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(54) Title: PRODRUGS OF 9-AMINOMETHYL TETRACYCLINE COMPOUNDS

(57) Abstract: The invention pertains to prodrugs of 9-aminomethyl substituted tetracycline compounds, methods of using the com-
pounds, and pharmaceutical compositions containing them.



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PRODRUGS OF 9-AMINOMETHYL TETRACYCLINE COMPOUNDS

Related Applications

This application claims priority to U.S. Provisional Patent Application No. 60/566,150, filed on April 27, 2004, U.S. Provisional Patent Application No. 60/530,123, filed on December 16, 2003, U.S. Provisional Patent Application No. 60/525,287, filed November 25, 2003, and U.S. Provisional Patent Application No. 60/486,017, filed on July 9, 2003. The entire contents of each of these patent applications are hereby incorporated herein by reference.

Background of the Invention

The development of the tetracycline antibiotics was the direct result of a systematic screening of soil specimens collected from many parts of the world for evidence of microorganisms capable of producing bacteriocidal and/or bacteriostatic compositions. The first of these novel compounds was introduced in 1948 under the name chlortetracycline. Two years later, oxytetracycline became available. The elucidation of the chemical structure of these compounds confirmed their similarity and furnished the analytical basis for the production of a third member of this group in 1952, tetracycline. A new family of tetracycline compounds, without the ring-attached methyl group present in earlier tetracyclines, was prepared in 1957 and became publicly available in 1967; and tetracycline was in use by 1972.

Recently, research efforts have focused on developing new tetracycline antibiotic compositions effective under varying therapeutic conditions and routes of administration. New tetracycline analogues have also been investigated which may prove to be equal to or more effective than the originally introduced tetracycline compounds. Examples include U.S. Patent Nos. 2,980,584; 2,990,331; 3,062,717; 3,165,531; 3,454,697; 3,557,280; 3,674,859; 3,957,980; 4,018,889; 4,024,272; and 4,126,680. These patents are representative of the range of pharmaceutically active tetracycline and tetracycline analogue compositions.

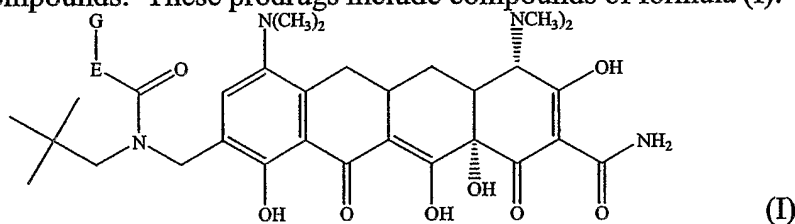
Historically, soon after their initial development and introduction, the tetracyclines were found to be highly effective pharmacologically against rickettsiae; a number of gram-positive and gram-negative bacteria; and the agents responsible for lymphogranuloma venereum, inclusion conjunctivitis, and psittacosis. Hence, tetracyclines became known as "broad spectrum" antibiotics. With the subsequent establishment of their in vitro antimicrobial activity, effectiveness in experimental infections, and pharmacological properties, the tetracyclines as a class rapidly became widely used for therapeutic purposes. However, this widespread use of tetracyclines for both major and minor illnesses and diseases led directly to the emergence of resistance

to these antibiotics even among highly susceptible bacterial species both commensal and pathogenic (*e.g.*, pneumococci and Salmonella). The rise of tetracycline-resistant organisms has resulted in a general decline in use of tetracyclines and tetracycline analogue compositions as antibiotics of choice.

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Summary of the Invention

The invention pertains, at least in part, to prodrugs of 9-substituted aminomethyl tetracycline compounds. These prodrugs include compounds of formula (I):



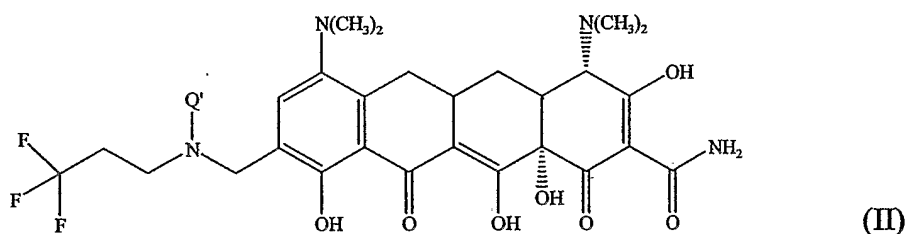
10 wherein

E is oxygen, nitrogen, or a covalent bond;

G is alkyl; heterocyclyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; alkoxyalkoxy carbonyloxyalkyl, and pharmaceutically acceptable salts thereof.

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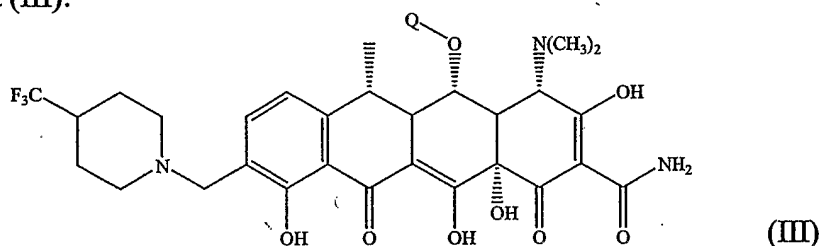
The invention also pertains, at least in part, to tetracycline compounds of formula (II):



wherein

20 Q' is a prodrug moiety and pharmaceutically acceptable salts thereof.

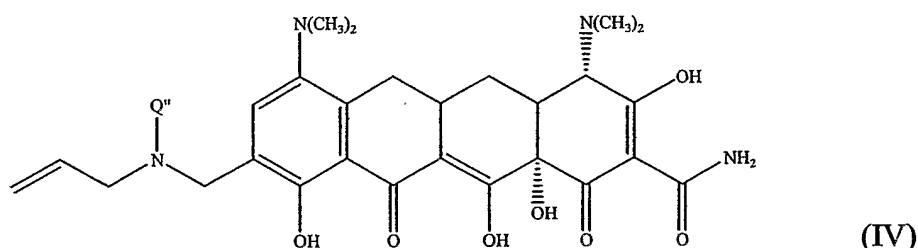
In addition, the invention also pertains, at least in part, to tetracycline compounds of the formula (III):



25 wherein:

Q is a prodrug moiety, and pharmaceutically acceptable salts thereof.

The invention also pertains, at least in part, to tetracycline compounds of the formula (IV):



5 wherein

Q'' is a prodrug moiety and pharmaceutically acceptable salts thereof.

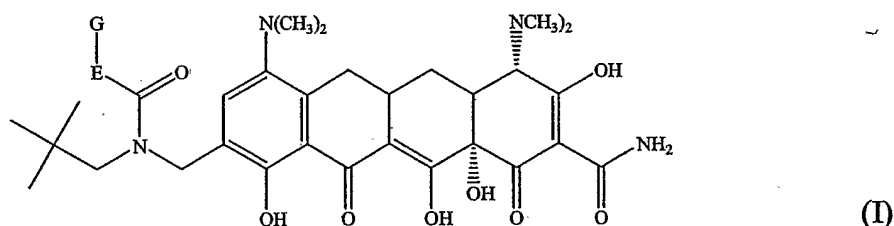
In another embodiment, the invention includes a method for treating a tetracycline responsive state in a subject, by administering to the subject a tetracycline compound of the invention. In a further embodiment, the tetracycline compound is metabolized *in vivo*.

The invention also pertains to pharmaceutical compositions comprising the compounds of the invention and a pharmaceutically acceptable carrier.

15 Detailed Description of the Invention

The invention pertains, at least in part, to prodrugs of tetracycline compounds. These compounds may be metabolized *in vivo*, to yield a desired tetracycline compound. The invention pertains, at least in part, to the tetracycline compounds described herein, to methods of using the tetracycline compounds, and pharmaceutical compositions comprising the tetracycline compounds.

In one embodiment, the invention pertains to tetracycline compounds of formula (I):



wherein

25 E is oxygen, nitrogen, or a covalent bond;

G is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; alkoxyalkoxyalkyl, and pharmaceutically acceptable salts thereof.

In one embodiment, E is a covalent bond. In a further embodiment, G is alkyl, e.g., methyl.

In another embodiment, E is nitrogen or NH. In a further embodiment, G is aryl, e.g., substituted or unsubstituted phenyl. In a further embodiment, G is substituted with, for example, a substituent which allows it to perform its intended function. Examples of possible substituents include alkyl (including substituted alkyl such as halogenated alkyl), nitro, halogen, or alkoxy.

In another embodiment, E is oxygen.

In a further embodiment, G is alkylcarbonyloxyalkyl. In yet a further embodiment, G is of the formula $-(CH_2)_g-O-(C=O)-R^1$, wherein g is 1-5 and R^1 is alkyl. R^1 may be substituted or unsubstituted alkyl. R^1 may be branched, straight, or cyclic. In a further embodiment, g is 1 or 2 and R^1 is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, $-(CH_2)_{10}-CH_3$, or $-(CH_2)_{11}CH_3$.

In another further embodiment, G is alkyl, e.g., methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, $-(CH_2)_{10}-CH_3$, or $-(CH_2)_{11}CH_3$.

In another embodiment, G is arylcarbonyloxyalkyl. In yet another further embodiment, G is of the formula: $-(CH_2)_f-O-(C=O)-R^2$, wherein f is 1-5 and R^2 is aryl. In a further embodiment, f is 1. Optionally, R^2 is substituted or unsubstituted phenyl. Examples of possible substituents include, for example, halogen, alkoxy, or alkyl (optionally substituted with, for example, halogens).

In another embodiment, G is alkyloxycarbonyloxyalkyl. In a further embodiment, G is of the formula $-(CH_2)_g-O-(C=O)-O-R^3$, wherein R^3 is alkyl and g is from 1-5. In a further embodiment, g is 1. In another further embodiment, R^3 is methyl, ethyl, propyl, butyl or pentyl.

In another embodiment, G is arylalkylcarbonyloxyalkyl. In another further embodiment, G is of the formula $-(CH_2)_h-O-(C=O)-(CH_2)_{h'}-R^4$, wherein h is 1-5, h' is 1-5, and R^4 is aryl. In a further embodiment, h' is 1 and h is 1 or 2. In another embodiment, R^4 is substituted or unsubstituted phenyl.

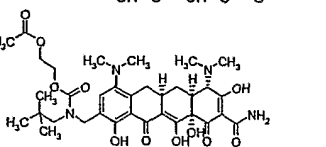
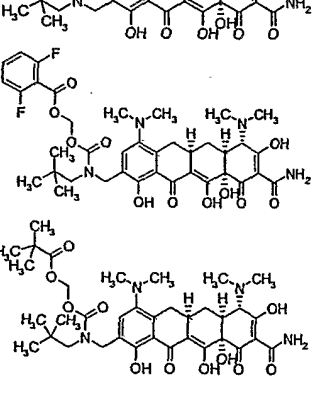
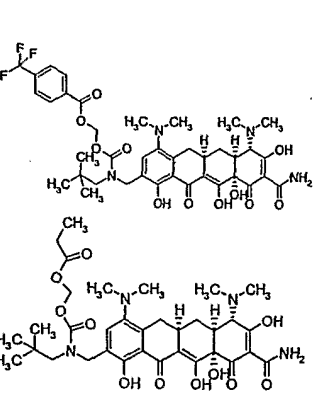
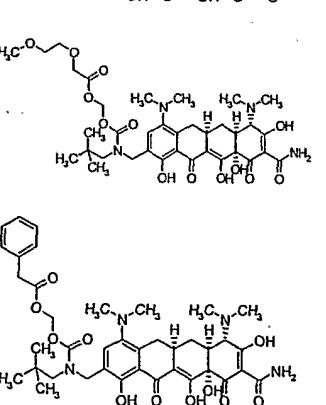
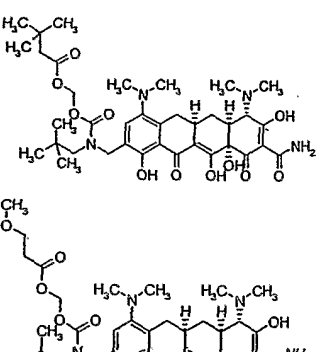
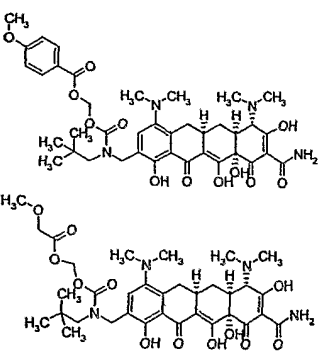
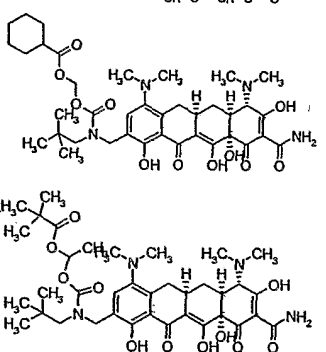
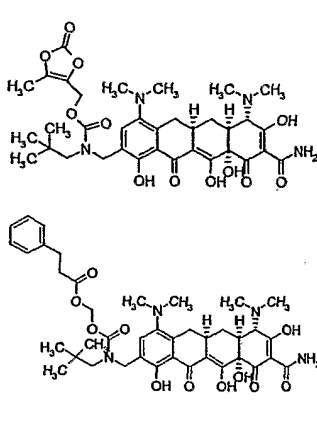
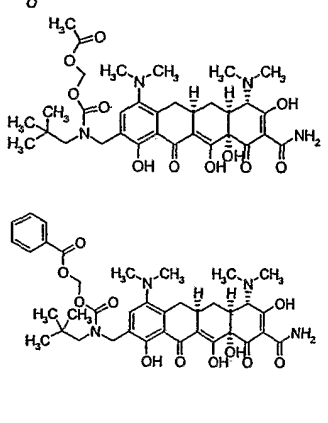
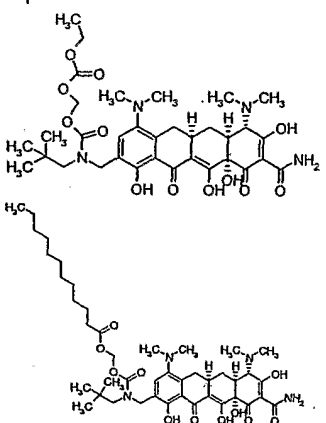
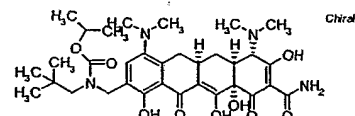
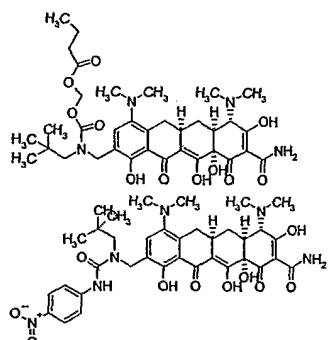
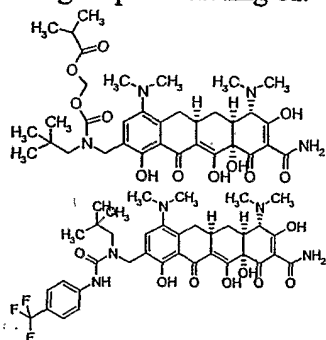
In another embodiment, G is alkyloxyalkylcarbonyloxyalkyl. In another further embodiment, G is of the formula $-(CH_2)_i-O-(C=O)-(CH_2)_{i'}-O-R^5$, wherein i and i' are each independently 1-5, and R^5 is alkyl. In yet a further embodiment i' is 1 and i is 1, 2, or 3. In a further embodiment, R^5 is methyl.

In yet another embodiment, G is alkoxyalkoxyalkylcarbonyloxyalkyl. Examples of G include moieties of the formula $-(CH_2)_j-O-(C=O)-(CH_2)_j-O-(CH_2)_k-O-R^6$, wherein j, j' and k are each independently 1-5, and R^6 is alkyl. In a further embodiment, j and j' are each 1 and k is 2. In another further embodiment, R^6 is methyl.

In a further embodiment, G is heterocyclic alkyl.

In a further embodiment, E is not oxygen, when G is $-(CH_2)_2-O-C(=O)-CH_3$ or $-CH_2-O-(C=O)-C(CH_3)_3$.

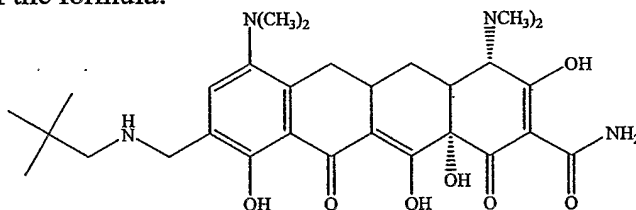
In a another further embodiment, the compound of the invention is selected from the group consisting of:



and pharmaceutically acceptable salts thereof. In another embodiment, the compounds of the invention do not include the compounds described in U.S.S.N. 10/384,855 or U.S.S.N. 10/412,656.

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In a further embodiment, E and G are selected such that after administration of the tetracycline compound to the subject, the tetracycline compound is metabolized *in vivo* to a compound of the formula:



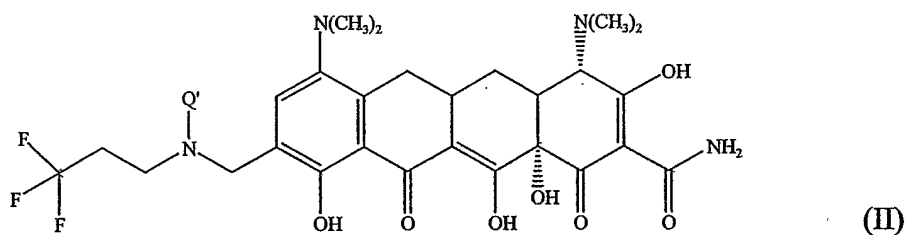
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(Ia)

The term "metabolized" includes any and all processes within a subject which would yield a compound of formula (Ia). The mechanisms may include, for example, enzymatic degradation, hydrolysis, cleavage by esterases, etc.

In an another embodiment, E and G are selected such that together they consist of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 atoms selected from the group consisting of oxygen, carbon, and nitrogen. E and G may further comprise hydrogen atoms or halogen atoms.

In another embodiment, the invention pertains to tetracycline compounds of the formula (II):

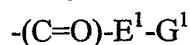


wherein

Q' is a prodrug moiety and pharmaceutically acceptable salts thereof.

The term "prodrug moiety" includes moieties which may be metabolized *in vivo* to form a desired tetracycline compound (e.g., a compound of formula Ia, IIa, IIIa, or IVa). Examples of prodrug moieties include carbonyl moieties, carbamates, amides, and the like. In one embodiment, the prodrug moiety consist of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 atoms selected from the group consisting of oxygen, carbon, and nitrogen. The prodrug moiety may further comprise hydrogen atoms, halogen atoms, or other substituents which allow the tetracycline compound to perform its intended function.

In a further embodiment, Q' is of the formula



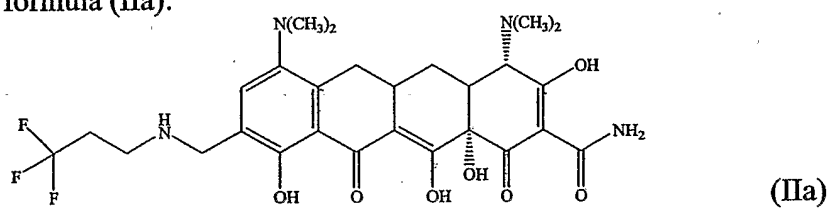
wherein

E¹ is oxygen, nitrogen, or a covalent bond;

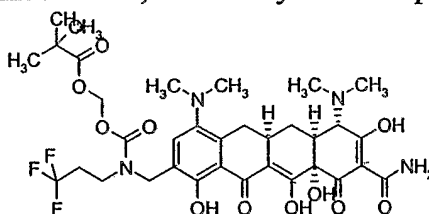
5 G¹ is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; or alkoxyalkoxycarbonyloxyalkyl.

In yet another further embodiment, E¹ is oxygen. In another further embodiment, G¹ is alkylcarbonyloxyalkyl. Examples of G¹ include moieties of the formula -(CH₂)_m-O-(C=O)-R⁷, wherein m is 1-5 and R⁷ is alkyl. In a further embodiment, m is 1. In another further embodiment, R⁷ is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, -(CH₂)₁₀-CH₃, or -(CH₂)₁₁CH₃.

In a further embodiment, Q' is selected such that after administration of the tetracycline compound to the subject, the tetracycline compound is metabolized *in vivo* to a compound of the formula (IIa):

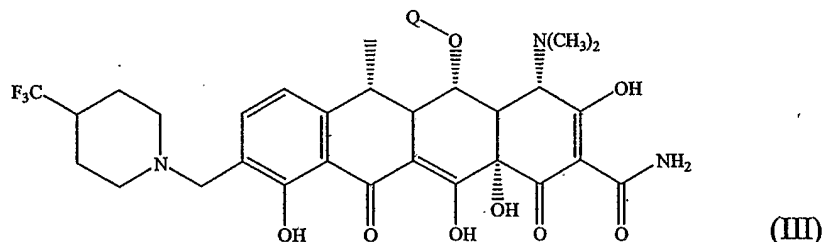


In another further embodiment, the tetracycline compound of the invention is:



20 or pharmaceutically acceptable salts thereof.

In another embodiment, the invention pertains to tetracycline compound of the formula (III):

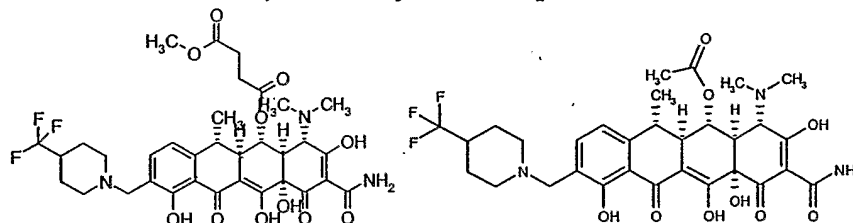


25 wherein:

Q is a prodrug moiety, and pharmaceutically acceptable salts thereof.

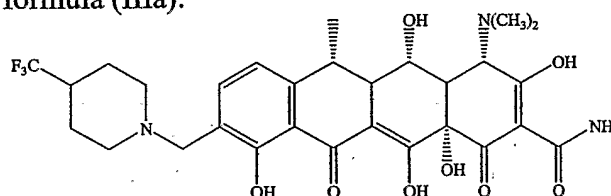
In a further embodiment, Q is $-(C=O)-G^2$. In another embodiment, G^2 is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; or alkoxyalkoxycarbonyloxyalkyl. In another embodiment, G^2 is alkyloxycarbonylalkyl or alkyl.

In another embodiment, the tetracycline compound of formula III is:



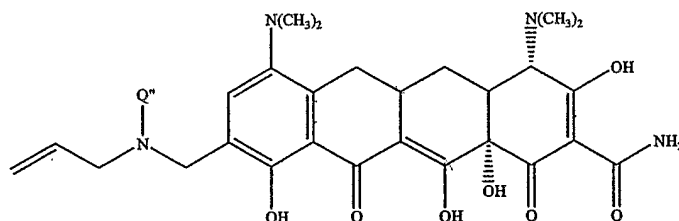
and pharmaceutically acceptable salts thereof.

In a further embodiment, Q is selected such that after administration of the tetracycline compound to the subject, the tetracycline compound is metabolized *in vivo* to a compound of the formula (IIIa):



(IIIa).

The invention also pertains, at least in part, to tetracycline compounds of the formula (IV):

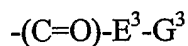


(IV)

wherein

Q'' is a prodrug moiety and pharmaceutically acceptable salts thereof:

In a further embodiment, Q'' is of the formula



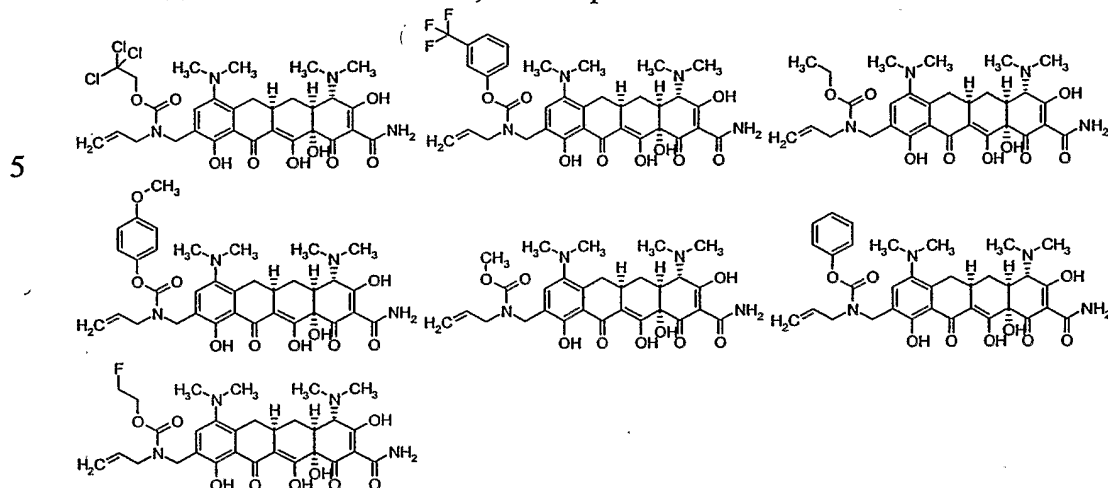
wherein

E^3 is oxygen, nitrogen, or a covalent bond;

G^3 is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; or alkoxyalkoxycarbonyloxyalkyl.

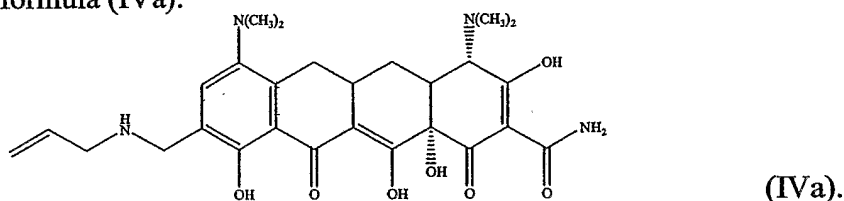
In another further embodiment, E³ is oxygen. In another further embodiment, G³ is substituted or unsubstituted alkyl (e.g., methyl, ethyl, propyl, etc.) or substituted or unsubstituted aryl (e.g., substituted or unsubstituted phenyl, etc.).

In a further embodiment, the compounds of the invention include:

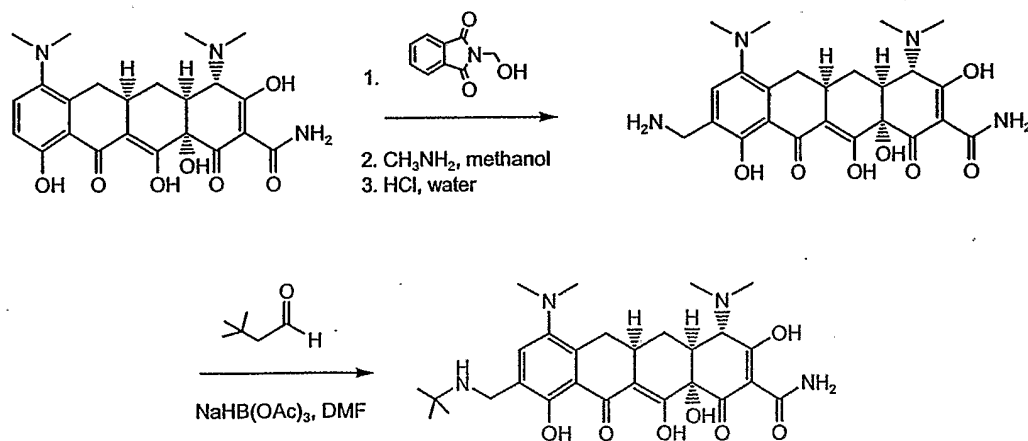


and pharmaceutically acceptable salts thereof.

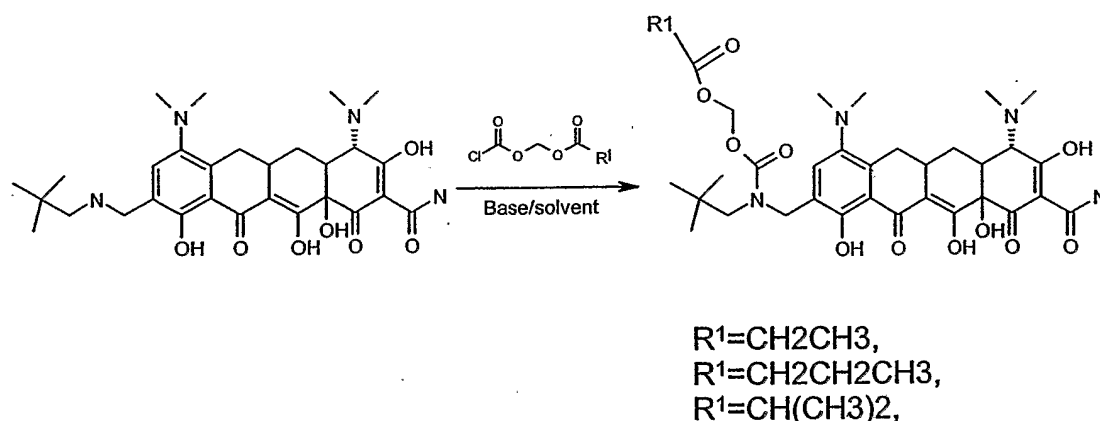
10 In a further embodiment, Q' is selected such that after administration of the tetracycline compound to the subject, the tetracycline compound is metabolized *in vivo* to a compound of the formula (IVa):



15 Compounds of the invention may be made as described below in the following Scheme and in the Examples, or with modifications to the procedure which are within the skill of those of ordinary skill in the art.



Scheme 1



Scheme 2

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The term "alkyl" includes saturated aliphatic groups, including straight-chain alkyl groups (*e.g.*, methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, *etc.*), branched-chain alkyl groups (isopropyl, tert-butyl, isobutyl, *etc.*), cycloalkyl (alicyclic) groups (cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl), alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. The term alkyl further includes alkyl groups, which can further include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone. In certain embodiments, a straight chain or branched chain alkyl has 6 or fewer carbon atoms in its backbone (*e.g.*, C₁-C₆ for straight chain, C₃-C₆ for branched chain), and more preferably 4 or fewer. Likewise, preferred cycloalkyls have from 3-8 carbon atoms in their ring structure, and more preferably have 5 or 6 carbons in the ring structure. The term C₁-C₆ includes alkyl groups containing 1 to 6 carbon atoms.

Moreover, the term alkyl includes both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonate, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Cycloalkyls can be further substituted, *e.g.*, with the substituents described above. An "alkylaryl" or an

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"arylalkyl" moiety is an alkyl substituted with an aryl (*e.g.*, phenylmethyl (benzyl)). The term "alkyl" also includes the side chains of natural and unnatural amino acids.

The term "aryl" includes groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, phenyl, pyrrole, furan, thiophene, thiazole, isothiazole, imidazole, triazole, tetrazole, pyrazole, oxazole, isooxazole, pyridine, pyrazine, pyridazine, and pyrimidine, and the like. Furthermore, the term "aryl" includes multicyclic aryl groups, *e.g.*, tricyclic, bicyclic, *e.g.*, naphthalene, benzoxazole, benzodioxazole, benzothiazole, benzoimidazole, benzothiophene, methylenedioxyphenyl, quinoline, isoquinoline, naphthridine, indole, benzofuran, purine, benzofuran, deazapurine, or indolizine. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heterocycles," "heteroaryls" or "heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy, carbonyloxy, carboxylate, alkylcarbonyl, alkylamino, arylalkyl aminocarbonyl, alkenylaminocarbonyl, alkylcarbonyl, arylcarbonyl, arylalkylcarbonyl, alkenylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonate, phosphinate, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfenyl, sulfonate, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (*e.g.*, tetralin).

The term "alkenyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double bond.

For example, the term "alkenyl" includes straight-chain alkenyl groups (*e.g.*, ethylenyl, propenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl, decenyl, *etc.*), branched-chain alkenyl groups, cycloalkenyl (alicyclic) groups (cyclopropenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl), alkyl or alkenyl substituted cycloalkenyl groups, and cycloalkyl or cycloalkenyl substituted alkenyl groups. The term alkenyl further includes alkenyl groups which include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone. In certain embodiments, a straight chain or branched chain alkenyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C₂-C₆ for straight chain, C₃-C₆ for branched chain). Likewise, cycloalkenyl groups may have from 3-8 carbon atoms in their ring structure,

and more preferably have 5 or 6 carbons in the ring structure. The term C₂-C₆ includes alkenyl groups containing 2 to 6 carbon atoms.

Moreover, the term alkenyl includes both "unsubstituted alkenyls" and "substituted alkenyls", the latter of which refers to alkenyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkyl groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonate, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "alkynyl" includes unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but which contain at least one triple bond.

For example, the term "alkynyl" includes straight-chain alkynyl groups (*e.g.*, ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl, decynyl, *etc.*), branched-chain alkynyl groups, and cycloalkyl or cycloalkenyl substituted alkynyl groups. The term alkynyl further includes alkynyl groups which include oxygen, nitrogen, sulfur or phosphorous atoms replacing one or more carbons of the hydrocarbon backbone. In certain embodiments, a straight chain or branched chain alkynyl group has 6 or fewer carbon atoms in its backbone (*e.g.*, C₂-C₆ for straight chain, C₃-C₆ for branched chain). The term C₂-C₆ includes alkynyl groups containing 2 to 6 carbon atoms.

Moreover, the term alkynyl includes both "unsubstituted alkynyls" and "substituted alkynyls", the latter of which refers to alkynyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, alkyl groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonate, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonate, sulfamoyl, sulfonamido,

nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to five carbon atoms in its backbone structure. "Lower alkenyl" and "lower alkynyl" have chain lengths of, for example, 2-5 carbon atoms.

The term "acyl" includes compounds and moieties which contain the acyl radical ($\text{CH}_3\text{CO}\cdot$) or a carbonyl group. The term "substituted acyl" includes acyl groups where one or more of the hydrogen atoms are replaced by for example, alkyl groups, alkynyl groups, halogens, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "acylamino" includes moieties wherein an acyl moiety is bonded to an amino group. For example, the term includes alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido groups.

The term "aroyl" includes compounds and moieties with an aryl or heteroaromatic moiety bound to a carbonyl group. Examples of aroyl groups include phenylcarboxy, naphthyl carboxy, *etc.*

The terms "alkoxyalkyl", "alkylaminoalkyl" and "thioalkoxyalkyl" include alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, *e.g.*, oxygen, nitrogen or sulfur atoms.

The term "alkoxy" includes substituted and unsubstituted alkyl, alkenyl, and alkynyl groups covalently linked to an oxygen atom. Examples of alkoxy groups include methoxy, ethoxy, isopropoxy, propoxy, butoxy, and pentoxy groups. Examples of substituted alkoxy groups include halogenated alkoxy groups. The alkoxy groups can be substituted with groups such as alkenyl, alkynyl, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxy carbonyloxy, carboxylate, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino,

arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfanyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moieties. Examples of halogen substituted alkoxy groups include, but
5 are not limited to, fluoromethoxy, difluoromethoxy, trifluoromethoxy, chloromethoxy, dichloromethoxy, trichloromethoxy, *etc.*

The term "amine" or "amino" includes compounds where a nitrogen atom is covalently bonded to at least one carbon or heteroatom. The term "alkyl amino" includes groups and compounds wherein the nitrogen is bound to at least one additional
10 alkyl group. The term "dialkyl amino" includes groups wherein the nitrogen atom is bound to at least two additional alkyl groups. The term "arylamino" and "diarylamino" include groups wherein the nitrogen is bound to at least one or two aryl groups, respectively. The term "alkylarylamino," "alkylaminoaryl" or "arylaminoalkyl" refers to an amino group which is bound to at least one alkyl group and at least one aryl group.
15 The term "alkaminoalkyl" refers to an alkyl, alkenyl, or alkynyl group bound to a nitrogen atom which is also bound to an alkyl group.

The term "amide" or "aminocarbonyl" includes compounds or moieties which contain a nitrogen atom which is bound to the carbon of a carbonyl or a thiocarbonyl group. The term includes "alkaminocarbonyl" or "alkylaminocarbonyl" groups which
20 include alkyl, alkenyl, aryl or alkynyl groups bound to an amino group bound to a carbonyl group. It includes arylaminocarbonyl groups which include aryl or heteroaryl moieties bound to an amino group which is bound to the carbon of a carbonyl or thiocarbonyl group. The terms "alkylaminocarbonyl," "alkenylaminocarbonyl," "alkynylaminocarbonyl," "arylamino," "alkylcarbonylamino,"
25 "alkenylcarbonylamino," "alkynylcarbonylamino," and "arylcarbonylamino" are included in term "amide." Amides also include urea groups (aminocarbonylamino) and carbamates (oxycarbonylamino).

The term "carbonyl" or "carboxy" includes compounds and moieties which contain a carbon connected with a double bond to an oxygen atom. Examples of
30 moieties which contain a carbonyl include aldehydes, ketones, carboxylic acids, amides, esters, anhydrides, *etc.*

The term "thiocarbonyl" or "thiocarboxy" includes compounds and moieties which contain a carbon connected with a double bond to a sulfur atom.

The term "ether" includes compounds or moieties which contain an oxygen
35 bonded to two different carbon atoms or heteroatoms. For example, the term includes "alkoxyalkyl" which refers to an alkyl, alkenyl, or alkynyl group covalently bonded to an oxygen atom which is covalently bonded to another alkyl group.

The term "ester" includes compounds and moieties which contain a carbon or a heteroatom bound to an oxygen atom which is bonded to the carbon of a carbonyl group. The term "ester" includes alkoxycarboxy groups such as methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, pentoxycarbonyl, *etc.* The alkyl, alkenyl, or alkynyl groups are as defined above.

The term "thioether" includes compounds and moieties which contain a sulfur atom bonded to two different carbon or hetero atoms. Examples of thioethers include, but are not limited to alkthioalkyls, alkthioalkenyls, and alkthioalkynyls. The term "alkthioalkyls" include compounds with an alkyl, alkenyl, or alkynyl group bonded to a sulfur atom which is bonded to an alkyl group. Similarly, the term "alkthioalkenyls" and alkthioalkynyls" refer to compounds or moieties wherein an alkyl, alkenyl, or alkynyl group is bonded to a sulfur atom which is covalently bonded to an alkynyl group.

The term "hydroxy" or "hydroxyl" includes groups with an -OH or -O⁻.

The term "halogen" includes fluorine, bromine, chlorine, iodine, *etc.* The term "perhalogenated" generally refers to a moiety wherein all hydrogens are replaced by halogen atoms.

The terms "polycyclyl" or "polycyclic radical" refer to two or more cyclic rings (*e.g.*, cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, *e.g.*, the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, alkylaminoacarbonyl, arylalkylaminocarbonyl, alkenylaminocarbonyl, alkylcarbonyl, arylcarbonyl, arylalkyl carbonyl, alkenylcarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, alkylsulfinyl, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" includes atoms of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

The term "prodrug moiety" includes moieties which can be metabolized *in vivo* to an active group and moieties which may advantageously remain attached *in vivo*. Preferably, the prodrugs moieties are metabolized *in vivo* by enzymes, *e.g.*, esterases or by other mechanisms to hydroxyl groups or other advantageous groups. Examples of

prodrugs and their uses are well known in the art (See, *e.g.*, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19). The prodrugs can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound with a suitable agent. Hydroxyl groups can be converted into
5 esters *via* treatment with a carboxylic acid. Examples of prodrug moieties include substituted and unsubstituted, branch or unbranched lower alkyl ester moieties, (*e.g.*, propionic acid esters), lower alkenyl esters, di-lower alkyl-amino lower-alkyl esters (*e.g.*, dimethylaminoethyl ester), acylamino lower alkyl esters (*e.g.*, acetyloxymethyl ester), acyloxy lower alkyl esters (*e.g.*, pivaloyloxymethyl ester), aryl esters (phenyl
10 ester), aryl-lower alkyl esters (*e.g.*, benzyl ester), substituted (*e.g.*, with methyl, halo, or methoxy substituents) aryl and aryl-lower alkyl esters, amides, lower-alkyl amides, di-lower alkyl amides, and hydroxy amides. Preferred prodrug moieties are propionic acid esters and acyl esters.

It will be noted that the structure of some of the compounds of this invention
15 includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (*e.g.*, all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis. Furthermore, the structures and other compounds
20 and moieties discussed in this application also include all tautomers thereof.

The invention also pertains to methods for treating a tetracycline responsive states in subjects, by administering to a subject an effective amount of a compound of the invention (*e.g.*, a compound of Formula (I), (II), (III), (IV) or otherwise described
25 herein), such that the tetracycline responsive state is treated.

The invention also pertains, at least in part, to administering to a subject an effective amount of a tetracycline compound of formula (I), (II), (III), or (IV) which is metabolized *in vivo* to a compound of formula (Ia), (IIa), (IIIa), or (IVa).

The language "tetracycline compound responsive state" or "tetracycline
30 responsive state" includes states which can be treated, prevented, or otherwise ameliorated by the administration of a compound of the invention, *e.g.*, a compound of Formula (I), (II), (III), (IV) or otherwise described herein. Tetracycline compound responsive states include bacterial, viral, and fungal infections (including those which are resistant to other tetracycline compounds), cancer (*e.g.*, prostate, breast, colon, lung
35 melanoma and lymph cancers and other disorders characterized by unwanted cellular proliferation, including, but not limited to, those described in U.S. 6,100,248), arthritis, osteoporosis, diabetes, cystic fibrosis, neurological disorders and other states for which tetracycline compounds have been found to be active (see, for example, U.S. Patent Nos.

5,789,395; 5,834,450; 6,277,061 and 5,532,227, each of which is expressly incorporated herein by reference). Compounds of the invention can be used to prevent or control important mammalian and veterinary diseases such as diarrhea, urinary tract infections, infections of skin and skin structure, ear, nose and throat infections, wound infection, mastitis and the like. In addition, methods for treating neoplasms using tetracycline compounds of the invention are also included (van der Bozert *et al.*, *Cancer Res.*, 48:6686-6690 (1988)). In a further embodiment, the tetracycline responsive state is not a bacterial infection. Other tetracycline compound responsive states include, for example, those described in U.S.S.N. 10/196,010.

10 Tetracycline compound responsive states also include inflammatory process associated states (IPAS). The term "inflammatory process associated state" includes states in which inflammation or inflammatory factors (e.g., matrix metalloproteinases (MMPs), nitric oxide (NO), TNF, interleukins, plasma proteins, cellular defense systems, cytokines, lipid metabolites, proteases, toxic radicals, adhesion molecules, *etc.*) are involved or are present in an area in aberrant amounts, e.g., in amounts which may be advantageous to alter, e.g., to benefit the subject. The inflammatory process is the response of living tissue to damage. The cause of inflammation may be due to physical damage, chemical substances, micro-organisms, tissue necrosis, cancer or other agents. Acute inflammation is short-lasting, lasting only a few days. If it is longer lasting
15 however, then it may be referred to as chronic inflammation.

IPAS's include inflammatory disorders. Inflammatory disorders are generally characterized by heat, redness, swelling, pain and loss of function. Examples of causes of inflammatory disorders include, but are not limited to, microbial infections (e.g., bacterial and fungal infections), physical agents (e.g., burns, radiation, and trauma), chemical agents (e.g., toxins and caustic substances), tissue necrosis and various types of immunologic reactions.

Examples of inflammatory disorders include, but are not limited to, osteoarthritis, rheumatoid arthritis, acute and chronic infections (bacterial and fungal, including diphtheria and pertussis); acute and chronic bronchitis, sinusitis, and upper
20 respiratory infections, including the common cold; acute and chronic gastroenteritis and colitis; acute and chronic cystitis and urethritis; acute and chronic dermatitis; acute and chronic conjunctivitis; acute and chronic serositis (pericarditis, peritonitis, synovitis, pleuritis and tendinitis); uremic pericarditis; acute and chronic cholecystitis; acute and chronic vaginitis; acute and chronic uveitis; drug reactions; insect bites; burns (thermal, chemical, and electrical); and sunburn.

35 Tetracycline compound responsive states also include NO associated states. The term "NO associated state" includes states which involve or are associated with nitric oxide (NO) or inducible nitric oxide synthase (iNOS). NO associated state includes

states which are characterized by aberrant amounts of NO and/or iNOS. Preferably, the NO associated state can be treated by administering tetracycline compounds of the invention, e.g., compounds of formula I, II, III, IV, or otherwise described herein. The disorders, diseases and states described in U.S. Patents Nos. 6,231,894; 6,015,804; 5,919,774; and 5,789,395 are also included as NO associated states. The entire contents of each of these patents are hereby incorporated herein by reference.

Other examples of NO associated states include, but are not limited to, malaria, senescence, diabetes, vascular stroke, neurodegenerative disorders (Alzheimer's disease, Huntington's disease), cardiac disease (reperfusion-associated injury following infarction), juvenile diabetes, inflammatory disorders, osteoarthritis, rheumatoid arthritis, acute and chronic infections (bacterial, viral, and fungal); cystic fibrosis, acute and chronic bronchitis, sinusitis, and respiratory infections, including the common cold; acute and chronic gastroenteritis and colitis; acute and chronic cystitis and urethritis; acute and chronic dermatitis; acute and chronic conjunctivitis; acute and chronic serositis (pericarditis, peritonitis, synovitis, pleuritis and tendinitis); uremic pericarditis; acute and chronic cholecystitis; acute and chronic vaginitis; acute and chronic uveitis; drug reactions; insect bites; burns (thermal, chemical, and electrical); and sunburn.

The term "inflammatory process associated state" also includes, in one embodiment, matrix metalloproteinase associated states (MMPAS). MMPAS include states characterized by aberrant amounts of MMPs or MMP activity. These are also include as tetracycline compound responsive states which may be treated using compounds of the invention, e.g., in formula (I), (II), (III), (IV) or otherwise described herein.

Examples of matrix metalloproteinase associated states ("MMPAS's") include, but are not limited to, arteriosclerosis, corneal ulceration, emphysema, osteoarthritis, multiple sclerosis (Liedtke *et al.*, *Ann. Neurol.* 1998, 44:35-46; Chandler *et al.*, *J. Neuroimmunol.* 1997, 72:155-71), osteosarcoma, osteomyelitis, bronchiectasis, chronic pulmonary obstructive disease, skin and eye diseases, periodontitis, osteoporosis, rheumatoid arthritis, ulcerative colitis, inflammatory disorders, tumor growth and invasion (Stetler-Stevenson *et al.*, *Annu. Rev. Cell Biol.* 1993, 9:541-73; Tryggvason *et al.*, *Biochim. Biophys. Acta* 1987, 907:191-217; Li *et al.*, *Mol. Carcinog.* 1998, 22:84-89), metastasis, acute lung injury, stroke, ischemia, diabetes, aortic or vascular aneurysms, skin tissue wounds, dry eye, bone and cartilage degradation (Greenwald *et al.*, *Bone* 1998, 22:33-38; Ryan *et al.*, *Curr. Op. Rheumatol.* 1996, 8:238-247). Other MMPAS include those described in U.S. Pat. Nos. 5,459,135; 5,321,017; 5,308,839; 5,258,371; 4,935,412; 4,704,383, 4,666,897, and RE 34,656, incorporated herein by reference in their entirety.

In another embodiment, the tetracycline compound responsive state is cancer. Examples of cancers which the tetracycline compounds of the invention may be useful to treat include all solid tumors, i.e., carcinomas e.g., adenocarcinomas, and sarcomas. Adenocarcinomas are carcinomas derived from glandular tissue or in which the tumor cells form recognizable glandular structures. Sarcomas broadly include tumors whose cells are embedded in a fibrillar or homogeneous substance like embryonic connective tissue. Examples of carcinomas which may be treated using the methods of the invention include, but are not limited to, carcinomas of the prostate, breast, ovary, testis, lung, colon, and breast. The methods of the invention are not limited to the treatment of these tumor types, but extend to any solid tumor derived from any organ system. Examples of treatable cancers include, but are not limited to, colon cancer, bladder cancer, breast cancer, melanoma, ovarian carcinoma, prostatic carcinoma, lung cancer, and a variety of other cancers as well. The methods of the invention also cause the inhibition of cancer growth in adenocarcinomas, such as, for example, those of the prostate, breast, kidney, ovary, testes, and colon.

In an embodiment, the tetracycline responsive state of the invention is cancer. The invention pertains to a method for treating a subject suffering or at risk of suffering from cancer, by administering an effective amount of a substituted tetracycline compound, such that inhibition cancer cell growth occurs, i.e., cellular proliferation, invasiveness, metastasis, or tumor incidence is decreased, slowed, or stopped. The inhibition may result from inhibition of an inflammatory process, down-regulation of an inflammatory process, some other mechanism, or a combination of mechanisms. Alternatively, the tetracycline compounds may be useful for preventing cancer recurrence, for example, to treat residual cancer following surgical resection or radiation therapy. The tetracycline compounds useful according to the invention are especially advantageous as they are substantially non-toxic compared to other cancer treatments. In a further embodiment, the compounds of the invention are administered in combination with standard cancer therapy, such as, but not limited to, chemotherapy.

The language "in combination with" another therapeutic agent or treatment includes co-administration of the tetracycline compound and with the other therapeutic agent or treatment, administration of the tetracycline compound first, followed by the other therapeutic agent or treatment and administration of the other therapeutic agent or treatment first, followed by the tetracycline compound. The other therapeutic agent may be any agent which is known in the art to treat, prevent, or reduce the symptoms of a tetracycline responsive state. Furthermore, the other therapeutic agent may be any agent of benefit to the patient when administered in combination with the administration of an tetracycline compound. In one embodiment, the cancers treated

by methods of the invention include those described in U.S. Patent Nos. 6,100,248; 5,843,925; 5,837,696; or 5,668,122, incorporated herein by reference in their entirety.

In another embodiment, the tetracycline compound responsive state is diabetes, e.g., juvenile diabetes, diabetes mellitus, diabetes type I, diabetes type II, diabetic ulcers, or other diabetic complications. In a further embodiment, protein glycosylation is not affected by the administration of the tetracycline compounds of the invention. In another embodiment, the tetracycline compound of the invention is administered in combination with standard diabetic therapies, such as, but not limited to insulin therapy. In a further embodiment, the IPAS includes disorders described in U.S. Patents Nos. 5,929,055; and 5,532,227, incorporated herein by reference in their entirety.

In another embodiment, the tetracycline compound responsive state is a bone mass disorder. Bone mass disorders include disorders where a subjects bones are disorders and states where the formation, repair or remodeling of bone is advantageous. For examples bone mass disorders include osteoporosis (e.g., a decrease in bone strength and density), bone fractures, bone formation associated with surgical procedures (e.g., facial reconstruction), osteogenesis imperfecta (brittle bone disease), hypophosphatasia, Paget's disease, fibrous dysplasia, osteopetrosis, myeloma bone disease, and the depletion of calcium in bone, such as that which is related to primary hyperparathyroidism. Bone mass disorders include all states in which the formation, repair or remodeling of bone is advantageous to the subject as well as all other disorders associated with the bones or skeletal system of a subject which can be treated with the tetracycline compounds of the invention. In a further embodiment, the bone mass disorders include those described in U.S. Patents Nos. 5,459,135; 5,231,017; 5,998,390; 5,770,588; RE 34,656; 5,308,839; 4,925,833; 3,304,227; and 4,666,897, each of which is hereby incorporated herein by reference in its entirety.

In another embodiment, the tetracycline compound responsive state is acute lung injury. Acute lung injuries include adult respiratory distress syndrome (ARDS), post-pump syndrome (PPS), and trauma. Trauma includes any injury to living tissue caused by an extrinsic agent or event. Examples of trauma include, but are not limited to, crush injuries, contact with a hard surface, or cutting or other damage to the lungs.

The invention also pertains to a method for treating acute lung injury by administering a tetracycline compound of the invention.

The tetracycline responsive states of the invention also include chronic lung disorders. The invention pertains to methods for treating chronic lung disorders by administering a tetracycline compound, such as those described herein. The method includes administering to a subject an effective amount of a substituted tetracycline

compound such that the chronic lung disorder is treated. Examples of chronic lung disorders include, but are not limited, to asthma, cystic fibrosis, and emphysema. In a further embodiment, the tetracycline compounds of the invention used to treat acute and/or chronic lung disorders such as those described in U.S. Patents No. 5,977,091; 5 6,043,231; 5,523,297; and 5,773,430, each of which is hereby incorporated herein by reference in its entirety.

In yet another embodiment, the tetracycline compound responsive state is ischemia, stroke, or ischemic stroke. The invention also pertains to a method for treating ischemia, stroke, or ischemic stroke by administering an effective amount of a substituted tetracycline compound of the invention. In a further embodiment, the 10 compounds of the invention are used to treat such disorders as described in U.S. Patents No. 6,231,894; 5,773,430; 5,919,775 or 5,789,395, incorporated herein by reference.

In another embodiment, the tetracycline compound responsive state is a skin wound. The invention also pertains, at least in part, to a method for improving the healing response of the epithelialized tissue (e.g., skin, mucosae) to acute traumatic 15 injury (e.g., cut, burn, scrape, etc.). The method may include using a tetracycline compound of the invention (which may or may not have antibacterial activity) to improve the capacity of the epithelialized tissue to heal acute wounds. The method may increase the rate of collagen accumulation of the healing tissue. The method may also decrease the proteolytic activity in the epithelialized tissue by decreasing the 20 collagenolytic and/or gelatinolytic activity of MMPs. In a further embodiment, the tetracycline compound of the invention is administered to the surface of the skin (e.g., topically). In a further embodiment, the tetracycline compound of the invention is used to treat a skin wound, and other such disorders as described in, for example, U.S. Patent 25 Nos. 5,827,840; 4,704,383; 4,935,412; 5,258,371; 5,308,839; 5,459,135; 5,532,227; and 6,015,804; each of which is incorporated herein by reference in its entirety.

Examples of tetracycline responsive states also include neurological disorders which include both neuropsychiatric and neurodegenerative disorders, but are not limited to, such as Alzheimer's disease, dementias related to Alzheimer's disease (such 30 as Pick's disease), Parkinson's and other Lewy diffuse body diseases, senile dementia, Huntington's disease, Gilles de la Tourette's syndrome, multiple sclerosis, amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy, epilepsy, and Creutzfeldt-Jakob disease; autonomic function disorders such as hypertension and sleep disorders, and neuropsychiatric disorders, such as depression, schizophrenia, schizoaffective disorder, 35 Korsakoff's psychosis, mania, anxiety disorders, or phobic disorders; learning or memory disorders, e.g., amnesia or age-related memory loss, attention deficit disorder, dysthymic disorder, major depressive disorder, mania, obsessive-compulsive disorder, psychoactive substance use disorders, anxiety, phobias, panic disorder, as well as bipolar

affective disorder, *e.g.*, severe bipolar affective (mood) disorder (BP-1), bipolar affective neurological disorders, *e.g.*, migraine and obesity. Further neurological disorders include, for example, those listed in the American Psychiatric Association's Diagnostic and Statistical manual of Mental Disorders (DSM), the most current version of which is incorporated herein by reference in its entirety.

In yet another embodiment, the tetracycline compound responsive state is an aortic or vascular aneurysm in vascular tissue of a subject (*e.g.*, a subject having or at risk of having an aortic or vascular aneurysm, etc.). The tetracycline compound may be effective to reduce the size of the vascular aneurysm or it may be administered to the subject prior to the onset of the vascular aneurysm such that the aneurysm is prevented. In one embodiment, the vascular tissue is an artery, *e.g.*, the aorta, *e.g.*, the abdominal aorta. In a further embodiment, the tetracycline compounds of the invention are used to treat disorders described in U.S. Patent Nos. 6,043,225 and 5,834,449, incorporated herein by reference in their entirety.

Bacterial infections may be caused by a wide variety of gram positive and gram negative bacteria. The compounds of the invention are useful as antibiotics against organisms which may be resistant to other tetracycline compounds. The antibiotic activity of the tetracycline compounds of the invention may be determined using the method discussed in Example 2, or by using the *in vitro* standard broth dilution method described in Waitz, J.A., *National Commission for Clinical Laboratory Standards, Document M7-A2*, vol. 10, no. 8, pp. 13-20, 2nd edition, Villanova, PA (1990). The compounds of the invention may also be used as antiinfectives and have antiparasitic, antiviral, antifungal, and/or antibiotic activities.

The tetracycline compounds of the invention may also be used to treat infections traditionally treated with tetracycline compounds such as, for example, rickettsiae; a number of gram-positive and gram-negative bacteria; and the agents responsible for lymphogranuloma venereum, inclusion conjunctivitis, psittacosis. The tetracycline compounds may be used to treat infections of, *e.g.*, *K. pneumoniae*, *Salmonella*, *E. hirae*, *A. baumannii*, *B. catarrhalis*, *H. influenzae*, *P. aeruginosa*, *E. faecium*, *E. coli*, *S. aureus* or *E. faecalis*. In one embodiment, the tetracycline compound is used to treat a bacterial infection that is resistant to other tetracycline antibiotic compounds. The tetracycline compound of the invention may be administered with a pharmaceutically acceptable carrier.

The language "effective amount" of the compound is that amount necessary or sufficient to treat or prevent a tetracycline compound responsive state. The effective amount can vary depending on such factors as the size and weight of the subject, the type of illness, or the particular compound. For example, the choice of the compound can affect what constitutes an "effective amount". One of ordinary skill in the art would

be able to study the aforementioned factors and make the determination regarding the effective amount of the tetracycline compound without undue experimentation.

The invention also pertains to methods of treatment against microorganism infections and associated diseases. The methods include administration of an effective amount of one or more tetracycline compounds to a subject. The subject can be either a plant or, advantageously, an animal, *e.g.*, a mammal, *e.g.*, a human.

In the therapeutic methods of the invention, one or more tetracycline compounds of the invention may be administered alone to a subject, or more typically a compound of the invention will be administered as part of a pharmaceutical composition in mixture with conventional excipient, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, oral or other desired administration and which do not deleteriously react with the active compounds and are not deleterious to the recipient thereof.

The invention also pertains to pharmaceutical compositions comprising a therapeutically effective amount of a tetracycline compound and, optionally, a pharmaceutically acceptable carrier.

The language "pharmaceutically acceptable carrier" includes substances capable of being coadministered with the tetracycline compound(s), and which allow both to perform their intended function, *e.g.*, treat or prevent a tetracycline responsive state. Suitable pharmaceutically acceptable carriers include but are not limited to water, salt solutions, alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, perfume oil, fatty acid monoglycerides and diglycerides, petroethral fatty acid esters, hydroxymethyl-cellulose, polyvinylpyrrolidone, *etc.* The pharmaceutical preparations can be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously react with the active compounds of the invention.

The tetracycline compounds of the invention that are basic in nature are capable of forming a wide variety of salts with various inorganic and organic acids. The acids that may be used to prepare pharmaceutically acceptable acid addition salts of the tetracycline compounds of the invention that are basic in nature are those that form non-toxic acid addition salts, *i.e.*, salts containing pharmaceutically acceptable anions, such as the hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, isonicotinate, acetate, lactate, salicylate, citrate, acid citrate, tartrate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate and palmoate [*i.e.*, 1,1'-

methylene-bis-(2-hydroxy-3-naphthoate)] salts. Although such salts must be pharmaceutically acceptable for administration to a subject, *e.g.*, a mammal, it is often desirable in practice to initially isolate a tetracycline compound of the invention from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert the
5 latter back to the free base compound by treatment with an alkaline reagent and subsequently convert the latter free base to a pharmaceutically acceptable acid addition salt. The acid addition salts of the base compounds of this invention are readily prepared by treating the base compound with a substantially equivalent amount of the chosen mineral or organic acid in an aqueous solvent medium or in a suitable organic
10 solvent, such as methanol or ethanol. Upon careful evaporation of the solvent, the desired solid salt is readily obtained. The preparation of other tetracycline compounds of the invention not specifically described in the foregoing experimental section can be accomplished using combinations of the reactions described above that will be apparent to those skilled in the art.

15 The preparation of other tetracycline compounds of the invention not specifically described in the foregoing experimental section can be accomplished using combinations of the reactions described above that will be apparent to those skilled in the art.

The tetracycline compounds of the invention that are acidic in nature are capable
20 of forming a wide variety of base salts. The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of those tetracycline compounds of the invention that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmaceutically acceptable cations such as alkali metal cations (*e.g.*, potassium
25 and sodium) and alkaline earth metal cations (*e.g.*, calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines. The pharmaceutically acceptable base addition salts of tetracycline compounds of the invention that are acidic in nature may be formed with pharmaceutically
30 acceptable cations by conventional methods. Thus, these salts may be readily prepared by treating the tetracycline compound of the invention with an aqueous solution of the desired pharmaceutically acceptable cation and evaporating the resulting solution to dryness, preferably under reduced pressure. Alternatively, a lower alkyl alcohol solution of the tetracycline compound of the invention may be mixed with an alkoxide of the
35 desired metal and the solution subsequently evaporated to dryness.

The preparation of other tetracycline compounds of the invention not specifically described in the foregoing experimental section can be accomplished using

combinations of the reactions described above that will be apparent to those skilled in the art.

The compounds of the invention and pharmaceutically acceptable salts thereof can be administered via either the oral, parenteral or topical routes. In general, these compounds are most desirably administered in effective dosages, depending upon the weight and condition of the subject being treated and the particular route of administration chosen. Variations may occur depending upon the species of the subject being treated and its individual response to said medicament, as well as on the type of pharmaceutical formulation chosen and the time period and interval at which such administration is carried out.

The pharmaceutical compositions of the invention may be administered alone or in combination with other known compositions for treating tetracycline responsive states in a subject, *e.g.*, a mammal. Preferred mammals include pets (*e.g.*, cats, dogs, ferrets, *etc.*), farm animals (cows, sheep, pigs, horses, goats, *etc.*), lab animals (rats, mice, monkeys, *etc.*), and primates (chimpanzees, humans, gorillas). The language "in combination with" a known composition is intended to include simultaneous administration of the composition of the invention and the known composition, administration of the composition of the invention first, followed by the known composition and administration of the known composition first, followed by the composition of the invention. Any of the therapeutically composition known in the art for treating tetracycline responsive states can be used in the methods of the invention.

The compounds of the invention may be administered alone or in combination with pharmaceutically acceptable carriers or diluents by any of the routes previously mentioned, and the administration may be carried out in single or multiple doses. For example, the novel therapeutic agents of this invention can be administered advantageously in a wide variety of different dosage forms, *i.e.*, they may be combined with various pharmaceutically acceptable inert carriers in the form of tablets, capsules, lozenges, troches, hard candies, powders, sprays, creams, salves, suppositories, jellies, gels, pastes, lotions, ointments, aqueous suspensions, injectable solutions, elixirs, syrups, and the like. Such carriers include solid diluents or fillers, sterile aqueous media and various non-toxic organic solvents, *etc.* Moreover, oral pharmaceutical compositions can be suitably sweetened and/or flavored. In general, the therapeutically-effective compounds of this invention are present in such dosage forms at concentration levels ranging from about 5.0% to about 70% by weight.

For oral administration, tablets containing various excipients such as microcrystalline cellulose, sodium citrate, calcium carbonate, dicalcium phosphate and glycine may be employed along with various disintegrants such as starch (and preferably corn, potato or tapioca starch), alginic acid and certain complex silicates, together with

granulation binders like polyvinylpyrrolidone, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tableting purposes. Solid compositions of a similar type may also be employed as fillers in gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspensions and/or elixirs are desired for oral administration, the active ingredient may be combined with various sweetening or flavoring agents, coloring matter or dyes, and, if so desired, emulsifying and/or suspending agents as well, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

For parenteral administration (including intraperitoneal, subcutaneous, intravenous, intradermal or intramuscular injection), solutions of a therapeutic compound of the present invention in either sesame or peanut oil or in aqueous propylene glycol may be employed. The aqueous solutions should be suitably buffered (preferably pH greater than 8) if necessary and the liquid diluent first rendered isotonic. These aqueous solutions are suitable for intravenous injection purposes. The oily solutions are suitable for intraarticular, intramuscular and subcutaneous injection purposes. The preparation of all these solutions under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art. For parenteral application, examples of suitable preparations include solutions, preferably oily or aqueous solutions as well as suspensions, emulsions, or implants, including suppositories. Therapeutic compounds may be formulated in sterile form in multiple or single dose formats such as being dispersed in a fluid carrier such as sterile physiological saline or 5% saline dextrose solutions commonly used with injectables.

Additionally, it is also possible to administer the compounds of the present invention topically when treating inflammatory conditions of the skin. Examples of methods of topical administration include transdermal, buccal or sublingual application. For topical applications, therapeutic compounds can be suitably admixed in a pharmacologically inert topical carrier such as a gel, an ointment, a lotion or a cream. Such topical carriers include water, glycerol, alcohol, propylene glycol, fatty alcohols, triglycerides, fatty acid esters, or mineral oils. Other possible topical carriers are liquid petrolatum, isopropylpalmitate, polyethylene glycol, ethanol 95%, polyoxyethylene monolaurate 5% in water, sodium lauryl sulfate 5% in water, and the like. In addition, materials such as anti-oxidants, humectants, viscosity stabilizers and the like also may be added if desired.

For enteral application, particularly suitable are tablets, dragees or capsules having talc and/or carbohydrate carrier binder or the like, the carrier preferably being lactose and/or corn starch and/or potato starch. A syrup, elixir or the like can be used

wherein a sweetened vehicle is employed. Sustained release compositions can be formulated including those wherein the active component is protected with differentially degradable coatings, *e.g.*, by microencapsulation, multiple coatings, *etc.*

5 In addition to treatment of human subjects, the therapeutic methods of the invention also will have significant veterinary applications, *e.g.* for treatment of livestock such as cattle, sheep, goats, cows, swine and the like; poultry such as chickens, ducks, geese, turkeys and the like; horses; and pets such as dogs and cats. Also, the compounds of the invention may be used to treat non-animal subjects, such as plants.

10 It will be appreciated that the actual preferred amounts of active compounds used in a given therapy will vary according to the specific compound being utilized, the particular compositions formulated, the mode of application, the particular site of administration, *etc.* Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art using conventional dosage determination tests conducted with regard to the foregoing guidelines.

15 In general, compounds of the invention for treatment can be administered to a subject in dosages used in prior tetracycline therapies. See, for example, the *Physicians' Desk Reference*. For example, a suitable effective dose of one or more compounds of the invention will be in the range of from 0.01 to 100 milligrams per kilogram of body weight of recipient per day, preferably in the range of from 0.1 to 50 milligrams per
20 kilogram body weight of recipient per day, more preferably in the range of 1 to 20 milligrams per kilogram body weight of recipient per day. The desired dose is suitably administered once daily, or several sub-doses, *e.g.* 2 to 5 sub-doses, are administered at appropriate intervals through the day, or other appropriate schedule.

25 It will also be understood that normal, conventionally known precautions will be taken regarding the administration of tetracyclines generally to ensure their efficacy under normal use circumstances. Especially when employed for therapeutic treatment of humans and animals *in vivo*, the practitioner should take all sensible precautions to avoid conventionally known contradictions and toxic effects. Thus, the conventionally recognized adverse reactions of gastrointestinal distress and inflammations, the renal
30 toxicity, hypersensitivity reactions, changes in blood, and impairment of absorption through aluminum, calcium, and magnesium ions should be duly considered in the conventional manner.

Furthermore, the invention also pertains to the use of a compound of formula I, II, III, IV, or otherwise described herein for the preparation of a medicament. The
35 medicament may include a pharmaceutically acceptable carrier and the compound is an effective amount, *e.g.*, an effective amount to treat a tetracycline responsive state.

EXEMPLIFICATION OF THE INVENTION

Compounds of the invention may be made as described below, with modifications to the procedure below within the skill of those of ordinary skill in the art.

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Example 1: Synthesis of 9-Aminomethyl Minocycline and derivatives thereof

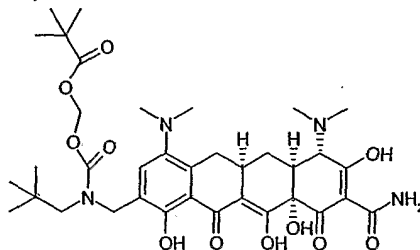
Trifluoroacetic acid (1L) was charged into a 2L flask under argon and tetracycline. HCl (200g, 1 eq) and N-hydroxymethylphthalimide (100g) were added to the flask while stirring. Once the entire solid dissolved, H₂SO₄ (200 mL) was added to the reaction. The reaction was heated to 40-50°C for 5-6 hours. N-hydroxymethylamine (100g) was added portionwise. When HPLC analysis confirmed that all the starting material was converted to 2,9-bis-aminomethylphthalimidominocycline, the mixture was precipitated out of 4 L of acetone. An exotherm of 15-20°C was observed. After 1 hour of stirring, the solid was filtered, washed with acetone (200 ml), and dried with the aid of a latex rubber dam. The solid was reslurried in a methanol (1L)/t-BME (2L) mixture and the pH was adjusted to 3 using triethylamine. The solid was filtered and washed with 50 mL of methanol. The yield was 97% of 2,9-bis-aminomethylphthalimidetetracycline.

2,9-bis-aminomethylphthalimideminocycline (100g) was suspended in 2M solution of methylamine in methanol (10 eq). The reaction was stirred at room temperature for 2-3 hours, at which point HPLC analysis confirmed total conversion of the starting material to 2,9-bis aminomethyltetracycline. The reaction mixture was poured into t-BME (5 volumes), and stirred for thirty minutes. Next, the suspension was filtered and washed with t-BME (200 mL) to isolate the desired product, 2,9-bis-aminomethyltetracycline.

2,9-bis-aminomethylminocycline (40g) was slurried in 200 mL water/methanol 1/9 and the pH was adjusted to 3 by the dropwise addition of trifluoroacetic acid. The mixture was heated to 40°C for 1-2 hours. When HPLC analysis confirmed the hydrolysis of 2,9-bis-aminomethylminocycline to 9-aminomethyltetracycline, the reaction was allowed to return to room temperature and the pH was adjusted to 7 using triethylamine. Isopropyl alcohol (200 mL) was added to precipitate out the solid. The product was filtered and washed with 50 mL IPA followed by 100 mL diethyl ether and dried under reduced pressure to isolate 9-aminomethylminocycline.

9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline dihydrochloride

9-dimethylaminomincycline (200 mg, 1 eq.), DMF, and trimethylacetaldehyde (45 μ l, 1 eq.) were combined in 40 mL flasks and stirred. Triethylamine (150 μ L, 3 eq.) was then added. After stirring at room temperature for several minutes, NaBH(OAc)₃ (175 mg, 2 eq.) and InCl₃ (9 mg, 0.1 eq.) was added. After one hour, the reactions were clear and red. Liquid chromatography showed a single product for the reaction. The reaction was quenched with methanol, the solvent was removed, and the product was purified using column chromatography.

Example 2: Synthesis of N-t-Butylcarbonyloxymethyl (9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline) carbamateThiocarbonic acid O-chloromethyl ester S-ethyl ester

To a solution of chloromethylchloroformate (32ml, 0.36mol) in dry ether (663ml) under an argon atmosphere, was added ethane thiol (27ml, 0.36mol) and triethyl amine (51ml, 0.36mol) in ether (147ml) at 0°C with stirring. After having added all of the ethane thiol and triethyl amine, the mixture was allowed to warm to room temperature and was stirred for an additional 2 hours. The mixture was then cooled over an ice bath and the solid was removed by filtration. The filtrate was concentrated yielding 55 grams of product which was used without further purification.

Thiocarbonic acid O-iodomethyl ester S-ethyl ester

The crude thiocarbonic acid O-chloromethyl ester S-ethyl ester (5g, 32.5mmol) was added to a solution of sodium iodide (7.3g, 48.7mmol) and stirred for 3 hours at 40 °C. The resulting solid was removed by filtration, and the filtrate was concentrated. The residue was partitioned at 0 °C between hexane and water (3:1, 100ml:30ml). The organic layer was separated, washed with 5% NaHCO₃, 1% Na₂S₂O₃ until colorless, and then it was washed with water. The organic layer was dried over MgSO₄. It was then filtered and concentrated, yielding 7 grams of crude material.

2,2-Dimethyl propionic acid ethylsulfanylcarbonyl oxymethyl ester

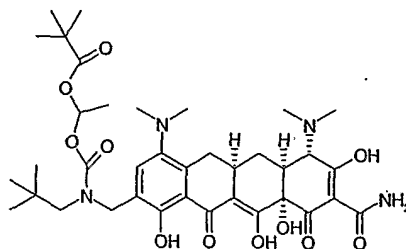
A mixture of NaHCO_3 (3.21g, 38.2mmol), Bu_4NHSO_4 (6.49g, 19.1mmol), BuCO_2H (1.95g, 19.1mmol), water (38.5ml) and dichloromethane (38.5ml) is stirred at room temperature for 1 hour. A solution of crude thiocarbonic acid O-iodomethyl ester S-ethyl ester (3.5g, 14.26mmol) in dichloromethane (7ml) was added over a 0.5 hour period and the temperature was maintained below 30°C . The mixture was then stirred for further 1.5 hours at room temperature. The organic layer was separated, washed with water twice, dried over Na_2SO_4 , and concentrated. The residue was stirred in ether for 16 hours, filtered, and washed with ether. The filtrate was then concentrated yielding 2g of product.

t-Butylcarbonyloxymethyl chloroformate

Sulfuryl chloride (0.68ml, 8.4mmol) was added to the 2,2-dimethyl propionic acid ethylsulfanylcarbonyl oxymethyl ester (1.84g, 8.4mmol) at $0-5^\circ\text{C}$ with stirring over 5 minutes. The solution was then stirred for another 45 minutes at room temperature. EtSCl was then removed by distillation at room temperature. The reagent was used without further purification.

N-t-Butylcarbonyloxymethyl (9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline) carbamate

To a mixture of 9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline (0.1g, 0.15mmol), NaHCO_3 (63mg, 0.75mmol) in water (1ml) and dichloromethane (20ml) was added the chloroformate (44mg, 0.225mmol) above. The reaction was monitored by HPLC. After the reaction was finished, the solvents were distilled off. The residue was redissolved in acetonitrile and purified by HPLC (C_{18} , MeCN-Water). The reaction yielded 25mg of product.

Example 3: Synthesis of N-t-Butylcarbonyloxy(1-ethyl) (9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline) carbamate

Thio carbonic acid O-isopropenyl ester S-ethyl ester

To a solution of isopropenylchloroformate (5g, 41.5mmol) in dry ether (70ml) under argon, was added ethane thiol (3.1ml, 41.5mmol) and triethyl amine (5.77ml, 41.5mmol) in ether (20ml) at 0°C with stirring. The mixture was then allowed to warm to room temperature and was stirred for an additional 2 hours. The mixture was then cooled over an ice bath. The solid was filtered off and the filtrate was concentrated, yielding 4.9g of product which was used without further purification.

10 Thio carbonic acid O-(1-chloroethyl) ester S-ethyl ester

A solution of the ester (4.8g, 33mmol) in ether-chloroform (100ml:100ml) was bubbled with HCl gas over an ice bath for 40 minutes. The solution was then allowed to warm to room temperature over night. Excessive HCl was removed by bubbling argon through the solution for 30 minutes. The solution was then concentrated and used without further purification.

2,2-Dimethyl propionic acid ethylsulfanylcarbonyl oxy(1-ethyl) ester

A mixture of pivalic acid (24g, 0.238mol), Hg(OAc)₂ (22.7g, 71.4mmol) and thio carbonic acid O-(1-chloroethyl) ester S-ethyl ester (4g, 23.8mmol) in dichloromethane (300ml) was stirred at room temperature for 48 hours. 100ml of water added and the organic layer was separated, washed with 0.5N NaOH till pH>7, brine, water, then dried over Na₂SO₄, filtered, and concentrated. The residue was then purified by silica gel (EtOAc:Hexane, 1:20), yielding 3 grams of product.

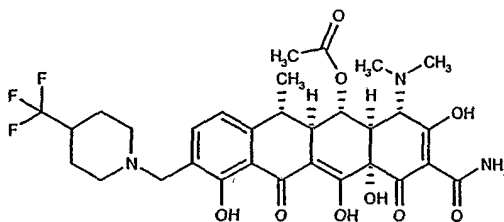
25 t-Butylcarbonyloxy(1-ethyl) chloroformate

Sulfuryl chloride (0.61ml, 7.5mmol) was added to the thio ester (1.47g, 6.28mmol) at 0-5 °C with stirring over 5 minutes. After all the reagent was added, the solution was stirred for another 45 minutes at room temperature. EtSCl was then removed by distillation at room temperature. The product was used in the next step without further purification.

N-t-Butylcarbonyloxy(1-ethyl) (9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline) carbamate

The chloroformate (0.45g, 2.16 mmol) was added to a mixture of 9-[(2,2, dimethyl-propyl amino)-methyl]-minocycline (0.6g, 1mmol), NaHCO₃ (0.42g, 2.7mmol) in water (6ml) and dichloromethane (60ml). Upon completion of the reaction, the solvents were removed by distillation. The residue was redissolved in acetonitrile and purified by HPLC (C18, MeCN-Water) yielding 100mg of product.

Example 4: Synthesis of 5-Acetyl-9-(4-Trifluoromethyl-Piperidin-1-ylmethyl)-Doxycycline



5 To a solution of 9-formyl doxycycline (472 mg, 1 mmol) in 30 mL of DMF under an argon atmosphere, indium trichloride (22 mg, 0.1 mmol) and trifluoromethylpiperidine HCl (379 mg, 2 mmol) were added. The pH of the reaction mixture was adjusted to 8.5 by adding triethylamine. The reaction mixture was then stirred at room temperature. The reaction was monitored by using HPLC/LCMS, and
10 was completed in 4 hours. The solvent was then evaporated and the crude material obtained was then precipitated using diethyl ether/ MeOH (100/10 mL). Filtration of the precipitate gave a yellow powder, which was used for the next step without further purification.

To a solution of 9-(4-trifluoromethyl-piperidin-1-ylmethyl)-doxycycline (300
15 mg) in 3 mL of glacial acetic acid, liquid HF (2 mL) was added at 0 °C. Reaction mixture was then left in a fume hood for 24 h. Completion of the reaction was confirmed by HPLC/LCMS. Excess HF was removed by flushing the reaction mixture with air. The crude material obtained was purified using preparative HPLC to afford the desired compound. LC-MS (M+1 650)

20

Example 5: *In vitro* Minimum Inhibitory Concentration (MIC) Assay

The following assay is used to determine the efficacy of compounds against common bacteria. 2 mg of each compound is dissolved in 100 µl of DMSO. The solution is then added to cation-adjusted Mueller Hinton broth (CAMHB), which results
25 in a final compound concentration of 200 µg per mL. The compound solutions are diluted to 50 µL volumes, with a test compound concentration of .098 µg/mL. Optical density (OD) determinations are made from fresh log-phase broth cultures of the test strains. Dilutions are made to achieve a final cell density of 1×10^6 CFU/mL. At OD=1, cell densities for different genera should be approximately:

30	<i>E. coli</i>	1×10^9 CFU/mL
	<i>S. aureus</i>	5×10^8 CFU/mL
	<i>Enterococcus sp.</i>	2.5×10^9 CFU/mL

50 µl of the cell suspensions are added to each well of microtiter plates. The final cell density should be approximately 5×10^5 CFU/mL. These plates are incubated at 35°C in

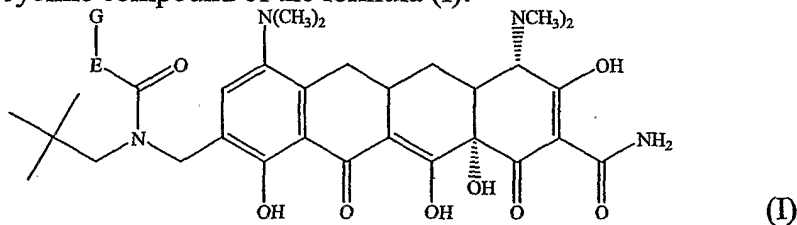
an-ambient air incubator for approximately 18 hr. The plates are read with a microplate reader and are visually inspected when necessary. The MIC is defined as the lowest concentration of the compound that inhibits growth.

5 EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of the present invention and are covered by the following claims. The contents of all references, patents, and
10 patent applications cited throughout this application are hereby incorporated by reference. The appropriate components, processes, and methods of those patents, applications and other documents may be selected for the present invention and embodiments thereof.

CLAIMS

1. A tetracycline compound of the formula (I):



wherein

E is oxygen, nitrogen, or a covalent bond;

G is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; alkoxyalkoxy carbonyloxyalkyl, and pharmaceutically acceptable salts thereof.

2. The tetracycline compound of claim 1, wherein E is a covalent bond.

3. The tetracycline compound of claim 2, wherein G is alkyl.

4. The tetracycline compound of claim 1, wherein E is nitrogen.

5. The tetracycline compound of claim 4, wherein G is aryl.

6. The tetracycline compound of claim 5, wherein G is substituted phenyl.

7. The tetracycline compound of claim 1, wherein E is oxygen.

8. The tetracycline compound of claim 7, wherein G is alkylcarbonyloxyalkyl.

9. The tetracycline compound of claim 8, wherein G is of the formula $-(CH_2)_g-O-(C=O)-R^1$, wherein g is 1-5 and R^1 is alkyl.

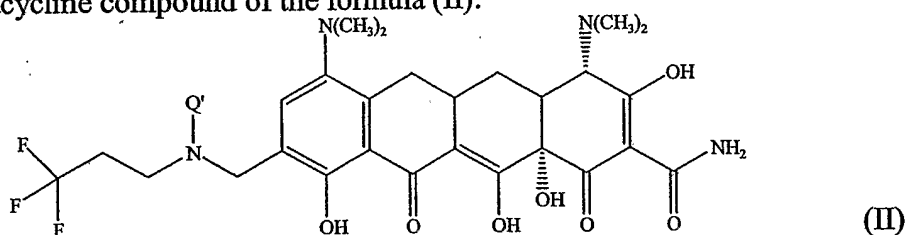
10. The tetracycline compound of claim 9, wherein g is 1.

11. The tetracycline compound of claim 10, wherein R^1 is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, $-(CH_2)_{10}-CH_3$, or $-(CH_2)_{11}CH_3$.

12. The tetracycline compound of claim 10, wherein R^1 is cycloalkyl.

13. The tetracycline compound of claim 9, wherein g is 2.
14. The tetracycline compound of claim 13, wherein R¹ is methyl, ethyl, propyl,
5 butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, -(CH₂)₁₀-CH₃, or -(CH₂)₁₁CH₃.
15. The tetracycline compound of claim 7, wherein G is alkyl.
16. The tetracycline compound of claim 14, wherein G is methyl, ethyl, propyl,
10 butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, -(CH₂)₁₀-CH₃, or -(CH₂)₁₁CH₃.
17. The tetracycline compound of claim 7, wherein G is arylcarbonyloxyalkyl.
18. The tetracycline compound of claim 17, wherein G is of the formula: -(CH₂)_f-O-
15 (C=O)-R², wherein f is 1-5 and R² is aryl.
19. The tetracycline compound of claim 18, wherein f is 1.
20. The tetracycline compound of claim 19, wherein R² is substituted or
20 unsubstituted phenyl.
21. The tetracycline compound of claim 20, wherein said phenyl is substituted with one or more substituents selected from the group consisting of halogen, alkoxy, or alkyl.
22. The tetracycline compound of claim 7, wherein G is alkyloxycarbonyloxyalkyl.
25
23. The tetracycline compound of claim 22, where G is of the formula -(CH₂)-O-(C=O)-O-R³, wherein R³ is alkyl.
24. The tetracycline compound of claim 23, wherein R³ is methyl, ethyl, propyl,
30 butyl or pentyl.
25. The tetracycline compound of claim 7, wherein G is arylalkylcarbonyloxyalkyl.
26. The tetracycline compound of claim 25, wherein G is of the formula -(CH₂)-O-(C=O)-(CH₂)_h-R⁴, wherein h is 1-5, and R⁴ is aryl.
35
27. The tetracycline compound of claim 26, wherein h is 1 or 2.

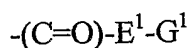
28. The tetracycline compound of claim 27, wherein R^4 is phenyl.
29. The tetracycline compound of claim 7, wherein G is
 5 alkyloxyalkylcarbonyloxyalkyl.
30. The tetracycline compound of claim 29, wherein G is of the formula $-(CH_2)_i-O-(C=O)-(CH_2)_i-O-R^5$, wherein i is 1-5, and R^5 is alkyl.
- 10 31. The tetracycline compound of claim 30, wherein i is 1, 2, or 3.
32. The tetracycline compound of claim 31, wherein R^5 is methyl.
33. The tetracycline compound of claim 7, wherein G is
 15 alkoxyalkoxyalkylcarbonyloxyalkyl.
34. The tetracycline compound of claim 33, wherein G is of the formula of the
 formula $-(CH_2)_i-O-(C=O)-(CH_2)_j-O-(CH_2)_k-O-R^6$, wherein j and k are each 1-5, and R^6
 is alkyl.
- 20 35. The tetracycline compound of claim 34, wherein j is 1 and k is 2.
36. The tetracycline compound of claim 35, wherein R^6 is methyl.
- 25 37. The tetracycline compound of claim 7, wherein G is heterocyclic alkyl.
38. A tetracycline compound of the formula (II):



wherein

- 30 Q' is a prodrug moiety and pharmaceutically acceptable salts thereof.

39. The tetracycline compound of claim 38, wherein Q' is of the formula



wherein

E^1 is oxygen, nitrogen, or a covalent bond;

G^1 is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxy carbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; or alkoxyalkoxy carbonyloxyalkyl.

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40. The tetracycline compound of claim 39, wherein E^1 is oxygen.

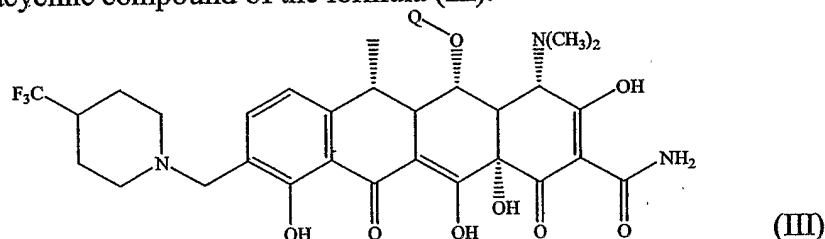
41. The tetracycline compound of claim 40, wherein G^1 is alkylcarbonyloxyalkyl.

10 42. The tetracycline compound of claim 41, wherein G^1 is of the formula $-(CH_2)_m-O-(C=O)-R^7$, wherein m is 1-5 and R^7 is alkyl.

43. The tetracycline compound of claim 42, wherein m is 1.

15 44. The tetracycline compound of claim 43, wherein R^7 is methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, $-(CH_2)_{10}-CH_3$, or $-(CH_2)_{11}CH_3$.

45. A tetracycline compound of the formula (III):



20

wherein:

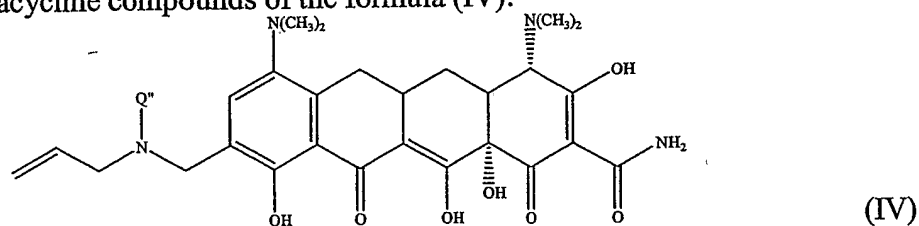
Q is a prodrug moiety, and pharmaceutically acceptable salts thereof.

46. The tetracycline compound of claim 45, wherein Q is $-(C=O)-G^2$.

25

47. The tetracycline compound of claim 45, wherein G^2 is alkyloxy carbonylalkyl or alkyl.

48. A tetracycline compounds of the formula (IV):



30

wherein

Q'' is a prodrug moiety and pharmaceutically acceptable salts thereof.

49. The tetracycline compound of claim 48, wherein Q'' is of the formula



wherein

E^3 is oxygen, nitrogen, or a covalent bond;

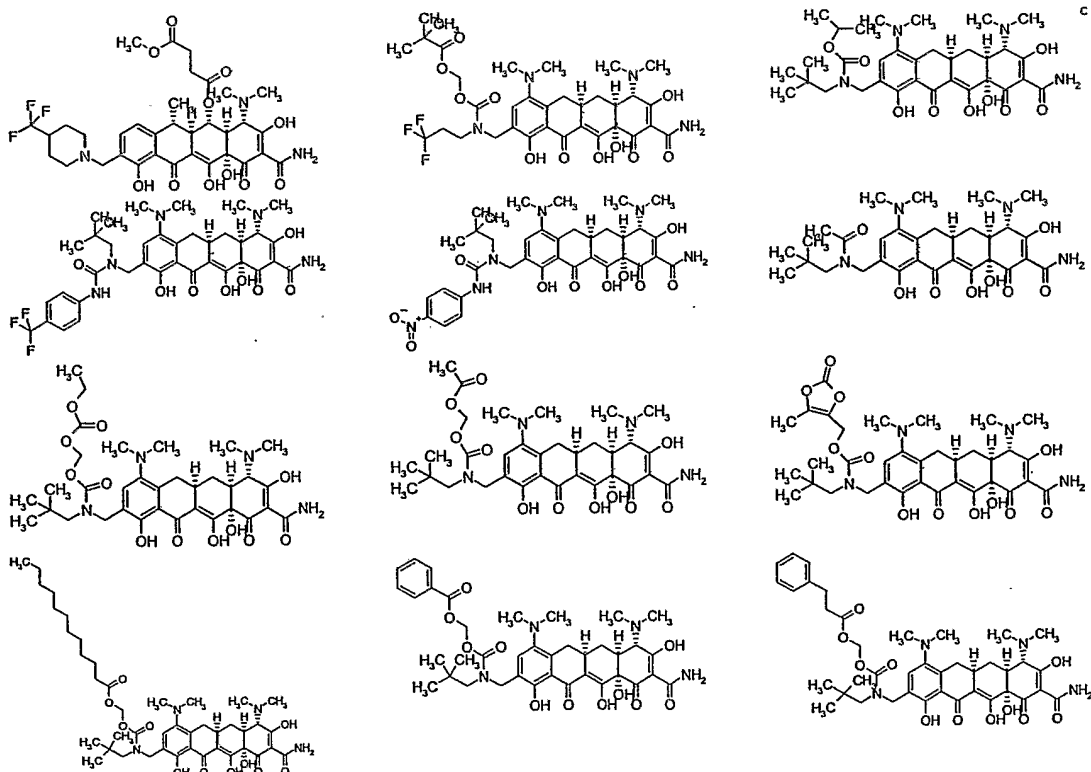
G^3 is alkyl; heterocyclicalkyl; aryl; alkylcarbonyloxyalkyl; arylcarbonyloxyalkyl; alkyloxycarbonyloxyalkyl; arylalkylcarbonyloxyalkyl; alkyloxyalkylcarbonyloxyalkyl; 10 or alkoxyalkoxycarbonyloxyalkyl.

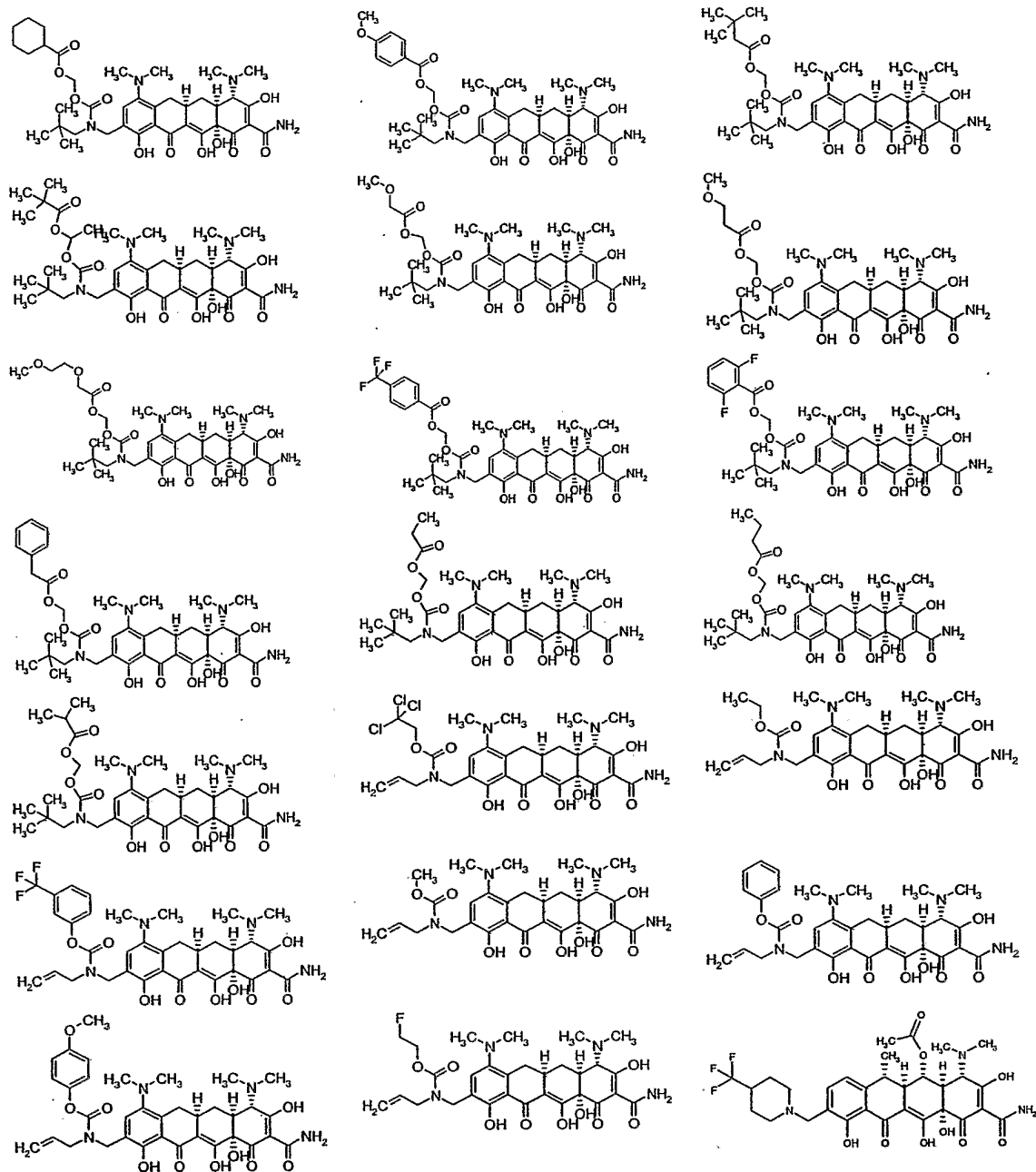
50. The tetracycline compound of claim 49, wherein E^3 is oxygen.

51. The tetracycline compound of claim 49, wherein G^3 is substituted or 15 unsubstituted alkyl.

52. The tetracycline compound of claim 49, wherein G^3 is substituted or unsubstituted aryl.

20 53. A tetracycline compound selected from the group consisting of:



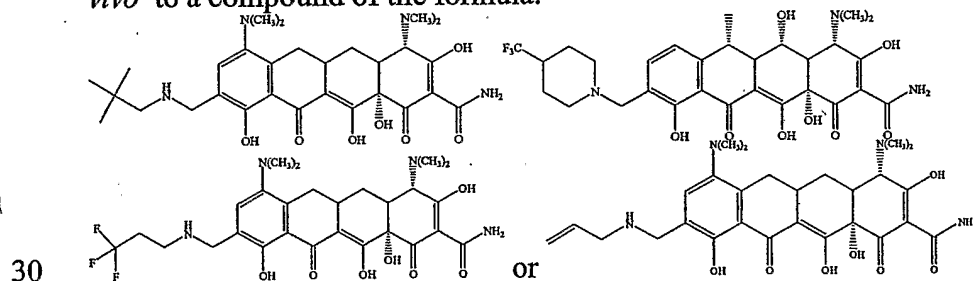


and pharmaceutically acceptable salts thereof.

54. A method for treating a tetracycline responsive state in a subject, comprising administering to said subject an effective amount of a tetracycline compound of any one of claims 1, 38, 45, 48, or 53, such that said subject is treated.

55. The method of claim 54, wherein said tetracycline responsive state is a bacterial infection, a viral infection, or a parasitic infection.

56. The method of claim 55, wherein said bacterial infection is associated with *E. coli*.
57. The method of claim 55, wherein said bacterial infection is associated with *S. aureus*.
58. The method of claim 55, wherein said bacterial infection is associated with *E. faecalis*.
59. The method of claim 54, wherein said bacterial infection is resistant to other tetracycline antibiotics.
60. The method of claim 55, wherein said bacterial infection is a gram positive bacterial infection.
61. The method of claim 55, wherein said bacterial infection is a gram negative bacterial infection.
62. The method of claim 54, wherein said subject is a human.
63. The method of claim 54, wherein said tetracycline compound is administered with a pharmaceutically acceptable carrier.
64. The method of claim 54, wherein said tetracycline compound is metabolized *in vivo*.
65. The method of claim 64, wherein said tetracycline compound is metabolized *in vivo* to a compound of the formula:



66. A pharmaceutical composition comprising a therapeutically effective amount of a tetracycline compound of any one of claims 1, 38, 45, 48, or 53 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/US2004/020305

 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07C237/26 A61K31/65

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 7 C07C

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 practical, search terms used)

EPO-Internal, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/04406 A (TUFTS COLLEGE ; PARATEK PHARMACEUTICALS INC (US)) 17 January 2002 (2002-01-17) in particular compound PE on page 43 in combination with claim 1 and definition of a prodrug moiety as provided on page 21, lines 1-16. the whole document	1-66
X,P	WO 03/075857 A (PARATEK PHARMACEUTICALS INC) 18 September 2003 (2003-09-18) prodrugs: cf. page 25, last paragraph table 1, pages 13 and 15 the whole document	1-3,7-9, 13,14, 53-66
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document 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.
- *G* document member of the same patent family

Date of the actual completion of the international search

21 December 2004

Date of mailing of the international search report

29/12/2004

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Seelmann, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/020305

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2004/038000 A (PARATEK PHARMACEUTICALS INC) 6 May 2004 (2004-05-06) in particular compounds cited in table 2 on pages 39 (last one), 40, 43-44, 73, 77 the whole document -----	1,7-11, 13-15, 48-66
X,P	WO 2004/038001 A (PARATEK PHARMACEUTICALS INC) 6 May 2004 (2004-05-06) in particular compounds on pages 40,41, 44, 45, 78 definition of prodrug moiety on page 94, lines 8-24 the whole document -----	1,7-11, 13-15, 48-66

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/020305

Box 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:

Although claims 54-65 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compounds of claims 1, 38, 45, 48 or 53.
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.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box 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 an additional fee, this Authority did not invite payment of any additional fee.
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.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/020305

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			US 2004214800 A1	28-10-2004
WO 2004038001	A	06-05-2004	WO 2004038001 A2	06-05-2004
			US 2004242548 A1	02-12-2004