounds of the formula



or pharmaceutically acceptable salts thereof, wherein:

X is H; and Y is OR_7; where R_7 is a monosaccharide or disaccharide, or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl;

Z is a monosaccharide or disaccharide, or a derivative thereof;

V is C(O), or $C(=NR_{11})$, wherein R_{11} is hydroxy or alkoxy;

W is H, F, Cl, Br, I, or OH;

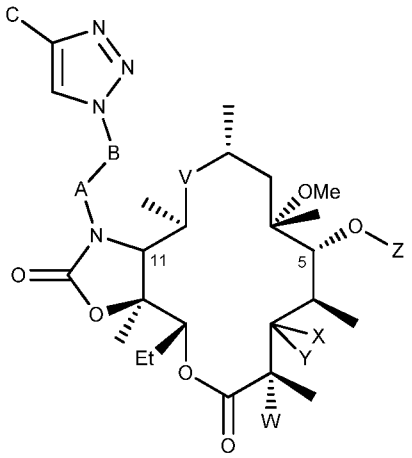
A is CH_2, C(O), C(O)O, C(O)NH, $S(O)_2$, $S(O)_2NH$, or $C(O)NHS(O)_2$;

B is $(CH_2)_n$ where n is an integer in the range from 0 to about 10, or B is C_2-C_{10} alkenyl or alkynyl; and

C is cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted;

where the compound is administered by inhalation to the endobronchial space of the patient.

2. A composition for administration by inhalation, the composition comprising one or more compounds of the formula



or pharmaceutically acceptable salts thereof, wherein:

X is H; and Y is OR_7; where R_7 is a monosaccharide or disaccharide, or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl;

Z is a monosaccharide or disaccharide, or a derivative thereof;

V is C(O), or $C(=NR_{11})$, wherein R_{11} is hydroxy or alkoxy;

W is H, F, Cl, Br, I, or OH;

A is CH_2, C(O), C(O)O, C(O)NH, $S(O)_2$, $S(O)_2NH$, or $C(O)NHS(O)_2$;

B is $(CH_2)_n$ where n is an integer in the range from 0 to about 10, or B is C_2-C_{10} alkenyl or alkynyl; and

C is cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted.

3. A unit dose of a therapeutically effective amount of the compound or composition of clause 2, the unit dose comprising a predetermined amount of the compound adapted for administering by inhalation.

4. A kit for treating a pulmonary or endobronchial disease in a host animal, the kit comprising a solid unit dose of a therapeutically effective amount of the compound or composition of any one of the preceding clauses, and an aerosolizer adapted or configured to aerosolize the pharmaceutical formulation and deliver it to the lower respiratory tract and

pulmonary compartment following intraoral administration, and instructions for use. It is appreciated that the solid unit dose may be administered as a dry powder or a metered-dose inhaler.

5. A kit for treating a pulmonary or endobronchial disease in a host animal, the kit comprising a solid unit dose of a therapeutically effective amount of the compound or composition of any one of the preceding clauses, and an aerosolizer adapted or configured to aerosolize the pharmaceutical formulation and deliver it to the nasal cavity following intranasal administration, and instructions for use. It is appreciated that the solid unit dose may be administered as a dry powder or a metered-dose inhaler.

6. A kit for treating a pulmonary or endobronchial disease in a host animal, the kit comprising a solid unit dose of a therapeutically effective amount of the compound or composition of any one of the preceding clauses, and a separate diluent, and instructions for use, including an instruction for reconstituting the solid unit dose using the diluent to prepare a liquid composition capable of being inhaled by the host animal.

7. The kit of any one of the preceding clauses further comprising a container.

8. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the composition is a dry powder adapted for inhalation by the host animal.

9. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the composition is a solution adapted for aerosolization and inhalation by the host animal.

10. The method, composition, unit dose, or kit of any one of the preceding clauses wherein compound is delivered to achieve a lung concentration, such as measured in epithelial lining fluid (ELF), sputum, ling tissues, bronchial lavage fluid, and the like, of at least about 2 μg/mL, at least about 4 μg/mL, at least about 8 μg/mL or at least about 16 μg.mL

11. The method, composition, unit dose, or kit of any one of the preceding clauses wherein X and Y are taken together with the attached carbon to form carbonyl.

12. The method, composition, unit dose, or kit of any one of the preceding clauses wherein Z is a monosaccharide.

13. The method, composition, unit dose, or kit of any one of the preceding clauses wherein Z is desosamine or a derivative thereof.

14. The method, composition, unit dose, or kit of any one of the preceding clauses wherein Z is desosamine.

15. The method, composition, unit dose, or kit of any one of the preceding clauses wherein V is C(O).

16. The method, composition, unit dose, or kit of any one of the preceding clauses wherein W is H or F.

17. The method, composition, unit dose, or kit of any one of the preceding clauses wherein W is F.

18. The method, composition, unit dose, or kit of any one of the preceding clauses wherein A is CH_2.

19. The method, composition, unit dose, or kit of any one of the preceding clauses wherein B is $(CH_2)_n$.

20. The method, composition, unit dose, or kit of any one of the preceding clauses wherein n is an integer from 2 to 4

21. The method, composition, unit dose, or kit of any one of the preceding clauses wherein n is 3.

22. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted.

23. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is substituted.

24. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is aryl or heteroarylalkyl, each of which is optionally substituted.

25. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is optionally substituted aryl or substituted aryl.

26. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is aminophenyl.

27. The method, composition, unit dose, or kit of any one of the preceding clauses wherein C is 3-aminophenyl.

28. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the compound is solithromycin, or a pharmaceutically acceptable salt, hydrate, solvate, or prodrug thereof

29. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the compound is solithromycin, or a pharmaceutically acceptable salt thereof

30. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the compound is solithromycin.

31. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the administration is performed using a nebulizer, and the composition or unit

dose is capable of producing a aerosol particle with an MMAD predominantly in the range from about 1 to about 5 μm.

32. The method, composition, unit dose, or kit of any one of the preceding clauses further comprising the step of administering a therapeutically effective amount of an aminoglycoside.

33. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the aminoglycoside is selected from the group consisting of tobramycin, amikacin, and a combination thereof.

34. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the compound is administered at a dose lower than that capable of antagonizing or significantly antagonizing the efficacy of the aminoglycoside.

35. The method, composition, unit dose, or kit of any one of the preceding clauses further comprising the step of administering a therapeutically effective amount of a fluoroquinolone antibiotic.

36. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the fluoroquinolone is levofloxacin

37. The method, composition, unit dose, or kit of any one of the preceding clauses further comprising the step of administering a therapeutically effective amount of aztreonam.

38. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease includes ventilator associated pneumonia (VAP), hospital acquired pneumonia (HAP), community acquired bacterial pneumonia (CABP), or a combination thereof.

39. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease includes cystic fibrosis, lung cancer, obstructive lung diseases, such as chronic obstructive pulmonary disease, asthma, chronic bronchitis, restrictive lung diseases, emphysema, primary and secondary ciliary dyskinesia, sinusitis, pneumonia, mesothelioma, or a combination thereof.

40. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease includes cystic fibrosis.

41. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the host animal is immunocompromised.

41. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by a gram-negative bacteria such as Pseudomonas spp., Stenotrophomonas maltophilia, Burkholderia cepacia, Aeromonas

hydrophilia, Escherichia coli, Citrobacter freundii, Salmonella spp., Shigella spp., Enterobacter spp., Klebsiella spp., Serratia marcescens, Francisella tularensis, Morganella morganii, Proteus spp., Providencia spp., Acinetobacter spp., Yersinia enterocolitica, Yersinia spp., Bordetella spp., Haemophilus spp., Pasteurella spp., Branhamella catarrhalis, Helicobacter pylori, Campylobacter spp., Borrelia spp., Legionella pneumophila, Listeria monocytogenes, Neisseria gonorrhoeae, Neisseria meningitidis, Kingella, Moraxella, Gardnerella vaginalis, Bacteroides spp., and the like.

42. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by a gram-positive bacteria such as Corynebacterium spp., Streptococcus spp., Enterococcus spp., Staphylococcus spp., and the like.

43. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by a gram-positive anaerobic bacteria such as Clostridium spp.

44. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by an acid-fast bacteria such as Mycobacterium tuberculosis, Mycobacterium avium, Mycobacterium intracellulare, and Mycobacterium leprae.

45. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by an atypical bacteria such as Chlamydia pneumoniae and Mycoplasma pneumoniae.

46. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by one or more strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus , including MRSA, or a combination thereof.

47. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by one or more CF strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, S. aureus , including MRSA, or a combination thereof.

48. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the mucoid Pseudomonas aeruginosa is pyocyanin positive.

49. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by one or more strains of Staphylococcus aureus, including susceptible and resistant strains, such as MRSA, B. anthracis, or a combination thereof.

50. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by one or more strains of MRSA.

51. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by one or more of clarithromycin resistant bacteria, including multi-resistance or pan-resistance, or a combination thereof.

52. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by inflammation.

53. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the disease is caused at least in part by inflammation arising from TNFα production, CXCL8 production, IL-8 production, MMP9 production, or a combination thereof.

54. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the host animal is a mammal.

55. The method, composition, unit dose, or kit of any one of the preceding clauses wherein the host animal is a human.

Illustrative inhalable compounds, compositions, and formulations include inhalable dry powders and inhalable aerosolizable solutions. It is to understood that the inhalable compounds, compositions, and formulations may provide additional benefits due to the direct delivery to the endobronchial site of infection, such as decreased toxicity compared to other systemic delivery, reduced cost, and better patient compliance, such as compared to IV antibiotic administration requiring an inpatient or outpatient visit.

Illustrative inhalable compounds, compositions, and formulations are adapted for delivery to the lungs and endobronchial space of the patient, such as by aerosolization or dry powder inhalation. Such compounds may be lyophilizates, or reconstitutable lypophilizates, as described in PCT International Publication No. WO 2011/112864, the disclosure of which is incorporated herein by reference. In another embodiment, the compounds are prepared as liposomes, including charged liposomes and antibody coated liposomes, nanoparticulate or microparticulate compositions, nanosuspensions, and the like. The inhalable compounds, compositions, and formulations may include one or more pharmaceutically acceptable carriers, excipients, suspending agents, diluents, fillers, salts, buffers, stabilizers, solubilizers, solvents, dispersion media, coatings, isotonic agents, and other materials. The inhalable compounds, compositions, and formulations may include potentiators, complexing agents, targeting agents, stabilizing agents, cosolvents, pressurized gases, or solubilizing conjugates.

Illustrative excipients include sugars such as lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium

caroxymethylcellulose, and/or polyvinylpyrrolidone (PVP). Preferred excipients include lactose, gelatin, sodium carboxymethyl cellulose, and low molecular weight starch products.

Illustrative suspending agents that can serve as valve lubricants in pressurized pack inhaler systems include oleic acid, simple carboxylic acid derivatives, and sorbitan trioleate.

Illustrative diluents include water, saline, phosphate-buffered citrate or saline solution, and mucolytic preparations. Other illustrative diluents include alcohol, propylene glycol, and ethanol. Other illustrative diluents have a tonicity and pH compatible with the alveolar apparatus. Other illustrative diluents include isotonic saline, phosphate buffered isotonic solutions whose tonicity have been adjusted with sodium chloride or sucrose or dextrose or mannitol.

Illustrative fillers include glycerin, propylene glycol, ethanol in liquid or fluid preparations. Illustrative fillers for dry powder inhalation systems include lactose, sucrose, dextrose, suitable amino acids, and derivatives of lactose. In another embodiment, the fillers include glycerin, propylene glycol, lactose and amino acids.

Illustrative salts include those that are physiologically compatible and provide the desired tonicity adjustment, including monovalent and divalent salts of strong or weak acids. In another embodiment, the salts include tartrates.

Illustrative buffers include phosphate or citrate buffers or mixed buffer systems of low buffering capacity. In another embodiment, the buffers include phosphate.

Illustrative coating agents to provide a hydrophobic sheath around the hydrophilic cores include caproic and lauric acids. During the preparation of liposomes the use of diphosphatidyl choline or diphosphatidyl myristyl choline or suitable such mixtures can be used to provide protection to the molecules or formulation.

Illustrative stabilizers include those that provide chemical or physical stability of the final preparations. Such stabilizers include antioxidants such a sodium metabisulfite, alcohol, polyethylene glycols, butylated hydroxyanisole, butylated hydroxytoluene, disodium edetate. In another embodiment, the stabilizers include sodium metabisulfite, disodium edetate and polyethylene glycols. In another embodiment, the stabilizers include cryoprotectants such as polyethylene glycols, sugars, and carrageenans.

Illustrative solubilizers include propylene glycol, glycerin, suitable amino acids, complexing agents such as cyclodextrins, sorbitol solution, or alcohol. In another embodiment, the solubilizers include ethanol, propylene glycol, glycerin, sorbitol, and cyclodetrins. In another embodiment, the solubilizers include propylene glycol, sorbitol, and cyclodextrins.

It is to be understood that the formulations described herein may include any and all selections from the above lists of components, in any combination.

In another embodiment, the active ingredients are formulated for inhalation with use of a suitable propellant such as dichlorodifluoromethane, dichloroflouromethane, dichlorotetrafluoroethane, carbon dioxide or other gas. In another embodiment, the propellants include non-CFC related class of propellants or related analogs.

In another embodiment, the active ingredients are dried into an inhalable dry powder by mixing with suitable adjuvants that are compatible with the compounds described herein and are biologically compatible. Illustrative methods of drying the pharmaceutical material for inhalation include spray drying, conventional bed drying, and/or super critical fluid processing. In another embodiment, spray drying and super critical fluid processing are used.

In another embodiment, the compounds, compositions, and formulations are adapted for aerosolization as concentrated solutions of the compounds, such as about 1 to about 5 mL solutions of about 100 to about 1,000 mg, about 200 to about 800 mg, about 400 to about 600 mg, about 400 to about 500 mg, about 200 to about 400 mg, about 200 to about 300 mg, about 100 to about 400 mg, about 100 to about 300 mg, or about 100 to about 200 mg of the compounds.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include cystic fibrosis (CF), ventilator associated pneumonia (VAP), hospital acquired pneumonia (HAP), community acquired bacterial pneumonia (CABP), and combinations thereof.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein also include lung cancer, obstructive lung diseases, such as chronic obstructive pulmonary disease, asthma, chronic bronchitis, restrictive lung diseases, emphysema, primary and secondary ciliary dyskinesia, sinusitis, pneumonia, mesothelioma, and combinations thereof.

In another embodiment, compounds, compositions, formulations, and methods are described herein for treating cystic fibrosis. In another embodiment, compounds, compositions, formulations, and methods are described herein for treating immunocompromised patients having a bacterial infection.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include diseases caused at least in part by one or more strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus , including MRSA, or a combination thereof.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include diseases caused at least in part by one or more CF strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, S. aureus , including MRSA, or a combination thereof. In one variation, the mucoid Pseudomonas aeruginosa is pyocyanin positive.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include diseases caused at least in part by one or more strains of Staphylococcus aureus, including susceptible and resistant strains, such as MRSA, B. anthracis, or a combination thereof

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include diseases caused at least in part by one or more strains of MRSA.

Illustrative diseases treatable with the compounds, compositions, formulations, and methods described herein include diseases caused at least in part by one or more of clarithromycin resistant bacteria, including multi-resistance and pan-resistance.

It has been reported that cystic fibrosis patients and other patients with chronic endobronchial infections may have a high incidence of bronchospastic or asthmatic airways. These airways are sensitive to hypotonic or hypertonic aerosols, to the presence of a permanent ion, particularly a halide such as chloride, as well as to aerosols that are acidic or basic. The effects of irritating the airways can be clinically manifested by cough or bronchospasm. It is therefore appreciated that the formulations described herein desirably have adjusted osmolality, tonicity, ionic strength and pH.

In another embodiment, the aerosolizable formulations have salinity adjusted to be well-tolerated by patients. In another embodiment, formulation has balanced osmolality ionic strength and chloride concentration. In another embodiment, the formulation has the smallest reasonable aerosolizable volume able to deliver the effective dose of the compounds to the site of the infection. In another embodiment, the formulation does not negatively impair airway function and does not cause any undesirable side effects.

In another embodiment, the aerosolizable solution formulations are physiologically acceptable solutions, such as saline and/or buffered saline solutions. Illustrative saline concentrations are physiological, such as about 0.9% saline, or sub-physiological, such as in the range from about 0.1% saline to less than about 0.9% saline, including about 0.225% saline (25% physiological saline) solutions. It is to be understood that the formulations may also contain bromide and/or iodide. In another embodiment, the pH of the solutions are in the range from about 4.2 to about 7.5, about 4.5 to about 7.5, about 4.5 to about 7, about 5.5 to

about 7, or about 5.5 to about 6.5, or at about 6.0. It is to be understood that the foregoing pHs may be buffered or unbuffered.

In another embodiment, the aerosolizable formulations are dry powders or liquids formed from or capable of forming small particles suitable for deep endobronchial entry, such as small particles having average diameter, such as a mass medium average diameter (MMAD) predominantly of about 10 μm or less, such as in the range of about 1 to about 10 μm; predominantly of about 5 μm or less, such as in the range from about 1 to about 5 μm, or in the range from about 2 to about 5 μm. In another embodiment, the formulations are capable of being aerosolized, such as by nebulization, and/or are capable of forming small particles suitable for deep endobronchial entry, such as having average diameter, such as a MMAD predominantly of about 5 μm or less, such as in the range from about 1 to about 5 μm. As used herein, predominantly or a majority of generally refers to about 70% or greater, about 80% or greater, or about 90% or greater of the particles are about 10 μm or less, such as in the range of about 1 to about 10 μm; or about 5 μm or less, such as in the range from about 1 to about 5 μm, or in the range from about 2 to about 5 μm. It is to be understood that standard deviations of less than or equal to about 3 μm, or less than or equal to about 2 μm are observed in the foregoing ranges. It is to be understood that the foregoing specifically describes each and all integral values in each range.

In another embodiment, the aerosolizable solution formulations have an osmolarity in the range from about 50 to about 1050 mOsm/L, or in the range from about 50 to about 550 mOsm/L, or in the range from about 100 to about 750 mOsm/L, or in the range from about 200 to about 750 mOsm/L, or in the range from about 200 to about 600 mOsm/L, or in the range from about 300 to about 600 mOsm/L, or in the range from about 300 to about 500 mOsm/L, or in the range from about 150 to about 250 mOsm/L, or in the range from about 165 to about 190 mOsm/L. In another embodiment, the formulations have an osmolality in the range from about 50 to about 550 mOsm/kg, or in the range from about 165 to about 190 mOsm/kg.

In another embodiment, the compounds described herein are administered as an aerosol suspension, such as an aerosol suspension of liposomes or other microscopic particles.

In another embodiment, the aerosol formulation is nebulized into particle sizes which can be delivered to all parts of the lung, throughout the endothelial tree including the bronchi and bronchioli and the terminal and respiratory bronchioles, and the alveoli, where the bacteria may be present. It is appreciated that Pseudomonas aeruginosa bacterium or other susceptible bacteria that reside in patients with cystic fibrosis may be located in the terminal

and respiratory bronchioles. It is further appreciated that during exacerbation of infection, bacteria can also be present in alveoli.

In another embodiment, the formulation or composition is a dry powder comprising a compound described herein. In one aspect, the dry powder is dispersed into an inhalable configuration that comprises or consists essentially of particles having a MMAD of about 5 μm or less, or in the range from about 1 to about 5 μm, or in the range from about 2 to about 5 μm, or in the range from about 3 to about 5 μm.

Dry powder formulations may be prepared using any conventional process, including but not limited to milling, including media milling, jet milling, and the like, lyophilizing, spray drying, precipitating into a fine powder, and the like.

Illustratively, spray drying is accomplished by suspending the compounds described herein in water, stirring and cooling. The solution is optionally purified using a charcoal and filtered. Subsequently, the solution is spray dried using any suitable spay-drying equipment, such as, for example Buchi Mini Spray Dryer B-191.

Particle size determinations may be made using a multi-stage cascade impactor or other suitable method. Illustratively, the Thermo Andersen Eight Stage Non-Viable Cascade Impactor is specifically cited within the US Pharmacopoeia Chapter 601 as a characterizing device for aerosols within metered-dose and dry powder inhalers. The Eight Stage Cascade Impactor utilizes eight jet stages enabling classification of aerosols from 9.0 micrometers to 0.4 micrometers (at 28.3 L/min) and allows airborne particulate to impact upon stainless steel impaction surfaces or a variety of filtration media substrates. A final filter collects all particles smaller than 0.4.

Illustratively, media milling is accomplished by placing the compounds described herein into a mill containing, for example, stainless steel or ceramic balls and rotating or tumbling the material until the desired drug particle size ranges are achieved. It is appreciated that the advantages of media milling may include good size control, narrow product size ranges, high efficiencies of recovery, and readily scalable processes.

Illustratively, jet milling uses very high pressure air streams to collide particles with one another, with fine particles of the desired size being recovered from the mill. It is appreciated that the advantages of jet milling may include rapidity of the manufacturing process and less energy transfer during milling, resulting in less temperature rise during the drug production. The jet milling process is generally completed in seconds to minutes.

Illustratively, precipitation and/or crystallization is accomplished by adding a co-solvent to a solution of one or more compounds described herein that decreases the solubility of compounds to a uniform drug solution results in solute precipitation and/or crystallization.

When sufficient co-solvent is added, the solubility of the compounds fall to the point where solid drug particles are formed which can be collected by filtration or centrifugation. It is appreciated that precipitation and/or crystallization may have the advantage of being highly reproducible, having a high yield of recovery and being able to be performed under low temperature conditions, which reduce degradation.

In another embodiment, the nebulization rate of the aerosolizable formulations is at least about 1 μL/sec, at least about 2 μL/sec, at least about 3 μL/sec, at least about 4 μL/sec, or at least about 5 μL/sec

In another embodiment, the delivered unit dose is about 5 mL, about 4.5 mL, about 4 mL, about 3.75 mL, or about 3.5 mL. It is to be understood that the packaging may include more material, but it is configured so that the delivered dose is a predetermined portion thereof.

Illustrative nebulizers capable of providing aerosols of the formulations described herein include atomizing, jet, electronic, and ultrasonic nebulizers, pressurized, vibrating porous plate or equivalent nebulizers or by dry powder inhalers which predominantly produce aerosols or dry powder particles between 1 and 5 μm. It is appreciated that such particle sizes are desirable for efficacious delivery of compounds described herein into the endobronchial space to treat bacterial infections. The compositions and formulations are delivered by aerosolization using a nebulizer capable of producing a particle, where the majority of the particles have an average diameter of about 5 μm or less, or in the range from about 1 to about 5 μm.

Illustrative nebulizers include dry powder inhalers, metered dose inhalers, micronebulizers, ultrasonic nebulizers, and jet nebulizers, including standard and breath-enhanced (ultrasonic nebulizer (Ultraneb 100/99; Sunrise Medical HHG; Somerset, PA) specific nebulizer (Pari LC Plus Jet Nebulizer; Pari; Midlothian, VA) and compressor (Pulmo-Aide; Sunrise Medical HHG).

Illustrative jet nebulizers, including breath enhanced jet nebulizers, are SIDESTREAM, PARI LC, PARI LC PLUS (Pari Respiratory Equipment, Richmond, Va), and the like.

Illustrative ultrasonic nebulizers include AEROSONIC (from DeVilbiss), ULTRAAIRE (from Omron), and the like.

In another embodiment, the formulation is administered on a daily dosing schedule, including but not limited to q.d., b.i.d., t.i.d., q 8h, and the like. In one embodiment, the daily dosing schedule is 1 to 4 times daily, t.i.d., q 8h, or b.i.d. In another embodiment, the

daily dosing schedule is t.i.d. during awake hours, such as t.i.d. at 7 hour intervals or t.i.d. at 6 hour intervals.

In another embodiment, the compounds described herein are co-administered with an aminoglycoside, where the compounds described herein are dosed in the alternative of the aminoglycoside, such as during the 28 day mandatory off period for tobramycin dosing per manufacturer labeling required to prevent permanent tobramycin resistance.

In another embodiment, the formulation is contained in a five-milliliter plastic vial, such as low-density polyethylene (LDPE) vial. The vials may be aseptically filled using a blow-fill-seal process. Additional alternative illustrative packages are described in U.S. Pat. Nos. 5,409,125, 5,379,898, 5,213,860, 5,046,627, 4,995,519, 4,979,630, 4,951,822, 4,502,616 and 3,993,223, the disclosures of which are incorporated herein by this reference. The unit dose containers may be designed to be inserted directly into a particular device adapted to allow inhalation of the compound. Illustratively, the vials are sealed in foil over-pouches, six per over-pouch.

Illustrative parenteral doses of the macrolides are described in U.S. Patent Application Serial No. 61/312417, the disclosure of which is incorporated herein by reference.

In another embodiment, the macrolides described herein are co-administered with one or more other antibiotics, such as one or more other aminoglycosides, one or more other fluoroquinolones, aztreonam, and/or fosfomycin. In one variation, such co-administration may be performed without antagonism of the action of the aminoglycosides. In one aspect, the macrolides are administered at a dose lower than that capable of antagonizing or significantly antagonizing the efficacy of the aminoglycoside. In another variation, such co-administration may be performed to synergize the activity of another antibiotic, such as an aminoglycoside, a fluoroquinolone, aztreonam, and/or fosfomycin.

Antibiotic aminoglycosides, such as tobramycin, have been an important addition to the available therapies for treating pseudomonal infections. Pseudomonas aeruginosa grows in the endobronchial space and is found in the sputum of patients suffering from or in need of relief from pathogenic bacterial infections. During exacerbations of infection, Pseudomonas aeruginosa growth may also occur in the alveoli of patients. Illustrative diseases caused by pathogenic endobronchial Pseudomonas aeruginosa include cystic fibrosis.

In another illustrative embodiment, methods for treating cystic fibrosis (CF) are described herein. In one aspect, the methods include the step of administering one or more macrolides described herein. In one variation, the methods also include the step of administering one or more aminoglycosides. In another variation, the methods also include the step of administering one or more fluoroquinolones. In another variation, the methods also

include the step of administering aztreonam. In another variation, the methods also include the step of administering fosfomycin. In one variation of any of the foregoing methods, the method includes the step of administering the fluoroquinolones, such as levofloxacin, aminoglycoside antibiotics, such as tobramycin, aztreonam, and/or fosfomycin by inhalation.

Illustrative aminoglycosides include, but are not limited to, amikacin, apramycin, arbekacin, astromicin, bekanamycin, dibekacin, framycetin, gentamicin, hygromycin B, isepamicin, kanamycin, neomycin, netilmicin, paromomycin, paromomycin sulfate, rhodostreptomycin, ribostamycin, sisomicin, spectinomycin, streptomycin, tobramycin, verdamicin, and combinations thereof.

In another embodiment, the aminoglycoside is selected from gentamycin, amikacin, kanamycin, streptomycin, neomycin, netilmicin and tobramycin, and combinations thereof.

Illustrative dosing regimens and protocols for aminoglycoside antibiotics that may be used in the methods described herein, are described in US Patent Nos. 5508269, 6083922, and 6890907, the disclosures of which are incorporated herein by reference. In one variation, the method includes the step of administering the aminoglycoside antibiotic, such as tobramycin, by inhalation.

Any conventional dosage unit, formulation, and/or method of administering aminoglycosides may be used herein, such as the dosage units, formulations, and/or methods of administering tobramycin as described in U.S. Patent Nos. 5508269, 6890907, 6083922, and 7696178, the disclosures of which are incorporated herein by reference.

Illustrative fluoroquinolone antibiotics include, but are not limited to oxolinic acid (Uroxin), piromidic acid (Panacid), pipemidic acid (Dolcol), rosoxacin (Eradacil), ciprofloxacin (Ciprobay, Cipro, Ciproxin), lomefloxacin (Maxaquin), nadifloxacin (Acuatim, Nadoxin, Nadixa), norfloxacin (Lexinor, Noroxin, Quinabic, Janacin), ofloxacin (Floxin, Oxaldin, Tarivid), pefloxacin (Peflacine), rufloxacin (Uroflox), balofloxacin (Baloxin), levofloxacin (Cravit, Levaquin), moxifloxacin (Avelox,Vigamox, pazufloxacin (Pasil, Pazucross), sparfloxacin (Zagam), tosufloxacin (Ozex, Tosacin), clinafloxacin, gemifloxacin (Factive), sitafloxacin (Gracevit), prulifloxacin (Quisnon), delafloxacin, and combinations thereof.

Any conventional dosage unit, formulation, and/or method of administering fluoroquinolone antibiotics may be used herein.

Any conventional dose, formulation, and/or method of administering aztreonam may be used herein, such as the dosage units, formulations, and/or methods of administering

aztreonam as described in U.S. Pat. Nos. 6660249 and 7214364, the disclosures of which are incorporated herein by reference.

In another embodiment, one or more macrolides described herein are administered in a suppression therapy protocol. In another embodiment, one or more macrolides described herein are administered in an adjunct therapy protocol for an acute exacerbation of the pulmonary disease, such as CF. In another embodiment, the protocol is capable of preventing or delaying chronic P. aeruginosa infection and/or colonization.

In another embodiment, one or more macrolides described herein, such as CEM-101, are co-administered with tobramycin.

In another embodiment, one or more macrolides described herein, such as CEM-101, are co-administered with levofloxacin.

In another embodiment, one or more macrolides described herein, such as CEM-101, are co-administered with aztreonam.

In another embodiment, CEM-101 is co-administered with tobramycin, where the CEM-101 is administered orally and the tobramycin is administered by inhalation. In another embodiment, CEM-101 is co-administered with aztreonam, where the CEM-101 is administered orally and the aztreonam is administered by inhalation. In another embodiment, CEM-101 is co-administered with tobramycin, where the CEM-101 is administered by inhalation and the tobramycin is administered by inhalation. In another embodiment, CEM-101 is co-administered with aztreonam, where the CEM-101 is administered by inhalation and the aztreonam is administered by inhalation. In one variation, the co-administration follows a protocol where the tobramycin or a aztreonam is administered during a first period of administration, for example days 1-28, and the CEM-101 is administered during a second period, for example days 29-56. The alternate periods may be repeated.

In another embodiment, illustrative daily oral doses of the macrolides described herein, such as CEM-101, are in the range from about 1 to about 25 mg/kg, about 1 to about 10 mg/kg, about 2 to about 8 mg/ kg, or about 4 to about 6 mg/kg of patient body weight. In another embodiment, illustrative daily adult human oral doses of the macrolides described herein, such as CEM-101, are in the range from about 100 to about 1,000 mg, about 200 to about 800 mg, or about 400 to about 600 mg. In another embodiment, the daily dose is single or divided and may be administered qd, bid, tid, and the like.

It is understood that dosing is desirably performed to achieve sputum concentrations at least about 10 times the MIC for one or more of the target organisms of the infection or disease. Without being bound by theory, it is believed that such illustrative dosages are sufficient to achieve lung levels of about 1 μg/mL or greater, about 2 μg/mL or greater,

about 4 μg/mL or greater, or about 8 μg/mL or greater, which may in most cases correspond to a concentration that is about 10 times the MIC or greater. Without being bound by theory, it is also believed that such illustrative dosages be sufficient to observe bactericidal activity against lung pathogens.

The term "therapeutically effective amount" as used herein, refers to that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a tissue system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician, which includes alleviation of the symptoms of the disease or disorder being treated. In one aspect, the therapeutically effective amount is that which may treat or alleviate the disease or symptoms of the disease at a reasonable benefit/risk ratio applicable to any medical treatment. However, it is to be understood that the total daily usage of the compounds and compositions described herein may be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors, including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, gender and diet of the patient: the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidentally with the specific compound employed; and like factors well known to the researcher, veterinarian, medical doctor or other clinician of ordinary skill.

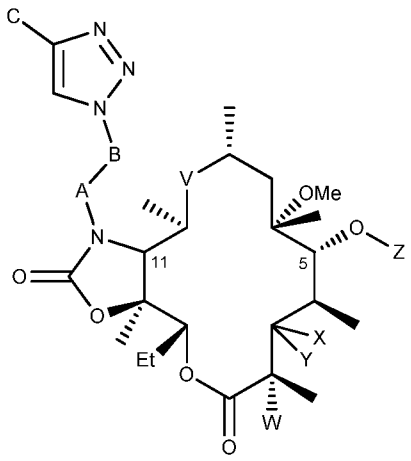
As used herein, the term therapeutically effective amount as applied to compositions, formulation, kits, methods, and the like, illustratively may include an amount that achieves sputum concentration of the compounds at about 10X the MIC or greater for the target organism.

It is appreciated that successful therapy may be monitored by any conventional method or endpoint, including but not limited to the decrease in the decline of or the improvement in forced expiratory volume (FEV), and/or forced vital capacity (FCV). It is further appreciated that successful therapy may be monitored by the decrease or the decrease in the growth of colony forming units (CFUs) of the target bacteria in sputum. It is further appreciated that successful therapy may be monitored by a decrease in persisters of Pseudomonas and/or Burkholderia species.

Illustrative target pathogenic bacteria include, but are not limited to, staphylococci, including coagulase-negative staphylococci and coagulase-positive staphylococci, streptococci, including Group A beta hemolytic streptococci, non-Group A beta hemolytic streptococci and viridans group streptococci, enterococci, Nesseria species,

Clostridium species, Bordetella species, Bacillus species and Corynebacterium species. In particular, the bacterial infection is an infection caused by bacteria selected from the group consisting of Staphylococcus aureus (methicillin-resistant and -susceptible), Staphylococcus epidermidis, Staphylococcus hemolyticus, Staphylococcus saprophyticus, Staphylococcus lugdunensis, Staphylococcus capitis, Staphylococcus caprae, Staphylococcus saccharolyticus, Staphylococcus simulans, Staphylococcus warneri, Staphylococcus hominis, Staphylococcus intermedius, Staphylococcus pseudointermedius, Staphylococcus lyricus, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae subspecies dysgalactiae, Streptococcus anginosus, Streptococcus mitis, Streptococcus salivarius, Streptococcus bovis, Streptococcus mutans, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Neisseria meningitidis, Bacillus anthracis, Bordetella pertussis, Burkholderia cepacia, Clostridium difficile, Enterococcus faecalis, Enterococcus faecium and Corynebacterium diphtheriae. In particular aspects, the bacterial infection is an infection caused by Staphylococcus aureus (methicillin-resistant or -susceptible

In another illustrative embodiment, the macrolides described herein are of the formula



and pharmaceutically acceptable salts, hydrates, solvates, esters, and prodrugs thereof, wherein:

X is H; and Y is OR_7; where R_7 is a monosaccharide or disaccharide, or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl;

Z is a monosaccharide or disaccharide, or a derivative thereof;

V is C(O), or $C(=NR_{11})$, wherein R_{11} is hydroxy or alkoxy;

W is H, F, Cl, Br, I, or OH;

A is CH_2, C(O), C(O)O, C(O)NH, $S(O)_2$, $S(O)_2NH$, or $C(O)NHS(O)_2$;

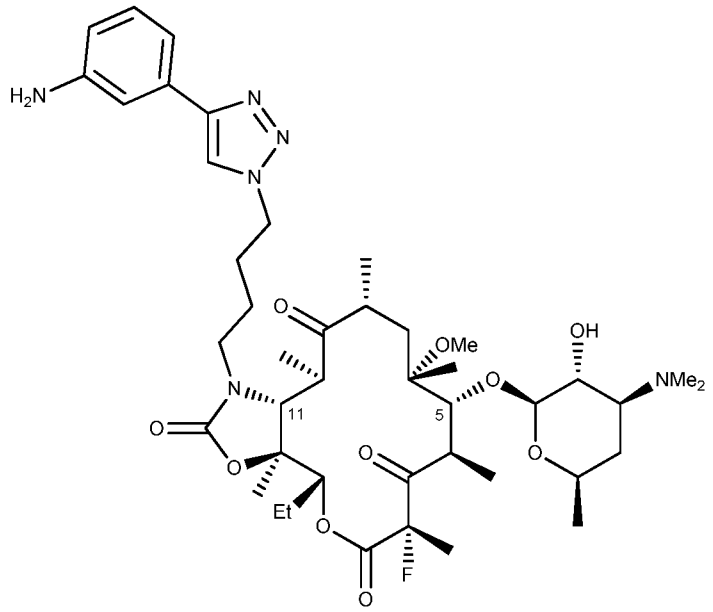
B is $(CH_2)_n$ where n is an integer in the range from 0 to about 10, or B is C_2-C_{10} alkenyl or alkynyl; and

C is cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted.

In another embodiment, X and Y are taken together with the attached carbon to form carbonyl. In another embodiment, Z is a monosaccharide or a derivative thereof. In another embodiment, Z is an amino-containing monosaccharide, such as an amino glucose or a derivative, analog, or stereoisomer thereof, including but not limited to desosamines and derivatives thereof, mycaminose and derivatives thereof, vancosamine and derivatives thereof, L-vancosamine, 3-desmethyl-vancosamine, 3-epi-vancosamine, 4-epi-vancosamine, acosamine, 3-amino-glucose, 4-deoxy-3-amino-glucose, actinosamine, daunosamine, 3-epi-daunosamine, ristosamine, N-methyl-D-glucamine, and the like. In another embodiment, Z is a desosamine or a derivative thereof. In another embodiment, Z is a mycaminose or a derivative thereof. In another embodiment, Z is a desosamine. In another embodiment, Z is a mycaminose. In another embodiment, V is C(O). In another embodiment, W is H or F. In another embodiment, W is F. In another embodiment, A is CH_2. In another embodiment, B is $(CH_2)_n$ where n is an integer in the range from about 2 to about 4. In another embodiment, B is $(CH_2)_3$. In another embodiment, C is optionally substituted aryl. In another embodiment, C is amino substituted aryl.

It is to be understood that each of the foregoing selections of X, Y, Z, W, A, B, and n may be combined without limitation, and therefore, such subgenera of compounds are specifically described herein. For example, in another embodiment, X and Y are taken together with the attached carbon to form carbonyl, and Z is a monosaccharide or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl, and V is C(O); or X and Y are taken together with the attached carbon to form carbonyl, W is F, and A is CH_2; or Z is a desosamine or a derivative thereof, V is C(O), A is CH_2, and B is $(CH_2)_n$ where n is an integer in the range from about 2 to about 4; and the like.

In another embodiment, the macrolide described herein is of the formula



also known as CEM-101 or solithromycin, or a pharmaceutically acceptable salt, hydrate, solvate, ester, or prodrug thereof.

The macrolides described herein may be prepared as described herein, or according to US Patent Application Publication No. 2006/0100164 and in PCT International Publication No. WO 2009/055557, the disclosures of which are incorporated herein by reference in their entirety.

As used herein, the term "alkyl" includes a chain of carbon atoms, which is optionally branched. As used herein, the term "alkenyl" and "alkynyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond or triple bond, respectively. It is to be understood that alkynyl may also include one or more double bonds. It is to be further understood that in certain embodiments, alkyl is advantageously of limited length, including C_1-C_{24}, C_1-C_{12}, C_1-C_8, C_1-C_6, and C_1-C_4. It is to be further understood that in certain embodiments alkenyl and/or alkynyl may each be advantageously of limited length, including C_2-C_{24}, C_2-C_{12}, C_2-C_8, C_2-C_6, and C_2-C_4. It is appreciated herein that shorter alkyl, alkenyl, and/or alkynyl groups may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior. Illustrative alkyl groups are, but not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, 2-pentyl, 3-pentyl, neopentyl, hexyl, heptyl, octyl and the like.

As used herein, the term "cycloalkyl" includes a chain of carbon atoms, which is optionally branched, where at least a portion of the chain in cyclic. It is to be understood that cycloalkylalkyl is a subset of cycloalkyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkyl include, but are not limited to, cyclopropyl, cyclopentyl, cyclohexyl, 2-methylcyclopropyl, cyclopentyleth-2-yl, adamantyl, and the like. As used herein,

the term "cycloalkenyl" includes a chain of carbon atoms, which is optionally branched, and includes at least one double bond, where at least a portion of the chain in cyclic. It is to be understood that the one or more double bonds may be in the cyclic portion of cycloalkenyl and/or the non-cyclic portion of cycloalkenyl. It is to be understood that cycloalkenylalkyl and cycloalkylalkenyl are each subsets of cycloalkenyl. It is to be understood that cycloalkyl may be polycyclic. Illustrative cycloalkenyl include, but are not limited to, cyclopentenyl, cyclohexylethen-2-yl, cycloheptenylpropenyl, and the like. It is to be further understood that chain forming cycloalkyl and/or cycloalkenyl is advantageously of limited length, including C_3-C_{24}, C_3-C_{12}, C_3-C_8, C_3-C_6, and C_5-C_6. It is appreciated herein that shorter alkyl and/or alkenyl chains forming cycloalkyl and/or cycloalkenyl, respectively, may add less lipophilicity to the compound and accordingly will have different pharmacokinetic behavior.

As used herein, the term "heteroalkyl" includes a chain of atoms that includes both carbon and at least one heteroatom, and is optionally branched. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. As used herein, the term "cycloheteroalkyl" including heterocyclyl and heterocycle, includes a chain of atoms that includes both carbon and at least one heteroatom, such as heteroalkyl, and is optionally branched, where at least a portion of the chain is cyclic. Illustrative heteroatoms include nitrogen, oxygen, and sulfur. In certain variations, illustrative heteroatoms also include phosphorus, and selenium. Illustrative cycloheteroalkyl include, but are not limited to, tetrahydrofuryl, pyrrolidinyl, tetrahydropyranyl, piperidinyl, morpholinyl, piperazinyl, homopiperazinyl, quinuclidinyl, and the like.

As used herein, the term "aryl" includes monocyclic and polycyclic aromatic groups, including aromatic carbocyclic and aromatic heterocyclic groups, each of which may be optionally substituted. As used herein, the term "carbaryl" includes aromatic carbocyclic groups, each of which may be optionally substituted. Illustrative aromatic carbocyclic groups described herein include, but are not limited to, phenyl, naphthyl, and the like. As used herein, the term "heteroaryl" includes aromatic heterocyclic groups, each of which may be optionally substituted. Illustrative aromatic heterocyclic groups include, but are not limited to, pyridinyl, pyrimidinyl, pyrazinyl, triazinyl, tetrazinyl, quinolinyl, quinazolinyl, quinoxalinyl, thienyl, pyrazolyl, imidazolyl, oxazolyl, thiazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiadiazolyl, triazolyl, benzimidazolyl, benzoxazolyl, benzthiazolyl, benzisoxazolyl, benzisothiazolyl, and the like.

As used herein, the term "amino" includes the group NH_2, alkylamino, and dialkylamino, where the two alkyl groups in dialkylamino may be the same or different, i.e. alkylalkylamino. Illustratively, amino includes methylamino, ethylamino, dimethylamino,

methylethylamino, and the like. In addition, it is to be understood that when amino modifies or is modified by another term, such as aminoalkyl, or acylamino, the above variations of the term amino are included therein. Illustratively, aminoalkyl includes H_2N-alkyl, methylaminoalkyl, ethylaminoalkyl, dimethylaminoalkyl, methylethylaminoalkyl, and the like. Illustratively, acylamino includes acylmethylamino, acylethylamino, and the like.

As used herein, the term "amino and derivatives thereof" includes amino as described herein, and alkylamino, alkenylamino, alkynylamino, heteroalkylamino, heteroalkenylamino, heteroalkynylamino, cycloalkylamino, cycloalkenylamino, cycloheteroalkylamino, cycloheteroalkenylamino, arylamino, arylalkylamino, arylalkenylamino, arylalkynylamino, acylamino, and the like, each of which is optionally substituted. The term "amino derivative" also includes urea, carbamate, and the like.

The term "optionally substituted" as used herein includes the replacement of hydrogen atoms with other functional groups on the radical that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, and/or sulfonic acid is optionally substituted.

As used herein, the term "optionally substituted aryl" includes the replacement of hydrogen atoms with other functional groups on the aryl that is optionally substituted. Such other functional groups illustratively include, but are not limited to, amino, hydroxyl, halo, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, nitro, sulfonic acids and derivatives thereof, carboxylic acids and derivatives thereof, and the like. Illustratively, any of amino, hydroxyl, thiol, alkyl, haloalkyl, heteroalkyl, aryl, arylalkyl, arylheteroalkyl, and/or sulfonic acid is optionally substituted.

Illustrative substituents include, but are not limited to, a radical $-(CH_2)_xZ^X$, where x is an integer from 0-6 and Z^X is selected from halogen, hydroxy, alkanoyloxy, including C_1-C_6 alkanoyloxy, optionally substituted aroyloxy, alkyl, including C_1-C_6 alkyl, alkoxy, including C_1-C_6 alkoxy, cycloalkyl, including C_3-C_8 cycloalkyl, cycloalkoxy, including C_3-C_8 cycloalkoxy, alkenyl, including C_2-C_6 alkenyl, alkynyl, including C_2-C_6 alkynyl, haloalkyl, including C_1-C_6 haloalkyl, haloalkoxy, including C_1-C_6 haloalkoxy, halocycloalkyl, including C_3-C_8 halocycloalkyl, halocycloalkoxy, including C_3-C_8 halocycloalkoxy, amino, C_1-C_6 alkylamino, (C_1-C_6 alkyl)(C_1-C_6 alkyl)amino, alkylcarbonylamino, N-(C_1-C_6 alkyl)alkylcarbonylamino, aminoalkyl, C_1-C_6 alkylaminoalkyl, (C_1-C_6 alkyl)(C_1-C_6 alkyl)aminoalkyl, alkylcarbonylaminoalkyl, N-(C_1-C_6 alkyl)alkylcarbonylaminoalkyl, cyano,

and nitro; or Z^X is selected from $-CO_2R^4$ and $-CONR^5R^6$, where R^4, R^5, and R^6 are each independently selected in each occurrence from hydrogen, C_1-C_6 alkyl, and aryl-C_1-C_6 alkyl.

The compounds described herein may contain one or more chiral centers, or may otherwise be capable of existing as multiple stereoisomers. It is to be understood that in one embodiment, the invention described herein is not limited to any particular stereochemical requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be optically pure, or may be any of a variety of stereoisomeric mixtures, including racemic and other mixtures of enantiomers, other mixtures of diastereomers, and the like. It is also to be understood that such mixtures of stereoisomers may include a single stereochemical configuration at one or more chiral centers, while including mixtures of stereochemical configuration at one or more other chiral centers.

Similarly, the compounds described herein may be include geometric centers, such as cis, trans, E, and Z double bonds. It is to be understood that in another embodiment, the invention described herein is not limited to any particular geometric isomer requirement, and that the compounds, and compositions, methods, uses, and medicaments that include them may be pure, or may be any of a variety of geometric isomer mixtures. It is also to be understood that such mixtures of geometric isomers may include a single configuration at one or more double bonds, while including mixtures of geometry at one or more other double bonds.

As used herein, the term "composition" generally refers to any product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combinations of the specified ingredients in the specified amounts. It is to be understood that the compositions described herein may be prepared from isolated compounds described herein or from salts, solutions, hydrates, solvates, and other forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various amorphous, non-amorphous, partially crystalline, crystalline, and/or other morphological forms of the compounds described herein. It is also to be understood that the compositions may be prepared from various hydrates and/or solvates of the compounds described herein. Accordingly, such pharmaceutical compositions that recite compounds described herein are to be understood to include each of, or any combination of, the various morphological forms and/or solvate or hydrate forms of the compounds described herein. Illustratively, compositions may include one or more carriers, diluents, and/or excipients. The compounds described herein, or compositions containing them, may be formulated in a therapeutically effective amount in any conventional dosage forms appropriate for the methods described herein. The compounds described herein, or compositions containing them, including such formulations, may be administered by a wide variety of conventional routes for the

methods described herein, and in a wide variety of dosage formats, utilizing known procedures (see generally, Remington: The Science and Practice of Pharmacy, (21st ed., 2005)).

The term "prodrug" as used herein generally refers to any compound that when administered to a biological system generates a biologically active compound as a result of one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof. In vivo, the prodrug is typically acted upon by an enzyme (such as esterases, amidases, phosphatases, and the like), simple biological chemistry, or other process in vivo to liberate or regenerate the more pharmacologically active drug. This activation may occur through the action of an endogenous host enzyme or a non-endogenous enzyme that is administered to the host preceding, following, or during administration of the prodrug. Additional details of prodrug use are described in U.S. Pat. No. 5,627,165; and Pathalk et al., Enzymic protecting group techniques in organic synthesis, Stereosel. Biocatal. 775-797 (2000). It is appreciated that the prodrug is advantageously converted to the original drug as soon as the goal, such as targeted delivery, safety, stability, and the like is achieved, followed by the subsequent rapid elimination of the released remains of the group forming the prodrug.

Prodrugs may be prepared from the compounds described herein by attaching groups that ultimately cleave in vivo to one or more functional groups present on the compound, such as -OH-, -SH, $-CO_2H$, $-NR_2$. Illustrative prodrugs include but are not limited to carboxylate esters where the group is alkyl, aryl, aralkyl, acyloxyalkyl, alkoxycarbonyloxyalkyl as well as esters of hydroxyl, thiol and amines where the group attached is an acyl group, an alkoxycarbonyl, aminocarbonyl, phosphate or sulfate. Illustrative esters, also referred to as active esters, include but are not limited to 1-indanyl, N-oxysuccinimide; acyloxyalkyl groups such as acetoxymethyl, pivaloyloxymethyl, β-acetoxyethyl, β-pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, (1 -aminoethyl)carbonyloxymethyl, and the like; alkoxycarbonyloxyalkyl groups, such as ethoxycarbonyloxymethyl, α-ethoxycarbonyloxyethyl, β-ethoxycarbonyloxyethyl, and the like; dialkylaminoalkyl groups, including di-lower alkylamino alkyl groups, such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl, diethylaminoethyl, and the like; 2-(alkoxycarbonyl)-2-alkenyl groups such as 2-(isobutoxycarbonyl) pent-2-enyl, 2-(ethoxycarbonyl)but-2-enyl, and the like; and lactone groups such as phthalidyl, dimethoxyphthalidyl, and the like.

Further illustrative prodrugs contain a chemical moiety, such as an amide or phosphorus group functioning to increase solubility and/or stability of the compounds described herein. Further illustrative prodrugs for amino groups include, but are not limited to, (C_3-C_{20})alkanoyl; halo-(C_3-C_{20})alkanoyl; (C_3-C_{20})alkenoyl; (C_4-C_7)cycloalkanoyl; (C_3-C_6)-

cycloalkyl(C_2-C_{16})alkanoyl; optionally substituted aroyl, such as unsubstituted aroyl or aroyl substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano, trifluoromethanesulphonyloxy, (C_1-C_3)alkyl and (C_1-C_3)alkoxy, each of which is optionally further substituted with one or more of 1 to 3 halogen atoms; optionally substituted aryl(C_2-C_{16})alkanoyl, such as the aryl radical being unsubstituted or substituted by 1 to 3 substituents selected from the group consisting of halogen, (C_1-C_3)alkyl and (C_1-C_3)alkoxy, each of which is optionally further substituted with 1 to 3 halogen atoms; and optionally substituted heteroarylalkanoyl having one to three heteroatoms selected from O, S and N in the heteroaryl moiety and 2 to 10 carbon atoms in the alkanoyl moiety, such as the heteroaryl radical being unsubstituted or substituted by 1 to 3 substituents selected from the group consisting of halogen, cyano, trifluoromethanesulphonyloxy, (C_1-C_3)alkyl, and (C_1-C_3)alkoxy, each of which is optionally further substituted with 1 to 3 halogen atoms. The groups illustrated are exemplary, not exhaustive, and may be prepared by conventional processes.

It is understood that the prodrugs themselves may not possess significant biological activity, but instead undergo one or more spontaneous chemical reaction(s), enzyme-catalyzed chemical reaction(s), and/or metabolic chemical reaction(s), or a combination thereof after administration in vivo to produce the compound described herein that is biologically active or is a precursor of the biologically active compound. However, it is appreciated that in some cases, the prodrug is biologically active. It is also appreciated that prodrugs may often serves to improve drug efficacy or safety through improved oral bioavailability, pharmacodynamic half-life, and the like. Prodrugs also refer to derivatives of the compounds described herein that include groups that simply mask undesirable drug properties or improve drug delivery. For example, one or more compounds described herein may exhibit an undesirable property that is advantageously blocked or minimized may become pharmacological, pharmaceutical, or pharmacokinetic barriers in clinical drug application, such as low oral drug absorption, lack of site specificity, chemical instability, toxicity, and poor patient acceptance (bad taste, odor, pain at injection site, and the like), and others. It is appreciated herein that a prodrug, or other strategy using reversible derivatives, can be useful in the optimization of the clinical application of a drug.

It is to be understood that in the methods described herein, the individual components of a co-administration, or combination can be administered by any suitable means, contemporaneously, simultaneously, sequentially, separately or in a single pharmaceutical formulation. Where the co-administered compounds or compositions are administered in separate dosage forms, the number of dosages administered per day for each compound may be the same or different. The compounds or compositions may be administered via the same or

different routes of administration. The compounds or compositions may be administered according to simultaneous or alternating regimens, at the same or different times during the course of the therapy, concurrently in divided or single forms.

Illustrative routes of oral administration include tablets, capsules, elixirs, syrups, and the like.

Illustrative routes for parenteral administration include intravenous, intraarterial, intraperitoneal, epidurial, intraurethral, intrasternal, intramuscular and subcutaneous, as well as any other art recognized route of parenteral administration. Illustrative means of parenteral administration include needle (including microneedle) injectors, needle-free injectors and infusion techniques, as well as any other means of parenteral administration recognized in the art. Parenteral formulations are typically aqueous solutions which may contain excipients such as salts, carbohydrates and buffering agents (preferably at a pH in the range from about 3 to about 9), but, for some applications, they may be more suitably formulated as a sterile non-aqueous solution or as a dried form to be used in conjunction with a suitable vehicle such as sterile, pyrogen-free water. The preparation of parenteral formulations under sterile conditions, for example, by lyophilization, may readily be accomplished using standard pharmaceutical techniques well known to those skilled in the art. Parenteral administration of a compound is illustratively performed in the form of saline solutions or with the compound incorporated into liposomes. In cases where the compound in itself is not sufficiently soluble to be dissolved, a solubilizer such as ethanol can be applied.

EXAMPLES

EXAMPLE. Inhalable formulation. The compounds described herein, such as CEM-101, are formulated in conventional dry powder or solution formulations. The compounds may be optionally formulated as pharmaceutically acceptable salts. The dry powder formulations are administered using a passive dry powder inhaler. The solution formulations are administered using a pressured metered dose inhaler, a nebulizer, or similar device. Illustrative dry powder formulations include, but are not limited to, Pulmosphere (PS) formulations (Inhale Therapeutic Systems, San Carlos, CA), and the like. PS formulations are prepared according to Dellamary et al., Hollow porous particles in metered dose inhalers, Pharm Res 17:168-174 (2000). The disclosure of the foregoing publication, and each additional publication cited herein, are each incorporated herein by reference.

EXAMPLE. Preparation of pseudomonal alginate. P. aeruginosa, such as NH57388A, is cultured in 50 mL Mueller-hinton broth (MHB) for 24 to 28 h at 37°C with shaking (170 rpm). The bacterial cells are harvested by centrifugation (23,000 x g, 30 min, 4°C) and resuspended in 3 to 6 mL of MHB. The supernatant is collected and placed in an 80°C

water bath for 30 min. Alginate is precipitated by adding the supernatant to 150 mL of ice-cold 99% ethanol. The precipitated alginate is collected with a sterile bacterial loop and washed several times in sterile saline. The purified alginate is then resuspended in 10 mL of sterile saline and stirred vigorously to form a homogeneous suspension. The alginate concentration is measured and adjusted to a concentration of 2 to 3 mg/mL.

EXAMPLE. Model of acute mouse lung infection. P. aeruginosa, such as ATCC 27853, is grown overnight in MHB at 35°C. The bacterial suspensions re adjusted to ca. 1 x 10^5 to 6 x 10^5 CFU/mL by correlation of the absorbance at 600 nm with predetermined plate counts. Female Swiss mice are made neutropenic by the i.p. injection of 150 mg/kg cyclophosphamide (Baxter, Deerfield, IL) on days 1 and 3. On day 4, the mice are infected by the intratracheal instillation of 0.05 mL of inoculum with a curved oral gavage tip attached to a 1-mL syringe. Antibiotic treatments are started at 24 h postinfection and are administered once or twice daily (BID) for 24 or 48 h. Antibiotics are aerosolized with a microspray aerosol device. All infections and aerosol treatments are performed while the mice are under isoflurane anesthesia (5% isoflurane in oxygen running at 4 L/min). An untreated group of mice (n = 8) is killed prior to the initiation of treatment to determine baseline bacterial counts. At 12 to 16 h following administration of the last antibiotic dose, the treated animals (n = 8) are killed by carbon dioxide asphyxiation. The lungs are removed aseptically and homogenized (Pro200 homogenizer; Pro Scientific, Monroe, CT) in 1 mL of sterile saline. Serial 10-fold dilutions of the homogenized lung are plated on Mueller-Hinton agar, and the colonies are counted. For the survival studies, mice (n = 10) are observed for 7 days after the end of treatment or for a total of 9 days postinfection.

EXAMPLE. Model of chronic mouse lung infection. P. aeruginosa, such as NH57388A, is cultured in 50 mL MHB for 24 to 28 h at 37°C with shaking (170 rpm). The bacterial cells are harvested by centrifugation (23,000 x g, 30 min, 4°C) and resuspended in 3 to 6 mL of MHB. The bacterial suspension is diluted (1:10) in the alginate suspension to yield about 10^8 CFU/mL. The initial establishment of infection is achieved by the establishment of a transient neutropenia by administration of a single 150-mg/kg i.p. dose of cyclophosphamide 4 days prior to infection. On day 4, the mice are infected by use of a curved bead-tipped oral gavage attached to a 1-mL syringe while the mice are under isoflurane anesthesia. Antibiotic treatments are started at 24 h postinfection and are administered BID for three consecutive days. Various concentrations of antibiotics are used, and they are administered either by an oral, i.p., or aerosol route with a microspray device. At 12 to 16 h following the last treatment, the mice are killed, and the colony counts in the lung are determined as described herein.

Statistical analysis. Survival and lung bacterial counts are analyzed by the log-rank test and the Mann-Whitney U test (GraphPad Prism, version 4.03), respectively. A P value of <0.05 is considered statistically significant.

EXAMPLE. The compounds described herein are poor substrates of efflux pumps expressed in P. aeruginosa (PA). MICs of 36 clinical and laboratory strains of PA with known efflux phenotype are measured by microdilution in cation-adjusted Muller-Hinton broth (CA-MHB) or in RPMI medium (commonly used in eukaryotic cell culture). Phe-Arg-β-naphthylamide (PaβN, 50 mg/L) and EGTA 5mM are used to inhibit efflux pumps and alter OM integrity, respectively. P. aeruginosa strain ATCC PAO1 is used as reference. PA12 is a clinical strain overexpressing the 4 main efflux systems (MexAB, MexCD, MexEF, MexXY), PA403 is a laboratory strain deleted in the genes coding for the 4 efflux systems. A series of reference strains or of clinical isolates for which the expression of genes coding for efflux pumps is known is also used for MICs determinations. MICs are measured by microdilution in MH broth or in RPMI medium (used for eukaryotic cells culture) supplemented with 10% of fetal calf serum, or in MH broth supplemented by increasing amounts of serum. EGTA (5mM) was used as a chelating agent (disrupting outer membrane integrity) and PaβN (50 mg/L) as an unspecific efflux inhibitor. The results are shown in Table 1.

Table 1.

Strains	Efflux expression	ERY		CLR		AZM		TEL		CEM-101	
		MHB	RPMI	MHB	RPMI	MHB	RPMI	MHB	RPMI	MHB	RPMI
12	AB+CD+EF+XY+	512	32	512	16	256	2	128	4	128	4
434	AB+CD+XY+	512	128	512	128	512	4	128	4	128	8
63	AB+ EF+XY+	512	64	512	32	256	2	128	4	64	4
207	AB+ EF+XY+	512	128	512	64	512	4	128	4	32	4
48	CD+EF+XY+	512	64	512	64	256	2	128	8	128	4
49	CD+EF+XY+	512	64	512	32	256	2	128	4	128	4
11	AB+CD+	256	16	512	16	128	2	64	1	16	2
266B	AB+CD+	512	64	512	64	256	2	256	4	256	4
333A	AB+ EF+	512	64	512	64	256	2	64	2	128	2
335	AB+ EF+	512	64	512	64	512	4	128	4	128	2
16	AB+ XY+	512	32	512	64	256	4	128	4	32	4
68	AB+ XY+	512	64	512	64	256	2	128	4	64	4
168B	CD+ XY+	512	256	512	256	512	4	256	4	128	4
133	EF+XY+	512	64	512	64	256	4	128	4	64	4
156	EF+XY+	512	16	512	32	512	4	128	2	64	2
1	AB+	512	8	512	32	128	4	128	4	128	2
21	AB+	512	64	512	64	256	2	64	2	128	4
2	CD+	512	256	512	128	512	4	256	16	256	8
41	CD+	512	64	512	64	256	2	256	4	256	4
3	EF+	256	8	256	16	64	2	64	0.25	8	1
40	EF+	256	32	256	16	256	2	64	2	32	4
4	XY+	512	32	512	32	256	2	128	4	128	4
22	XY+	512	32	512	32	256	2	128	2	64	4
PAO1	REFERENCE	512	32	512	32	256	4	256	2	128	4
397	AB-	16	2	16	2	8	1	8	0.03	2	1
392	CD-	256	16	256	16	128	2	32	0.5	16	1
398	CD-	16	4	32	4	16	1	8	0.25	4	2
391	EF-	256	32	256	16	128	2	64	2	32	2
394	XY-	512	32	512	32	256	4	64	1	32	4
400	XY-	16	4	16	4	8	2	8	0.25	4	1
395	HI-	256	32	256	32	128	2	32	2	64	2
396	ompH-	128	16	64	16	64	2	16	0.25	8	2
401	ompH-	8	4	8	2	8	2	4	1	2	2
399	AB – EF -	16	4	16	4	16	2	4	0.25	8	1
403	AB –CD – EF – XY -	16	4	16	4	8	2	4	0.5	4	1
405	AB –CD – EF – XY -	8	4	8	4	8	2	8	0.25	4	1

Table 2 shows the results obtained with PAO1 (wild-type), PA12 (overexpressing 4 efflux pumps), and PA403 (disrupted for genes coding for 5 efflux pumps).

Table 2.

	PAO1 (a)						PA12 (b)					
	CA-MHB			RPMI			CA-MHB			RPMI		
	CT	PABN	EGTA	CT	PaBN	EGTA	CT	PaBN	EGTA	CT	PaBN	EGTA
ERY	512	16	256	32	32	32	512	32	512	32	32	2
CLR	512	8	256	32	4	32	512	16	256	16	16	2
AZI	128	4	8	2	4	0.25	256	2	256	2	2	0.5
TEL	128	4	32	2	4	1	128	4	32	4	4	1
CEM-101	32	8	8	2	2	1	32	4	16	4	4	1

	P403 ©					
	CA-MHB			RPMI		
	CT	PaBN	EGTA	CT	PaBN	EGTA
ERY	16	ND	16	4	4	0.5
CLR	16	ND	32	4	4	0.5
AZI	8	ND	2	2	2	0.125
TEL	4	ND	2	0.5	1	0.06
CEM-101d	4	ND	1	1	2	0.25

MIC (mg/L) in control conditions (CT), in the presence of PaβN 50 mg/L or EGTA 5 mM. (a) wild type strain; (b) clinical isolate overexpressing MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM; (c) Δ(MexAB-OprM), Δ(MexCD-OprJ), Δ(MexEF-OprN), Δ(MexJK), Δ(MexXY). MICs of all molecules were high against PAO1 and PA12 in CA-MHB but reduced if tested in either RPMI or in the presence of PaβN (reaching values close to those of PA403). EGTA reduced the MICs of ketolides in CA-MHB and had an additive effect in RPMI. CEM-101 showed a smaller difference in the presence of efflux pump inhibitors.

EXAMPLE. Several in vivo protocols wherein CEM-101 is repeatedly dosed in toxicology studies in rodents and non human primates have demonstrated tissue levels of CEM-101 between about 17 and about 100X higher than peak plasma levels. CEM-101 accumulated in tissues and concentrations were highest in liver, spleen, lung, and salivary gland. This relationship was confirmed in rodent ADME studies using radiolabeled CEM-101. When administered orally at 100 mg/kg, lung tissue to plasma radioactivity ratios of about 13:1 were observed in male and female animals. After IV dosing at 20 mg/kg, the data was more variable and lung/plasma ratios of 17.6 for males and 6.2 for females were observed. Cmax and AUC ranged from 0.022 μg/mL and 0.04 μg•h/mL to 1.96 μg/mL and 28.60 μg•h/mL across the dose range. The mean CEM-101 tmax increased from 1.5 to 6.0 hours and the mean terminal half-life increased from 2.2 to 7.9 hours over the 50 to 1600 mg dose range.

EXAMPLE. Tissue distribution. CEM-101 is well absorbed and distributed to the tissue. In the rat at 250 mg/kg/d, mean lung and liver concentrations 5 of CEM-101 were 17 and 15-fold higher than in plasma. Lung and liver concentrations were 503 and 711-fold higher than plasma concentrations at the 200 mg/kg/d dose in monkeys. Concentrations of CEM-101 in the heart were significantly lower than levels found in lung or liver with levels 5 and 54-fold higher than plasma concentrations in rat and monkey, respectively.

EXAMPLE. Activity of CEM-101 Alone and in Combination with Tobramycin and Amikacin, against *P. aeruginosa*, MRSA, and *B. cepacia*. Cystic fibrosis is a congenital genetic abnormality commonly encountered in the US. As a result of this disease patients, who may live through early to late adulthood, suffer from recurrent bouts of pneumonia caused by *Pseudomonas aeruginosa* (often mucoid), *Burkholderia cepacia*, and MRSA, and other pathogens. The recurrent nature of these infectious attacks leads to multi-resistance and sometimes pan-resistance, with combination therapy the only therapeutic alternative. There is a dearth of new experimental agents active against resistant Gram-negative and Gram-positive strains in general, and CF strains in particular.

CEM-101 is tested against *P. aeruginosa,* MRSA, and *B. cepacia* strains isolated from CF strains isolated at Hershey Medical Center, alone and in combination with amikacin and tobramycin.

Strains. Two strains each of mucoid *P. aeruginosa* (both pyocyanin positive) and 40 MRSA (only one strain with gold colonies) isolated from patients at a CF clinic were tested. Additionally, 2 *B.cepacia* strains were acquired, from Hershey Medical Center. All strains were identified by standard methods. Only one strain per patient was tested. MLVA was done on all strains to examine clonality, and confirmed that examination is not taking place on only one or a few clones. Strains will be stored in skim milk at –70°C until use.

Susceptibility testing. Original MICs of each strain to CEM-101 and other comparators were tested by CLSI microdilution methodology. Trays were obtained from Trek, Inc., Cleveland, OH. Time-kill macrobroth MIC dilution by CLSI was performed for all synergy testing.

Synergy testing. Two of the MRSA strains were chosen and tested for synergy, together with the 4 Gram-negative strains mentioned above. Broth macrodilution formed the basis of MICs used in time-kill experiments, as detailed below. The kill kinetics of each drug was tested alone by incubating an initial inoculum of 5 x 10^5 to 5 x 10^6 cfu/mL with drug concentrations at the MIC, three dilutions above and three dilutions below the MIC (1/2, 1/4 and 1/8 x MIC). Viability counts were performed after 0, 3, 6, 12 and 24 h incubation at 37°C in a shaking water bath by plating onto trypticase soy-5% sheep blood agar plates.

After initial time-kills with compounds alone are performed, CEM-101 was combined with amikacin and tobramycin. Combinations were tested 1-2 dilutions below the MIC (1/2 X MIC and ¼ X MIC) of each drug. Inocula and time-kill methodology were as above when the compounds alone are tested. Concentrations in synergy time-kill tests were selected such that one of the two drugs yields a growth curve similar to that of the drug-free control, while the other drug was more active.

MICs were assayed by standard methodology. Synergy was defined as a $\geq$2 $\log_{10}$ decrease in cfu/mL between the combination and its most active constituent after 3, 6, 12 and 24 h, with the number of surviving organisms in the presence of the combination $\geq$2 $\log_{10}$ cfu/mL below the starting inoculum. At least one of the compounds in the combination was present in a concentration which did not significantly affect the growth curve of the organism when used alone. Antagonism was defined as a $\geq$2 $\log_{10}$ increase in cfu/mL between the combination and its most active constituent after 3, 6, 12 and 24 h, with the number of surviving organisms in the presence of the combination $\geq$2 $\log_{10}$ cfu/mL above the starting inoculum.

Results. Each individual strain tested proved to be an individual clone. Compiled *S. aureus* (MRSA) MICs (μg/mL) are listed in Table 3.

TABLE 3. Microdilution MICs (μg/mL) of all compounds against 40 MRSA strains from CF patients.

Drug	Range	MIC_{50}	MIC_{90}
CEM-101	0.06-≥16	0.25	≥16
Vancomycin	0.5-1	0.5	1
Teicoplanin	0.25-1	0.5	1
Daptomycin	0.5-1	0.5	1
Tigecycline	0.12-0.25	0.12	0.25
Azithromycin	1-≥32	≥32	≥32
Clarithromycin	0.25-≥32	≥32	≥32
Linezolid	1-4	2	2
Quinupristin/dalfopristin	0.25-1	0.5	1

CEM-101 was active (MICs 0.06-0.25) against 21 of the 40 strains (52.5%), with MICs against the remaining organisms ≥16 μg/mL. Vancomycin and teicoplanin were also active at MICs 0.25-1, linezolid at MICs 1-4 and quinupristin/dalfopristin at MICs 0.25-1. Most strains (38 of 40) were resistant (>32) to azithromycin and clarithromycin. Microbroth MICs for the 4 Gram-negative rods are presented in Tables 4 and 5, and time-kill macrobroth MIC data are shown in Table 6.

TABLE 4. Macrobroth Dilution MICs (μg/mL) of all compounds against 2 *P. aeruginosa* strains from cystic fibrosis patients.

Drug	Range
CEM-101	64
Amikacin	2-8
Tobramycin	0.25-1.0

TABLE 5. Macrobroth Dilution MICs (μg/mL) of all compounds against 2 *B. cepacia* strains from cystic fibrosis patients.

Drug	Range
CEM-101	8-32
Amikacin	256
Tobramycin	128

TABLE 6. Time-kill Macrobroth MICs (μg/mL) of all compounds against 6 strains from cystic fibrosis patients.

Strain	CEM-101	Tobramycin	Amikacin
SA 2230	0.125	4.0	32.0
SA 2232	0.125	NT[a]	64.0
PSAR 461	64.0	2.0	8.0
PSAR 468	32.0	1.0	4.0
BCEP 953	8.0	128	512
BCEP 954	32.0	128	256

[a]NT; not tested

Synergy time-kill data are shown in Tables 7 and 8.

Table 7. Results of In Vitro Antimicrobial Combinations with CEM101 Studied by Time-kill

	CEM-101/Tobramycin[c]				CEM-101/Amikacin			
	3h[a]	6h[a]	12h[a]	24h[a]	3h	6h	12h	24h
Synergy	0[b]	0	0	1	0	0	0	1
Indifference	5	5	4	4	6	6	4	5
Antagonism	0	0	1	0	0	0	2	0

[a] time-point (hours)
[b] number of strains (strains tested)
[c]one strain (MRSA 2232) not tested (MIC >512 μg/mL)

Table 8. Results of In Vitro Antimicrobial Combinations with CEM101 Studied by Time-kill

	CEM-101/Tobramycin				CEM-101/Amikacin			
	3h[a]	6h[a]	12h[a]	24h[a]	3h	6h	12h	24h
SA2230	IND	IND	IND	SYN[b] (0.03/2)	IND	IND	IND	IND
SA2232	NT[c]	NT	NT	NT	IND	IND	IND	SYN (0.06/ 32)
PSAR461	IND	IND	IND	IND	IND	IND	ANT	IND

	CEM-101/Tobramycin				CEM-101/Amikacin			
PSAR468	IND	IND	IND	IND	IND	IND	IND	IND
BCEP953	IND	IND	ANT[b]	IND	IND	IND	ANT	IND
BCEP954	IND	IND	IND	IND	IND	IND	IND	IND

[a] time-point (hours)
[b] IND- indifference; SYN-synergy; ANT-antagonism
[c] NT; not tested (MIC >512 µg/mL)

Briefly, synergy was found with CEM-101/tobramycin at (0.03/2) concentration at 24 h for one MRSA strain and at 0.06/32 µg/mL for CEM-101/amikacin at 24h for the second MRSA strain. All other time points and combinations were indifferent for the 2 MRSA strains. One strain of MRSA was not tested with tobramycin in combination because of its very high MIC (>512 µg/mL). One of the 2 *P. aeruginosa* strains showed antagonism at 12 h with the CEM-101/amikacin combination (16/4 µg/mL). All other time points and combinations were indifferent with the 2 *P. aeruginosa* strains. One *B. cepacia* strain was antagonistic at 12 h with the CEM-101/tobramycin and CEM-101/amikacin combinations (2/64 and 2/256 µg/mL, respectively). The 2 *B. cepacia* strains were indifferent at all other time points and drug combinations.

No correlation between pigment and any MRSA results was found. When both mucoid *P. aeruginosa* strains were subcultured for a few days, viscosity disappeared but reappeared when they were re-exposed to all combinations. CEM-101 showed low MICs against approximately ½ of MRSA strains tested. Synergy was not found in the Gram negative rods tested. For MRSA, clinically achievable synergy was observed with strain SA 2230, with CEM-101 combined with tobramycin. Synergy against MRSA SA 2230 is shown in FIG. 1.

EXAMPLE. Intrapulmonary Penetration of CEM-101 in Healthy Adult Subjects. CEM-101 is evaluated for the treatment of patients with community-acquired bacterial pneumonia. The penetration of CEM-101 into the epithelial lining fluid (ELF) and alveolar macrophages (AM) is assessed in a Phase 1 clinical study.

Methods: 30 subjects received 400 mg of CEM-101 orally daily for 5 days. On Day 5, each subject underwent a single bronchoscopy and bronchoalveolar lavage at 1 of 5 time points (3, 6, 9, 12 or 24 h post-dose) to obtain ELF and AM samples (6 subjects/time point). Plasma samples were collected pre-dose on Days 1 to 5 and serially post-dose on Day 5 and 6. The samples collected were assayed for CEM-101 using LC/MS/MS. Urea in the plasma and ELF was used to correct the ELF CEM-101 concentrations. Noncompartmental pharmacokinetic (PK) analysis using the median concentrations at each time point was used to calculate Day 5 AUC0- 24. In addition, a population PK model (PPM) was used to determine

Day 5 AUC0-24 for each subject in plasma and ELF. Intrapulmonary penetration of CEM-101 into the ELF and AM was determined by dividing the Day 5 AUC0-24 of each matrix by the Day 5 plasma AUC0-24.

Results: CEM-101 penetrated well into ELF and AM. CEM-101 achieved higher exposures in ELF (>8 times) and AM (>180 times) compared to plasma concentrations during the 24 hour period after drug administration in healthy adults. CEM-101 provides a good intrapulmonary penetration profile for the treatment of bacterial pathogens associated with lower respiratory tract infections.

EXAMPLE. Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis of CEM-101 Against *Streptococcus pneumoniae* Using Data from a Murine-Lung Infection Model. Using a murine-lung infection model, epithelial lining fluid (ELF) and plasma PK-PD measures most closely associated with CEM-101 efficacy against *S. pneumoniae* and targets based on PK-PD relationships for such indices were identified.

Methods: CEM-101 PK data were obtained from healthy mice administered single CEM-101 doses ranging from 0.625 to 40 mg/kg. Plasma and ELF were collected over 24 h (3 mice/time point) and assayed for CEM-101. Urea in plasma and ELF was used to correct ELF concentrations. Neutropenic mice infected with 108 CFU of 1 of 5 *S. pneumoniae* isolates via inhalation were administered daily CEM-101 doses (0.156 to 160 mg/kg) via oral gavage. Dose-fractionation was performed for 1 isolate; CEM-101 was administered to the other 4 isolates as a Q6h or Q12h regimen. PK and PK-PD were evaluated using S-ADAPT 1.56.

Results: A 3-compartment model with a parallel first-order and capacity-limited clearance and a capacity-limited first pass effect with fitted lag-times best described the plasma and ELF data (r2 = 0.98 and 0.83 for observed vs fitted concentrations, respectively). ELF to total- and free-drug (*f*) plasma (based on protein binding of 91.8% in mice) AUC0-24 ratios were 0.22 and 2.7, respectively. ELF and *f* plasma AUC0-24:MIC ratios were most predictive of efficacy (r2 = 0.85 for ELF and *f* plasma). ELF and *f* plasma AUC0-24:MIC ratios associated with net bacterial stasis and a 1- and 2-log10 CFU reduction from baseline were 1.26 and 1.65, 15.1 and 6.31, and 59.8 and 12.8, respectively. AUC0-24:MIC ratio was the PK-PD index most predictive of efficacy for CEM-101. PK-PD targets based on these relationships will inform dose selection for future clinical studies.

EXAMPLE. Compounds described herein exhibit potent anti-inflammatory activity.

Cells. The human monocytic cell line U937 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). PBMCs from COPD patients were obtained

from Brompton hospital and separated by AccuSPIN (Sigma–Aldrich). Cells were cultured in complete growth medium (RPMI 1640) (Sigma–Aldrich) supplemented with 10% fetal bovine serum (FBS) and 1% L-glutamine at 37°C in a humidified atmosphere with 5% CO_2. U937 cells were differentiated into adherent macrophage-like morphology by exposure to PMA (50 ng/mL) for 48 hrs in complete growth medium. Cell viability was assessed microscopically by trypan blue staining. Cell toxicity was determined by MTT assay as needed. This study was approved by the ethics committee of the Royal Brompton Hospitals, and all subjects gave written informed consent.

Cell Lysis. Whole cell extracts were prepared as previously described (Kobayashi et al., 2011). Briefly, cell protein extracts were prepared using modified RIPA buffer (50 mM Tris HCl pH 7.4, 0.5% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl with freshly added complete protease inhibitor cocktail (Roche, Mannheim, Germany)). Protein concentration was determined using the BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA).

Cytokine ELISA. TNFα and IL-8 concentrations in the supernatant of cell cultures were determined by sandwich ELISA according to the manufacturer's instructions (R&D Systems Europe, Abingdon, UK).

Zymography. MMP9 enzyme activity was measured by gelatin zymography. Cell culture supernatants were diluted with equal amount of Laemli sample buffer (Bio-Rad, Hertfordshire, UK) and loaded on a Novex® 10 % Zymogram (Gelatin) gel (Invitrogen Ltd, Paisley, UK). After electrophoresis, gels were incubated and rinsed with Novex® zymogram renaturing buffer (Invitrogen) for 30 min at room temperature. The gels were then rinsed in Novex® zymogram developing buffer (Invitrogen) for 30 min at room temperature prior to overnight incubation in the developing buffer at 37 °C. After incubation, the gels were stained using a Colloidal Blue Staining Kit (Invitrogen) to visualize the zymogen bands.

NF-κB activity. The activation of NF-κB (p65 binding activity to NF-κB binding sequence) was determined using a TransAM NF-κB p65 Assay kit (Active Motif, Inc., Carlsbad, CA) according to the manufacturer's instruction.Whole cell extracts were prepared from PMA-differentiated U937 cells, and 20 μL of each extract was used for this study. Results were determined by measuring the spectrophotometric absorbance at 450 nm with a reference wavelength of 655 nm.

Statistical analysis. The results were expressed as the mean ± SEM. Comparisons of data in two groups were performed using the Student's *t* test or the Wilcoxon signed rank test. Multiple comparisons were made by one-way ANOVA with *post hoc* test

(Dunnett's) as appropriate. The difference was considered significant at $p < 0.05$. IC_{50} values (50% inhibitory concentration) for macrolides for production of cytokines or MMP9 were calculated using Prism 4.0 (GraphPad Software Inc., San Diego, CA).

Anti-inflammatory effects of CEM-101 in U937 cells. LPS significantly increased TNFα and IL-8 production in PMA-differentiated U937 cells (TNFα, 63.1 ± 2.6 fold in LPS vs. non-stimulated; and CXCL8, 2.0 ± 0.1 fold in LPS vs. non-stimulated cells, n=3). CEM-101 significantly inhibited both TNFα and CXCL8 at 100 μM (FIGS 3 and 4). Although clarithromycin showed modest effects on both TNFα and IL-8 production at a higher concentration (333 μM), erythromycin and azithromycin did not inhibit them. Telithromycin at 100 μM did not inhibit production of TNFα and CXCL8. The IC_{50} values for CEM-101 on TNFα and CXCL8 release were 41.6 ± 1.9 μM and 78.2 ± 9.5 μM, respectively, and were superior to those for clarithromycin (IC_{50}, 426.3 ± 63.9 μM for TNFα and 506.5 ± 44.0 μM for CXCL8) (Table 9).

The effects of macrolides on MMP9 activity was also investigated, which were clearly elevated by PMA stimulation in U937 cells (9.9 ± 2.0 fold in PMA vs. non-stimulation, n=3). CEM-101 remarkably reduced MMP9 activity, with an IC_{50} of 14.9 ± 3.1 μM (FIG. 2 and Table 9). In contrast, clarithromycin and azithromycin showed 10-fold lower inhibitory effects than CEM-101 whereas erythromycin showed no effect (FIG. 2 and Table 9). Telithromycin also inhibited MMP9 activity, although to lesser extent than CEM-101, with an IC_{50} of 97.9 μM.

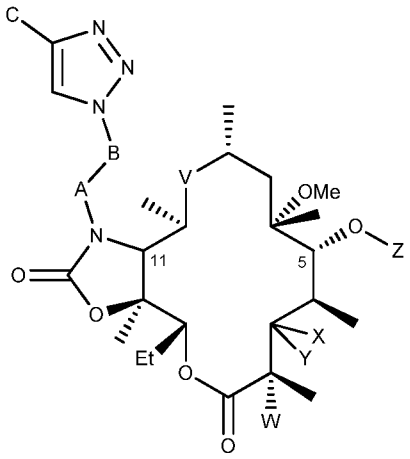
Table 9. Effect of macrolides on inhibition of LPS-induced IL-8 and TNFα release, and PMA-induced MMP9 activation in U937 cells.

	IC_{50} (μM)				
	Solithro-mycin	Erythro-mycin	Clarithro-mycin	Azithro-mycin	Telithro-mycin
LPS-induce IL-8 release	78.2	NE at 333μM	506.5	NE at 333μM	NE at 100μM
LPS-induced TNFα release	41.6	NE at 333μM	426.3	NE at 333μM	NE at 100μM
PMA-induced MMP9 activation	14.9	NE at 333μM	118.0	212.1	97.9

NE: no effect

WHAT IS CLAIMED IS:

1. A method for treating a pulmonary or endobronchial disease in a host animal, the method comprising the step of administering to the host animal a therapeutically effective amount of one or more compounds of the formula



or pharmaceutically acceptable salts thereof, wherein:

X is H; and Y is OR_7; where R_7 is a monosaccharide or disaccharide, or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl;

Z is a monosaccharide or disaccharide, or a derivative thereof;

V is C(O), or $C(=NR_{11})$, wherein R_{11} is hydroxy or alkoxy;

W is H, F, Cl, Br, I, or OH;

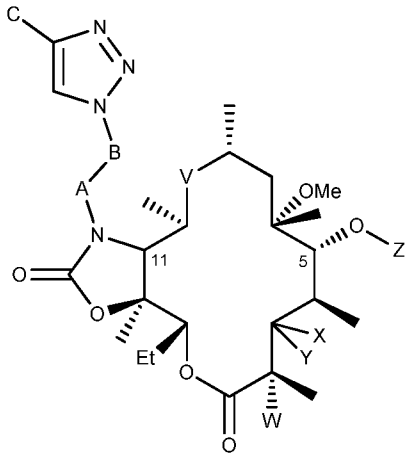
A is CH_2, C(O), C(O)O, C(O)NH, $S(O)_2$, $S(O)_2NH$, or $C(O)NHS(O)_2$;

B is $(CH_2)_n$ where n is an integer in the range from 0 to about 10, or B is C_2-C_{10} alkenyl or alkynyl; and

C is cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted;

where the compound is administered by inhalation to the endobronchial space of the patient.

2. A composition for administration by inhalation, the composition comprising one or more compounds of the formula



or pharmaceutically acceptable salts thereof, wherein:

X is H; and Y is OR_7; where R_7 is a monosaccharide or disaccharide, or a derivative thereof; or X and Y are taken together with the attached carbon to form carbonyl;

Z is a monosaccharide or disaccharide, or a derivative thereof;

V is C(O), or $C(=NR_{11})$, wherein R_{11} is hydroxy or alkoxy;

W is H, F, Cl, Br, I, or OH;

A is CH_2, C(O), C(O)O, C(O)NH, $S(O)_2$, $S(O)_2NH$, or $C(O)NHS(O)_2$;

B is $(CH_2)_n$ where n is an integer in the range from 0 to about 10, or B is C_2-C_{10} alkenyl or alkynyl; and

C is cycloalkyl, cycloheteroalkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted.

3. The composition of claim 2 wherein the composition is a solution adapted for aerosolization and inhalation by the host animal.

4. A unit dose of a therapeutically effective amount of the compound or composition of claim 2, the unit dose comprising a predetermined amount of the compound adapted for administering by inhalation.

5. A kit for treating a pulmonary or endobronchial disease in a host animal, the kit comprising a solid unit dose of a therapeutically effective amount of the compound or composition of any one of the preceding claims, and a separate diluent, and instructions for use, including an instruction for reconstituting the solid unit dose using the diluent to prepare a liquid composition capable of being inhaled by the host animal.

6. The kit of claim 4 further comprising a container.

7. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein X and Y are taken together with the attached carbon to form carbonyl.

8. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein Z is a monosaccharide.

9. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein Z is desosamine or a derivative thereof.

10. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein Z is desosamine.

11. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein V is C(O).

12. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein W is H or F.

13. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein A is CH_2.

14. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein B is $(CH_2)_n$.

15. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein n is an integer from 2 to 4

16. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein n is 3.

17. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein C is aryl, arylalkyl, heteroaryl, or heteroarylalkyl, each of which is optionally substituted.

18. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein C is aryl or heteroarylalkyl, each of which is optionally substituted.

19. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein C is substituted aryl.

20. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein C is aminophenyl.

21. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein C is 3-aminophenyl.

22. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the compound is solithromycin, or a pharmaceutically acceptable salt thereof

23. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the administration is performed using a nebulizer, and the composition or unit dose is capable of producing a aerosol particle with an MMAD predominantly in the range from about 1 to about 5 μm.

24. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the host animal is immunocompromised.

25. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the disease includes cystic fibrosis.

26. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the disease is caused by one or more strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, Staphylococcus aureus , including MRSA, or a combination thereof.

27. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the disease is caused by one or more CF strains of Pseudomonas aeruginosa, mucoid Pseudomonas aeruginosa, Burkholderia cepacia, S. aureus , including MRSA, or a combination thereof.

28. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the disease is caused by one or more clarithromycin resistant bacteria.

29. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the disease is caused by inflammation.

30. The method, composition, unit dose, or kit of any one of claims 1 to 6 further comprising the step of administering a therapeutically effective amount of an aminoglycoside.

31. The method, composition, unit dose, or kit of any one of claims 1 to 6 wherein the compound is administered at a dose lower than that capable of antagonizing or significantly antagonizing the efficacy of the aminoglycoside.

32. The method, composition, unit dose, or kit of any one of claims 1 to 6 further comprising the step of administering a therapeutically effective amount of a fluoroquinolone antibiotic.

33. The method, composition, unit dose, or kit of any one of claims 1 to 6 further comprising the step of administering a therapeutically effective amount of aztreonam.

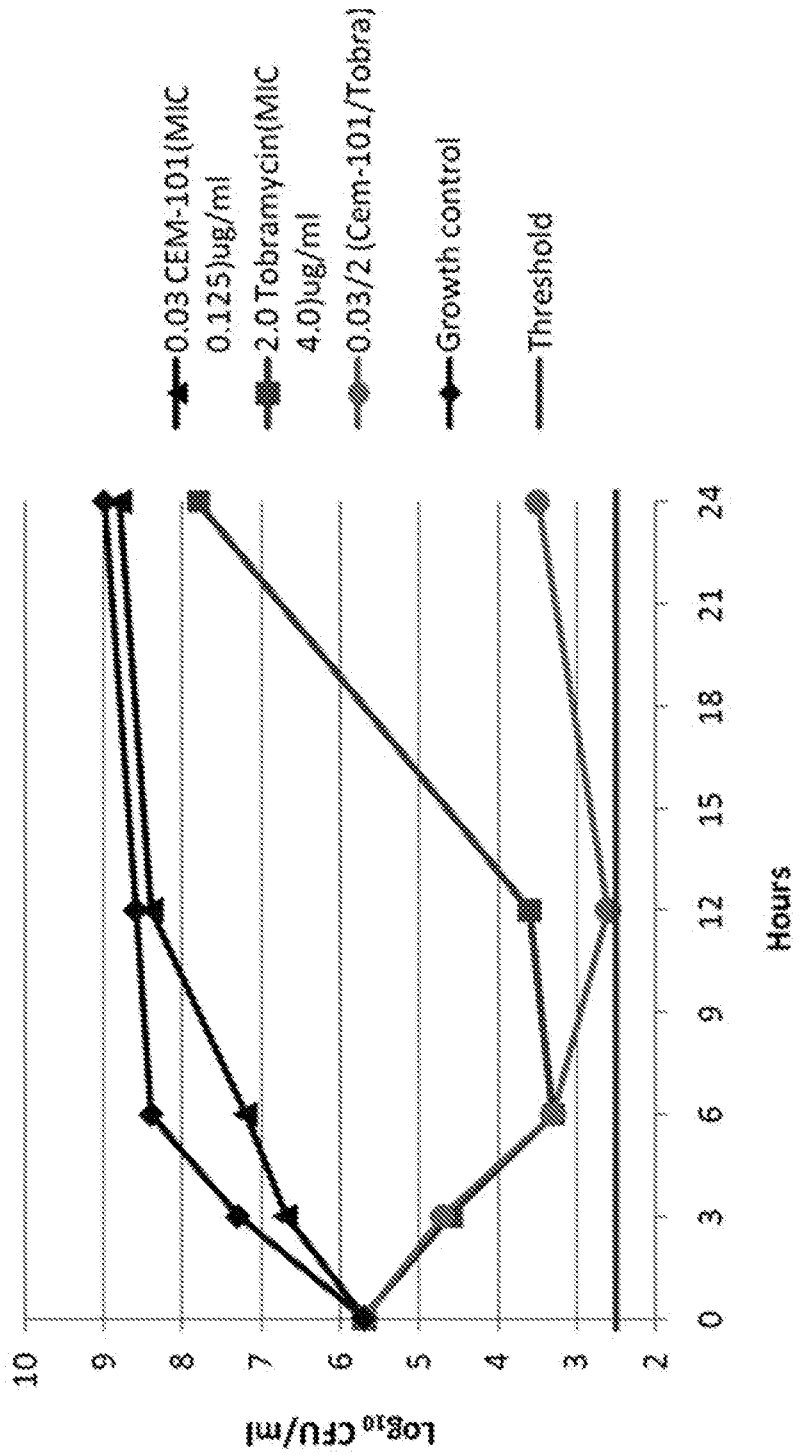

0.03 CEM-101(MIC 0.125)ug/ml
2.0 Tobramycin(MIC 4.0)ug/ml
0.03/2 (Cem-101/Tobra)
Growth control
Threshold
Log10 CFU/ml
Hours
10
9
8
7
6
5
4
3
2
0
3
6
9
12
15
18
21
24

FIG. 1

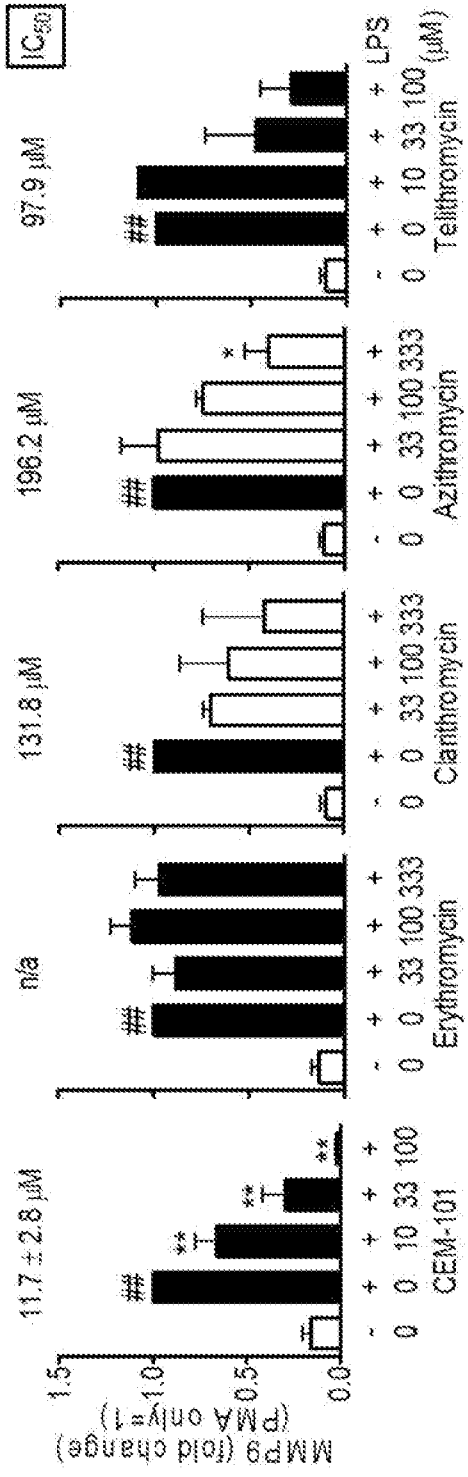

IC50
11.7 ± 2.8 µM
n/a
131.8 µM
196.2 µM
97.9 µM
MMP9 (fold change)
(PMA only=1)
1.5
1.0
0.5
0.0
##
**
CEM-101
Erythromycin
Clarithromycin
Azithromycin
Telithromycin
LPS
(µM)
0 0 10 33 100
0 0 33 100 333
0 0 33 100 333
0 0 10 33 100

FIG. 2

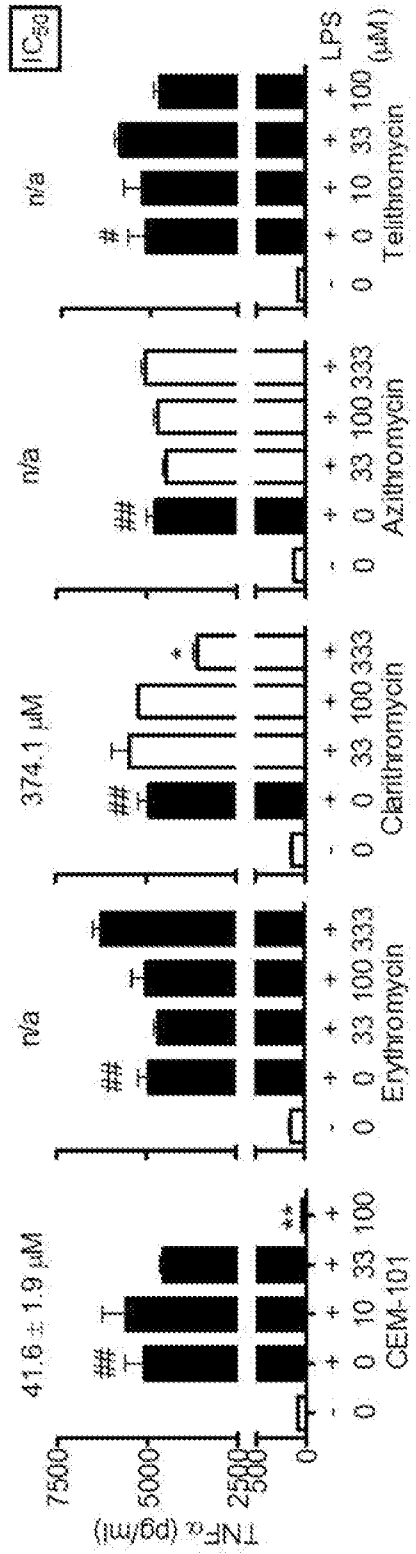

IC50
41.6 ± 1.9 µM
n/a
374.1 µM
n/a
TNFα (pg/ml)
7500
5000
2500
0
##
**
CEM-101
Erythromycin
Clarithromycin
Azithromycin
Telithromycin
LPS
(µM)
0 0 10 33 100
0 0 33 100 333
0 0 33 100 333
0 0 10 33 100

FIG. 3

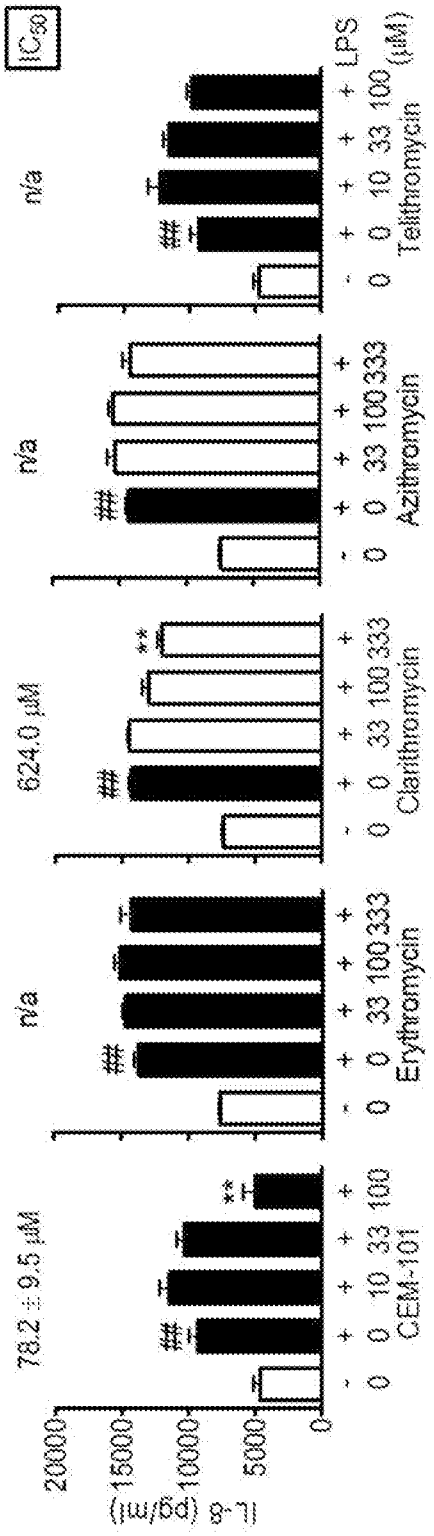

IC50
78.2 ± 9.5 μM
n/a
624.0 μM
n/a
n/a
IL-8 (pg/ml)
20000
15000
10000
5000
0
CEM-101
Erythromycin
Clarithromycin
Azithromycin
Telithromycin
(μM)
LPS
##
**

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/027214

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61M 15/00 (2014.01)
USPC - 128/200.14
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) - A61K 9/72; A61M 11/00, 15/00 (2014.01)
USPC - 128/200.14, 203.16; 514/29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 9/0073, 9/0078, 9/008; A61M15/0065 (2014.06)

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 2012/0071429 A1 (DUFFIELD et al) 22 March 2012 (22.03.2012) entire document	2, 4 ---------- 1, 3, 5, 6
Y	US 2003/0143162 A1 (SPEIRS et al) 31 July 2003 (31.07.2003) entire document	1, 3, 6
Y	US 2009/0087389 A1 (LEONARD et al) 02 April 2009 (02.04.2009) entire document	5

☐ 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
24 June 2014	18 JUL 2014
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: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/027214

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.: 7-33
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.

(19)中华人民共和国国家知识产权局

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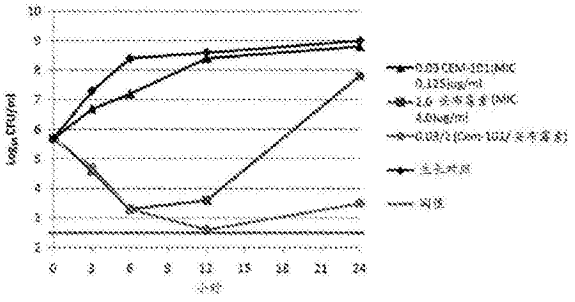
(54)发明名称

用于治疗呼吸道疾病的方法及其制剂

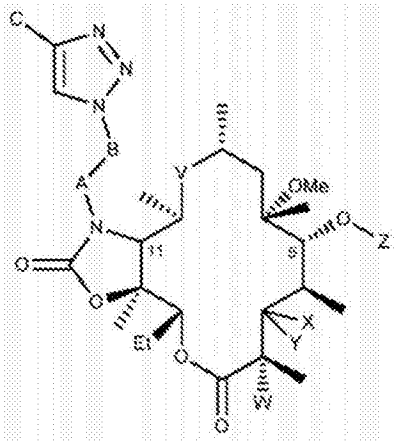
(57)摘要

本文描述了用于治疗呼吸道疾病（包括囊性纤维化）的化合物、组合物、方法和用途。本文还描述了大环内酯类抗生素的吸入制剂。细菌感染的治疗一直是药物研发的重要努力方向。对目前可用抗生素的细菌抗药性现象始终存在，因此，需要新的改善的化合物、药物制剂、治疗方法和治疗方案。



CN 105163785 A

1.一种用于治疗宿主动物中的肺或支气管疾病的方法,所述方法包括向所述宿主动物施用治疗有效量的一种或多种下式的化合物或其药学上可接受的盐的步骤：



其中：

X是H;并且Y是OR_7;其中R_7是单糖或二糖或者其衍生物;或者X和Y与所连接的碳一起形成羰基；

Z是单糖或二糖或者其衍生物；

V是C(O)或C(=NR_{11}),其中R_{11}是羟基或烷氧基；

W是H、F、Cl、Br、I或OH；

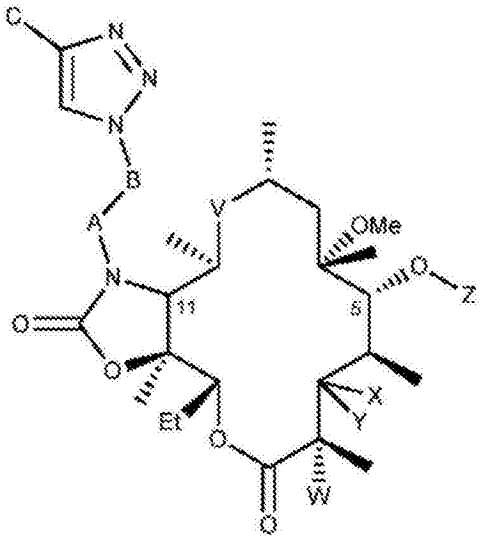
A是CH_2、C(O)、C(O)O、C(O)NH、$S(O)_2$、$S(O)_2NH$或$C(O)NHS(O)_2$;

B是$(CH_2)_n$,其中n是0至约10的整数,或者B是C_2-C_{10}烯基或炔基;并且

C是环烷基、环杂烷基、芳基、芳基烷基、杂芳基或杂芳基烷基,其各自任选地被取代；

其中所述化合物通过吸入施用到所述患者的支气管内空间。

2.用于通过吸入施用的组合物,所述组合物包含一种或多种下式的化合物或其药学上可接受的盐：



其中：

X是H;并且Y是OR_7;其中R_7是单糖或二糖或者其衍生物;或者X和Y与所连接的碳一起形成羰基；

Z是单糖或二糖或者其衍生物；

V 是 C(O) 或 C(= NR_{11})，其中 R_{11}是羟基或烷氧基；

W 是 H、F、Cl、Br、I 或 OH；

A 是 CH_2、C(O)、C(O)O、C(O)NH、$S(O)_2$、$S(O)_2NH$ 或 $C(O)NHS(O)_2$；

B 是 $(CH_2)_n$，其中 n 是 0 至约 10 的整数，或者 B 是 C_2-C_{10}烯基或炔基；并且

C 是环烷基、环杂烷基、芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代。

3. 根据权利要求 2 所述的组合物，其中所述组合物是适于雾化并且被所述宿主动物吸入的溶液。

4. 治疗有效量的根据权利要求 2 所述的化合物或组合物的单位剂型，所述单位剂型包含预定量的适于通过吸入施用的所述化合物。

5. 一种用于治疗宿主动物中的肺或支气管疾病的试剂盒，所述试剂盒包含：治疗有效量的根据前述权利要求中任一项所述的化合物或组合物的固体单位剂型，以及分开的稀释剂，以及使用说明书，所述使用说明书包含用于使用所述稀释剂重构所述固体单位剂型以制备能够被所述宿主动物吸入的液体组合物的说明。

6. 根据权利要求 4 所述的试剂盒，其还包含容器。

7. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 X 和 Y 与所连接的碳一起形成羰基。

8. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是单糖。

9. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是德糖胺或其衍生物。

10. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是德糖胺。

11. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 V 是 C(O)。

12. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 W 是 H 或 F。

13. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 A 是 CH_2。

14. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 B 是 $(CH_2)_n$。

15. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 n 是 2 至 4 的整数

16. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 n 是 3。

17. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 C 是芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代。

18. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 C 是芳基或杂芳基烷基，其各自任选地被取代。

19. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 C 是被

取代的芳基。

20. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 C 是氨基苯基。

21. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中 C 是 3- 氨基苯基。

22. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述化合物是索利霉素或者其药学上可接受的盐

23. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述施用使用雾化器进行，并且所述组合物或单位剂型能够产生 MMAD 主要为约 1 至约 5 μm 的气雾剂颗粒。

24. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述宿主动物是免疫功能低下的。

25. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病包括囊性纤维化。

26. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌、洋葱伯克霍尔德氏菌、金黄色葡萄球菌包括 MRSA 或其组合的一种或多种菌株引起的。

27. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌、洋葱伯克霍尔德氏菌、金黄色葡萄球菌包括 MRSA 或其组合的一种或多种 CF 菌株引起的。

28. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是由一种或多种克拉霉素抗性细菌引起的。

29. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是由炎症引起的。

30. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氨基糖苷类的步骤。

31. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其中以低于能够拮抗或显著拮抗氨基糖苷类的效力的剂量施用所述化合物。

32. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氟喹诺酮类抗生素的步骤。

33. 根据权利要求 1 至 6 中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氨曲南的步骤。

用于治疗呼吸道疾病的方法及其制剂

[0001] 相关申请的交叉引用

[0002] 本申请根据 35U.S.C. § 119(e) 要求于 2013 年 3 月 14 日提交的美国临时申请号 61/781,197 的权益和优先权，其通过引用整体并入本文。

技术领域

[0003] 本文描述的本发明涉及治疗呼吸道疾病，包括囊性纤维化。本文描述的本发明还涉及大环内酯类抗生素的吸入制剂。

[0004] 背景和发明概述

[0005] 细菌感染的治疗一直是药物研发的重要努力方向。对目前可用抗生素的细菌抗药性现象始终存在，因此，需要新的改善的化合物、药物制剂、治疗方法和治疗方案。另外，细菌感染本身存在于多种组织中，并且在许多情况下，这些组织对于成功治疗造成了特定挑战。例如，需要对呼吸系统的细菌感染（包括急性和慢性肺和支气管感染）的新的治疗。

[0006] 许多抗生素不能取得有效用于治疗和 / 或预防急性和慢性肺和支气管疾病的充分高的肺浓度。例如，已经报道渗透到支气管分泌物中的氨基糖苷类很少，大体上仅为峰值血浆浓度的约 12% (Rev. Infect. Dis., 3:67(1981))。另外，已经报道，由于高离子强度以及二价阳离子的存在，痰本身抑制氨基糖苷类的生物活性。(Advances in Pediatric Infections Diseases, 8:53(1993))。痰还含有与氨基糖苷类结合的粘蛋白糖蛋白和 DNA。还报道，为了克服抑制活性，需要将痰中氨基糖苷类的浓度增加到特定靶病原体如铜绿假单胞杆菌 (Pseudomonas aeruginosa) 分离株的最小抑菌浓度的约 10 倍 (J. Infect. Dis., 148:1069(1983))。

[0007] 还已经报道，囊性纤维化 (CF) 的治疗特别困难，这是一种以肺组织的炎症和进行性破坏为特征的常见的遗传病。CF 患者中肺的衰弱与由于病原菌如流感嗜血杆菌 (H. influenzae)、金黄色葡萄球菌 (Staphylococcus aureaus) 和铜绿假单胞杆菌等引起的慢性支气管感染而产生的浓痰的累积相关。几乎所有患有 CF 的个体最终都死于呼吸衰竭。

[0008] 由于某些抗生素如氨基糖苷类很难渗透到痰中以在痰中达到治疗浓度，所以需要高剂量的肠胃外施用。由于血清含有高氨基糖苷类浓度，这样的剂量方案增加了全身毒性（包括耳毒性和肾毒性）的风险。静脉内注射还可能增加患者的痛苦，并且需要住院治疗，这增加了治疗成本并且使患者暴露于潜在的其他感染。应理解的是，在感染期间，细菌可能主要存在于较小气道中，例如末端细支气管和呼吸性细支气管中，并且细菌可能主要在较大气道中繁殖。已经报道当通过吸入和其他支气管内途径施用阿奇霉素时，咽和肺中的半衰期太长，导致更高可能性产生抗性。

[0009] 妥布霉素吸入性溶液剂是当前唯一经批准用于治疗 CF 患者中的细菌感染的气雾剂抗生素。已经报道以气雾剂施用妥布霉素降低了潜在的全身毒性。但是，还报道长期使用与多抗生素抗性铜绿假单胞杆菌菌株相关。因此，需要开发不同的治疗，包括用于治疗 CF 患者中的慢性肺感染的气雾剂抗生素种类。

[0010] 已经出乎意料地发现，不同于阿奇霉素，本发明的含三唑的大环内酯类在咽和肺

中具有最佳的半衰期，其允许治疗肺中疾病的效力并且具有低的形成抗性的潜力。还出乎意料地发现，本文所述含三唑的大环内酯类可通过吸入施用，包括鼻内吸入和经口吸入，以及其他鼻、鼻窦、呼吸道、肺和支气管途径。还出乎意料地发现，本文所述含三唑的大环内酯类表现出大的分布体积。

[0011] 已经发现，本文所述大环内酯类可用于治疗呼吸道感染(RTI)。已经出乎意料的发现，在口服施用后，本文所述化合物还取得了出乎意料的高肺水平。因此，本文描述了用于治疗和/或预防急性和慢性肺和支气管疾病的方法，其中该方法包括向宿主动物施用或共施用一种或多种本文所述大环内酯类的步骤。大环内酯类可以通过多种途径施用，包括但不限于口服、肠胃外、吸入等施用途径。不受理论的约束，本文相信本文所述大环内酯类的实用性至少部分地是由于施用(包括口服施用和肠胃外施用)后出乎意料的高肺组织化合物水平。还出乎意料地发现，该化合物不需要通过吸入施用以取得有效的肺水平。

[0012] 还已经发现本文所述大环内酯类可用于治疗和/或预防急性和慢性肺和支气管疾病，例如由细菌引起或加剧的疾病，该细菌包括发现于CF患者、慢性支气管炎和支气管扩张中的铜绿假单胞杆菌。已经发现本文所述大环内酯类具有强效抗炎活性，因此可用于治疗多种肺和支气管疾病如CF中的炎症部分。

[0013] 还已经发现本文所述大环内酯可以与其他抗生素如氨基糖苷类、氟喹诺酮类、氨曲南、磷霉素等共施用，并且这样的共施用具有意料之外的高效力。不受理论的约束，本文相信意料之外的高效力可能是由于大环内酯类的一个或更多个特性。一个这样的特性可能在于，已经发现在共施用时大环内酯类不拮抗其抗生素如已经被报道用于其他抗菌剂的氨基糖苷类抗生素的活性。另一个这样的特性可能在于，大环内酯出人意料地对其他抗生素如氨基糖苷类抗生素的活性具有协同作用。

[0014] 还已经发现本文所述大环内酯类可用于治疗至少部分地由以下细菌引起的疾病：大肠杆菌(Escherichia coli)、肠杆菌物种(Enterobacteria species)、克雷伯氏炎菌(Klebsiella pneumoniae)、产酸克雷伯氏菌(K.oxytoca)、奇异变形杆菌(Proteus mirabilis)、铜绿假单胞杆菌、粘质沙雷氏菌(Serratia marcescens)、流感嗜血杆菌(Haemophilus influenzae)、洋葱伯克霍尔德氏菌(Burkholderia cepacia)、嗜麦芽糖寡养单孢菌(Stenotrophomonas maltophilia)、木糖氧化产碱杆菌(Alcaligenes xylosoxidans)、多重耐药性铜绿假单胞杆菌。该单环内酯类可以单独施用或者与其他抗生素如氨基糖苷类、氟喹诺酮类、氨曲南、磷霉素等组合施用。

[0015] 本文描述了化合物、组合物、制剂、制备药物的用途，以及用于治疗呼吸道感染及相关疾病包括囊性纤维化(CF)，至少部分地由鸟分枝杆菌复合物(MAC)或人分枝杆菌(Mycobacterium hominus，MAH)引起的疾病，患有具HIV、AIDS和/或AIDS相关疾病的感染的患者以及其他患有感染的免疫功能低下患者的方法。不受理论的约束，本文相信治疗疾病如CF的效力至少部分地是由于所施用化合物的抗菌和抗炎活性的组合。

[0016] 附图简述

[0017] 图1表明利用与妥布霉素共施用的CEM-101的可实现水平观察到了对抗MRSA SA 2230的协同作用。

[0018] 图2示出了多种大环内酯类对PMA诱导产生MMP9的影响。大环内酯类对U937细胞中佛波醇12-肉豆蔻酸13-醋酸酯(PMA)诱导的MMP9激活的影响。用CEM-101(10至

100μM) 或红霉素、克拉霉素、阿奇霉素或泰利霉素 (33 至 333μM) 预处理细胞 1 小时，然后用 PMA(50ng/mL) 刺激 48 小时。48 小时后，收集上清液用于酶谱法。通过明胶酶谱法测量 MMP9 酶活性。数据相对于标准表示。值表示为 CEM-101 的四个实验以及红霉素、克拉霉素、阿奇霉素和泰利霉素各自的三个实验的平均值 ±SEM。##p<0.01(相比于未处理对照)，*p<0.05，**p<0.019(相比于仅用 PMA 处理)。

[0019] 图 3 示出了多种大环内酯类对 LPS 诱导产生 TNFα 的影响。大环内酯类对 PMA 分化的 U937 细胞中脂多糖 (LPS) 诱导的 TNFα 释放的影响。用 CEM-101(10 至 100μM) 或红霉素、克拉霉素、阿奇霉素或泰利霉素 (33 至 333μM) 预处理细胞 1 小时，然后用 LPS(100ng/mL) 刺激 4 小时。通过 ELISA 评估 LPS 诱导的 TNFα 释放。值表示为三个实验的平均值 ±SEM。##p<0.01(相比于未处理对照)，*p<0.05，**p<0.01(相比于仅用 LPS 处理)。

[0020] 图 4 示出了多种大环内酯类对 LPS 诱导产生 IL-8 的影响。大环内酯对 PMA 分化的 U937 细胞中脂多糖 (LPS) 诱导的 CXCL8 释放的影响。用 CEM-101(10 至 100μM) 或红霉素、克拉霉素、阿奇霉素或泰利霉素 (33 至 333μM) 预处理细胞 1 小时，然后用 LPS(100ng/mL) 刺激 4 小时。通过 ELISA 评估 LPS 诱导的 CXCL8 释放。值表示为三个实验的平均值 ±SEM。##p<0.01(相比于未处理对照)，*p<0.05，**p<0.01(相比于仅用 LPS 处理)。

[0021] 发明详述

[0022] 在一个实施方案中，化合物、组合物、制剂和方法包括一种或多种本文所述大环内酯类。在另一个实施方案中，化合物、组合物和制剂适于口服施用。在另一个实施方案中，化合物、组合物和制剂适于肠胃外施用。在另一个实施方案中，化合物、组合物和制剂适于通过吸入施用。在另一个实施方案中，该方法包括口服施用。在另一个实施方案中，该方法包括肠胃外施用。在另一个实施方案中，该方法包括通过吸入施用。

[0023] 本文出乎意料地发现，含三唑的酮内酯抗生素及其氟代衍生物如 CEM-101 和相关化合物是有效的抗炎剂并且因此有效治疗 CF。特别地，本文所述含三唑的酮内酯抗生素及其氟代衍生物有效治疗 CF 的细菌和炎症方面。

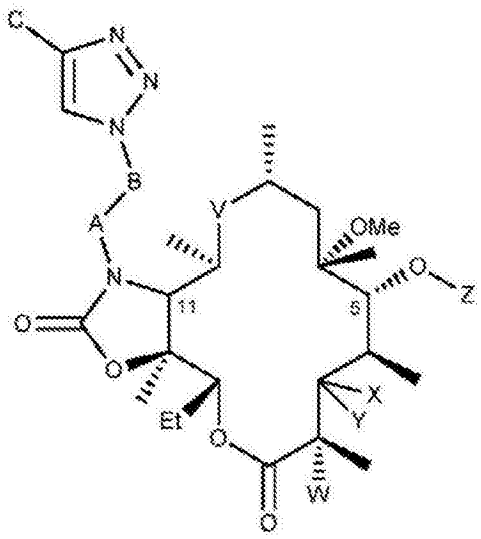
[0024] 本文还发现，本文所述化合物即使是在长期储存期间也表现出高溶液稳定性。

[0025] 在另一个实施方案中，本文描述了用于治疗包括细菌和炎症方面二者的 CF 的化合物、组合物和方法。

[0026] 以下列举的非限制性项目进一步举例说明了本文描述的本发明：

[0027] 1. 一种用于治疗宿主动物中的肺或支气管疾病的方法，所述方法包括向所述宿主动物施用治疗有效量的一种或多种下式的化合物或其药学上可接受的盐的步骤：

[0028]



[0029] 其中：

[0030] X 是 H；并且 Y 是 OR_7；其中 R_7 是单糖或二糖或者其衍生物；或者 X 和 Y 与所连接的碳一起形成羰基；

[0031] Z 是单糖或二糖或者其衍生物；

[0032] V 是 C(O) 或 C(= NR_{11})，其中 R_{11} 是羟基或烷氧基；

[0033] W 是 H、F、Cl、Br、I 或 OH；

[0034] A 是 CH_2、C(O)、C(O)O、C(O)NH、$S(O)_2$、$S(O)_2NH$ 或 $C(O)NHS(O)_2$；

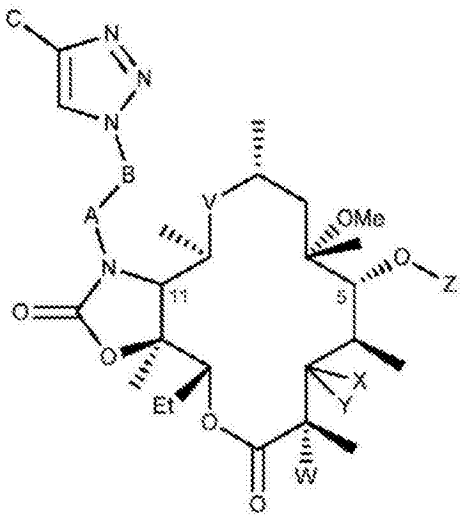
[0035] B 是 $(CH_2)_n$，其中 n 是 0 至约 10 的整数，或者 B 是 C_2-C_{10} 烯基或炔基；并且

[0036] C 是环烷基、环杂烷基、芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代；

[0037] 其中所述化合物通过吸入施用到所述患者的支气管内空间。

[0038] 2. 用于通过吸入施用的组合物，所述组合物包含一种或多种下式的化合物或其药学上可接受的盐：

[0039]



[0040] 其中：

[0041] X 是 H；并且 Y 是 OR_7；其中 R_7 是单糖或二糖或者其衍生物；或者 X 和 Y 与所连接的碳一起形成羰基；

[0042] Z 是单糖或二糖或者其衍生物；

[0043] V 是 C(O) 或 C(= NR_{11})，其中 R_{11}是羟基或烷氧基；

[0044] W 是 H、F、Cl、Br、I 或 OH；

[0045] A 是 CH_2、C(O)、C(O)O、C(O)NH、$S(O)_2$、$S(O)_2NH$ 或 C(O)NHS$(O)_2$；

[0046] B 是 $(CH_2)_n$，其中 n 是 0 至约 10 的整数，或者 B 是 C_2-C_{10}烯基或炔基；并且

[0047] C 是环烷基、环杂烷基、芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代。

[0048] 3. 治疗有效量的根据项目 2 所述的化合物或组合物的单位剂型，所述单位剂型包含预定量的适于吸入施用的所述化合物。

[0049] 4. 一种用于治疗宿主动物中的肺或支气管疾病的试剂盒，所述试剂盒包含：治疗有效量的根据前述项目中任一项所述的化合物或组合物的固体单位剂型，以及被调整为或配置为雾化所述药物制剂并且在口内施用后将其递送到下呼吸道和肺隔室的雾化器，以及使用说明书。应理解的是，所述固体单位剂型可以由干粉吸入器或计量剂量吸入器施用。

[0050] 5. 一种用于治疗宿主动物中的肺或支气管疾病的试剂盒，所述试剂盒包含：治疗有效量的根据前述项目中任一项所述的化合物或组合物的固体单位剂型，以及被调整为或配置为雾化所述药物制剂并且在鼻内施用后将其递送到鼻腔的雾化器，以及使用说明书。应理解的是，所述固体单位剂型可以作由干粉吸入器或计量剂量吸入器施用。

[0051] 6. 一种用于治疗宿主动物中的肺或支气管疾病的试剂盒，所述试剂盒包含：治疗有效量的根据前述项目中任一项所述的化合物或组合物的固体单位剂型，以及分开的稀释剂，以及使用说明书，所述使用说明书包含用于使用所述稀释剂重构所述固体单位剂型以制备能够被所述宿主动物吸入的液体组合物的说明。

[0052] 7. 根据前述项目中任一项所述的试剂盒，其包含容器。

[0053] 8. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述组合物是适于被所述宿主动物吸入的干粉。

[0054] 9. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述组合物是适于雾化并且被所述宿主动物吸入的溶液。

[0055] 10. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中递送化合物以取得以下肺浓度，例如在上皮内衬液 (ELF)、痰、内衬组织、支气管灌洗液等中测量的至少约 2 μg/mL、至少约 4 μg/mL、至少约 8 μg/mL 或至少约 16 μg.mL。

[0056] 11. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中 X 和 Y 与所连接的碳一起形成羰基。

[0057] 12. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是单糖。

[0058] 13. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是德糖胺或其衍生物。

[0059] 14. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中 Z 是德糖胺。

[0060] 15. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中 V 是 C(O)。

[0061] 16. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中W是H或F。

[0062] 17. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中W是F。

[0063] 18. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中A是CH_2。

[0064] 19. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中B是$(CH_2)_n$。

[0065] 20. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中n是2至4的整数

[0066] 21. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中n是3。

[0067] 22. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代。

[0068] 23. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是芳基、芳基烷基、杂芳基或杂芳基烷基，其各自是被取代的。

[0069] 24. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是芳基或杂芳基烷基，其各自任选地被取代。

[0070] 25. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是任选被取代的芳基或被取代的芳基。

[0071] 26. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是氨基苯基。

[0072] 27. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中C是3-氨基苯基。

[0073] 28. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述化合物是索利霉素(solithromycin)或者其药学上可接受的盐、水合物、溶剂合物、或前药。

[0074] 29. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述化合物是索利霉素或者其药学上可接受的盐。

[0075] 30. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述化合物是索利霉素。

[0076] 31. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述施用使用雾化器进行，并且所述组合物或单位剂型能够产生MMAD为约1至约5μm的气雾剂颗粒。

[0077] 32. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氨基糖苷类的步骤。

[0078] 33. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述氨基糖苷类选自由妥布霉素、阿米卡星及其组合组成的组。

[0079] 34. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中以低于拮抗或显著拮抗所述氨基糖苷类的效力的剂量施用所述化合物。

[0080] 35. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氟喹诺酮类抗生素的步骤。

[0081] 36. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述氟喹诺酮类是左氟沙星

[0082] 37. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其还包括施用治疗有效量的氨曲南的步骤。

[0083] 38. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病包括呼吸机相关肺炎 (VAP)、医院获得性肺炎 (HAP)、社区获得性细菌性肺炎 (CABP) 或其组合。

[0084] 39. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病包括囊性纤维化、肺癌、阻塞性肺病如慢性阻塞性肺病、哮喘、慢性支气管炎、限制性肺病、肺气肿、原发性和继发性纤毛运动障碍、鼻窦炎、肺炎、间皮瘤或其组合。

[0085] 40. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病包括囊性纤维化。

[0086] 41. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述宿主动物是免疫功能低下的。

[0087] 41. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由革兰氏阴性菌引起的，例如假单胞菌属 (Pseudomonas spp.)、嗜麦芽糖寡养单孢菌 (Stenotrophomonas maltophilia)、洋葱伯克霍尔德氏菌 (Burkholderia cepacia)、亲水性气单孢菌 (Aeromonas hydrophilia)、大肠杆菌 (Escherichia coli)、弗氏柠檬酸杆菌 (Citrobacter freundii)、沙门氏菌属 (Salmonella spp.)、志贺氏菌属 (Shigella spp.)、肠杆菌属 (Enterobacter spp.)、克雷伯氏菌属 (Klebsiella spp.)、粘质沙雷氏菌 (Serratia marcescens)、土拉弗朗西斯菌 (Francisella tularensis)、摩氏摩根氏菌 (Morganella morganii)、变形杆菌属 (Proteus spp.)、普罗维登斯菌属 (Providencia spp.)、不动杆菌属 (Acinetobacter spp.)、小肠结肠炎耶尔森氏菌 (Yersinia enterocolitica)、耶尔森氏菌属 (Yersinia spp.)、博德特氏菌 (Bordetella spp.)、嗜血杆菌属 (Haemophilus spp.)、巴斯德菌属 (Pasteurella spp.)、粘膜布兰汉氏菌 (Branhamella catarrhalis)、幽门螺杆菌 (Helicobacter pylori)、弯曲杆菌属 (Campylobacter spp.)、疏螺旋体属 (Borrelia spp.)、嗜肺军团菌 (Legionella pneumophila)、单核细胞增生李斯特氏菌 (Listeria monocytogenes)、淋病奈瑟氏菌 (Neisseria gonorrhoeae)、脑膜炎奈瑟氏菌 (Neisseria meningitidis)、金氏菌属 (Kingella)、莫拉氏菌属 (Moraxella)、阴道加德纳氏菌 (Gardnerella vaginalis)、拟杆菌属 (Bacteroides spp.) 等。

[0088] 42. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病至少部分地由革兰氏阳性菌如棒状杆菌属 (Corynebacterium spp.)、链球菌属 (Streptococcus spp.)、肠球菌属 (Enterococcus spp.)、葡萄球菌属 (Staphylococcus spp.) 等引起的。

[0089] 43. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由革兰氏阳性菌如梭菌属 (Clostridium spp.) 引起的。

[0090] 44. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由抗酸细菌如结核杆菌 (Mycobacterium tuberculosis)、鸟分支感觉

(Mycobacterium avium)、胞内分枝杆菌(Mycobacterium intracellulare)和麻风分枝杆菌(Mycobacterium leprae)引起的。

[0091] 45. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由非典型细菌如肺炎衣原体和肺炎支原体引起的。

[0092] 46. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌(mucoid Pseudomonas aeruginosa)、洋葱伯克霍尔德氏菌、金黄色葡萄球菌(Staphylococcus aureus)包括MRSA及其组合的一种或多种菌株引起的。

[0093] 47. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病至少部分地由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌、洋葱伯克霍尔德氏菌、金黄色葡萄球菌包括MRSA及其组合的一种或多种CF菌株引起的。

[0094] 48. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述粘液样铜绿假单胞杆菌是绿脓菌素阳性的。

[0095] 49. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由金黄色葡萄球菌包括易感性菌株和耐药性菌株如MRSA、炭疽芽孢杆菌或其组合的一种或多种菌株引起的。

[0096] 50. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由MRSA的一种或多种菌株引起的。

[0097] 51. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由克拉霉素抗性细菌包括多重耐药性或全耐药性或其组合的一种或多种菌株引起的。

[0098] 52. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由炎症引起的。

[0099] 53. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述疾病是至少部分地由TNFα产生、CXCL8产生、IL-8产生、MMP9产生或其组合引起的炎症引起的。

[0100] 54. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述宿主动物是哺乳动物。

[0101] 55. 根据前述项目中任一项所述的方法、组合物、单位剂型或试剂盒，其中所述宿主动物是人。

[0102] 示例性可吸入化合物、组合物和制剂包括可吸入干粉和可吸入可气雾化溶液。应理解的是，由于直接递送到感染的支气管内部位，可吸入化合物、组合物和制剂可提供额外的益处，例如与其他系统性递送相比降低毒性、降低成本以及更好的患者依从性，例如与需要住院护理或门诊访问的静脉内抗生素施用相比。

[0103] 示例性可吸入化合物、组合物和制剂适于向患者的肺和支气管内空间递送，例如通过气雾化或干粉吸入。这样的化合物可以是冻干剂或可重构冻干剂，如PCT国际公开号WO 2011/112864中所述，其公开内容通过引用并入本文。在另一个实施方案中，化合物制备成脂质体，包括带电脂质体和抗体涂覆的脂质体，纳米颗粒或微颗粒组合物，纳米混悬液等。可吸入化合物、组合物和制剂可包含一种或多种药学上可接受的载体、赋形剂、助悬剂、

稀释剂、填充剂、盐、缓冲剂、稳定剂、增溶剂、溶剂、分散介质、包衣、等渗剂以及其他材料。可吸入化合物、组合物和制剂可包含增效剂、络合剂、靶向剂、稳定剂、助溶剂、压缩气体或增溶缀合物。

[0104] 示例性赋形剂包括：糖，例如乳糖、蔗糖、甘露醇或山梨醇；纤维素制备物，例如玉米淀粉、小麦淀粉、大米淀粉、马铃薯淀粉、明胶、黄蓍胶、甲基纤维素、羟丙基甲基纤维素、羧甲基纤维素钠和/或聚乙烯吡咯烷酮(PVP)。优选赋形剂包括乳糖、明胶、羧甲基纤维素钠和低分子量淀粉产品。

[0105] 可用作加压包装吸入器系统中的阀润滑剂的示例性助悬剂包括油酸、简单羧酸衍生物和脱水山梨醇三油酸酯。

[0106] 示例性稀释剂包括水、盐水、磷酸盐缓冲的柠檬酸盐或盐水溶液和粘液溶解制剂。其他示例性稀释剂包括醇、丙二醇和乙醇。其他示例性稀释剂具有与肺泡装置相容的张力和pH。其他示例性稀释剂包括等渗盐水、磷酸盐缓冲的等渗溶液，其张力已经用氯化钠或蔗糖或葡萄糖或甘露醇调节。

[0107] 示例性填充剂包括液体或流体制剂中的甘油、丙二醇、乙醇。用于干粉吸入系统的示例性填充剂包括乳糖、蔗糖、葡萄糖、合适的氨基酸以及乳糖衍生物。在另一个实施方案中，填充剂包括甘油、丙二醇、乳糖和氨基酸。

[0108] 示例性盐包括生理上相容并且提供期望的张力调节的那些，包括强酸或弱酸的单价和二价盐。在另一个实施方案中，盐包括酒石酸盐。

[0109] 示例性缓冲剂包括磷酸盐或柠檬酸盐缓冲剂或者低缓冲能力的混合的缓冲体系。在另一个实施方案中，缓冲剂包括磷酸盐。

[0110] 在亲水核周围提供疏水鞘的包衣剂包括己酸和月桂酸。在脂质体的制备过程中，二磷脂酰胆碱或二磷脂酰肉豆蔻基胆碱或其合适的混合物可用于向分子或制剂提供保护。

[0111] 示例性的稳定剂包括为最终制剂提供化学或物理稳定性的那些。这样的稳定剂包括抗氧化剂，例如焦亚磷酸钠、醇、聚乙二醇、丁基化羟基茴香醚、丁基化羟基甲苯、依地酸二钠。在另一个实施方案中，稳定剂包括焦亚磷酸钠、依地酸二钠和聚乙二醇。在另一个实施方案中，稳定剂包括冷冻保护剂，例如乙二醇、糖和卡拉胶。

[0112] 示例性增溶剂包括丙二醇、甘油、合适的氨基酸、络合剂如环糊精、山梨醇溶液或醇。在另一个实施方案中，增溶剂包括乙醇、丙二醇、甘油、山梨醇和环糊精。在另一个实施方案中，增溶剂包括丙二醇、山梨醇和环糊精。

[0113] 应理解本文所述制剂可包含任意组合的来自上文组分列表的任何或所有选择。

[0114] 在另一个实施方案中，活性成分被配制为用于利用合适的推进剂吸入，推进剂例如二氯二氟甲烷、二氯氟甲烷、二氯四氟乙烷、二氧化碳或其他气体。在另一个实施方案中，推进剂包括非CFC相关类型的推进剂或相关类似物。

[0115] 在另一个实施方案中，通过与同本文所述化合物相容并且生物学相容的合适的佐剂混合来将活性成分干燥为可吸入干粉。用于吸入的药物材料的示例性干燥方法包括喷雾干燥、常规床干燥和/或超临界流体处理。在另一个实施方案中，使用喷雾干燥和超临界流体处理。

[0116] 在另一个实施方案中，化合物、组合物和制剂适于作为所述化合物的浓溶液来气雾化，例如约100至约1,000mg、约200至约800mg、约400至约600mg、约400至约500mg、

约 200 至约 400mg、约 200 至约 300mg、约 100 至约 400mg、约 100 至约 300mg、或约 100 至约 200mg 化合物的约 1 至约 5mL 溶液。

[0117] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括囊性纤维化 (CF)、呼吸机相关肺炎 (VAP)、医院获得性肺炎 (HAP)、社区获得性细菌性肺炎 (CABP) 及其组合。

[0118] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病还包括肺癌、阻塞性肺病如慢性阻塞性肺病、哮喘、慢性支气管炎、限制性肺病、肺气肿、原发性和继发性纤毛运动障碍、鼻窦炎、肺炎、间皮瘤及其组合。

[0119] 在另一个实施方案中，本文描述了化合物、组合物、制剂和方法，其用于治疗囊性纤维化。在另一个实施方案中，本文描述了的化合物、组合物、制剂和方法，其用于治疗具有细菌感染的免疫功能低下患者。

[0120] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括至少部分地由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌、洋葱伯克霍尔德氏菌、金黄色葡萄球菌包括 MRSA 或其组合的一种或多种菌株造成疾病。

[0121] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括至少部分地由铜绿假单胞杆菌、粘液样铜绿假单胞杆菌、洋葱伯克霍尔德氏菌、金黄色葡萄球菌包括 MRSA 或其组合的一种或多种 CF 菌株造成疾病。在一种变化中，粘液样铜绿假单胞杆菌是绿脓菌素阳性。

[0122] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括至少部分地由金黄色葡萄球菌的一种或多种菌株造成的疾病，包括易感性菌株和耐药性菌株，例如 MRSA，炭疽芽孢杆菌或其组合。

[0123] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括至少部分地由 MRSA 的一种更多种菌株造成的疾病。

[0124] 可以利用本文所述化合物、组合物、制剂和方法治疗的示例性疾病包括至少部分地由克拉霉素耐药菌的一种或多种菌株造成的疾病，包括多重耐药性或全耐药性。

[0125] 已经报道囊性纤维化患者以及具有慢性支气管感染的其他患者可能具有高发生率的支气管痉挛或哮喘气道。这些气道对低渗或高渗气雾剂、对永久离子特别是卤化合物如氯化物、以及对酸性或碱性气雾剂敏感。刺激气道的效果在临床上可以通过咳嗽或支气管痉挛证明。因此，应理解的是，本文所述制剂理想地具有调节的摩尔渗透压、张力、离子强度和 pH。

[0126] 在另一个实施方案中，可气雾化制剂具有调节到被患者良好耐受的盐度。在另一个实施方案中，制剂具有平衡的摩尔渗透压强度和氯化物浓度。在另一个实施方案中，制剂具有能够向感染部位递送有效剂量的化合物的最小合理可气雾体积。在另一个实施方案中，制剂不会不利地影响气道功能并且不造成任何不利副作用。

[0127] 在另一个实施方案中，可气雾化溶液制剂是生理上可接受的溶液，例如盐水和 / 或缓冲的盐水溶液。示例性盐水浓度是生理性的，例如约 0.9％盐水，或亚生理性的，例如约 0.1％盐水至小于约 0.9％盐水的范围，包括约 0.225％盐水 (25％生理盐水) 溶液。应理解的是，制剂还可以包含溴化物和 / 或碘化物。在另一个实施方案中，溶液的 pH 为约 4.2 至约 7.5、约 4.5 至约 7.5、约 4.5 至约 7、约 5.5 至约 7、或约 5.5 至约 6.5，或者约 6.0。应理

解是，前述 pH 可以是经缓冲的或未经缓冲的。

[0128] 在另一个实施方案中，可气雾化制剂是干粉或液体，干粉或液体形成自或能够形成适合于进入支气管深处的小颗粒，例如主要为以下平均直径例如质量中位平均直径(mass medium average diameter，MMAD)的小颗粒：约 10μm 或更小，例如约 1 至约 10μm；主要为约 5μm 或更小，例如约 1 至约 5μm，或约 2 至约 5μm。在另一个实施方案中，制剂能够气雾化，例如通过雾化，和 / 或能够形成适于进入支气管深处的小颗粒，例如主要为以下平均直径例如 MMAD：约 5μm 或更小，例如约 1 至约 5μm。本文使用的主要或大部分通常是指约 70％或更多、约 80％或更多、或者约 90％或更多的颗粒为约 10μm 或更小，例如约 1 至约 10μm；或者约 5μm 或更小，例如约 1 至约 5μm，或约 2 至约 5μm。应理解的是，在前述范围内观察到小于或等于约 3μm，或者小于或等于约 2μm 的标准差。应理解的是，前文特别描述了每个范围内的每个和所有整数值。

[0129] 在另一个实施方案中，可气雾化溶液的摩尔渗透压为约 50 至约 1050mOsm/L、或者约 50 至约 550mOsm/L、或者约 100 至约 750mOsm/L、或者约 200 至约 750mOsm/L、或者约 200 至约 600mOsm/L、或者约 300 至约 600mOsm/L、或者约 300 至约 500mOsm/L、或者约 150 至约 250mOsm/L、或者约 165 至约 190mOsm/L。在另一个实施方案中，制剂的摩尔渗透压为约 50 至约 550mOsm/kg、或者约 165 至约 190mOsm/kg。

[0130] 在另一个实施方案中，本文所述化合物作为气雾剂混悬剂例如脂质体或其他微观颗粒的气雾剂混悬剂施用。

[0131] 在另一个实施方案中，气雾剂制剂被雾化为可以递送到肺的贯穿内皮树的所有可能存在细菌的部分的粒径，包括支气管和细支气管以及末端和呼吸性细支气管，以及肺泡。应理解的是，存在于囊性纤维化患者中的铜绿假单胞杆菌细菌或其他易感性细菌可以位于末端和呼吸性细支气管中。还应理解的是，在感染恶化期间，细菌还可能存在于肺泡中。

[0132] 在另一个实施方案中，制剂或组合物是包含本文所述化合物的干粉。在一个方面，干粉分散在可吸入制剂中，可吸入制剂包含 MMAD 为约 5μm 或更小、或者约 1 至约 5μm、或者约 2 至约 5μm、或者约 3 至约 5μm 的颗粒或者基本上由其组成。

[0133] 干粉制剂可以使用任何常规方法制备，包括但不限于研磨（包括介质研磨、喷射研磨等），冻干、喷雾干燥、沉淀成细粉等。

[0134] 示例性喷雾干燥通过将本文所述化合物混悬在水中、搅拌并且冷却来实现。溶液任选地使用炭以及过滤纯化。随后，使用任何合适的喷雾干燥设备如 Buchi 微型喷雾干燥机 B-191 对溶液进行喷雾干燥。

[0135] 可以使用多级级联冲击仪或其他合适的方法测定颗粒尺寸。示例性地，美国药典第 601 章特别引用了 Thermo Andersen 八阶段不能活动的级联冲击器作为用于计量剂量吸入器和干粉吸入器中的气雾剂的特性装置。八阶段级联冲击器使用 8 个能够将气雾剂分类为 9.0 微米至 0.4 微米 (28.3L/ 分钟）的喷射阶段，并且允许空气颗粒冲击在不锈钢冲击表面上或多个过滤介质基材上。最终过滤器收集小于 0.4 的所有颗粒。

[0136] 示例性地，介质研磨通过将本文所述化合物放在包括例如不锈钢或陶瓷球的磨机中并且旋转或翻转材料直到取得期望的药物粒径范围来实现。应理解的是，介质研磨的优点可能包括良好的尺寸控制、狭窄的产品尺寸范围、高回收效率和容易扩展加工。

[0137] 示例性地，喷射研磨使用非常高压的空气流使颗粒彼此碰撞，从磨机中回收期望

尺寸的细颗粒。应理解的是，喷射研磨的优点可以包括迅速的制造过程和研磨期间少的能量传递，导致药物生产期间较少的温度上升。喷射研磨处理通常在数秒至数分钟完成。

[0138] 示例性地，通过将共溶剂添加到本文所述一种或多种化合物的溶液中来实现沉淀和/或结晶，其降低化合物的溶解度至均匀药物溶液，导致溶质沉淀和/或结晶。当添加足够共溶剂时，化合物的溶解度降至形成可以通过过滤或离心收集的固体药物颗粒的点。应理解的是，沉淀和/或结晶可能具有以下优点：高可重复性，具有高回收率以及能够在低温度条件（其会减少分解）下进行。

[0139] 在另一个实施方案中，可气雾化制剂的雾化速率为至少约1μL/秒、至少约2μL/秒、至少约3μL/秒、至少约4μL/秒或至少约5μL/秒。

[0140] 在另一个实施方案中，递送的单位剂量为约5mL、约4.5mL、约4mL、约3.75mL或约3.5mL。应理解的是，包装可能包含更多材料，但是其被配置以使得递送剂量为其预定部分。

[0141] 能够提供本文所述制剂的气雾剂的示例性雾化器包括雾化、喷射、电子和超声波雾化器、增压的震动的多孔板或等效雾化器，或者干粉吸入器，其主要产生1至5μm的气雾剂或干粉颗粒。应理解的是，这样的粒径是对于有效递送本文所述化合物到支气管内空间以治疗细菌感染所期望的。使用能够产生颗粒的雾化器通过气雾化递送组合物和制剂，其中大部分颗粒的平均直径为约5μm或更小、或者约1至约5μm。

[0142] 示例性雾化器包括干粉吸入器、计量剂量吸入器、微型雾化器、超声雾化器和喷射雾化器，包括标准和呼吸增强的（超声雾化器(Ultraneb 100/99；Sunrise Medical HHG；Somerset,PA)、特殊雾化器(Pari LC Plus Jet Nebulizer；Pari；Midlothian,VA)和压缩器(Pulmo-Aide；Sunrise Medical HHG)。

[0143] 示例性喷射雾化器（包括呼吸增强的喷射雾化器）是SIDESTREAM,PARI LC,PARI LC PLUS(Pari Respiratory Equipment,Richmond,Va)等。

[0144] 示例性超声雾化器包括AEROSONIC(来自DeVilbiss)、ULTRA AIRE(来自Omron)等。

[0145] 在另一个实施方案中，以每日给药方案施用该制剂，包括但不限于：每日四次、每日两次、每日三次、每8小时1次等。在一个实施方案中，每日给药方案为每日1至4次，每日三次、每8小时1次等或每日两次。在另一个实施方案中，每日给药方案是醒时每日三次，例如以7小时间隔每日三次或者以6小时间隔每日三次。

[0146] 在另一个实施方案中，本文所述化合物与氨基糖苷类共施用，其中本文所述化合物与氨基糖苷类交替给药，例如根据为了防止永久性布托霉素抗性所需的制造商标记，在用于布托霉素给药的28天强制间歇期期间。

[0147] 在另一个实施方案中，制剂包含在5毫升塑料小瓶中，例如低密度聚乙烯(LDPE)小瓶。小瓶可以使用吹入-填充-密封过程来无菌填充。额外的可选示例性包装描述在美国专利号5,409,125、5,379,898、5,213,860、5,046,627、4,995,519、4,979,630、4,951,822、4,502,616和3,993,223中，其公开内容通过引用并入本文。单位剂量容器可以被设计为直接插入适于允许吸入化合物的特定装置中。示例性地，小瓶密封在箔外袋(over-pouch)中，每个外袋中6个。

[0148] 大环内酯类的示例性肠胃外剂量描述在美国专利申请系列号61/312417中，其公开内容通过引用并入本文。

[0149] 在另一个实施方案中，本文所述大环内酯类与一种或多种其他抗生素共施用，例如一种或多种其他氨基糖苷类、一种或更独中其他氟喹诺酮类、氨曲南和/或磷霉素。在一种变化中，可以进行这样的共施用而不拮抗氨基糖苷类的作用。在一个方面，以低于能够拮抗或显著拮抗氨基糖苷类的效力的剂量施用大环内酯类。在另一个变化中，可以进行这样的共施用以加强另一种抗生素的活性，例如氨基糖苷类、氟喹诺酮类、氨曲南和/或磷霉素。

[0150] 抗生素氨基糖苷类如妥布霉素大大增加了治疗假单胞菌感染的可用治疗。铜绿假单胞杆菌在支气管空间内生长，并且发现于患有或需要缓解致病菌感染的患者的痰中。在感染加剧期间，铜绿假单胞杆菌生长还可以发生在患者的肺泡中。致病性支气管内铜绿假单胞杆菌造成的示例性疾病包括囊性纤维化。

[0151] 在另一个示例性实施方案中，本文描述了用于治疗囊性纤维化(CF)的方法。在一个方面，该方法包括施用一种或多种本文所述大环内酯类的步骤。在一个变化中，该方法还包括使用一种或多种氨基糖苷类的步骤。在另一个变化中，该方法还包括施用一种或多种氟喹诺酮类的步骤。在另一个变化中，该方法还包括施用氨曲南的步骤。在另一个变化中，该方法还包括施用磷霉素的步骤。在任意前述方法的一个变化中，该方法包括通过吸入施用氟喹诺酮类如左氟沙星、氨基糖苷抗生素如妥布霉素、氨曲南和/或曲霉素的步骤。

[0152] 示例性氨基糖苷类包括但不限于阿米卡星、阿普拉霉素、阿贝卡星、阿司米星、卡那霉素B、地贝卡星、新霉素B、庆大霉素、潮霉素B、异帕米星、卡那霉素、新霉素、奈替米星、巴龙霉素、巴龙霉素硫酸盐、红链霉素(rhodostreptomycin)、核糖霉素、西索米星、大观霉素、链霉素、妥布霉素、甲基姿苏霉素(verdamicin)及其组合。

[0153] 在另一个实施方案中，氨基糖苷类选自庆大霉素、阿米卡星、卡那霉素、链霉素、新霉素、奈替米星和妥布霉素及其组合。

[0154] 可用于本文所述方法的氨基糖苷类抗生素的示例性给药方法和方案描述在美国专利号5508269、6083922和6890907中，其公开内容通过引用并入本文。在一个变化中，该方法包括通过吸入施用氨基糖苷类抗生素如妥布霉素的步骤。

[0155] 施用氨基糖苷类的任何常规剂量单位、制剂和/或方法均可以用在本文中，例如美国专利号5508269、6890907、6083922和7696178中描述的施用妥布霉素的剂量单位、制剂和/或方法，其公开内容通过引用并入本文。

[0156] 示例性氟喹诺酮类抗生素包括但不限于：恶喹酸(Uroxin)、吡咯米酸(Panacid)、吡哌酸(Dolcol)、rosoxacin(Eradacil)、环丙沙星(Ciprobay、Cipro、Ciproxin)、洛美沙星(Maxaquin)、那氟沙星(Acuatim、Nadoxin、Nadixa)、诺氟沙星(Lexinor、Noroxin、Quinabic、Janacin)、氧氟沙星(Floxin、Oxaldin、Tarivid)、台氟沙星(Peflacine)、芦氟沙星(Uroflox)、巴洛沙星(Baloxin)、左氟沙星(Cravit、Levaquin)、莫西沙星(Avelox,Vigamox)、帕珠沙星(Pasil、Pazucross)、司帕沙星(Zagam)、托氟沙星(Ozex、Tosacin)、克林沙星、吉米沙星(Factive)、西他沙星(Gracevit)、普卢利沙星(Quisnon)、德拉沙星及其组合。

[0157] 施用氟喹诺酮类的任何常规剂量单位、制剂和/或方法均可用于本文。

[0158] 施用氨曲南的任何常规剂量单位、制剂和/或方法均可用于本文，例如美国专利号6660249和7214364中所述施用氨曲南的剂量单位、制剂和/或方法，其公开内容通过引

用并入本文。

[0159] 在另一个实施方案中，在抑制治疗方案中施用一种或多种本文所述大环内酯类。在另一个实施方案中，在用于肺病如 CF 的急性加剧的辅助治疗方案中施用一种或多种本文所述大环内酯类。在另一个实施方案中，方案能够阻止或延迟慢性铜绿假单胞菌感染和/或定殖。

[0160] 在另一个实施方案中，一种或多种本文所述大环内酯类如 CEM-101 与妥布霉素共施用。

[0161] 在另一个实施方案中，一种或多种本文所述大环内酯类如 CEM-101 与左氟沙星共施用。

[0162] 在另一个实施方案中，一种或多种本文所述大环内酯类如 CEM-101 与氨曲南共施用。

[0163] 在另一个实施方案中，CEM-101 与妥布霉素共施用，其中 CEM-101 口服施用，妥布霉素通过吸入施用。在另一个实施方案中，CEM-101 与氨曲南共施用，其中 CEM-101 口服施用，氨曲南通过吸入施用。在另一个实施方案中，CEM-101 与妥布霉素共施用，其中 CEM-101 通过吸入施用，妥布霉素通过吸入施用。在另一个实施方案中，CEM-101 与氨曲南共施用，其中 CEM-101 通过吸入施用，氨曲南通过吸入施用。在一个变化中，根据以下方案共施用，其中在第一施用周期施用妥布霉素或氨曲南，例如第 1 至 28 天，在第二时期施用 CEM-101，例如第 29 至 56 天。交替的时期可以重复。

[0164] 在另一个实施方案中，本文所述大环内酯类如 CEM-101 的示例性每日口服剂量为约 1 至约 25mg/kg、约 1 至约 10mg/kg、约 2 至约 8mg/kg 或约 4 至约 6mg/kg 患者体重。在另一个实施方案中，本文所述大环内酯类如 CEM-101 的示例性每日成人口服剂量为约 100 至约 1,000mg、约 200 至约 800mg、或约 400 至约 600mg。在另一个实施方案中，每日剂量是单词或分开的，并且可以每日四次、每日二次、每日三次等施用。

[0165] 应理解的是，理想地进行给药以取得为用于一个或更多个被感染或患病靶生物的 MIC 的至少约 10 倍的痰浓度。不受理论的约束，相信这样的示例性剂量足以取得约 1μg/mL 或更多、约 2μg/mL 或更多、约 4μg/mL 或更多、或约 8μg/mL 或更多的肺水平，其在大部分情况下可能相当于 MIC 的约 10 倍或更多的浓度。不受理论的约束，还相信这样的示例性剂量足以观察对抗肺病原体的杀菌活性。

[0166] 本文使用的术语“治疗有效量”是指在研究人员、兽医、医生或其他临床医师所探寻的组织系统、动物或人中引起生物学或药学反应的活性化合物或药物制剂的量，该反应包括缓解被治疗疾病或紊乱的症状。在一个方面，治疗有效量是在适于任何医学治疗的合理利益/风险比例下治疗或减轻疾病或疾病的症状的量。但是，应理解的是，本文所述化合物和组合物的总每日使用可由主治医生在合理的医学判断内决定。用于任何热定患者的治疗有效剂量水平取决于多种因素，包括被治疗疾病和疾病的严重程度；所使用特定化合物的活性；所使用的特定组合物；患者的年龄、体重、总体健康、性别和饮食；所使用特定化合物的施用时间、施用途径和排泄速率；治疗的持续时间；与所使用的特定化合物联合或共同使用的药物等普通技术的研究人员、兽医、医生或其他临床医生周知的因素。

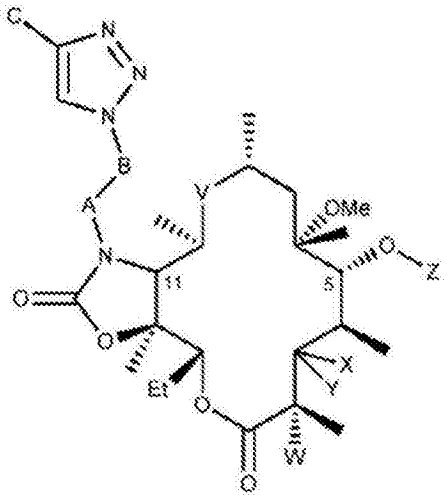
[0167] 本文示例性应用于组合物、制剂、试剂盒、方法等所使用的术语治疗有效量包括实现靶生物的 MIC 的 10X 或更多的化合物的痰浓度的量。

[0168] 应理解的是，成功的治疗可以通过任何常规方法或终点检测，包括但不限于用力呼气量 (FEV) 和 / 或肺活量 (FCV) 下降的减少或改善。还应理解的是，成功的治疗可以通过痰中靶细菌的菌落形成单位 (CFU) 的减少或生长的降低来监测。还应理解的是，成功的治疗可以通过假单胞菌属和 / 或伯克氏菌属的幸存菌的减少来监测。

[0169] 示例性靶致病菌包括但不限于葡萄球菌，包括凝固酶阴性葡萄球菌和凝固酶阳性葡萄球菌，链球菌，包括 A 组 β 溶血链球菌、非 A 组 β 溶血链球菌和绿色组链球菌，肠球菌、奈瑟菌属、梭菌属、博德特氏菌属、芽孢杆菌属和棒状杆菌属。特别地，细菌感染是由选自以下成员组成的组的细菌引起的感染 :金黄色葡萄球菌 (甲氧西林耐受性和甲氧西林易感性)、表皮葡萄球菌 (Staphylococcus epidermidis)、溶血葡萄球菌 (Staphylococcus hemolyticus)、腐生葡萄球菌 (Staphylococcus saprophyticus)、里昂葡萄球菌 (Staphylococcus lugdunensis)、头状葡萄球菌 (Staphylococcus capitis)、山羊葡萄球菌 (Staphylococcus caprae)、解糖葡萄球菌 (Staphylococcus saccharolyticus)、模拟葡萄球菌 (Staphylococcus simulans)、沃氏葡萄球菌 (Staphylococcus warneri)、人葡萄球菌 (Staphylococcus hominis)、中间型葡萄球菌 (Staphylococcus intermedius)、假中间型葡萄球菌 (Staphylococcus pseudointermedius)、Staphylococcus lyricus、酿脓链球菌 (Streptococcus pyogenes)、无乳链球菌 (Streptococcus agalactiae)、停乳链球菌停乳亚种 (Streptococcus dysgalactiae subspecies dysgalactiae)、咽峡炎链球菌 (Streptococcus anginosus)、缓症链球菌 (Streptococcus mitis)、唾液链球菌 (Streptococcus salivarius)、牛链球菌 (Streptococcus bovis)、变异链球菌 (Streptococcus mutans)、铜绿假单胞杆菌、淋病奈瑟菌 (Neisseria gonorrhoeae)、脑膜炎链球菌 (Neisseria meningitidis)、炭疽杆菌 (Bacillus anthracis)、百日咳杆菌 (Bordetella pertussis)、洋葱伯克霍尔德氏菌、艰难梭菌 (Clostridium difficile)、粪肠球菌 (Enterococcus faecalis)、屎肠球菌 (Enterococcus faecium) 和白喉杆菌 (Corynebacterium diphtheriae)。在特定方面，细菌感染是由金黄色葡萄球菌 (甲氧西林耐受性或甲氧西林易感性) 造成的感染。

[0170] 在另一个示例性实施方案中，本文所述大环内酯类是下式

[0171]



[0172] 或其药学上可接受的盐、水合物、溶剂合物、酯和前药，其中 :

[0173] X是H；并且Y是OR_7；其中R_7是单糖或二糖或者其衍生物；或者X和Y与所连接的碳一起形成羰基；

[0174] Z是单糖或二糖或者其衍生物；

[0175] V是C(O)或C(=NR_{11})，其中R_{11}是羟基或烷氧基；

[0176] W是H、F、Cl、Br、I或OH；

[0177] A是CH_2、C(O)、C(O)O、C(O)NH、$S(O)_2$、$S(O)_2NH$或C(O)NHS$(O)_2$；

[0178] B是$(CH_2)_n$，其中n是0至约10的整数，或者B是C_2-C_{10}烯基或炔基；并且

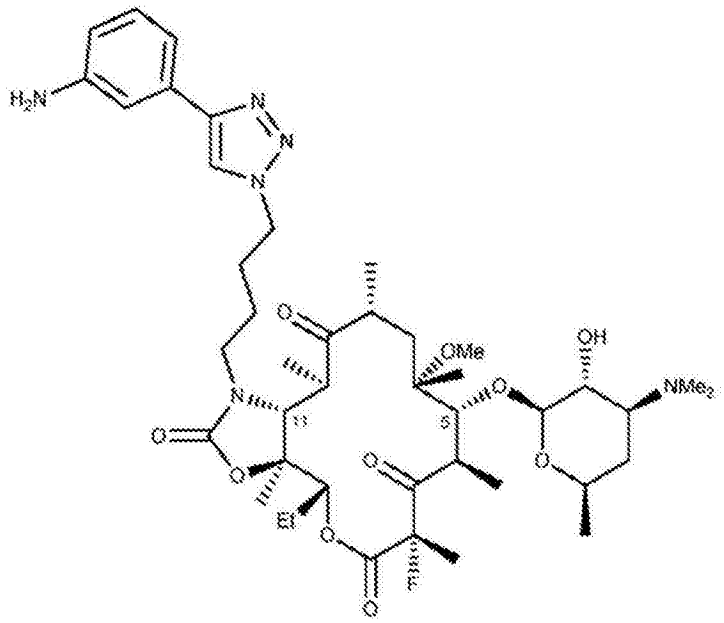
[0179] C是环烷基、环杂烷基、芳基、芳基烷基、杂芳基或杂芳基烷基，其各自任选地被取代。

[0180] 在另一个实施方案中，X和Y与所连接的碳一起形成羰基。在另一个实施方案中，Z单糖或其衍生物。在另一个实施方案中，Z是含氨基的单糖，例如氨基葡萄糖或其衍生物、类似物或立体异构体，包括但不限于德糖胺及其衍生物、碳霉糖(mycaminose)及其衍生物、万古糖胺及其衍生物、L-万古糖胺、3-脱甲基-万古糖胺、3-表-万古糖胺、4-表-万古糖胺、acosamine、3-氨基-葡萄糖、4-脱氧-3-氨基-葡萄糖、放线糖胺(actinosamine)、道诺糖胺(daunosamine)、3-表-道诺糖胺、瑞斯托糖胺(ristosamine)、N-甲基-D-葡萄糖胺等。在另一个实施方案中，Z是德糖胺及其衍生物。在另一个实施方案中，Z是碳霉糖或其衍生物。在另一个实施方案中，Z是德糖胺。在另一个实施方案中，Z是碳霉糖。在另一个实施方案中，V是C(O)。在另一个实施方案中，W是H或F。在另一个实施方案中，W是F。在另一个实施方案中，A是CH_2。在另一个实施方案中，B是$(CH_2)_n$，其中n是约2至约4的整数。在另一个实施方案中，B是$(CH_2)_3$。在另一个实施方案中，C是任选经取代的芳基。在另一个实施方案中，C是氨基取代的芳基。

[0181] 应理解的是，X、Y、Z、W、A、B和n的每一前述选择可以不受限制地组合，因此本文具体描述了这样的化合物的亚类。例如，在另一个实施方案中，X和Y与所连接的碳一起形成羰基，并且Z是单糖或其衍生物；或者X和Y与所连接的碳一起形成羰基，并且V是C(O)；或者X和Y与所连接的碳一起形成羰基，W是F，并且A是CH_2；或者Z是德糖胺或其衍生物，V是C(O)，A是CH_2，并且B是$(CH_2)_n$，其中n是约2至约4的整数；等等。

[0182] 在另一个实施方案中，本文所述大环内酯类是式

[0183]



[0184] 也称为CEM-101或索利霉素(solithromycin),或者其药学上可接受的盐、水合物、溶剂合物、酯或前药。

[0185] 本文所述大环内酯类可以如本文所述制备或者根据美国专利申请公开号2006/0100164和PCT国际公开号WO 2009/055557制备,其公开内容通过引用整体并入本文。

[0186] 本文使用的术语“烷基”包括任选地分支的碳原子链。本文使用的术语“烯基”和“炔基”包括任选地分支并且分别包含至少一个双键或三键的碳原子链。应理解的是,炔基还可以包含一个或更多个双键。还应该理解的是,在某些实施方案中,烷基有利地为有限长度,包括C_1-C_{24}、C_1-C_{12}、C_1-C_8、C_1-C_6和C_1-C_4。还应该理解的是,在某些实施方案中,烯基和/或炔基各自可以有利地为有限长度,包括C_2-C_{24}、C_2-C_{12}、C_2-C_8、C_2-C_6和C_2-C_4。应理解的是,本文较短的烷基、烯基和/或炔基可以为化合物添加较少亲油性,因此具有不同的药物代谢动力学行为。示例性烷基是但不限于甲基、乙基、正丙基、异丙基、正丁基、异丁基、仲丁基、叔丁基、戊基、2-戊基、3-戊基、新戊基、己基庚基、辛基等。

[0187] 本文使用的术语“环烷基”包括任选地分支的碳原子链,其中链的至少一部分是环形。应理解的是,环烷基烷基是环烷基的子集。应理解的是,环烷基可以是多环的。示例性环烷基包括但不限于环丙基、环戊基、环己基、2-甲基环丙基、环戊基乙-2-基、金刚烷基等。本文使用的术语“环烯基”包括任选地分支并且包含至少一个双键的碳原子链,其中链的至少一部分是环形。应理解的是,一个或更多个双键可以在环烯基的环部分中和/或在环烯基的非环部分中。应理解的是,环烯基烷基和环烷基烯基各自是环烯基的子集。应理解的是,环烷基可以是多环的。示例性环烯基包括但不不限于:环戊烯基、环己基乙烯-2-基、环庚烯基丙烯基等。还应理解的是,形成环烷基和/或环烯基的链有利地为有限长度,包括C_3-C_{24}、C_3-C_{12}、C_3-C_8、C_3-C_6和C_5-C_6。应理解的是,本文较短的分别形成环烷基和/或环烯基的烷基和/或烯基链可以为化合物添加较少亲油性,因此具有不同的药物代谢动力学行为。

[0188] 本文使用的术语“杂烷基”包括包含碳原子和至少一个杂原子二者并且任选地分支的原子链。示例性杂原子包括氮、氧和硫。在某些变化中,示例性杂原子还可以包括磷和

硒。本文使用的术语“环杂烷基”包括杂环基和杂环，包括包含碳和至少一个杂原子（例如杂烷基）并且任选地分支的原子链，其中链的至少部分是环形。示例性杂原子包括氮、氧和硫。在某些变化中，示例性杂原子还包括磷和硒。示例性环杂烷基包括但不限于四氢呋喃基、吡咯烷基、四氢吡喃基、哌啶基、吗啉基、哌嗪基、高哌嗪基、奎宁环基等。

[0189] 本文使用的术语“芳基”包括单环和多环芳基，包括芳族碳环和芳族杂碳环基，其各自可以任选地被取代。本文使用的术语“胺甲萘（carbaryl）”包括芳族碳环基团，其各自可任选地被取代。本文所述示例性芳族碳环基团包括但不限于苯基、萘基等。本文使用的术语“杂芳基”包括芳族杂环基团，其各自可以任选地被取代。示例性芳族杂环基团包括但不限于吡啶基、嘧啶基、吡嗪基、三嗪基、四嗪基、喹啉基、喹唑啉基、喹喔啉基、噻吩基、吡唑基、咪唑基、噁唑基、噻唑基、异噁唑基、异噻唑基、噁二唑基、噻二唑基、三唑基、苯并咪唑基、苯并噁唑基、苯并噻唑基、苯并异噁唑基、苯并异噻唑基等。

[0190] 本文使用的术语“氨基”包括基团 NH_2、烷基氨基和二烷基氨基，其中二烷基氨基中的两个烷基可以是相同的或不同的，即烷基烷基氨基。示例性氨基包括甲氨基、乙氨基、二甲氨基、甲基乙基氨基等。此外，应理解的是，当氨基修饰另一术语或被另一术语修饰时，例如氨基烷基或酰氨基，术语氨基的上述变化均包含在本文中。示例性氨基烷基包括 H_2N- 烷基、甲基氨基烷基、乙基氨基烷基、二甲基氨基烷基、甲基乙基氨基烷基等。示例性酰胺基包括酰基甲基氨基、酰基乙基氨基等。

[0191] 本文使用的术语“氨基及其衍生物”包括本文所述氨基，以及烷基氨基、烯基氨基、炔基氨基、杂烷基氨基、杂烯基氨基、杂炔基氨基、环烷基氨基、环烯基氨基、环杂烷基氨基、环杂烯基氨基、芳基氨基、芳基烷基氨基、芳基烯基氨基、芳基炔基氨基、酰胺基等，其各自任选地被取代。术语“氨基衍生物”包括脲、氨基甲酸盐 / 酯等。

[0192] 本文使用的术语“任选地被取代”包括将任选地被取代的基团上的氢原子替换成其他官能团。这样的其他官能团示例性包括但不限于：氨基、羟基、卤素、硫醇基、烷基、卤代烷基、杂烷基、芳基、芳基烷基、芳基杂烷基、硝基、磺酸及其衍生物、羧酸及其衍生物等。示例性地，任意氨基、羟基、硫醇基、烷基、卤代烷基、杂烷基、芳基、芳基烷基、芳基杂烷基和 / 或磺酸任选地被取代。

[0193] 本文使用的术语“任选被取代的芳基”包括将任选被取代的芳基上的氢原子替换成其他官能团。这样的其他官能团示例性包括但不限于氨基、羟基、卤素、硫醇基、烷基、卤代烷基、杂烷基、芳基、芳基烷基、芳基杂烷基、硝基、磺酸及其衍生物、羧酸及其衍生物等。示例性地，任意氨基、羟基、硫醇基、烷基、卤代烷基、杂烷基、芳基、芳基烷基、芳基杂烷基和 / 或磺酸任选地被取代。

[0194] 示例性取代基包括但不限于基团 $-(CH2)_xZ^X$，其中 x 是 0-6 的整数，且 Z^X 选自卤素、羟基、烷酰氧基（包括 C_1-C_6 烷酰氧基）、任选被取代的芳酰氧基、烷基（包括 C_1-C_6 烷基）、烷氧基（包括 C_1-C_6 烷氧基）、环烷基（包括 C_3-C_8 环烷基）、环烷氧基（包括 C_3-C_8 环烷氧基）、烯基（包括 C_2-C_6 烯基）、炔基（C_2-C_6 炔基）、卤代烷基（包括 C_1-C_6 卤代烷基）、卤代烷氧基（包括 C_1-C_6 卤代烷氧基）、卤代环烷基（包括 C_3-C_8 卤代环烷基）、卤代环烷氧基（包括 C_3-C_8 卤代环烷氧基）、氨基、C_1-C_6 烷基氨基、(C_1-C_6 烷基)(C_1-C_6 烷基) 氨基、烷基羰基氨基、N-(C_1-C_6 烷基) 烷基羰基氨基、氨基烷基、C_1-C_6 烷基氨基烷基、(C_1-C_6 烷基)(C_1-C 烷基) 氨基烷基、烷基羰基氨基烷基、N-(C_1-C_6 烷基) 烷基羰基氨基烷基、氰基和硝基；或者 Z^x

选自 $-CO_2R^4$和 $-CONR^5R^6$，其中 R^4、R^5和 R^6每次出现时都各自独立地选自氢、C_1-C_6烷基和芳基 $-C_1$-C_6烷基。

[0195] 本文所述化合物可以包含一个或更多个手性中心，或者另外可以以多种立体异构体存在。应理解是，在一个实施方案中，本文所述发明不限于任何特定立体化学要求，并且化合物以及包含它们的组合物、方法、用途和药物可以是光学纯的，或者可以是多种立体异构体的混合物，包括外消旋混合物以及其他对映体混合物，其他非对映体混合物等。还应理解的是，这样的立体异构体混合物可以包括一个或更多个手性中心处的单一立体异构体构型，同时包括一个或更多个其他手性中心处的立体异构体构型的混合物。

[0196] 同样地，本文所述化合物可以包含几何中心，例如顺式、反式、E 和 Z 双键。应理解的是，在另一个实施方案中，本文所描述的本发明不限于任何特定的几何异构体要求，并且化合物以及包含它们的组合物、方法、用途和药物可以是纯的，或者可以是任何多种几何异构体的混合物。还应理解的是，这样的几何异构体混合物可以包括一个或更多个双键处的单一构型，同时包括一个或更多个其他双键处的几何异构体的混合物。

[0197] 本文使用的术语“组合物”一般是指包含特定量的特定成分的任何产物，以及直接或间接地来自特定量的特定成分的组合的任何产物。应理解的是，本文所述组合物可以有本文所述分离的化合物或由本文所述化合物的盐、溶液、水合物、溶剂合物和其他形式制备。还应理解的是，组合物可以由本文所述化合物的多种无定形、非无定形、部分结晶、结晶和 / 或其他形态形式制备。还应理解的是，组合物可以由本文所述化合物的多种水合物和 / 或溶剂合物制备。因此，还应理解的是，具有本文所述化合物的这样的药物组合物包含本文所述化合物的多种形态形式和 / 或溶剂合物或水合物形式中的任一种或任意组合。示例性地，组合物可包含一种或多种载体、稀释剂和 / 或赋形剂。本文所述化合物或含该化合物的组合物可以以治疗有效量配制在适合于本文所述方法的任何常规剂型中。本文所述化合物或包含该化合物的组合物（包括这样的制剂）可使用已知程序通过用于本文所述方法的多种常规途径并且以多种剂型施用。用于本文所述方法（一般参见，Remington:The Science and Practice of Pharmacy,（第 21 版，2005））。

[0198] 本文使用的术语“前药”通常是指当施用到生物系统中时由于一种或多种自发化学反应、酶促化合物反应和 / 或代谢化学反应或其组合而产生生物活性化合物的任何化合物。在体内，前药通常通过酶（例如，酯酶、酰胺酶、磷酸酶等）、简单生物化学或体内的其他过程作用以释放或再生药学活性更高的药物。该激活可通过内源宿主酶或在施用前药之前、之后或同时向宿主施用的非内源酶作用。前药用途的另外的细节描述在下：美国专利号 5,627,165；以及 Pathalk 等，Enzymic protecting group techniques in organic synthesis,Stereosel.Biocatal.775-797(2000)。应理解的是，有利地一旦目标如靶向递送、安全性、稳定性等实现就将前药转化成原药，然后迅速消除形成前药之基团的残余。

[0199] 前药可以由本文所述化合物通过向存在于化合物上的一个或更多个官能团（例如，-OH-、-SH、$-CO_2H$、$-NR_2$）上连接最终将在体内切割的官能团来制备。示例性前药包括但不限于羧酸酯，其中基团是烷基、芳基、芳烷基、酰基氧基烷基、烷氧基羰氧基烷基，以及羟基的酯、硫醇和胺，其中连接的基团是酰基、烷氧基羰基、氨基羰基、磷酸盐或硫酸盐。示例性酯（也称为活性酯）包括但不限于 1- 茚满基、N- 氧基琥珀酰亚胺；酰基氧基烷基，例如乙酰氧基甲基、新戊酰氧基甲基、β - 乙酰氧基乙基、β - 新戊酰氧基乙基、1-（环己基羰基氧

基）丙 -1- 基、(1 - 氨基乙基）氧基氧基甲基等；烷氧基羰基氧基烷基，例如乙氧基羰基氧基甲基、α - 乙氧基羰基氧基乙基、β - 乙氧基羰基氧基乙基等；二烷基氨基烷基基团，包括低级烷基氨基烷基基团，例如二甲基氨基甲基、二甲基氨基乙基、二乙基氨基甲基、二乙基氨基乙基等；2-（烷氧基羰基）-2- 烯基基团，例如 2-（异丁氧基羰基）戊 -2- 烯基、2-（乙氧基羰基）丁 -2- 烯基等；以及内酯基团，例如酞基、二甲氧基酞基等。

[0200] 另一些示例性前药包含化学部分如酰胺或磷官能团以增加本文所述化合物的溶解性和 / 或稳定性。用于氨基的另一些示例性前药包括但不限于 (C_3-C_{20}) 烷酰基；卤代 -(C_3-C_{20}) 烷酰基；(C_3-C_{20}) 烷酰基；(C_4-C_7) 环烷酰基；(C_3-C_6)- 环烷基 (C_2-C_{16}) 烷酰基；任选被取代的芳酰基，例如未被取代的芳酰基或被 1 至 3 个选自由以下成员组成的组的取代基取代的芳酰基：卤素、氰、三氟甲烷磺酰基氧基、(C_1-C_3) 烷基和 (C_1-C_3) 烷氧基，其各自任选地被 1 至 3 个卤素原子中的一个或更多个取代；任选被取代的芳基 (C_2-C_{16}) 烷酰基，例如未被取代的芳基或被 1 至 3 个选自由以下成员组成的组的取代基取代的芳基：卤素、(C_1-C_3) 烷基和 (C_1-C_3) 烷氧基，其各自任选地进一步被 1 至 3 个卤素原子取代；以及任选被取代的杂芳基烷酰基，其在杂芳基部分中具有选自 O、S 和 N 的 1 至 3 个杂原子的以及在烷酰基部分中具有 2 至 10 个碳原子，例如，未被取代的或被 1 至 3 个选自由以下成员组成的组的取代基取代的杂芳基：卤素、氰、三氟甲烷磺酰基氧基、(C_1-C_3) 烷基和 (C_1-C_3) 烷氧基，其各自任选地进一步被 1 至 3 个卤素原子取代。示出的基团是示例性并且非详尽的，并且可以通过常规方法制备。

[0201] 应理解的是，前药本文可以不具有显著的生物活性，但是在体内施用后经历一个或更多个自发化合反应、酶促化学反应和 / 或代谢化学反应或其组合以产生本文所述生物活性化合物或生物活性化合物的前体。但是，应理解的是，在一些情况下，前药是生物活性的。还应理解的是，前药经常可用于通过提高口服生物利用度、药代动力学半衰期等提高药物效力或安全性。前药还指本文所述化合物的包含简单掩蔽不希望的药物特性或提高药物递送的基团的衍生物。例如，一种或多种本文所述化合物可表现出不希望的特性，该特性可以不利地阻断或最小化，可能变成临床药物应用中的药理学、制药学或药物代谢动力学障碍，例如低的口服药物吸收、缺少部位特异性、化学不稳定性、毒性以及差的患者接收（坏的味道、注射部位的疼痛等）等。应理解的是，前药或其他使用可逆衍生物的策略可用于优化药物的临床应用。

[0202] 应理解的是，在本文所述方法中，共施用的单独组分或组合可通过合适的方式同时、先后、分开或在单一药物制剂中施用。当共施用的化合物或组合物以分开的剂型施用时，每种化合物每日施用的剂量数目可以相同或不同。化合物或组合物可以通过相同或不同施用途径施用。可以根据相同的或交替的方案，在治疗过程的相同时间或不同时间并且以分开的形式或单一形式施用该化合物或组合物。

[0203] 示例性口服施用途径包括片剂、胶囊剂、酊剂、糖浆剂等。

[0204] 示例性肠胃外施用途径包括静脉内、动脉内、覆膜内、硬膜外、尿道内、胸骨内、肌内和皮下，以及本领域中已知的任何其他肠胃外施用途径。示例性肠胃外施用方式包括针式（包括微针）注射器、无针注射器和输注技术，以及本领域已知的其他肠胃外施用方式。肠胃外制剂通常是水溶液，其可以包含赋形剂如盐、碳水化合物和缓冲剂（优选约 3 至约 9 范围的 pH），但是对于一些应用，其可能更适合配制成无菌无水溶液或待与合适的载体如无

菌无热原水结合使用的干燥形式。无菌条件（例如，通过冻干）下肠胃外制剂的制备可以使用本领域技术人员周知的标准制药技术实现。化合物的肠胃外施用示例性地以盐水溶液或并入到脂质体化合物的形式进行。在溶解本身不充分可溶的化合物的情况下，可以使用增溶剂，例如乙醇。

实施例

[0205] 实施例。可吸入制剂。将本文所述化合物如 CEM-101 配制成常规干粉或溶液制剂。化合物可任选地配制成药学上可接受的盐。干粉制剂可使用被动式干粉吸入器施用。溶液制剂使用加压计量剂量吸入器、雾化器或类似装置施用。示例性干粉制剂包括但不限于 Pulmosphere(PS) 制剂 (Inhale Therapeutic Systems, San Carlos, CA) 等。PS 制剂根据 Dellamary 等，Hollow porous particles in metered dose inhalers, Pharm Res 17:168-174(2000) 制备。前述出版物以及本文引用的每一份另外的出版物的公开内容通过引用并入本文。

[0206] 实施例。假单胞菌藻酸盐的制备。将铜绿假单胞菌如 NH57388A 在 37℃下于 50mL Mueller-hinton 肉汤 (MHB) 中震荡 (170rpm) 培养 24 至 28 小时。通过离心 (23,000xg, 30 分钟，4℃）收集细菌细胞并且重悬在 3 至 6mL MHB 中。收集上清液并且放置在 80℃水浴中 30 分钟。通过将上清液添加到 150mL 冰冷的 99％乙醇中使藻酸盐沉淀。用无菌细菌环收集沉淀的海藻盐并且在无菌盐水中洗涤多重。然后纯化的藻酸盐重悬在 10mL 无菌盐水中并且强力搅拌以形成均匀混悬液。测量海藻盐浓度并且调节到 2 至 3mg/mL 的浓度。

[0207] 实施例。急性小鼠肺部感染模型。将铜绿假单胞杆菌如 ATCC27853 在 MHB 中于 35℃下培养过夜。通过 600nm 的吸光度与预定的平板计数之间的相关性将细菌悬液重新调整到约 1×10^{5}至 6×10^{5}CFU/mL。在第 1 天和第 3 天，通过覆膜内注射 150mg/kg 环磷酰胺 (Baxter, Deerfield, IL) 使雌性 Swiss 小鼠的嗜中性白细胞减少。在第 4 天，利用与 1mL 注射器连接的弯曲的口饲器尖端通过气管内滴注 0.05mL 接种物来感染小鼠。在感染后 24 小时开始抗生素治疗并且每天施用一次或两次 (BID)，持续 24 小时或 48 小时。抗生素利用微喷雾气雾剂装置雾化。所有感染和气雾剂治疗在小鼠处于异氟烷麻醉（氧气中 5％异氟烷以 4L/ 分钟输送）的情况下进行。未治疗小鼠组 (n ＝ 8) 在开始治疗之前处死以确定基线细菌计数。在施用最后一次抗生素剂量后 12 至 16 小时，通过二氧化碳窒息处死经治疗动物 (n ＝ 8)。无菌切下肺并且在 1mL 无菌盐水中均化 (Pro200 均化器；Pro Scientific, Monroe, CT)。经均化肺的连续 10 倍稀释液在 Mueller-Hinton 琼脂上布板，并且对菌落计数。对于存活研究，在治疗结束后观察小鼠 (n ＝ 10)7 天或感染后总计 9 天。

[0208] 实施例。慢性小鼠肺感染模型。将铜绿假单胞杆菌如 NH57388A 在 50mL MHB 中于 37℃下震荡 (170rpm) 培养 24 至 28 小时。通过离心 (23,000xg, 30 分钟，4℃）收集细菌细胞并且重悬在 3 至 6mL MHB 中。将细菌悬液在海藻盐悬液中稀释 (1:10) 以产生约 10^{8}CFU/mL。通过在感染前 4 天覆膜内施用单次 150-mg/kg 剂量的环磷酰胺建立短暂的嗜中性白细胞减少来实现最初的感染建立。在第 4 天，在小鼠用异氟烷麻醉的情况下，使用与 1mL 注射器连接的弯曲的焊接有珠的口饲器感染小鼠。在感染后 24 小时开始抗生素治疗，每日两次施用，连续三天。使用多个浓度的抗生素，并且通过口服、覆膜内或利用微型喷雾装置通过气雾剂途径施用。在最后一次治疗后 12 至 16 小时，处死小鼠，并且如本文中确定肺中的菌

落计数。

[0209] 统计学分析。分别通过对数-秩检验法和 Mann-Whitney U 检验法 (GraphPad Prism,4.03 版）进行存活和肺细菌计数分析。认为 <0.05 的 P 值是统计学显著的。

[0210] 实施例。本文所述化合物是铜绿假胞杆菌 (PA) 中表达的流出泵的差的底物。在阳离子调节的 Muller-Hinton 肉汤 (CA-MHB) 或 RPMI 培养基（常用于真核细胞培养）中，通过微量稀释测量 36 个具有已知流出表型的 PA 临床和实验室菌株的 MIC。分别使用 Phe-Arg-β-萘基酰胺 (PaβN,50mg/L) 和 EGTA 5mM 抑制流出泵和改变 OM 完整性。使用铜绿假单胞杆菌菌株 ATCC PAO1 作为参照。PA12 是过表达 4 种主要流出系统 (MexAB、MexCD、MexEF、MexXY) 的临床菌株，PA403 是缺失编码 4 种流出系统的基因的实验室菌株。还使用已知表达流出泵的基因的一系列参照菌株或临床分离株用于 MIC 测定。在 MH 肉汤中或在补充有 10％胎牛血清的 RPMI 培养基（用于真核细菌培养）中或在补充有增加量的血清的 MH 肉汤中，通过微量稀释测量 MIC。使用 EGTA(5mM) 作为螯合剂（破坏外膜完整性），并且使用 PaβN(50mg/L) 作为非特异性流出抑制剂。结果示出在表 1 中。

[0211] 表 1

[0212]

菌株	流出表达	ERY		CLR		AZM		TEL		CEM-101	
		MHB	RPMI	MHB	RPMI	MHB	RPMI	MHB	RPMI	MHB	RPMI
12	AB+CD+EF+XY+	512	32	512	16	256	2	128	4	128	4
434	AB+CD+XY+	512	128	512	128	512	4	128	4	128	8
83	AB+ EF+XY+	512	64	512	32	256	2	128	4	64	4
267	AB+ EF+XY+	512	128	512	64	512	4	128	4	32	4
48	CD+EF+XY+	512	64	512	64	256	2	128	8	128	4
49	CD+EF+XY+	512	64	512	32	256	2	128	4	128	4
11	AB+CD+	256	16	512	16	128	2	64	1	16	2
286B	AB+CD+	512	64	512	64	256	2	256	4	256	4
333A	AB+ EF+	512	64	512	64	256	2	64	2	128	2
336	AB+ EF+	512	64	512	64	512	4	128	4	128	2
16	AB+ XY+	512	32	512	64	256	4	128	4	32	4
88	AB+ XY+	512	64	512	64	256	2	128	4	64	4
168B	CD+ XY+	512	256	512	256	512	4	256	4	128	4
133	EF+XY+	512	64	512	64	256	4	128	4	64	4
156	EF+XY+	512	16	512	32	512	4	128	2	64	2
1	AB+	512	8	512	32	128	4	128	4	128	2
21	AB+	512	64	512	64	256	2	64	2	128	4
2	CD+	512	256	512	128	512	4	256	16	256	8
41	CD+	512	64	512	64	256	2	256	4	256	4
3	EF+	256	8	256	16	64	2	64	0.25	8	1
40	EF+	256	32	256	16	256	2	64	2	32	4
4	XY+	512	32	512	32	256	2	128	4	128	4
22	XY+	512	32	512	32	256	2	128	2	64	4
PAO1	参照	512	32	512	32	256	4	256	2	128	4
397	AB-	16	2	16	2	8	1	8	0.03	2	1
392	CD-	256	16	256	16	128	2	32	0.5	16	1
398	CD-	16	4	32	4	16	1	8	0.25	4	2
391	EF-	256	32	256	16	128	2	64	2	32	3
394	XY-	512	32	512	32	256	4	64	1	32	4
400	XY-	16	4	16	4	8	2	8	0.25	4	1
395	MI-	256	32	256	32	128	2	32	2	64	2
396	ompH-	128	16	64	16	64	2	16	0.25	8	2
401	ompH-	8	4	8	2	8	2	4	1	2	2
399	AB-EF-	16	4	16	4	16	2	4	0.25	8	1
403	AB-CD-EF-XY-	16	4	16	4	8	2	4	0.5	4	1
405	AB-CD-EF-XY-	8	4	8	4	8	2	8	0.25	4	1

[0213] 表 2 示出了利用 PA01（野生型）、PA12（过表达 4 种流出泵）和 PA403（编码 5 种流出泵的基因被破坏）获得的结果。

[0214] 表 2

[0215]

	PAO1 (a)						PA12 (b)					
	CA-MHB			RPMI			CA-MHB			RPMI		
	CT	PAβN	EGTA	CT	PaβN	EGTA	CT	PaβN	EGTA	CT	PaβN	EGTA
ERY	512	16	256	32	32	32	512	32	512	32	32	2
CLR	512	8	256	32	4	32	512	16	256	16	16	2
AZI	128	4	8	2	4	0.25	256	2	256	2	2	0.5
TEL	128	4	32	2	4	1	128	4	32	4	4	1
CEM-101	32	8	8	2	2	1	32	4	16	4	4	1

[0216]

	P403 ©					
	CA-MHB			RPMI		
	CT	PaβN	EGTA	CT	PaβN	EGTA
ERY	16	ND	16	4	4	0.5
CLR	16	ND	32	4	4	0.5
AZI	8	ND	2	2	2	0.125
TEL	4	ND	2	0.5	1	0.06
CEM-101	4	ND	1	1	2	0.25

[0217] 在PaβN 50mg/L或EGTA 5mM的存在下，控制条件(CT)下的MIC(mg/L)。(a)野生型菌株；(b)过表达MexAB-OprM、MexCD-OprJ、MexEF-OprN,、MexXY-OprM的临床分离株；(c)Δ(MexAB-OprM)、Δ(MexCD-OprJ)、Δ(MexEF-OprN)、Δ(MexJK)、Δ(MexXY)。对于CA-MHB中的PAO1和PA12，所有分子的MIC较高，但是如果在RPMI中或在PaβN的存在下测试，MIC降低（达到接近于PA403的那些的值）。EGTA降低CA-MHB中酮内酯的MIC并且在RPMI中具有累加效果。CEM-101在流出泵抑制剂的存在性表现出较小的差异。

[0218] 实施例。在啮齿类动物和非人灵长类动物中的毒理学研究中，其中CEM-101重复给药的多个体内方案表现出为峰值血浆水平的约17至约100倍的CEM-101组织水平。CEM-101在组织中积累，并且肝、脾、肺和唾液腺中的浓度最高。这种关系在使用放射性标记的CEM-101的啮齿类动物ADME研究中得到确认。当以100mg/kg口服施用时，在雄性和雌性动物中观察到约13:1的肺组织和血浆的放射性比例。在以20mg/kg静脉内给药后，观察到数据变化更大，肺/血浆比例在雄性中为17.6，雌性中为6.2。在剂量范围内，Cmax和AUC范围为0.022μg/mL和0.04μg·h/mL至19.6μg/mL和28.60μg·h/mL。在50至1600mg剂量范围，平均CEM-101tmax从1.5小时增加到6.0小时，平均最终半衰期从2.2小时增加9.9小时。

[0219] 实施例。组织分布。CEM-101良好吸收并且在组织中分布。在大鼠中，在250mg/kg/天之下，CEM-101的平均肺和肝浓度为5，为血浆的约17和15倍。在猴中，在200mg/kg/天的剂量下，肺和肝浓度为血浆浓度的503和711倍。心脏中CEM-101的浓度显著低于在肺和肝中发现的水平，分别为大鼠和猴中血浆浓度的5倍和54倍的水平。

[0220] 实施例。单独时以及与妥布霉素和阿米卡星组合时CEM-101抗铜绿假单胞杆菌、MRSA和洋葱伯克霍尔德氏菌的活性。囊性纤维化是美国常见的先天性遗传异常。由于这种疾病，患者（可能在成年期早期至晚期经历）经受由铜绿假单胞杆菌（通常粘液样）、洋葱伯克霍尔德氏菌和MRSA以及其他病原体造成的肺炎的反复发作。这些感染攻击反复发作的性质导致作为唯一治疗选择的联合疗法的多重抗药性和有时候的全抗性。缺少具抗一般的革兰氏阴性菌和革兰氏阳性菌以及特别的CF菌株之活性的新的实验药剂。

[0221] 在分离在Hershey Medical Center的从CF菌株分离的铜绿假单胞杆菌、MRSA和洋葱伯克霍尔德氏菌菌株上测试单独的以及与阿米卡星和妥布霉素组合的CEM-101。

[0222] 菌株。测试从CF诊所的患者分离的粘液样铜绿假单胞杆菌（两个均为绿脓菌素

阳性）和 40MRSA（仅一个菌株为金色菌落）的各自两个菌株。另外，从 Hershey Medical Center 获得 2 个洋葱伯克霍尔德氏菌菌株。所有菌株通过标准方法鉴定。仅测试每位患者的一个菌株。在所有菌株上进行 MLVA 以检查科隆性，并且确认了在一个或少数菌落不进行检查。菌株储保存在 -70℃的脱脂牛奶中直到使用。

[0223] 敏感性测试。通过 CLSI 微量稀释方法测试每一个菌株对于 CEM-101 和其他比较菌株的原始 MIC。托盘获自 Trek, Inc., Cleveland, OH。对于所有协同性测试，通过 CLSI 进行时间杀灭大量肉汤 MIC 稀释 (Time-kill macrobroth MIC dilution)。

[0224] 协同性测试。选择两个 MRSA 菌株并且与上述 4 种革兰氏阴性菌一起测试协同性。肉汤大量稀释形成了下述时间杀灭试验中使用的 MIC 的基础。通过在 MIC 下、高于 MIC 的三个稀释度下和低于 MIC 的三个稀释度 (1/2、1/4 和 1/8x MIC) 下的药物浓度下孵育 5×10^5 至 5×10^6cfu/mL 的初始接种物来单独测试每一种药物的杀灭动力学。在震荡水浴中于 37℃孵育 0、3、6、12 和 24 小时后，通过在 5％胰蛋白酶解酪蛋白大豆绵羊血琼脂平板上布板来进行存活率计数。

[0225] 在进行单独化合物的初始时间杀灭之后，将 CEM-101 与阿米卡星和妥布霉素组合。测试每种药物的 MIC 下的 1 至 2 个稀释度 (1/2X MIC 和 1/4X MIC) 的组合。孵育和时间杀灭方法如上文测试单独化合物时。选择协同时间杀灭测试中的浓度，以使得两种药物中的一种产生与无药物对照类似的生长曲线，而另一种药物活性更高。

[0226] 通过标准方法测试 MIC。协同作用定义为 3、6、12 和 24 小时后组合与最高活性组分之间 cfu/mL 降低≥ $2\log_{10}$，并且在大于≥ $2\log_{10}$cfu/mL 的组合的存在下存活生物体的数目低于初始接种物。组合中至少一种化合物以在单独使用时不显著影响生物体的生长曲线的浓度存在。拮抗作用定义为 3、6、12 和 24 小时后组合与最高活性组分之间 cfu/mL 的增加≥ $2\log_{10}$，并且在大于≥ $2\log_{10}$cfu/mL 的组合的存在下存活生物体的数目大于初始接种物。

[0227] 结果。测试的每个单个菌株被证明是单个菌落。表 3 中列出了编辑的金黄色葡萄球菌 (MRSA)MIC（μg/mL）。

[0228] 表 3. 所有化合物对抗来自 CF 的 40 个 MRSA 菌株的微量稀释 MIC（μg/mL）。

[0229]

药物	范围	MIC_{50}	MIC_{90}
CEM-101	0.06- ≥ 16	0.25	≥ 16
万古霉素	0.5-1	0.5	1
替考拉宁	0.25-1	0.5	1
达托霉素	0.5-1	0.5	1
替加环素	0.12-0.25	0.12	0.25
阿奇霉素	1- ≥ 32	≥ 32	≥ 32

克拉霉素	0.25- ≥ 32	≥ 32	≥ 32
利奈唑胺	1-4	2	2
奎奴普丁 / 达福普丁	0.25-1	0.5	1

[0230] CEM-101 具有对抗 40 个菌株中的 21 个 (52.5%) 的活性 (MIC0.06-0.25),而对抗其余生物体的 MIC > 16 μg/mL。万古霉素和替考拉宁也以 MIC 0.25-1 具有活性,利奈唑胺 MIC 1-4,奎奴普丁 / 达福普丁 MIC 0.25-1。大部分菌株 (40 中 38 个) 耐受 (> 32) 阿奇霉素和万古霉素。4 种革兰氏阴性杆菌的大量肉汤 MIC 示出在表 4 和 5 中,时间杀灭大量肉汤 MIC 示出在表 6 中。

[0231] 表 4. 所有化合物对抗来自囊性纤维化患者的 2 个铜绿假单胞杆菌的大量肉汤稀释 MIC(μg/mL)

[0232]

药物范围
CEM-10164
阿米卡星 2-8
妥布霉素 0.25-1.0

[0233] 表 5. 所有化合物对抗来自囊性纤维化患者的 2 个洋葱伯克霍尔德氏菌的大量肉汤稀释 MIC(μg/mL)

[0234]

药物	范围
CEM-101	8-32
阿米卡星	256
妥布霉素	128

[0235] 表 6. 所有化合物对抗来自囊性纤维化患者的 6 个菌株的时间杀灭大量肉汤 MIC(μg/mL)。

[0236]

菌株	CEM-101	妥布霉素	阿米卡星
SA 2230	0.125	4.0	32.0
SA 2232	0.125	NT[a]	64.0
PSAR 461	64.0	2.0	8.0

PSAR 468	32.0	1.0	4.0
BCEP 953	8.0	128	512
BCEP 954	32.0	128	256

[0237] [a]NT :未测试

[0238] 协同时间杀灭数据示出在表 7 和表 8 中。

[0239] 表 7. 通过时间杀灭研究的与 CEM101 的体外抗菌组合的结果。

[0240]

	CEM-101/妥布霉素[c]				CEM-101/阿米卡星			
	3h[a]	6h[a]	12h[a]	24h[a]	3h	6h	12h	24h
协同作用	0[b]	0	0	1	0	0	0	1
无差异	5	5	4	4	6	6	4	5
拮抗作用	0	0	1	0	0	0	2	0

[0241] [a]时间点（小时）

[0242] [b]菌株数（测试菌株）

[0243] [c]未测试的一个菌株 (MRSA 2232)(MIC ＞ 512 μg/mL)

[0244] 表 8. 通过时间杀灭研究的与 CEM101 的体外抗菌组合的结果。

[0245]

	CEM-101/妥布霉素				CEM-101/阿米卡星			
	3h[a]	6h[a]	12h[a]	24h[a]	3h	6h	12h	24h
SA2230	IND	IND	IND	SYN[b] (0.03/2)	IND	IND	IND	IND
SA2232	NT[c]	NT	NT	NT	IND	IND	IND	SYN (0.06/32)
PSAR461	IND	IND	IND	IND	IND	IND	ANT	IND

[0246]

	CEM-101/妥布霉素				CEM-101/阿米卡星			
PSAR468	IND	IND	IND	IND	IND	IND	IND	IND
BCEP953	IND	IND	ANT[b]	IND	IND	IND	ANT	IND
BCEP954	IND	IND	IND	IND	IND	IND	IND	IND

[0247] [a]时间点（小时）

[0248] [b]IND- 无差异 ;SYN- 协同作用 ;ANT- 拮抗作用

[0249] [c]NT :未测试 (MIC>512 μg/mL)

[0250] 简单地说，在 (0.03/2) 浓度下 24 小时发现了 CEM-101/ 妥布霉素对于一个 MRSA 菌株的协同作用，并且在 0.06/32 μg/mL 下发现了 CEM-101/ 阿米卡星对于第二 MRSA 菌株的协同作用。所有其他时间点和组合对于 2 个 MRSA 菌株无差异。用组合中的妥布霉素测试一个 MRSA 菌株的原因是其非常高的 MIC(>512 μg/mL)。利用 CEM-101/ 阿米卡星组合 (16/4 μg/mL)，两个铜绿假单胞杆菌中的一个在 12 小时表现出拮抗作用。所有其他时间点和其他组合对于 2 个铜绿假单胞杆菌株无差异。利用 CEM-101/ 妥布霉素和 CEM-101/ 阿米卡星组合（分别为 2/64 和 2/256 μg/mL)，在 12 小时一个洋葱伯克霍尔德氏菌是拮抗的。

在所有其他时间点和药物组合下，2 个洋葱伯克霍尔德氏菌株无差异。

[0251] 没有发现着色和任何 MRSA 结果之间的相关性。当将两个粘液样铜绿假单胞杆菌再次培养数天时，粘度消失，但是当其再次暴露于所有组合时粘度再次出现。CEM-101 对约 $^{1}/_{2}$的受试 MRSA 菌株表现出低 MIC。在受试革兰氏阴性杆菌中未发现协同作用。对于 MRSA，利用与妥布霉素组合的 CEM-101 观察到了对菌株 SA 2230 的临床上可实现的协同作用。对于 MRSA SA 2230 的协同作用示出在图 1 中。

[0252] 实施例。健康成年对象中 CEM-101 的肺内渗透。评估 CEM-101 对于患有摄取获得性细菌性肺炎的患者的治疗。在阶段 1 临床研究中评估了 CEM-101 向上皮内衬液 (ELF) 和肺泡巨噬细胞 (AM) 中的渗透。

[0253] 方法 :30 位对象每天口服接收 400mg CEM-101，持续 5 天。在第 5 天，每位对象在 5 个时间点（给药后 3、6、9、12 或 24 小时）中的 1 个经受单次支气管镜检查和支气管肺泡灌洗，以获得 ELF 和 AM 样品 (6 位患者 / 时间点）。在第 1 至 5 天给药前以及连续地第 5 和 6 天给药后收集血浆样品。使用 LC/MS/MS 分析收集的样品中的 CEM-101。使用血浆和 ELF 中的尿素校正 ELF CEM-101 浓度。将使用每个时间点的中值浓度的肺区室药物代谢动力学 (PK) 分析用于计算第 5 天 AUC0-24。另外，使用群体 PK 模型 (PPM) 确定血浆和 ELF 中的每位对象的第 5 天 AUC0-24。通过第 5 天血浆 AUC0-24 分开每种基质的第 5 天 AUC0-24，确定进入 ELF 和 Am 中的 CEM-101 的肺内渗透性。

[0254] 结果 :CEM-101 良好渗透进了 ELF 和 AM。在健康成人中施用药物后 24 小时的时期，与血浆浓度相比，CEM-101 在 ELF(>8 倍）或 AM(>180 倍）中获得的较高的暴露。CEM-101 提供了用于治疗与下呼吸道感染相关的细菌病原体的好的肺内渗透谱。

[0255] 实施例。使用来自小鼠肺感染模型的数据进行 CEM-101 对抗肺炎链球菌的药物代谢动力学 - 药效学 (PK-PD) 分析。使用小鼠肺感染模型，鉴定与 CEM-101 对抗肺炎链球菌和基于这样的指数的 PK-PD 关系的靶标的效力最相关的上皮内衬液 (ELF) 和血浆 PK-PD 测量值。

[0256] 方法 : 从单次施用剂量范围为 0.625 至 40mg/kg 的 CEM-101 的健康小鼠获得 CEM-101PK 数据。经过 24 小时 (3 只小鼠 / 时间点）收集血浆和 ELF 并且分析 CEM-101。使用血浆和 ELF 中的尿素校正 ELF 浓度。向通过吸入感染有 5 个肺炎链球菌分离株中的一个的嗜中性粒细胞减少小鼠通过经口管饲法每日施用 CEM-101 剂量 (0.156 至 160mg/kg)。对 1 个分离株进行剂量分割 (Dose-fractionation) ;向其他 4 个分离株以每 6 小时一次或每 12 小时一次的方案施用 CEM-101。使用 S-ADAPT 1.56 评估 PK 和 PK-PD。

[0257] 结果 : 具有平行的一阶和有限能力的清理以及具有拟合的滞后时间的有限能力的首次通过效应的 3 隔室模型最佳描述了血浆和 ELF 数据 (r2 = 0.98 和 0.83，分别为观察的浓度 vs. 拟合浓度）。ELF 与总药物和游离药物 (f) 血浆（基于小鼠中 91.8％的蛋白质结合）AUC0-24 比率分别为 0.22 和 2.7。ELF 和 f 血浆 AUC0-24:MIC 比率对效力最具预测性（对于 ELF 和 f 血浆 r2 = 0.85)。ELF 和 / 或血浆 AUC0-24:MIC 比率与净细菌淤积和从基线减少 1 和 2log 10 的 CFU 减少分别为 1.26 和 1.65、15.1 和 6.31 以及 59.8 和 12.8。AUC0-24:MIC 比率是对 CEM-101 效力的最具预测性 PK-PD 指数。基于这些关系的 PK-PD 目标将报告用于未来临床的剂量选择。

[0258] 实施例。本文所述化合物表现出强效抗炎活性。

[0259] 细胞。人单核细胞系U937获自美国模式培养物保藏中心(ATCC,Rockville,MD)。来自COPD患者的PBMC获自Brompton医院并且通过AccuSPIN(Sigma-Aldrich)分离。将细胞在37℃的具有5% CO_2的湿润气氛下培养在补充有10%胎牛血清(FBS)和1% L-谷氨酰胺的完全生长培养基(RPMI 1640)(sigma-Aldrich)中。U937细胞在完全培养基中通过暴露于PMA(50ng/mL)48小时分化为粘附的巨噬细胞样形态。通过台盼蓝染色用显微镜评估细胞存活。根据需要通过MTT分析测定细胞毒性。该研究经过Royal Brompton Hospital伦理委员会批准,并且所有对象给出了书面的知情同意书。

[0260] 细胞溶解。如以前的描述制备全细胞提取物(Kobayashi等,2011)。简单地说,使用修改的RIPA缓冲液(50mM Tris HCl pH 7.4,0.5% NP-40,0.25%脱氧胆酸钠,150mM NaCl,具有新加入的完全蛋白酶抑制剂混合物(Roche,Mannheim,Germany))制备细胞蛋白质提取物。使用BCA Protein Assay(Thermo Fisher Scientific,Waltham,MA)测定蛋白质浓度。

[0261] 细胞因子ELISA。通过夹心ELISA根据制造商说明书(R&D Systems Europe,Abingdon,UK)确定细胞培养物上清液中TNFα和IL-8的浓度。

[0262] 酶谱法。通过明胶酶谱法测量MMP9酶活性。将细胞培养物上清液用等量Laemli样品缓冲液(Bio-Rad,Hertfordshire,UK)稀释并且装载到Novex® 10% Zymogram(Gelatin)凝胶(Invitrogen Ltd,Paisley,UK)上。电泳后,将凝胶用Novex®酶谱复性缓冲液(Invitrogen)在室温下孵育和漂洗30分钟。凝胶然后在Novex®酶谱显影缓冲液(Invitrogen)中于室温下漂洗30分钟,之后在显影缓冲液中于37℃过夜孵育。孵育后,用Colloidal Blue Staining Kit(Invitrogen)对凝胶染色以显现酶原条带。

[0263] NF-κB活性。使用TransAM NF-κB p65分析试剂盒(Active Motif,Inc.,Carlsbad,CA)根据制造商的说明测定NF-κB的活性(NF-κB结合序列的p65结合活性)。由PMA分化的U937细胞制备全细胞提取物,在该研究中每种提取物使用20μL。结果由450nm的分光光度吸光度确定,以655nm作为参考波长。

[0264] 统计分析。结果表示为平均值±SEM。使用Student's t检验或Wilcoxon符号秩检验进行两组数据的比较。通过单因子ANOVA进行多重比较,根据需要进行事后检验(Dunnett's)。$p<0.05$时认为差异是显著的。使用Prism 4.0(GraphPad Software Inc.,San Diego,CA)计算大环内酯类对于细胞因子或MMP9之产生的IC_{50}值(50%抑制浓度)。

[0265] CEM-101在U937细胞中的抗炎效果。LPS显著提高PMA分化的U937细胞中TNFα和IL-8的产生(TNFα,LPS中63.1±2.6倍相较于未刺激的;CXCL8,LPS中2.0±0.1倍相较于未刺激的细胞,n = 3)。CEM-101在100μM显著抑制TNFα和CXCL8二者(图3和图4)。虽然克拉霉素在更高浓度(333μM)下表现出对TNFα和IL8二者的中度效果,但是红霉素和阿奇霉素并不抑制它们。泰利霉素在100μM下不抑制TNFα和CXCL8的产生。CEM-101对于TNFα和CXCL8释放的IC_{50}分别为41.6±1.9μM和78.2±9.5μM,优于克拉霉素(IC_{50},对于TNFα为426.3±63.9μM,对于CXCL8为506.5±44.0μM)(表9)。

[0266] 还在U937细胞中研究了大环内酯类对于MMP9活性的效果,其被PMA刺激显著提高(与未刺激相比PMA为9.9±2.0倍,n = 3)。CEM-101显著降低MMP9活性,IC_{50}为14.9±3.1μM(图2和表9)。相比之下,克拉霉素和阿奇霉素表现出为CEM-101的1/10

的抑制效果，而红霉素未表现出效果（图 2 和表 9）。泰利霉素也抑制 MMP9 活性，但是小于 CEM-101 的程度，IC_{50}为 97.9 μM。

[0267] 表 9. 单环内酯类对于 U937 细胞中 LPS 诱导的 IL-8 和 TNFα 释放和 PMA 诱导的 MMP9 活化的抑制效果。

[0268]

	IC_{50} (μM)				
	索利霉素	红霉素	克拉霉素	阿奇霉素	泰利霉素
LPS 诱导的 IL-8 释放	78.2	333μM 无效果	506.5	333μM 无效果	100μM 无效果
LPS 诱导的 TNFα 释放	41.6	333μM 无效果	426.3	333μM 无效果	100μM 无效果
PMA 诱导的 MMP9 激活	14.9	333μM 无效果	118.0	212.1	97.9

[0269] NE：无效果

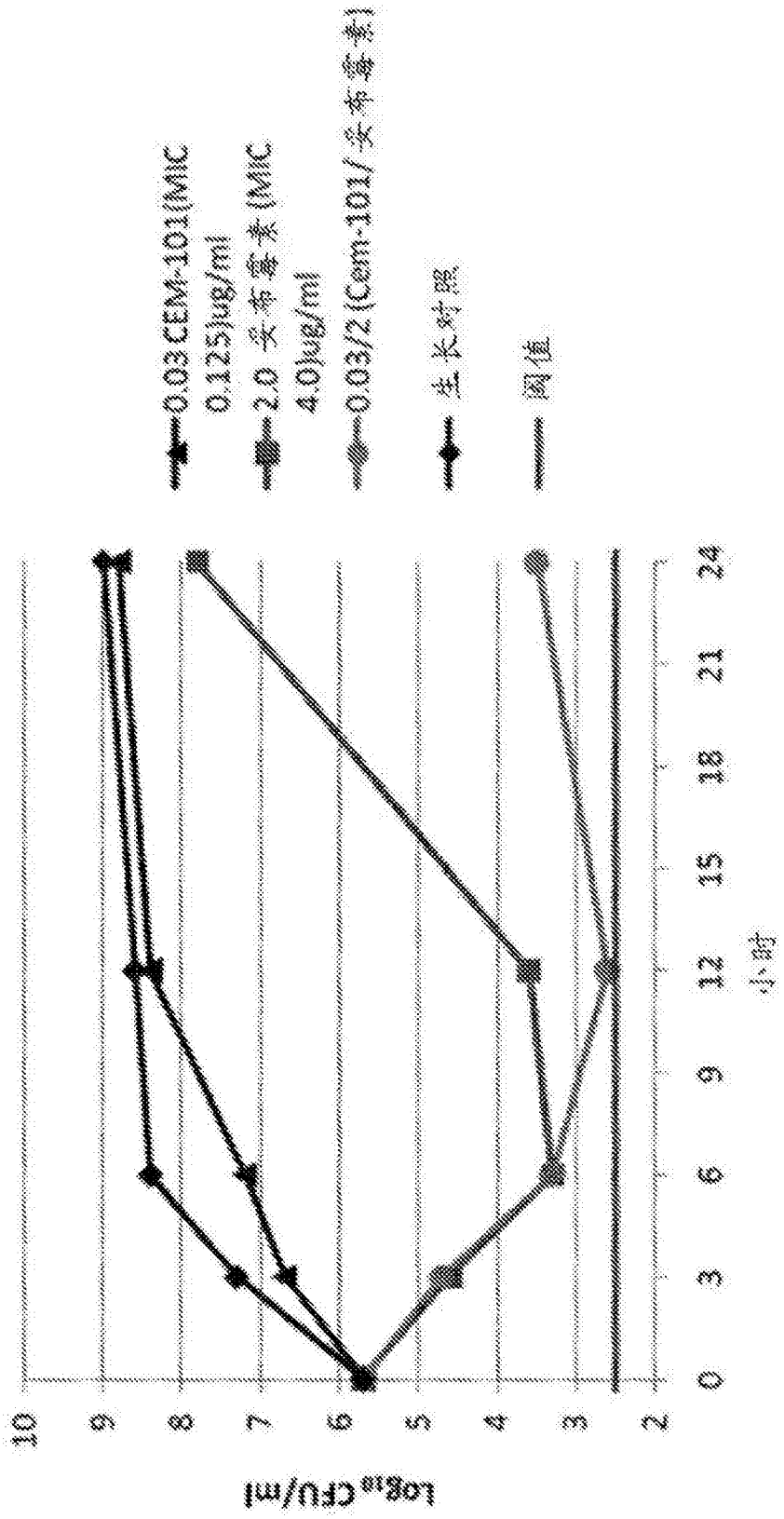

0.03 CEM-101(MIC 0.125ug/ml
2.0 安布雷素 (MIC 4.0ug/ml
0.03/2 (Cem-101/安布雷素)
生长对照
阈值
log10 CFU/ml
10
9
8
7
6
5
4
3
2
0
3
6
9
12
15
18
21
24
小时

图 1

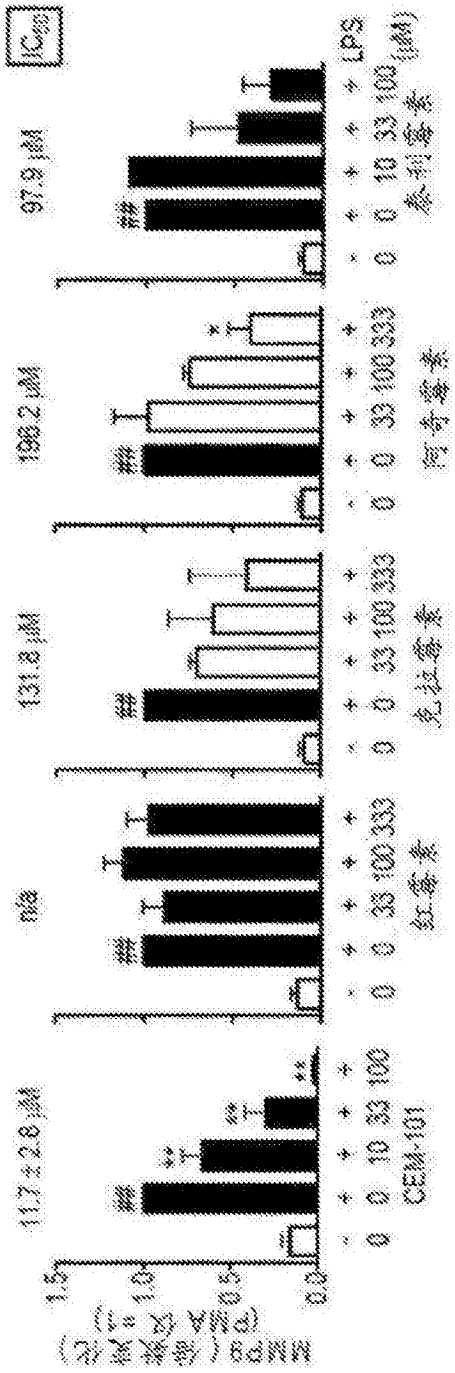

MMP9（相对表达）
(PMA (X = 1))
1.5
1.0
0.5
0.0
11.7 ± 2.8 μM
n/a
131.8 μM
196.2 μM
97.9 μM
CEM-101
红霉素
克拉霉素
阿奇霉素
泰利霉素
LPS
(μM)

图 2

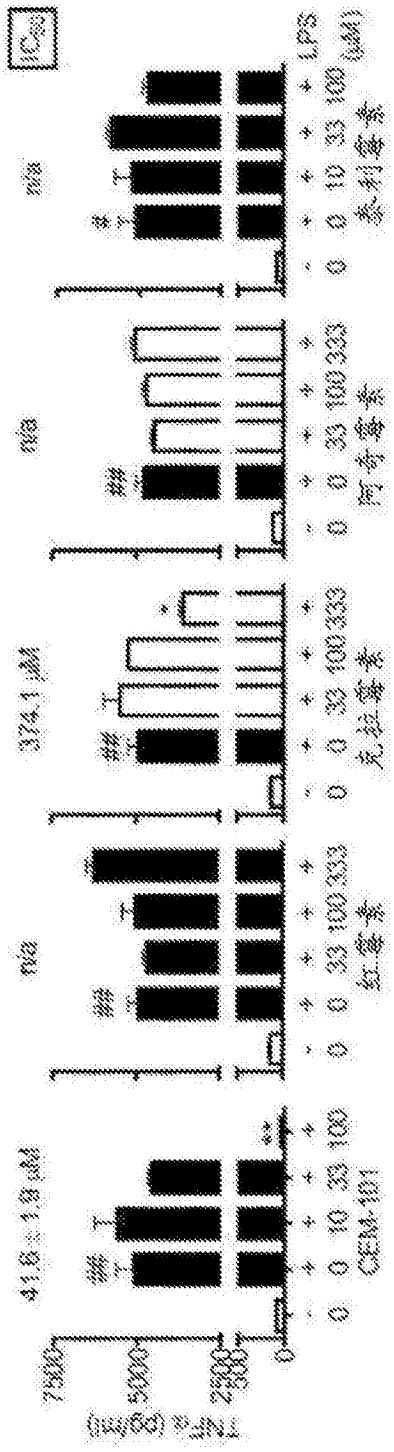

TNFα (pg/ml)
7500
5000
2500
0
41.8 ± 1.9 μM
n/a
374.1 μM
n/a
CEM-101
红霉素
克拉霉素
阿奇霉素
泰利霉素
LPS
(μM)

图 3

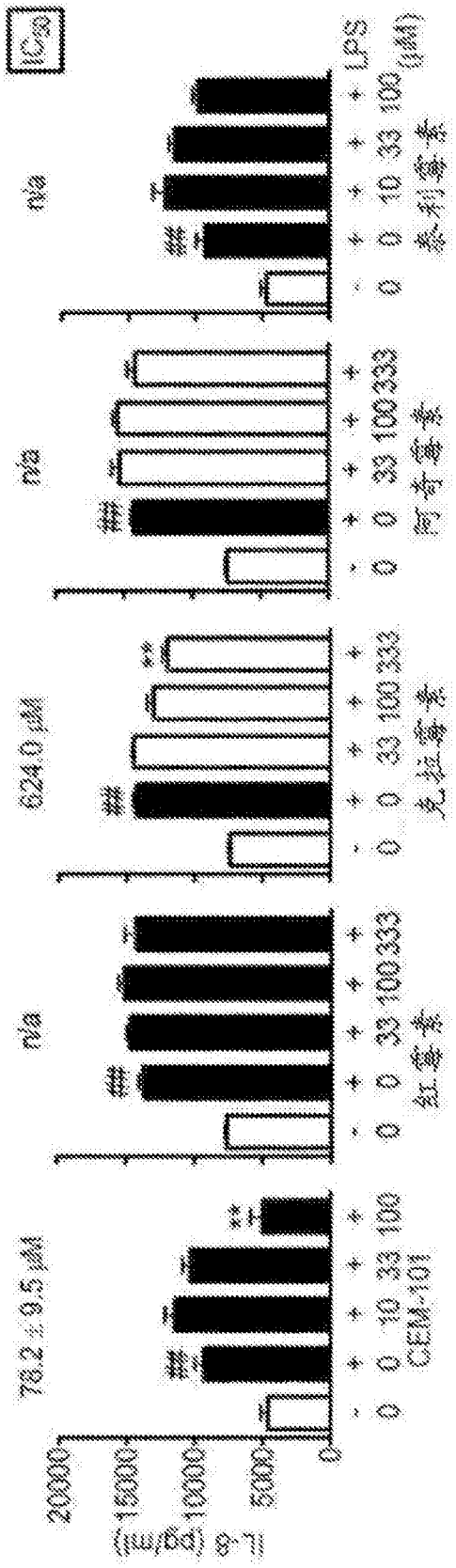

IC50
n/a
n/a
624.0 μM
n/a
78.2 ± 9.5 μM
IL-8 (pg/ml)
20000
15000
10000
5000
0
CEM-101
红霉素
克拉霉素
阿奇霉素
泰利霉素
LPS
(μM)

图 4