Abstract:
The present invention relates to pharmaceutical compositions comprising bicyclomycin, a bacteriostatic antibiotic, combined with other antibiotics and a pharmaceutically acceptable carrier, including methods to treat a subject with a Gram negative bacterial infection.
SYNTHETIC AND ENHANCED BACTERIAL KILLING
INVOLVING BICYCLOMYCIN

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority of U.S. Provisional Application No. 61/805,228 filed on Mar. 26, 2012, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under 1DP2-OD007423-01, 1RO1-AI073491, and 1R21-AI103781 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The Gram stain test, developed in the 1800s by Hans Christian Gram, is a method for classifying different types of bacteria using a chemical stain and viewing through a microscope the results on the bacteria's protective cell wall. Most bacteria are classified into two groups—Gram-positive or Gram-negative—depending on whether they retain a specific stain color. Gram-positive bacteria retain a purple-colored stain, while Gram-negative bacteria appear pinkish or red.

[0004] Gram-negative bacteria can cause many types of infections and are spread to humans in a variety of ways. Several species, including Escherichia coi, are common causes of food-borne disease. Vibrio cholerae—the bacteria responsible for cholera—is a waterborne pathogen. Gram-negative bacteria can also cause respiratory infections, such as certain types of pneumonia, and sexually transmitted diseases, including gonorrhea. Yersinia pestis, the Gram-negative bacterium responsible for plague, is transmitted to people through the bite of an infected insect or handling an infected animal.

[0005] Certain types of Gram-negative bacteria have become increasingly resistant to available antibiotic drugs. Some strains are now resistant to many, most, or all available treatments resulting in increased illness and death from bacterial infections, and contributing to escalating healthcare costs.

[0006] Treating Gram-negative bacterial infections can be difficult because of several unique features of these bacteria. For example, the unique nature of their cell wall makes them resistant
to several classes of antibiotics. Infections have typically been treated with broad-spectrum antibiotics, such as beta-lactams followed by earhapenems. However, even these drugs have become ineffective against some bacteria, leaving healthcare providers no choice but to use older drugs, such as colistin, which can have toxic side effects.

[0007] Bicyclomycin is another antibiotic that has shown potency against Gram-negative pathogens. However, due to weak or lack of bactericidal activity, poor oral absorption, and high minimal inhibitory concentration (MIC) relative to that of agents such as the fluoroquinolones, bicyclomycin has been marginally used. Furthermore, bactericidal antibiotics are not generally co-administered with bacteriostatic antibiotics because in many cases the bacteriostatic antibiotic will interfere with the effectiveness of the primary bactericidal antibiotic, resulting in reduced bacterial killing. For example, treatment with chloramphenicol and rifampicin, usually antagonizes killing by bactericidal agents, such as quinolones, beta-lactams, and aminoglycosides, when combined as a treatment. A need exists for new antibiotics and pharmaceutical compositions that are lethal, and not simply bacteriostatic to control infections caused by multi-drug resistant Gram-negative bacteria.

SUMMARY OF THE INVENTION

[0008] The present invention relates to pharmaceutical compositions comprising bicyclomycin, a bacteriostatic antibiotic, combined with other antibiotics and a pharmaceutically acceptable carrier, including methods to treat a subject with a Gram negative bacterial infection.

[0009] In an embodiment, the invention provides a method of treating a Gram-negative bacterial infection comprising administering to a subject in need thereof an effective amount of bicyclomycin and at least one agent that inhibits bacterial UNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide is administered prior to the administration of bicyclomycin or concurrently administered with bicyclomycin. The concentration of bicyclomycin may be about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell may be about 1 MIC or more. In certain embodiments, the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell may be a bacteriostatic agent. In certain embodiments, bicyclomycin and the bacteriostatic agent
are coadministered to the subject, wherein the combination of the bicyclomycin and the basferiostatik agent are bactericidal.

[0010] In certain embodiments, the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell may be a tetracycline, aminoglycoside, lincosamide, streptogramin, glycyclycline, amphenicol, pleuromutilin, macrolide, oxazolidinone and EF-G inhibitor.

[0011] In certain embodiments, the tetracycline may be demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, chlortetracycline, clomocycline, lyniecycline, mecloycline, metacycline, peniniepicylliiie, and rolitetracycline. In certain embodiments, the aminoglycoside may be streptomycin, dihydrostreptomycin, neomycin, framyctin, paromomycin, ribostamycin, kanamycin, anikacin, arbekacin, bekanamycin, dibekacin, tobramycin, spectinomycin, hygromycin, paromomycin, gentamicin, netilmicin, sisomicin, sepamicin, verdamicin, and atrimicin. In certain embodiments, the lincosamide may be clindamycin, lincomycin, and pirlimycin. In certain embodiments, the streptogramin may be qukupristm/dalfopristin, pristinamycin, and virginianycin. In certain embodiments, the glycyclycline may be tigecycline. H1 certain embodiments, the amphenicol may be chloramphenicol, azidamfenicol, ihianphenicol, and florfencol. In certain embodiments, the pleuromutilin is selected from the group consisting of retapamulin, tiamumi, and valnemulin. In certain embodiments, the macrolide may be azithromycin, claritiromycin, diritnoinyein, eithromycin, fiurithromycin, josamycin, midecamycin, micamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, troleandomycin, tylosin, ketohdes, telithromycin, cethromycin, and solitriomyciiii. In certain embodiments, the oxazolidinone may be eperezolid, linezolid, posizolid, radezolid, ranbezolid, sutezolid, and tedizolid. H1 certain embodiments, the EF-G inhibitor may be fusidic acid.

[0012] In certain embodiments, the Gram-negative bacteria may be Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Shigella dysenteriae, or Salmonella typhimurium.

[0013] In another embodiment, the invention provides a pharmaceutical composition comprising bicyclomycin, at least one agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell, and a pharmaceutically acceptable carrier, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is released earlier than the bicyclomycin or concurrently with
bicyclomycin. In a further embodiment, the concentration of bicyclomycin is about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is about 1 MIC or more. In certain embodiments, the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell may be a bacteriostatic agent. In a further embodiment, the bicyclomycin and bacteriostatic agent are co-administered to the subject, wherein the combination of bicyclomycin and bacteriostatic agent are bactericidal.

[0014] In another embodiment, the invention provides a kit comprising a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of bicyclomycin, and a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of at least one agent that inhibits the synthesis of protein in a bacterial cell wherein the two pharmaceutically acceptable dose units can optionally take the form of a single pharmaceutically acceptable dose unit. In a further embodiment, the concentration of bicyclomycin is about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is about 1 MIC or more. In certain embodiments, the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is a bacteriostatic agent. In certain embodiments, the kit containing bicyclomycin and bacteriostatic agent, the combination of bicyclomycin and the bacteriostatic agent is bactericidal.

BRIEF DESCRIPTION OF THE DRAWINGS
[0015] Fig. 1 depicts the structure of bicyclomycin (BCM).
[0016] Fig. 2 depicts the stimulation of bicyclomycin-mediated lethal activity by inhibitors of bacterial RNA transcription or the translation of bacterial mRNA to a peptide. Fig. 2A shows the effect of bicyclomycin concentration on survival of Escherichia coli. Fig. 2B shows the Rho-specific, bicyclomycin-mediated synthetic lethality with tetracycline.
[0017] Fig. 3 shows bicyclomycin synthetic lethality with various Grain-negative bacterial species. Fig. 3A. Klebsiella pneumoniae. Fig. 3B. Salmonella typhimurium. Fig. 3C. Acinetobacter baumannii, and Fig. 3D. Shigella dysenteriae.
[0018] Fig. 4 depicts the enhancement of bicyclomycin-mediated killing by various inhibitors of protein synthesis.
Fig. 5 shows pretreatment with bicyclomycin (BCM) blocks synthetic Lethality associated with tetracycline (Tet) co-treatment. Fig. 5A shows the effect of bicyclomycin pretreatment on synthetic lethality. Fig. 5B shows stability of putative protective factors produced by bicyclomycin treatment. Fig. 5C shows bacteria are actively growing after bicyclomycin pretreatment.

DETAILED DESCRIPTION OF THE INVENTION

Bicyclomycin is an antibiotic that has shown potency against Gram-negative pathogens. It has weak or lack of bactericidal activity, poor oral absorption, and high minimal inhibitory concentration (MIC) relative to that of agents such as the fluoroquinolones. However, the present invention addresses the need for new lethal antibiotic treatments, bicyclomycin in combination with certain other antibiotics provides a new bactericidal treatment for treating Gram-negative infections. The present invention provides pharmaceutical compositions comprising bicyclomycin combined with at least one other antibiotic that targets bacterial gene expression, and a pharmaceutically acceptable carrier. The present invention further includes methods to treat a subject with a Gram-negative bacterial infection.

DEFINITIONS

An antibiotic that targets gene expression refers to an antibiotic that inhibits RNA transcription or the translation of mRNA to a peptide ("translation"), also refined to "protein synthesis".

As used herein, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

The term "about", as used here, refers to +/- 10% of a value.

As used herein, the term "subject" refers to any animal (e.g., a bird, a fish, a mammal), including, but not limited to humans, non-human primates, rodents, dogs, cats, horses, cows, goats, pigs, livestock, and the like, which is to be the recipient of a particular treatment. Typically, the terms "subject" and "patient" are used interchangeably herein in reference to a human subject.

The term "effective amount," "therapeutically effective amount" or "therapeutic effect" refers to an amount of an antibiotic or combination of antibiotics, or other dmg effective to "treat" a disease or disorder in a subject or mammal. In the case of a bacterial infection, the therapeutically effective amount of the dmg has a therapeutic effect and as such can reduce the
number of bacterial cells; decrease the multiplication of bacterial cells, relieve to some extent one or more of the symptoms associated with the bacterial infection; reduce morbidity and mortality; improve quality of life; or a combination of such effects.

0026 Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to both 1) therapeutic measures that cwe, slow down, lessen symptoms of, and/or halt progression of a -diagnosed pathologic condition or disorder and 2) prophylactic or preventative measures that prevent and/or slow the development of a targeted pathologic condition or disorder. Thus those in need of treatment include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented. In certain embodiments, a subject is successfully "treated" according to the methods of the present invention if the subject shows one or more of the following: a reduction in the number of or complete absence of bacterial cells; inhibition of growth or multiplication of bacteria; or some combination of effects.

0027 "Bactericidal" is used herein to refer to an agent that causes the death of bacteria and is used interchangeably with "lethality", "lethal" or "bacterial killing".

0028 "Bacteriostatic" is used herein to refer to an agent that inhibits growth or multiplication of bacteria.

0029 "Synthetic lethality" is used herein to refer bacterial killing observed when a largely bacteriostatic agent, e.g. bicyclomycin, is combined with another bacteriostatic agent to treat bacteria.

0030 "Enhanced lethality" is used herein to refer improved bacterial killing observed when a largely bacteriostatic agent, e.g. bicyclomycin, is combined with a bactericidal agent but at non-weak cidal concentrations to treat bacteria, such concentrations can be determined by one with ordinary skill in the art, which may be dependent upon the bacterial species being treated.

0031 "Antibiotic" is used herein to refer to an agent that kills or inhibits the growth of bacteria.

0032 The term "bacterial gene expression inhibitor" and "bacterial gene expression antibiotic" are used interchangeably.

0033 "Treatment effective amount" is used herein to mean that amount which results in a sufficient concentration of bicyclomycin and an inhibitor of bacterial gene expression at an infected site to therapeutically ameliorate or reduce the effects of the infection. The infection being treated can be the first occurrence or a subsequent reoccurrence of the infection in the subject.
"MIC" is used herein to mean minimum inhibitory concentration, as known in the art for the antibiotic referred to.

**Pharmaceutical Compositions and Methods of Use**

[0035] The invention provides pharmaceutical compositions comprising bicyclomycin, at least one oilier antibiotic, and a pharmaceutically acceptable carrier. In a further embodiment, bicyclomycin includes derivatives and pharmaceutically acceptable salts thereof. The present invention is also directed to the use of the pharmaceutical compositions to treat a Gram negative bacterial infection in a subject.

[0036] Bicyclomycin targets and binds the transcription factor Rho. In a preferred embodiment, the pharmaceutical composition comprises bicyclomycin and at least one antibiotic that inhibits bacterial gene expression ("bacterial gene expression antibiotic"), either transcription, protein synthesis or both. In a further embodiment, the pharmaceutical composition is formulated to release the antibiotic that inhibits bacterial gene expression earlier than bicyclomycin. In a further embodiment, the MIC concentration of the bacterial gene expression antibiotic is released prior to the release of bicyclomycin, wherein the release profile reflects that the MIC concentration of the bacterial gene expression antibiotic is reached in the subject prior to the release of the bicyclomycin. In another embodiment, the pharmaceutical composition is formulated to release the antibiotic that inhibits gene expression concurrently with bicyclomycin, wherein the release profile reflects that the MIC concentration of the bacterial gene expression antibiotic is reached in the subject prior to the release of the bicyclomycin. In certain embodiments, the pharmaceutical composition is formulated, whereby the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr. In a further embodiment, the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr, while the bacterial gene expression antibiotic is below its MIC. One with ordinary skill in the art can determine the proper concentration of bicyclomycin and the proper concentration of the bacterial gene expression antibiotic for a pharmaceutical composition formulation, wherein the protective effect of bicyclomycin in a subject is avoided. In certain embodiments, the concentration of bicyclomycin and the concentration of the antibiotic that inhibits gene expression is higher than the standard MIC (of either agent) known in the art to treat a Gram negative infection in a subject independently and the pharmaceutical composition is bactericidal. For illustrative purposes, when bicyclomycin or
tetracycline is used alone, even at concentrations of multiple times of MIC, the treatment is bacteriostatic. However, when combining bicyclomycin at the concentration slightly above its MIC and tetracycline at the concentration slightly above its MIC, the pharmaceutical composition is synthetically lethal, as opposed to being bacteriostatic.

[0037] In one embodiment, antibiotics that inhibit transcription include rifaniyehi, rifabutin, lifapentme, rifaximin, derivatives thereof, and pharmaceutically acceptable salts thereof that are known in the art.

[0038] Protein synthesis inhibiting antibiotics generally refer to antibiotics that target ribosomal translation of peptides. Protein synthesis inhibiting antibiotics generally interfere with the processes at the 30S subunit or 50S subunit of the 70S bacterial ribosome, in particular, (1) the formation of the 30S initiation complex (made up of mRNA, the 30S ribosomal subunit, and foniyl-inetliioiyl-transfer RNA), (2) the formation of the 70S ribosome by the 30S initiation complex and the 50S ribosome, and (3) the elongation process of assembling amino acids into a polypeptide/ premature termination of a peptide. Protein synthesis inhibiting antibiotics include without limitation, tetracycline, aminoglycoside, lincosamide, streptogranin, glycylcyciine, amphemcol, pleuromiitilin, macrohde, oxazolidmoiie, EF-G inhibitors, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0039] Tetracyclines inhibit bacterial cell growth by inhibiting translation by binding to the 16S part of the 30S ribosomal subunit and preventing the amino-acyl tRNA from binding to the A site of the ribosome. Tetracyclines include without limitation demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, doxycycline, chlortetracycline, clomocycline, lymecycline, mecloycline, metacycline, penimepicycline, rolitetracycline, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0040] In certain embodiments, the tetracycline is demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, diloitetracycline, clomocycline, lymecycline, mecloycline, metacycline, penimepicycline, rolitetracycline, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0041] Aminoglycosides are compounds that are characterized by the presence of an aminocyclitol ring linked to aminosugars in their structure. Aminoglycosides primarily act by binding to the aminoacyl site of 16S ribosomal RNA within the 30S ribosomal subunit, leading to misreading of the genetic code and inhibition of translocation. Aminoglycosides include
without limitation, streptomycin, dmydrostreptomycin, neomycin, iramyeetin, paromomycin, ribostamycciiL kaiiamycin,. amikacin, arbekacin, bekanamycin, dibekacin, tobramycin, spechnomycm, hygromycin b, paromomycin, gentamicin, netilmicin, sisomiciein, sepaniiein, verdamicin, astromicin, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0042] In certain embodiments, the aminoglycoside is streptomycin, dihydrostreptomycin, neomycin, fiamycetin, paromomycin, ribostamycm, kanamycin, amikacin, arbekacin, bekanamycin, dibekacin, tobramycin, spectinomycin, hygromycin b, paromomycin, gentamicin, netilmicin, sisomicin, sepanamicin, verdamicin, astromicin, derivatives, and pharmaceutically acceptable salts thereof.

[0043] Lincosamides interfere with the synthesis of proteins by binding to the 23s portion of the SOS subunit of bacterial ribosomes and causing premature dissociation of the peptidyl-tRNA from the ribosome. Liiicosaimdes include without limitation, clindamycin, lincomycin, pirlimycin, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0044] In certain embodiments, the lincosamide is clindamycin, lincomycin, pMiinyein, derivatives, derivatives thereof and pharmaceutically acceptable salts thereof.

[0045] Streptograminis include streptogamin A and B, and may be a mixture. Streptogramin A binds to the peptidyl transferase domain of the 50s ribosomal subunit, preventing elongation. Streptogramin B prevents protein chain extension and can initiate the release of incomplete peptides. Streptogamins include without limitation, quinupristin/L"dalfopristin, pristinamycin, virginiamycin, derivatives thereof, and pharmaceutically acceptable salts thereof.

[0046] In certain embodiments, the streptogamin is streptogamin A, streptogamin B, qimiupristin/dalfopristm, pristinamycm, virginiamycin, derivatives thereof and pharmaceutically acceptable salts thereof.

[0047] Glycylcycleniies are antibiotics derived from tetracycline. These tetracycline analogues are specifically designed to overcome two common mechanisms of tetracycline resistance, resistance mediated by acquired efflux pumps and/or ribosomal protection. Glycylcyclmes include without limitation, tigecycline, N,N-dimethylglycyl-amhio derivative of nnoclycline (DMG-MINO), and 6-dimethyl-0-deec\tetacycline (DMG-DMDOT), derivatives thereof and pharmaceutically acceptable salts thereof.
In certain embodiments, the glycylcyclae is tigecyelme, N,N-diineiliyIglycycl-aniiiio derivative of minoclyciine (DMG-MINQ), and 6-dimethyi-6-deeocytetracyeiine (DMG-DMDOT), derivatives thereof, and pharmaceutically acceptable salts thereof.

Amphenieois block the enzyme peptidy iraiisèrase on the SOS ribosome subunit of bacteria. Amphenieois include without limitation, chloramphenicol, azidamfenicol, thiamphenicol, derivatives thereof and pharmaceutically acceptable salts thereof.

In certain embodiments, the amphenicol is chloramphenicol, azidamfenicol, thiamphenicol, florfenicol, derivatives thereof, and pharmaceutically acceptable salts thereof.

Pleuromutilins inhibit protein synthesis in bacteria by binding to the peptidy iraiisèrase component of the 50S summit of ribosome. Pleuromutilins include without limitation, retapamuiin, tiamulin, vaheimilin, derivatives thereof and pharmaceutically acceptable salts thereof.

In certain embodiments, the pleuromeutilin is retapamuiini, tiamulin, vaheimilin, derivatives thereof, and pharmaceutically acceptable salts thereof.

Maciolides contain a macrolide ring and bind to the P site on the subunit 50S of the bacterial ribosome. Macrolides include without limitation, azitlrromycin, clarithromycin, diriltriromycin, erythromycin, flurithromycin, josamycin, midecamycin, mioeamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, ^oleandomycin, tylosin, ketohdes, tehthromycin, cethromycin, solitlrromycin, derivatives thereof and pharmaceutically acceptable salts thereof.

In certain embodiments, the macrolide is azithromycin, clarithromycin, diriltriromycin, erythromycin, flurithromycin, josamycin, midecamycin, mioeamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, ^oleandomycin, tylosin, ketohdes, tehthromycin, cethromycin, solitlrromycin, derivatives thereof, and pharmaceutically acceptable salts thereof.

Oxazolidinones inhibit protein synthesis by binding at the P site at the ribosomal 50S subunit. Oxazolidinones include without limitation, eperezolid, linezolid, posizolid, radezolid, ranbezolid, sutezolid, tedizolid, derivatives thereof and pharmaceutically acceptable salts thereof.

In certain embodiments, the oxazolidhione is eperezolid, linezolid, posizolid, radezolid, ranbezolid, sutezolid, tedizolid, derivatives thereof, and pharmaceutically acceptable salts thereof.
Elongation factor G (EF-G) is a translational GTPase catalysing two different steps of protein synthesis. First, EF-G is needed for translocation of tRNAs and mRNA with respect to the ribosomal 30S subunit to make a new mRNA. codon available for decoding. Second, EF-G acts together with ribosome recycling factor (RRF) in splitting of the ribosomal post-termination complex. EF-G inhibitors are known in the art and include fusidic acid, derivatives thereof and pharmaceutically acceptable salts thereof.

In certain embodiments, the EF-G inhibitor is fusidic acid, derivatives thereof and pharmaceutically acceptable salts thereof.

To administer the pharmaceutical composition to a subject, it is preferable to formulate the molecules in a composition comprising one or more pharmaceutically acceptable carriers. The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce allergic, or other adverse reactions when administered using routes well-known in the art. "Pharmaceutically acceptable carriers" include any and all clinically useful solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, modified release components, and the like.

The carrier may be formed of any suitable pharmaceutically acceptable or therapeutically acceptable material, which is well known. The carrier may comprise of a metal, glass, lipid, protein, polymer or any combinations thereof.

The pharmaceutical composition of the present invention can be in the form of discrete pellets or particles contained in a capsule, or particles embedded in a tablet or suspended in a liquid suspension. In a further embodiment, the pharmaceutical composition may be formulated as a delayed release formulation, as is known in the art.

In an embodiment of the present invention, the formulation contains a modified release component. Such component can be in the form of a modified release particle, coating, layer, or matrix. By "modified release", it is meant that the component allows for a release of an active compound (bicyclomycin and/or bacterial gene expression antibiotic) from the component that is (immediate or) not immediate. For example, the release may be controlled or it may be delayed in part and immediate in other parts. By "controlled release" it is meant that the release of the agent is characterized by a specific release profile in which, for a specific period of time, a specific rate of release is achieved. Various different rates of release may be achieved at different periods of time. By "delayed release" it is meant that the agent is released after a period of delay...
in which the agent is not released. The agent may be released immediately following the period of delay, in which case the component is considered to be a "delayed immediate release" either in a particle or a layer or alike. Alternatively, the agent may be released on a controlled release basis following the initial delay period, in which case the particle is considered to be a "delayed controlled release" particle.

[0063] In an embodiment of the present invention, an agent of interest (bicyclomycin and/or bacterial gene expression antibiotic) is released from the formulation in a "pulsatile" manner. A pulsatile release profile is one in which, over the course of time; at least two periods in which there are relatively high blood plasma concentrations of the agent ("peaks") are separated by a blood plasma concentration level of the agent (a "trough"). Pulsatile release profiles in which there are two peaks are called "bimodal" release profiles. A bimodal release profile may be achieved, for example, by the combination of particles which allow for the immediate release of the agent of interest with particles which allow for the delayed release of the agent after a period of time. Additional populations containing particles which allow for the delayed release of the agent after differing periods of time may be used to create a release profile with additional higher blood plasma concentration "peaks".

[0064] In another embodiment, an agent of interest (bicyclomycin and/or bacterial gene expression antibiotic) is released from the formulation in a "continuous" manner. In such a release, the agent of interest is released in continuously, either at a constant or a variable rate. This may be achieved by the use of modified release particles, including two or more different populations of modified release particles with each population releasing the agent of interest at different rates.

[0065] To allow for modified release of the agent of interest (bicyclomycin and/or bacterial gene expression antibiotic), the particle may contain a modified release coating or a modified release matrix. The coating or matrix serves to retard the release of the agent from the particle. The release characteristics of a particle may be adjusted by adjusting the amount of the coating or matrix, for example, by applying a thicker coating to the particle, or by adjusting the ingredients of the coating or matrix.

[0066] Any coating material which modifies the release of the agent of interest (bicyclomycin and/or bacterial gene expression antibiotic) in the desired manner may be used. Examples of coating materials which are suitable for use in the practice of the present invention include:
polymer coating materials, such as cellulose acetate phthalate, cellulose acetate trimethylate, hydroxy propyl methylcellulose phthalate, polyvinyl acetate phthalate, ammonio metiiacrylate copolymers such as those sold under the trademark Eudragit® RS and RL, poly acrylic acid and poly acrylate and metliaciylate copolymers such as those sold under the trademark Eudragit® S and L, polyvinyl acetaldimethylamino acetate, hydroxypropyl methylcellulose acetate succinate, and shellac; hydrogels and gel-forming materials, such as carboxyvinyl polymers, sodium alginate, sodium carrageen, calcium carmellose, sodium carboxymethyl starch, poly vinyl alcohol, hydroxyethyl cellulose, methyl cellulose, gelatin, starch, and cellulose based cross-linked polymers—in which the degree of crosslinking is low so as to facilitate adsorption of water and expansion of the polymer matrix, hydroxypropyl cellulose, IiydiOxypropynmethylecellulose (HPMC), polyvinylpyrrolidone, crosslinked starch, niiciOcrystaliine cellulose, cliitin, aminoacryl-methacrylate copolymer (Eudragit® RS-PM, Rohm & Haas), pitiHulan, collagen, casein, agar, gum arabic, sodium carboxymethyl cellulose, (swellable hydrophilic polymers) poly(hydroxyalkyl methacrylate), polyvinylpyrrolidone, anionic and cationic hydrogels, polyvinyl alcohol having a low acetate residual, a swellable mixture of agar and carboxymethyl cellulose, copolymers of inalec anhydride and styrene, ethylene, propylene or isobutylene, pectin (m. wt. about 30 k-300 k), polysaccharides such as agar, acacia, karaya, tragacanth, algins and guar, poiyacrylamides, AquaKeep® acrylate polymers, diesters of polyglucan, crosslinked polvinyl alcohol and poly N-vinyl-2-pyrrolidone, sodium starch ghicolate: hydrophilic polymers such as polysaccharides, methyl cellulose, sodium or calcium carboxymethyl cellulose, nitre cellulose, carboxymethyl cellulose, cellulose ethers, polyethylene oxides (e.g. Polyox®, Union Carbide), methyl ethyl cellulose, etliyliiiodroxy ethylcelMose, cellulose acetate, cellulose butyrate, cellulose propionate, gelatin, collagen, starch, maltodextrin, pullulan, polyvinyl pyiTolidone, polyvinyl alcohol, polyvinyl acetate, glycerol fatty acid esters, polyacrylamide, polyacrylic acid, copolymers of memacracylic acid or methacrylic acid (e.g. Eudragit®, Rohm and Haas), other acrylic acid derivatives, sorbitan esters, natural gums, lecithins, pectin, alginates, ammonia alginate, sodium, calcium, potassium alginates, propylene glycol alginate, agar, and gums such as arabic, karaya, locust bean, tragacanth, carrageens, guar, xanthan, scleroglucan and mixtures and blends thereof.

[0067] As will be appreciated by the person skilled in the art, excipients such as plasticisers, lubricants, solvents and the like may be added to the coating. Suitable plasticisers include for
example acetylated monoglycerides; butyl plithalyl butyl glycolate; dibutyl tartrate; diethyl phthalate; dimethyl phthalate; ethyl plithalyl ethyl glycolate; glycerin; propylene glycol; triacetin; citrate; hipropion; diacetin; dibutyl phthalate: acetyl monoglyceride; polyethylene glycols; castor oil; triethyl citrate; polyhydric alcohols; glycerol, acetate esters, glycerol triacetate, acetyl triethyl citrate, dibenzyl phthalate, dihexyl phthalate, butyl octyl phthalate, diisonooyl phthalate, butyl octyl phthalate, dioctyl azelate, epoxidised tallate, triisocetyl trimellitate, diethylhexyl phthalate, di-n-octyl phthalate, di-i-octyl phthalate, di-i-decyl phthalate, di-n-undeeyl phthalate, di-n-irideeyl phthalate, tri-2-ethylhexyl trimellitate, di-2-ethylhexyl adipate, di-2-ethylhexyl sebacate, di-2-ethylhexyl azelate, dibutyl sebacate. Suitable solvents include acetone and isopropyl alcohol.

[0068] In an embodiment in which a delayed immediate release is desired, the coating used may be enteric. Enteric coatings comprise pH sensitive polymers. Typically, these polymers are carboxylated and interact sparingly with water at low pH. However, at a high pH, the polymer ionizes which causes swelling or the dissolution of the polymers. Such coatings may, therefore, remain intact in the acidic environment of the stomach and then dissolve in the more alkaline environment of the intestine.

[0069] Any matrix material which modifies the release of the agent of interest (bicyclomyecm and/or bacterial gene expression antibiotic) in the desired manner may be used. Examples of matrix materials which are suitable for use in the practice of the present invention include: hydrophilic polymers, hydrophobic polymers and mixtures thereof which are capable of modifying the release of the agent of interest dispersed therein in vitro or in vivo: Modified-release matrix materials suitable for the practice of the present invention include but are not limited to microcystalline cellulose, sodium carboxymethylcellulose, hydroxyalkylcelluloses such as lihydroxypropylmethylicelulose (HPMC) and hydroxypropylcellulose, polyethylene oxide, alkycelluloses such as methylcellulose and ethylcellulose, polyethylene glycol, polyvinylpyrroolidone, cellulose acetate, cellulose acetate butyrate, cellulose acetate phthalate, cellulose acetate trimellitate, polyvinylacetate phthalate, polyalkylmethacrylates, polyvinyl acetate and mixture thereof.

[0070] In an embodiment of the invention, the formulation releases the bacterial gene expression antibiotic in such a manner, that the duration of action of the bacterial gene expression antibiotic matches that of bicyclomyecm (simultaneous release), or prolonged (bacterial gene expression
aniibioiic released first followed by the release of bicyclomycin). This may be accomplished by, for example; using modified release particles which comprise the active ingredient and/or modified release particles which comprise the second active ingredients. The release is modiied such that the release of one active compound is over a period of time such that the duration of action of that compound matches that of the other active compound. In such an embodiment, the release of the second active compound may also be modiied.

[0071] An immediate release layer or particle may be made, for example, by coating a solution comprising the agent of interest onto an inert bead (for example, a sugar sphere). Following coating, the solvent dries off, leaving the immediate release particle.

[0072] A modified release particle may be made, for example, by coating an immediate release particle such as that described above with a solution comprising the agents of a modified release coating. Following coating, the solvent dries off, leaving the modified release particle.

[0073] The particles described above may be combined to form a larger solid dosage form, for example a tablet, a capsule, a lozenge, etc.

The invention provides a method for the treatment of pain comprising the step of delivering to the patient a formulation comprising a narcotic analgesic and a non-narcotic analgesic.

[0074] The invention also provides methods for treating a Gram-negative bacterial infection in a subject in need of such treatment comprising administeriig effective amounts of bicyclomycin with a bacterial gene expression antibiotic to said subject. In a preferred embodiment, the bacterial gene expression antibiotic is administered earlier than bicyclomycin. In a further embodiment the MIC concentration of the bacterial gene expression antibiotic is reached in the subject prior to the administration of the bicyclomycin. In a further embodiment, the concentration of the bicyclomycin in a subject falls below the bicyclomycin MIC concentration prior to the re-administration of a bacterial gene expression antibiotic. One with ordinary skill in the art may be able to implement a dosing regimen, whereby the concentration of bicyclomycin in a subject is below the bicyclomycin MIC concentration in a subject before the MIC concentration of a bacterial gene expression antibiotic in a subject is reached, to avoid the protective effect of bicyclomycin. In certain embodiments, the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr. In a further embodiment the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr. while the bacterial gene expression antibiotic is below its
MIC. In a preferred embodiment, the concentration of the bicyclomycin in a subject will not stay above the bacterial gene expression antibiotic MIC concentration. In certain embodiments, the concentration of bicyclomycin and the concentration of the antibiotic that inhibits gene expression is higher than the standard MIC (of either agent) known in the art to treat a Gram negative infection in a subject independently and the combination is bactericidal.

[0075] In another embodiment, the bacterial gene expression antibiotic can be administered concurrently with bicyclomycin, wherein the MIC concentration of the bacterial gene expression antibiotic is reached in the subject prior to the MIC concentration of bicyclomycin. One with ordinary skill in the art can determine the proper dose of bicyclomycin and the proper dose of the bacterial gene expression antibiotic for a subject, wherein the protective effect of bicyclomycin in a subject is avoided. In certain embodiments, the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr. In a further embodiment, the concentration of bicyclomycin in a subject will not be above the bicyclomycin MIC for more than 5, 2, 1, or .1 hr. while the bacterial gene expression antibiotic is below its MIC. In a preferred embodiment, the concentration of the bicyclomycin in a subject will not stay above the bacterial gene expression antibiotic MIC concentration. In certain embodiments, the concentration of bicyclomycin and the concentration of the antibiotic that inhibits gene expression is higher than the standard MIC (of either agent) known in the art to treat a Gram negative infection in a subject independently and the combination is bactericidal.

[0076] Gram-negative bacteria are a class of bacteria that do not take up the crystal violet stain used in the Gram staining method of bacterial differentiation, making positive identification possible. Gram-negative bacteria have a cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharide.

[0077] The Gram-negative bacterial infection may be caused by Escherichia coli (E. coli), Salmonella, Shigella, Pseudomonas, Moraxella, Helicobacter, Stenatrophomcmas, Bdellovibrio, Acinetobacter, Vibrio, Yersinia, Legionella, Enterobacter, Brucella, Campylobacter, Neisseria, Neisseria gonorrhoeae, Neisseria meningitidis, Moraxello catarrhalis, Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens, Helicobacter pylori, Salmonella enteritidis, Salmonella typhi, and Acinetobacter baumannii.
In certain embodiments, the Gram-negative infection is caused by *E. coli*, *Klebsiella*, *Klebsiella pneumonia*, *Acinetobacter*, *Acinetobacter baumannii*, *Shigella*, *Shigella flexneri*, *Salmonella*, or *Salmonella typhi*.

Depending on the nature of the Gram-negative bacterial infection, the pharmaceutical compositions of the instant invention may be administered by routes independently selected from the group consisting of oral administration, intravenous administration, intraarterial administration, intramuscular administration, intracranial administration, intrathecal administration, intraventricular administration, intraurethral administration, intravaginal administration, subcutaneous administration, intracocular administration, intranasal administration, locally (e.g., powders, ointments or drops), or as a buccal or nasal spray, and any combinations thereof.

In the present specification, parenteral includes subcutaneous injection, intravenous injection, intramuscular injection, intraperitoneal injection, drip or topical administration (transdermal administration, transocular administration, transpulmonary or bronchial administration, transnasal administration, transrectal administration and the like) and the like.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carders, diluents, solvents, or vehicles including water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The dose of the pharmaceutical composition of the present invention is determined according to the age, body weight, general health condition, sex, diet, administration time, administration method, clearance rate, and the level of disease for which patients are undergoing treatments at that time, or further in consideration of other factors. While the daily dose of the agent of the present invention varies depending on the condition and body weight of patient, the kind of the agent, administration route and the like, it is parenterally administered at, for example, 0.01 to 5000 mg/patient/day by subcutaneous, intravenous, intramuscular, transdermal, transocular, transpulmonary bronchial, or transnasal administration.
Oral dosage forms may include capsules, tablets, emulsions and aqueous suspensions, dispersions, and solutions. In the case of tablets, commonly used carriers include, but are not limited to, lactose and corn starch. Lubricating agents, such as, but not limited to, magnesium stearate, also are typically added. For oral admixture in a capsule form, useful diluents include, but are not limited to, lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase combined with emulsifying or suspending agents. If desired, certain sweetening, flavoring, or coloring agents can be added. In a preferred embodiment, the oral dosage is in the form of a controlled release formulation; such formulations are known in the art.

In particular examples, a dosage range is from about 1.0 to about 5000 mg/kg body weight administered in single or divided doses, including from about 1.0 to about 2000 mg/kg body weight, from about 1.0 to about 500 mg/kg body weight, from about 1.0 to about 25 mg/kg body weight (assuming an average body weight of approximately 70 kg; values adjusted accordingly for persons weighing more or less than average).

For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the bicyclomycin for the symptomatic adjustment of the dosage to the subject being treated.

For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the tetracycline for the symptomatic adjustment of the dosage to the subject being treated.

For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the aminoglycoside for the symptomatic adjustment of the dosage to the subject being treated.
75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the lincosamide for the symptomatic adjustment of the dosage to the subject being treated.

[0089] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the streptogramin for the symptomatic adjustment of the dosage to the subject being treated.

[0090] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin. particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the glycyclcline for the symptomatic adjustment of the dosage to the subject being treated.

[0091] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the amphenicol for the symptomatic adjustment of the dosage to the subject being treated.

[0092] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the pieuromutilin for the symptomatic adjustment of the dosage to the subject being treated.

[0093] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the macrolide for the symptomatic adjustment of the dosage to the subject being heated.

[0094] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg,
about 1000 mg or 2000 mg of the oxazolidone for the symptomatic adjustment of the dosage to the subject being treated.

[0095] For oral administration, the compositions are, for example, provided in the form of a tablet containing from about 50 to about 5000 mg of the bicyclomycin, particularly about 75 mg, about 100 mg, about 200 mg, about 400 mg, about 500 mg, about 600 mg, about 750 mg, about 1000 mg or 2000 mg of the EF-G inhibitor for the symptomatic adjustment of the dosage to the subject being treated.

Kits Containing Bicyclomycin and a Bacterial Gene Expression Antibiotic

[0096] In another embodiment, the invention provides kits comprising a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of bicyclomycin, and a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of a bacterial gene expression antibiotic. In a further embodiment, the pharmaceutically acceptable dose unit is formulated to release the bacterial gene expression antibiotic before bicyclomycin, or formulated to simultaneously release the active the bacterial gene expression antibiotic and bicyclomycin. The two pharmaceutically acceptable dose units can optionally take the form of a single pharmaceutically acceptable dose unit.

[0097] The other gene expression inhibitors include without limitation a tetracycline, aminoglycoside, lineosamide, streptogramin, glycyclcline, amphenicol, pleuromutilin, macrolide, oxazolidinone and EF-G inhibitor. The kits of the invention may further comprise a set of instructions that provide guidance on the use of the dose units for treatment of a grain-negative bacterial infection by simultaneous or sequential adminishation, and that the bacterial gene expression antibiotic be administered earlier than bicyclomycin or simultaneously administered with bicyclomycin.

EXAMPLES

Methods and Materials:

[0098] Bacterial strains, culture conditions, and reagents. Strains were grown at 37 °C in LB liquid medium and on LB agar. Relative turbidity of bacterial cultures, measured with a Klett-Summerson colorimeter, was used to measure growth. Bicyclomycin. Rifampicin, chloramphenicol, nalidixic acid, tetracycline, tobramycin, neomycin, doxycycline, ampicillin, thiourea, and 2,2′-bipyridyl were obtained from Sigma Chemical Co.. Ciprofloxacin was from Bayer Healthcare. Streptolydigin was a generous gift from Arkady Mustaev.
Susceptibility testing. Inhibition of growth (MIC) was measured by broth dilution. About $10^9$ to $10^5$ cells were applied to broth cultures containing dings at various concentrations that differed by 2-fold increments. MIC (Table 1) was taken as the minimal concentration that blocked visible growth of liquid cultures following overnight incubation. Lethal activity was measured with liquid cultures in which aliquots were incubated for various times and drug concentrations, as indicated in figure legends. The number of surviving cells was determined by dilution and plating on drug-free agar followed by incubation at 37 °C for 24 hrs. Percent survival was determined relative to an untreated control sampled at the time of drug addition.

DNA synthesis inhibition assay. Rate of DNA synthesis was measured as known in the art. Aliquots (200µl) of E. coli cultures were incubated in the presence of $^3$H-thymidine (0.1µCi) for 2 min at 37 °C followed by addition of ice-cold 10% trichloroacetic acid to precipitate high molecular weight DNA. Precipitates were collected on filter paper disks (Fislierbrand 4.25 diameter, catalogue number 09-801J), and precipitated radioactivity was determined by scintillation spectrometry.

Viscosity assay. E. coli cell lysates were obtained as known in the art for isolation of bacterial nucleoids. Cells were treated with lysozyme and non-ionic detergents at 20°C for 2-3 min, the cell lysates were divided into aliquots, and several dilutions were distributed to 10 X 75 mm glass tubes. Samples were then incubated at 80°C for 2 min to unfold chromosomal DNA, chilled on ice, and brought to 20°C in a water bath. A 0.025 ml glass micropipet (Kimble Glass, Cat. no. 71900-25) was placed in each tube, and the time required to fill the capillary, less the time for buffer alone, was taken as an empirical measure of lysate viscosity. That value was normalized to DNA concentration for comparison of drug treatments.

Results:

Bicyclomycin interferes with the Rli transcription terminator, and Fig. 2A shows that bicyclomycin is bacteriostatic. Fig. 2A shows the effect of bicyclomycin concentration on survival of Escherichia coli. An exponentially growing culture of E. coli was treated for 2 hrs with the indicated concentrations of bicyclomycin alone (empty circles) or in the presence of bacteriostatic concentrations (2-times MIC) of tetracycline (filled circles), chloramphenicol (empty triangles), or rifampicin (empty squares). Tetracycline, chloramphenicol, or rifampicin alone does not cause bacterial killing (not shown). Inhibition of protein synthesis by a bacteriostatic agent (tetracycline) with bicyclomycin resulted in synthetic lethality (the
combination of the two bacteriostatic agents becomes bactericidal), thus lethal with E. coli (filled circles; Fig. 2A). Fig. 2B shows the Rho-specific, bicyclomycin-mediated synthetic lethality with tetracycline. Exponentially growing cultures of wild-type E. coli (circles) or bicyclomycin-resistant (Rho G337S) mutant (squares) were heated for 2 hrs with the indicated concentrations of bicyclomycin alone (empty symbols) or in the presence of bacteriostatic concentrations (2-times MIC) of tetracycline (filled symbols). The lethal activity achieved by bicyclomycin-tetracycline combination involves bicyclomycin targeting of Rho, since a point mutation in rho that confers resistance to bicyclomycin also eliminated lethal action of the combination (Fig. 2B).

Fig. 3 shows bicyclomycin synthetic lethality with various Gram-negative bacterial species. Exponentially growing cultures of bacteria were treated for 2 hrs with the indicated concentrations of bicyclomycin alone (empty circles) or in the presence of bacteriostatic concentrations (2-times MIC) of tetracycline (filled circles), chloramphenicol (empty triangles), or rifampicin (empty squares). Tetracycline, chloramphenicol, or rifampicin alone does not cause bacterial killing (not shown). Fig. 3A. Klebsiella pneumoniae, Fig. 3B. Salmonella typhimurium. Fig. 3C. Acinetobacter baumannii, and Fig. 3D. Shigella dysenteriae.

Cotreatment with bicyclomycin also enhanced killing for translational inhibitors (neomycin, tobramycin, doxycycline, and tigecycline) (Fig. 4). Exponentially growing cultures of E. coli were treated for the indicated times with bacteriostatic concentrations (e.g. 3 times MIC) of neomycin (MIC = 5 µg/ml. Fig. 4A), tobramycin (MIC = 1.2 µg/µl, Fig. 4C), doxycycline (MIC = 1.2 µg/µl. Fig. 4E) or tigecycline (MIC = 1.25 µg/ml. Fig. 4G). Cells were also treated with the indicated concentrations of each drug for 2 hrs (Fig. 4B: neomycin. Fig. 4D: tobramycin, Fig. 4F: doxycycline, and Fig. 4H: tigecycline). Cells were heated with protein synthesis inhibitors alone (empty circles) or in the presence of 2-times MIC of bicyclomycin (filled circles) added 10 min after addition of protein synthesis inhibitors.

Bicyclomycin administered 1.5 hour before tetracycline, eliminated the synthetic lethal effect (Fig. 5A). Fig. 5 shows pretreatment with bicyclomycin (BCM) blocks syntietic lethality associated with tetracycline (Tet) co-treatment. Fig. 5A shows the effect of bicyclomycin pretreatment on synthetic lethality. Exponentially growing cultures of wild-type E. coli were treated for 2 hrs with bicyclomycin alone at 2-times MIC, with tetracycline alone at 2-times MIC, with both bicyclomycin and tetracycline, each at 2-times MIC, as indicated (first 3 black
bars on the left). In addition, cultures were preheated with bicyclomycin for the indicated times (underlined portion) before tetracycline (black bars) or bicyclomycin plus tetracycline (grey bars) were added for an additional 2-hr incubation. The far-left 3 black bars indicate samples without bicyclomycin pre-treatment. Fig. 5B shows stability of putative protective factors produced by bicyclomycin treatment. Exponentially growing cultures of E. coli were treated with 2-times MIC bicyclomycin for 1 hr, drug was removed, and incubation at 37°C was continued. At the indicated times samples were taken for cfu determination (to monitor growth, inset) and heated with tetracycline and bicyclomycin (both at 2-times MIC) for an additional 2 hrs. A control sample, taken before bicyclomycin pretreatment, was treated with tetracycline and bicyclomycin for 2 hr. Full protection from bicyclomycin-tetracycline-mediated synthetic lethality was seen 2 hours after pre-induction of the protective function by bicyclomycin (Fig. 5B). Fig. 5C shows bacteria are actively growing after bicyclomycin pretreatment.

[0106] The embodiments within the specification provide an illustration of embodiments of the invention and should not be construed to limit the scope of the invention. The skilled artisan readily recognizes that many other embodiments are encompassed by the invention. All publications, patent applications and U.S. Patents cited in this disclosure are incorporated herein by reference in their entireties. The citation of any references herein is not an admission that such references are prior art to the present invention.

[0107] The invention has been described via the specific embodiments and examples provided above which, however, do not limit the invention in any way.
Claims:
1. A method of treating a Gram-negative bacterial infection comprising administering to a subject in need thereof an effective amount of bicyclomycin and at least one agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide is administered prior to the administration of bicyclomycin or concurrently administered with bicyclomycin.
2. The method of claim 1, wherein the concentration of bicyclomycin is about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is about 1 MIC or more.
3. The method according to claim 1 or 2, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is a bacteriostatic agent.
4. The method of claim 3, wherein the bicyclomycin and the bacteriostatic agent are co-administered to the subject, wherein the combination of the bicyclomycin and the bacteriostatic agent are bactericidal.
5. The method according to claims 1-3, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is selected from the group consisting of tetracycline, aminoglycoside, lincosamide, streptogramin, glycyclcycline, amphenicol, pleuromutilin, macrolide, oxazolidinone and EF-G inhibitor.
6. The method of claim 5, wherein the tetracycline is selected from the group consisting of demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, chlorotetracycline, clomocycline, lymecycline, meclocycline, metacycline, penicycline, and rolitetracycline.
7. The method of claim 5, wherein the aminoglycoside is selected from the group consisting of streptomycin, dihydrostreptomycin, neomycin, framycetin, paromomycin, ribostamycin, fcanamycin, amikacin, arbekacin, bekanaamycin, dibekacin, tobramycin, spectinomycin, hygromycin b, paromomycin, gentamicin, netilmicin, sisomicin, sepamicin, verdamicin, and astromicin.
8. The method of claim 5, wherein the lincosamide is selected from the group consisting of clindamycin, lincomycin, and pirlimycin.
9. The method of claim 5, wherein the streptogranjin is selected from the group consisting of quinupristin/dalfopristin, pristinamycin, and miamphenicol.

10. The method of claim 5, wherein the glycyrrhizic acid is tigecycline.

11. The method of claim 5, wherein the amphenicol is selected from the group consisting of chloramphenicol, azidamfenicol, miamphenicol, and florfenicol.

12. The method of claim 5, wherein the pleuromutilin is selected from the group consisting of retapamulin, tiamulin, and valnemulin.

13. The method of claim 5, wherein the macrolide is selected from the group consisting of azithromycin, clarithromycin, dirithromycin, erythromycin, liuramycin, josamycin, romidecamycin, miocamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, troleandomycin, tyllosin, fefolides, telimation, cetromycin, and solithromycin.

14. The method of claim 5, wherein the oxazolidinone is selected from the group consisting of eperezolid, liiiezolid, posizolid, radezolid, ranbezolid, sutezolid, and tedizolid.

15. The method of claim 5, wherein the EF-G inhibitor is fusidic acid.

16. The method of claim 1, wherein the Gram-negative bacteria is Escherichia coli, Klebsiella pneumonia, Acinetobacter baumannii, Shigella dysenteriae, or Salmonella typhimurium.

17. A pharmaceutical composition comprising bicyclomycin, at least one agent that bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell, and a pharmaceutically acceptable carrier, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is released earlier than the bicyclomycin or concurrently with bicyclomycin.

18. The pharmaceutical composition of claim 17, wherein the concentration of bicyclomycin is about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is about 1 MIC or more.

19. The pharmaceutical composition according to claim 17 or 18, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is a bacteriostatic agent.
20. The pharmaceutical composition according to claims 17-19, wherein the bicyclo- 
mycin and bacteriostatic agent are co-administered to the subject, wherein the combination of 
bicyclo- 
mycin and bacteriostatic agent are bactericidal.

21. The pharmaceutical composition according to claims 17-20, wherein the agent that 
inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a 
bacterial cell is selected from the group consisting of a tetracycline, aminoglycoside, 
linco- 
samide, streptogranin, grycylcycline, amphenicol, pleuromutilin, macrolide, oxazolidinone 
and EF-G inhibitor.

22. The pharmaceutical composition of claim 21, wherein the tetracycline is selected from 
the group consisting of demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, 
chlorotetacycline, clomocycline. lymecycline, meclo- 
cycline, metacycline, penimepicycline, and rolitetracycline.

23. The pharmaceutical composition of claim 21, wherein the aminoglycoside is selected 
from the group consisting of streptomycin, dihydrostreptomycin, neomycin, framycetin, 
paromomycin, ribostamycin, kanamycin, amikacin, arbekacin, bekanamycin, dibekacin, 
tobramycin, speetinomycm, hygromycin b, paromomycin, gentamicin, netilmicin, sisomicin, 
sepamicin, verda- 
micin, and astromicin.

24. The pharmaceutical composition of claim 21, wherein the linco- 
samide is selected from the group consisting of clindamycin, lincomycin, and pirlimycin.

25. The pharmaceutical composition of claim 21, wherein the streptogranin is selected from 
the group consisting of quinupristin/dalfopristin, pristamycin, and virginia- 
ycin.

26. The pharmaceutical composition of claim 21, wherein the glycylcycline is tigecycline.

27. The pharmaceutical composition of claim 21, wherein the amphenicol is selected from 
the group consisting of chloramphenicol, azidamfenieol. thiamphenicol, and flora- 
fenicol.

28. The pharmaceutical composition of claim 21, wherein the pleuromutilin is selected from 
the group consisting of retapamulin, fiamulin, and valnemulin.

29. The pharmaceutical composition of claim 21, wherein the macrolide is selected from the 
group consisting of azithromycin, clarithromycin, dirithromycin, erythromycin, flurithromycin, 
josamycin, midecamycin, mo- 
camycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, 
troleandomycin, tylosin, ketolides, telithromycin, cethromycin, and solithromycin.
30. The pharmaceutical composition of claim 21, wherein the oxazolidinone is selected from the group consisting of eperezolid, liriezolid, posizolid, radezoiiid, ranSbezoiiid, sutezolid, and tedizolid.

31. The pharmaceutical composition of claim 21, wherein the EF-G inhibitor is fiisidic acid.

32. A kit comprising a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of bicycimycin, and a pharmaceutically acceptable dose unit of a pharmaceutically effective amount of at least one agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell, wherein the two pharmaceutically acceptable dose units can optionally take the form of a single phamaceutically acceptable dose unit.

33. The kit of claim 32, wherein the concentration of bicycimycin is about 1 MIC or more, and the concentration of the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is about 1 MIC or more.

34. The kit of claim 32 or 33 wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is a bacteriostatic agent.

35. The kit according to claims 32-34, wherein the bicycimycin and bacteriostatic agent are co-administered to the subject, wherein the combination of the bicycimycin and the bacteriostatic agent is bactericidal.

36. The kit according to claims 32-35, wherein the agent that inhibits bacterial RNA transcription or the translation of bacterial mRNA to a peptide in a bacterial cell is selected from the group consisting of a tetracycline, aminoglycoside, lincosamide, streptogramin, glycyclcline, amphenicol, pleuromutilin, rnaclrolide, oxazolidinone and EF-G inhibitor.

37. The kit of claim 36, wherein the tetracycline is selected from the group consisting of demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, chlortetracycline, clomocycline, lymecycline, meclocycline, metacycline, peniniepicychne, and rolitetracycline.

38. The kit of claim 36, wherein the aminoglycoside is selected from the group consisting of streptomycin, dillydrostreptomycin, neomycin, framycetin, paromomycin, ribostamycin, kanamycin, amikacin, arbekacin, bekanamycin, dibekacin, tobramycin, spectinomycin, hygromycin, paromomycin, gentamicin, netilmicin, sisomicicii, sepamycin, verdamicin, and atrimicm.

39. The kit of claim 36, wherein the lincosamide is selected from the group consisting of clindamycin, lincomycin, and pirlinicycm.
40. The kit of claim 36, wherein the streptogramin is selected from the group consisting of quinupristin/daJfopristin, pristinamycin, and virginiamycin.

41. The kit of claim 36, wherein the glycycline is tigecycline.

42. The kit of claim 36, wherein the amphenicol is selected from the group consisting of chloramphenicol, azidanrifencol, hiamphenicoL and florfenicol.

43. The kit of claim 36, wherein the pleuropastilin is selected from the group consisting of retaparniilin, tiainliii, and vaïnenuïin.

44. The kit of claim 36, wherein the inacrolide is selected from the group consisting of azithromycin, clarithromycin, diritiromyciii, erytliromycin, lllmithromyein, josamycin, midecamycin, miocamycin, oleandomycin, rokitamycin, roxithromycin, spiramycin, tioleandomycin, tylosin, ketolides, telitlironiyciii, cethromwem, and solitlirornycin.

45. The kit of claim 36, wherein the oxazolidinone is selected from the group consisting of eperezolid, linezolid, posizolid, radezolid, ranbezolid, sutezolid, and tedizoLid.

46. The kit of claim 36, wherein the EF-G inhibitor is fusidic acid.
Figs. 2A-B
Figs. 3A-D
Figs. 4A-H
Figs. 5A-C
INTERATIONAL SEARCH REPORT

INTERNATIONAL SEARCH REPORT

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01M 43/50; C07D 498/00 (2014.01)
USPC - 514/249, 248, 247, 183, 1; 540/456, 455, 453, 454, 451, 450, 1
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): A01M 43/50; C07D 498/00; A61K31/495 (2014.01)
USPC: 514/249, 248, 247, 183, 1; 540/456, 455, 453, 454, 451, 450, 1

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 6328989 B1 (MATSUMOTO, Y et al.) December 11, 2001; abstract; column 2, lines 2-5; column 2, lines 19-25; column 2, lines 59-64; column 5, lines 17-23; Table 1; Claims 3-4</td>
<td>1, 2, 31, 32, 43/1, 43/2, 16-18, 19-17, 19-18, 32, 33, 34/32, 34/33</td>
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<tr>
<td>Y</td>
<td>WO 1995/08344 (COHEN, J et al.) March 30, 1995; abstract; page 8, lines 3-7; page 12, lines 1-12; page 20, lines 25-29; page 32, lines 15-16; page 74, Table 15A; Claims 62, 63</td>
<td>1, 2, 31, 32, 43/1, 43/2, 16-18, 19-17, 19-18, 32, 33, 34/32, 34/33</td>
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<tr>
<td>Y</td>
<td>US 5589470 A (LEVY, SB) December 31, 1996; abstract; column 3, line 61; column 11, lines 29-32</td>
<td>43/1, 43/2</td>
</tr>
</tbody>
</table>

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 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 persons skilled in the art
  "Z" document member of the same patent family

Date of the actual completion of the international search
08 August 2014 (08.08.2014)

Date of mailing of the international search report
29 AUG 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: Shane Thomas
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

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

   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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.