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(54) **DNA METHYL TRANSFERASE INHIBITORS**

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(57) **ABSTRACT**

This invention provides broad-spectrum antibiotics that are inhibitors of bacterial adenine DNA methyltransferases.

Figure 1 - Proposed Active Site of Adenine DNA Methyltransferase.

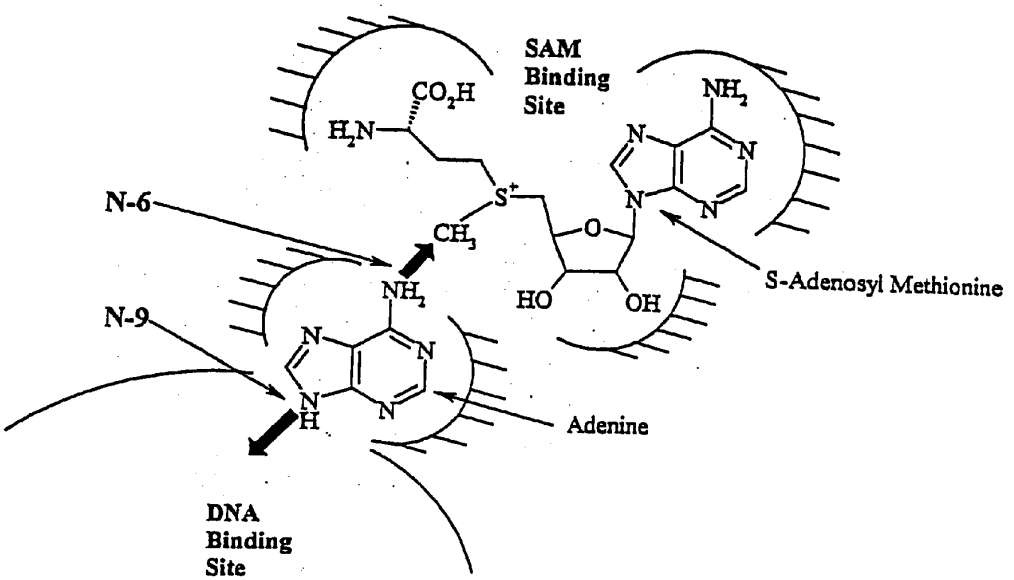
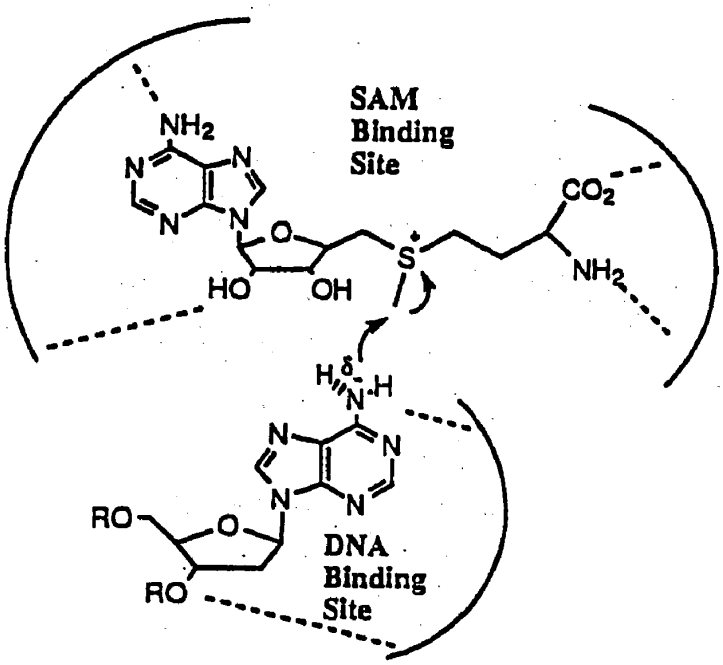


Figure 2 - Mechanism of Adenine DNA methyltransferase



DNA METHYL TRANSFERASE INHIBITORS

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates to the field of antibiotics and particularly antibacterial compounds. The invention specifically relates to antibiotics targeted to DNA modification enzymes, in particular adenine DNA methyltransferases, that are the components of a broad variety of different bacterial pathogens including those that are essential for bacterial cell growth. The invention particularly provides inhibitors of such adenine DNA methyltransferases having little or no inhibitory effects on cytosine methyltransferases, and hence having limited antibiotic effect on eukaryotic, particularly mammalian, cells. Methods for preparing and using the adenine DNA methyltransferase inhibitors of the invention, and pharmaceutical compositions thereof, are also provided.

[0003] 2. Background of the Invention

[0004] One hallmark of the modern era of medicine has been the decline in morbidity and mortality associated with bacterial infections. The development of a variety of antibiotic drugs in the early and middle parts of the twentieth century provided medical practitioners for the first time with effective treatments for a variety of infectious diseases.

[0005] However, misuse of conventional antibiotics and natural selection of the infectious bacterial population has resulted in the development of varying degrees of drug resistance by most bacterial infectious agents to most antibiotic agents. In severe cases, such as MRSA Multidrug-Resistant StaphA, one or only a few antibiotics are currently effective. In addition, the existence of immunodeficiency syndromes results in additional incidence of opportunistic infections requiring intensive antibiotic treatment.

[0006] Thus, there is an increasing need in the art for novel, more effective antibiotic compounds for treating bacterial infections that are resistant to currently available therapies.

[0007] Most bacteria modify their genomic DNA by methylation of specific nucleotide bases. DNA methylation is critical to gene regulation and repair of mutational lesions (see Jost & Soluz, 1993, DNA METHYLATION, MOLECULAR BIOLOGY AND BIOLOGICAL SIGNIFICANCE, Birhauser Verlag: Basel, Switzerland; Palmer & Marinus, 1994, *Gene* 143: 1-12; Dryden, 1999, "Bacterial DNA Methyltransferases," in S-ADENOSYLMETHIONINE-DEPENDENT METHYLTRANSFERASES: STRUCTURES AND FUNCTIONS, X. Cheng and R. M. Blumenthal (eds.), World Scientific Publishing, p.283-340 for review). DNA methylation is catalyzed by a class of enzymes having different sequences specificities. There are those DNA methyltransferases for example (dam) that methylate adenine residues in GATC sequences or cytosine (dcm) residues in CCAGG or CCTGG sequences which are not contained in the recognition site of a cognate restriction enzyme. There are those DNA methyltransferases that methylate residues contained in the recongnition site of a cognate restriction enzyme (for example, ApaI, AvaII, BclI, ClaI, DpnII, EcoRI, HaeIII, HhaI, MboI, and MspI; see, Marinus & Morris, 1973, *J. Bacteriol.* 114: 1143-1150; May & Hatman, 1975, *J. Bacteriol.* 123: 768-770; Heitman, 1993, *Genet. Eng.* 15: 57-108). In addition, the instant inventors have discovered an adenine DNA methyltransferase from *Caulobacter cresentus* that methylates the adenine residue in the sequence GANTC, as disclosed in International Application Publication No. WO98/12206. This methyltransferase is cell-cycle regulated and essential for successful bacterial cell growth; inhibition of the enzyme makes the bacteria non-viable. Similar methyltransferases have also been discovered in *Brucella abortus, Helicobacter pylori, Agrobacterium tumefaciens* and *Rhizobium meliloti*. In contrast with bacterial cells, DNA methylation in eukaryotic, and particularly mammalian cells, is limited to cytosine methylation at sites comprising the sequence CpG (Razin & Riggs, 1980, *Science* 210: 604-610; Jost & Bruhat, 1997, *Prog. Nucleic Acid Res. Molec. Biol.* 57: 217-248).

[0008] Thus, the existence of DNA methylation, in particular, the cell-cycle regulated adenine DNA methyltransferase found by the inventors in certain bacterial species, addresses the need in the art for novel targets for antibiotic activity.

SUMMARY OF THE INVENTION

[0009] The invention provides antibiotic compounds capable of inhibiting adenine DNA methyltransferases in bacterial cells. The antibiotic compounds of the invention specifically inhibit adenine-specific bacterial DNA methyltransferases, and do not inhibit bacterial or eukaryotic, particularly mammalian and most particularly human, cytosine-specific DNA methyltransferases. The compounds of the invention also inhibit adenine-specific DNA methyltransferases in plants. The antibiotic compounds are also provided as pharmaceutical compositions capable of being administered to an animal, most preferably a human, for treatment of a disease having a bacterial etiology, or an opportunistic infection with a bacteria in an animal, most preferably a human, in an immunologically compromised or debilitated state of health.

[0010] The invention also provides methods for preparing the antibiotic compounds and pharmaceutical compositions thereof, and methods of using said antibiotics therapeutically. Kits and packaged embodiments of the antibiotic compounds and pharmaceutical compositions of the invention are also provided.

[0011] Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

DESCRIPTION OF THE DRAWINGS

[0012] **FIGS. 1 and 2** depict schematic diagrams of the "active site" of bacterial adenine DNA methyltransferases.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0013] This invention provides antibiotics, and specifically antibacterial compounds, that are inhibitors of bacterial adenine DNA methyltransferases. The compounds of the invention exhibit antibacterial, growth-inhibitory properties against any bacterial species that produces an adenine DNA methyltransferase. These include adenine DNA methyltransferases that are components of bacterial restriction/modifi-

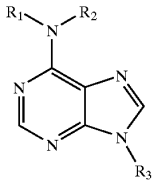
cation systems as understood in the art, as well as cell-cycle regulated adenine DNA methyltransferases (CcrM), such as those disclosed in International Application Publication No. WO98/12206, incorporated by reference. Thus, inhibitors of adenine DNA methyltransferases are particularly provided by the invention.

[0014] The adenine DNA methyltransferase inhibitors of the invention comprise a novel class of broad-spectrum antibiotics. Most bacterial species possess a DNA methyltransferase that is part of a modification apparatus, typically associated with a restriction enzyme, that preserves the integrity of cellular DNA while providing a defense against foreign (most typically viral) DNA. In addition, certain bacteria produce an adenine DNA methyltransferase that is essential for bacterial cell growth. Medically-important bacterial species that provide appropriate targets for the antibacterial activity of the inhibitors of the invention include gram-positive bacteria, including cocci such as *Staphylococcus* species and *Streptococcus* species; bacilli, including *Bacillus* species, *Corynebacterium* species and *Clostridium* species; filamentous bacteria, including *Actinomyces* species and *Streptomyces* species; gram-negative bacteria, including cocci such as *Neisseria* species; *bacilli*, such as *Pseudomonas* species, *Brucella* species, *Agrobacterium* species, *Bordetella* species, *Escherichia* species, *Shigella* species, *Yersinia* species, *Salmonella* species, *Klebsiella* species, *Enterobacter* species, *Hemophilus* species, *Pasteurella* species, and *Streptobacillus* species; spirochetal species, *Campylobacter* species, *Vibrio* species; and intracellular bacteria including *Rickettsiae* species and *Chlamydia* species.

[0015] Specific bacterial species that are targets for the adenine DNA methyltransferase inhibitors of the invention include *Staphylococcus aureus; Staphylococcus saprophyticus; Streptococcus pyrogenes; Streptococcus agalactiae; Streptococcus pneumoniae; Bacillus anthracis; Corynebacterium diphtheria; Clostridium perfringens; Clostridium botulinum; Clostridium tetani; Neisseria gonorrhoeae; Neisseria meningitidis; Pseudomonas aeruginosa; Legionella pneumophila; Escherichia coli; Yersinia pestis; Hemophilus influenzae; Helicobacter pylori; Campylobacter fetus; Vibrio cholerae; Vibrio parahemolyticus; Trepomena pallidum; Actinomyces israelii; Rickettsia prowazekii; Rickettsia rickettsii; Chlamydia trachomatis; Chlamydia psittaci; Brucella abortus* and *Agrobacterium tumefaciens.*

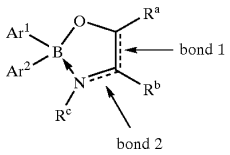
[0016] It is an important property of the adenine DNA methyltransferase inhibitors of the invention that the level of activity of these substances with cytosine-specific DNA methyltransferases is low. This is because cytosine-specific DNA methyltransferases occur in mammalian, most particularly human, cells, and it is an advantageous property of the adenine DNA methyltransferases of the invention to have little or no inhibitory activity against mammalian methyltransferases. This property confers upon the molecules provided by the invention the beneficial property of being bacterial cell specific, and having little antibiotic activity against mammalian, most preferably human, cells. Preferably, the IC_{50} of these compounds for cytosine-specific DNA methyltransferases is greater than 500 μM.

[0017] The inhibitory compounds provided by the invention are represented by Formula I:



[0018] where R^1, R^2 and R^3 are the same or different and are independently hydrogen, lower alkyl, aryl or substituted aryl, lower alkoxy, lower alkoxyalkyl, or cycloalkyl or cycloalkyl alkoxy, where each cycloalkyl group has from 3-7 members, where up to two of the cycloalkyl members are optionally hetero atoms selected from oxygen and nitrogen, and where any member of the alkyl, aryl or cycloalkyl group is optionally substituted with halogen, lower alkyl or lower alkoxy, aryl or substituted aryl, and where R^3 can be ribose, deoxyribose or phosphorylated derivatives thereof, including phosphorothioates, phosphoramidites and similar derivatives known in the art, provided that R^1, R^2 and R^3 are not all hydrogen, and where R^3 is ribose, deoxyribose or phosphorylated derivatives thereof, R^1 and R^2 are not both hydrogen. In preferred embodiments, R^1 is H, R^2 is (2-diphenylborinic ester)ethyl or diphenylpropyl, and R^3 is H, 2-(4-morpholinyl)-ethyl, 3-(N-phthaloyl)-aminopropyl, 2-(2-(2-hydroxyethoxy)ethoxy)ethyl, or ethyl-2-(acrylate)-methyl. In additional preferred embodiments, R^1 is H, R^2 is (S-homocysteinyl)methyl and R^3 is ribose, 5'phosphorylribose, deoxyribose or 5' phosphoryl deoxyribose. In other preferred embodiments, R^3 is H and R^1 and R^2 are together 2-(diphenylmethyl)cyclopentyl or 2-(diphenylhydroxymethyl)cyclopentyl. In further preferred embodiments, R^1 is H_1. R^2 is alanylbutyl ester, 2-carboximido-2-aminoethyl, 2-aminoethyl or mono- or bisubstituted 2-amino ethyl, and R^3 is 2-(4-morpholinyl)-ethyl.

[0019] The invention also provides compounds of Formula II:



[0020] wherein bonds 1 and 2 can be double or single, Ar^1 and Ar^2 can be the same or different and are each independently aryl or heteroaryl, or aryl or heteroaryl substituted at one or a plurality of positions with halogen, nitro, nitroso, lower alkyl, aryl or substituted aryl, lower alkoxy, lower alkoxyalkyl, or cycloalkyl or cycloalkyl alkoxy, where each cycloalkyl group has from 3-7 members, where up to two of the cycloalkyl members are optionally hetero atoms selected from sulfur, oxygen and nitrogen, and where any member of the alkyl, aryl or cycloalkyl group is optionally substituted with halogen, lower alkyl or lower alkoxy, aryl or substituted

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