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(54) Title: METHODS OF PREPARING SUBSTITUTED TETRACYCLINES WITH TRANSITION METAL-BASED CHEMISTRIES

(57) Abstract: The present invention relates to novel chemistries which allow for heretofore unobtainable substituted tetracycline compounds which exhibit significant antibacterial activity. The methods disclosed herein utilize reactive tetracycline-based precursor compounds, reactive organic substituent precursors and transition metal catalysts under conditions such that a tetracycline compound substituted with the desired organic substituent is formed. In one embodiment of the invention, a substituted tetracycline compound may be prepared by combining a reactive tetracycline-based precursor compound such as an arene tetracycline diazonium salt, and a reactive organic substituent precursor, e.g., alkenes, substituted alkenes, vinyl monomers, aromatics and heteroaromatics, in the presence of a transition metal catalyst, such as palladium chloride, under conditions such that a tetracycline compound substituted with the organic substituent is formed. Such compounds may optionally act as intermediates for making other compounds, e.g., hydrogenation of unsaturated groups on the substituent.

METHODS OF PREPARING SUBSTITUTED TETRACYCLINES WITH TRANSITION METAL-BASED CHEMISTRIES

RELATED APPLICATIONS

This application is related to copending U.S. Provisional Application No(s). 60/154,701, filed on September 14, 1999, and U.S. Provisional Application, Attorney Docket No. PKZ-018-2, filed September 12, 2000, the entire contents of both of which are 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 detailed elucidation of the chemical structure of these agents confirmed their similarity and furnished the analytical basis for the production of a third member of this group in 1952, tetracycline. By 1957, a new family of tetracycline compositions characterized chemically by the absence of the position 6 ring-attached OH group present in the earlier compositions was prepared and became publicly available in 1967; and minocycline was in use by 1972. Individual tetracycline-type agents are structurally compared within Table I below, with reference made the following structural formula:

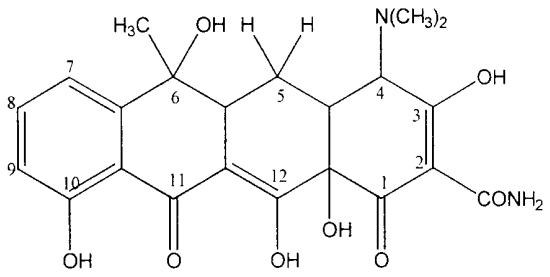


Table I

Congener	Substituent(s)	At Carbon Position Nos.
Chlortetracycline	-Cl	(7)
Oxytetracycline	-OH,-H	(5)
Demeclocycline	-OH,-H;-Cl	(6;7)
Methacycline	-OH,-H;=CH_2	(5;6)
Doxycycline	-OH,-H;-CH_3,-H	(5;6)
Minocycline	-H,-H;-$N(CH_3)_2$	(6;7)

More recent research efforts have focused on developing new tetracycline antibiotic compositions effective under varying therapeutic conditions and routes of administration; and for developing new tetracycline analogues which might prove to be equal or more effective then the originally introduced tetracycline families beginning in 1948. Representative of such developments include U.S. Patent Nos. 3,957,980; 3,674,859; 2,980,584; 2,990,331; 3,062,717; 3,557,280; 4,018,889; 4,024,272; 4,126,680; 3,454,697; and 3,165,531. It will be understood that these issued patents are merely representative of the range of diversity of investigations seeking tetracycline and tetracycline analogue compositions which are pharmacologically active.

Historically, soon after their initial development and introduction, the tetracyclines, regardless of specific formulation or chemical structure, 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 - as for example *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.

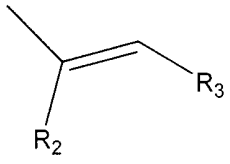
SUMMARY OF THE INVENTION

The present invention relates to novel chemistries which allow for the production of substituted tetracycline compounds including substituted tetracycline compounds which exhibit

significant antibacterial activity. The methods disclosed herein utilize reactive tetracycline-based precursor compounds, reactive organic substituent precursors and transition metals or transition metal catalysts under conditions such that a tetracycline compound substituted with the desired organic substituent is formed. In one embodiment of the invention, a substituted tetracycline compound may be prepared by combining a reactive tetracycline-based precursor compound such as an arene tetracycline diazonium salt, and a reactive organic substituent precursor, e.g., alkenes, substituted alkenes, vinyl monomers, aromatics and heteroaromatics, in the presence of a transition metal catalyst, such as palladium chloride, under conditions such that a tetracycline compound substituted with the organic substituent is formed. In another embodiment, a substituted tetracycline compound may be prepared by contacting a reactive tetracycline chemical complex comprising a reactive tetracycline-based precursor compound and a transition metal or transition metal catalyst forming a reactive chemical intermediate with a reactive organic substituent precursor under conditions such that a tetracycline compound substituted with the organic substituent is formed.

The invention relates in another embodiment to reactive tetracycline chemical complexes comprising a reactive tetracycline-based precursor compound and a transition metal catalyst forming a chemical intermediate, which can advantageously be used in the methods of the invention.

In yet another embodiment substituted tetracycline analogs are disclosed, wherein the substituent (denoted herein as "Z") at the desired position, e.g., 7, 9, 13, is connected with a -C-C- linkage, and wherein the substituent comprises an aromatic or heteroaromatic moiety. The substituent may also comprise a -C=C- bond adjacent to the -C-C- linkage, e.g.,



(Z)

,

wherein R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, aryloxycarbonyl; or R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring having 5 to 15 atoms in the ring.

The methods and chemical intermediates disclosed herein allow for novel substituted tetracycline-type compounds and therapeutic methods and pharmaceutical compositions that comprise such compounds.

The method of the invention includes providing Z substituents, above, on the basic tetracycline ring structure through a process involving forming a reactive intermediate (comprising a tetracycline arenediazonium salt in a preferred embodiment) at the desired position and adding a reactive compound, e.g., a π-bond containing compound in the presence of a transition metal catalyst to that position. The reactive intermediate may be formed *in situ.* In an advantageous embodiment such substituents are provided on the D ring of the basic tetracycline ring structure, e.g., positions 7 and/or 9. In another advantageous embodiment, such substitutions may be made at position 13. Such synthetic schemes are heretofore new in this art and advantageously allow for direct substitution of different and/or heretofore complex substituent groups at desired positions.

Compounds of the invention are active against susceptible microorganisms, including tetracycline-sensitive bacteria as well as tetracycline-resistant bacteria. Particularly preferred compounds of the invention exhibit 24-hr minimum inhibitory concentration (MIC) values of about 10 μg/mL or less, more preferably about 1 μg/mL or less, against tetracycline-resistant *E. coli, S. aureus* and *E. faecalis* strains such as *E. coli* pHCM1, *S. aureus* RN4250 and *E. faecalis* pMV158. Preferred compounds of the invention also include those that exhibit such MIC values against tetracycline-sensitive *E. coli, S. aureus* and *E. faecalis* strains such as *E. coli* D31m4, *S. aureus* RN450 and *E. faecalis* ATCC9790.

The invention provides methods of treatment against susceptible microorganisms such as bacteria, fungi, rickettsia, parasites and the like, and diseases associated with such microorganisms. These therapeutic methods in general comprise administration of a therapeutically effective amount of one or more compounds of the invention to a living subject that is suffering from or susceptible to infection by a susceptible microorganism such as bacteria, fungi, rickettsia and the like. Suitable subjects for treatment include animals, particularly a mammal such as human, or plants.

Pharmaceutical compositions comprising one or more compounds of the invention and a suitable carrier are also provided.

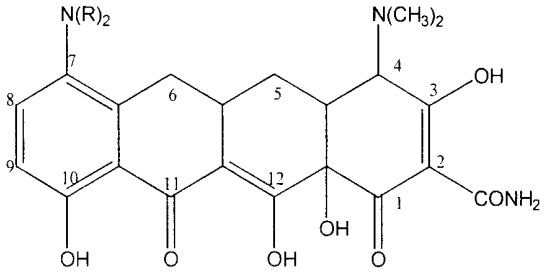
DETAILED DESCRIPTION OF THE INVENTION

The present invention will be more fully illustrated by reference to the definitions set forth below.

"Tetracycline" or "tetracycline-type" is intended to include tetracycline and other tetracycline family members such as oxytetracycline; chlortetracycline; demeclocycline; doxycycline; chelocardin; minocycline; rolitetracycline; lymecycline; sancycline; methacycline; apicycline; clomocycline; guamecycline; meglucycline; mepylcycline; penimepicycline; pipacycline; etamocycline; penimocycline, etc. as well as other tetracycline compounds having the characteristic naphthacene A-B-C-D ring structure noted in the Background Of The Invention. Additionally, numbered tetracycline ring positions as referred to herein are the same as designated in the above structural formula.

"Reactive tetracycline-based precursor compound" or "RT-based precursor compound" includes tetracyclines which have a reactive position on the tetracycline ring structure, e.g., at 7, 9 or 13, such that substitution of the reactive tetracycline-based precursor compound may be accomplished as disclosed herein to form a substituted tetracycline compound. Examples of RT-based precursor compounds include derivatives from art-recognized tetracycline compound families. Without limitation, such tetracycline compound families include minocycline, doxycycline and sancycline compounds.

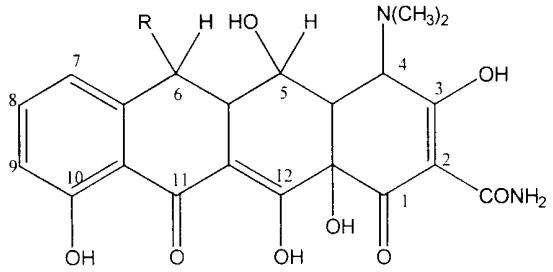
"Minocycline-based precursor compound" is intended to include compounds having the core structure of minocycline, which differs from the core structure of tetracycline by the presence of a dimethylamino group at position 7, and the absence of methyl and hydroxyl groups at position 6, and the absence of a hydroxyl group at position 5. The core structure of minocycline-based precursor compounds is shown below for the purposes of illustration:



It should be understood that minocycline-based precursor compounds can be substituted, unsubstituted or derivatized, e.g., at positions other than positions 5 and 6. For

example, other positions in the core structure, e.g., position 8, can be substituted or unsubstituted and others can be substituted or derivatized, such as the 2-position amido group. Suitable substituents include moieties such as hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, carboxamido, carboxy ester, alkoxycarbonyl, aryloxycarbonyl, carbocyclic or heterocyclic groups, and combinations thereof. Other substituent groups will be recognized by those of skill in the art. Further, R in the above formula can represent a group other than methyl, e.g., lower alkyl such as ethyl, propyl, etc. Reactive minocycline-based precursor compounds include, without limitation, 9-diazonium minocycline-based compounds, 9-iodo minocycline-based compounds, 9-bromo minocycline-based compounds, and 9-chloro minocycline-based compounds.

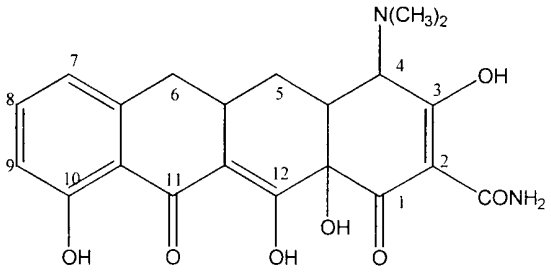
"Doxycycline-based precursor compound" is intended to include compounds having the core structure of doxycycline, which differs from the core structure of tetracycline by the substitution of a hydrogen for a hydroxyl at position 6, and the substitution of a hydroxyl for a hydrogen at position 5. The core structure of doxycycline-based precursor compounds is shown below for the purposes of illustration:



It should be understood that doxycycline-based precursor compounds can be substituted, unsubstituted or derivatized, e.g., at positions 7, 8 and/or 9. For example, other positions in the core structure, e.g., position 8, can be substituted or unsubstituted and others can be substituted or derivatized, such as the 5-position hydroxyl group or the 2-position amido group. Suitable substituents include moieties such as hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, carboxamido, carboxy ester, alkoxycarbonyl, aryloxycarbonyl, carbocyclic or heterocyclic groups, and combinations thereof. Other substituent groups will be recognized by those of skill in the art. Further, R in the above formula can represent a group other than methyl, e.g., lower alkyl such as ethyl, propyl, etc.

Reactive doxycycline-based precursor compounds include, without limitation, 7- and/or 9-diazonium doxycycline compounds, 7- and/or 9-iodo doxycycline compounds, 7- and/or 9-bromo doxycycline compounds, and 7- and/or 9-chloro doxycycline compounds.

"Sancycline-based precursor compound" is intended to include compounds having the core structure of sancycline, which differs from the core structure of tetracycline by the substitution of a hydrogen for a methyl group and hydrogen for a hydroxyl at position at position 6. The core structure of sancycline-based precursor compounds is shown below for the purposes of illustration:



It should be understood that sancycline-based precursor compounds can be substituted, unsubstituted or derivatized, e.g., at positions 7, 8 and/or 9. For example, other positions in the core structure, e.g., position 8, can be substituted or unsubstituted and others can be substituted or derivatized, such as the 2-position amido group. Suitable substituents include moieties such as hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, carboxamido, carboxy ester, alkoxycarbonyl, aryloxycarbonyl, carbocyclic or heterocyclic groups, and combinations thereof. Other substituent groups will be recognized by those of skill in the art. Reactive sancycline-based precursor compounds include, without limitation, 7- and/or 9-diazonium sancycline compounds, 7- and/or 9-iodo sancycline compounds, 7- and/or 9-bromo sancycline compounds, and 7- and/or 9-chloro sancycline compounds.

In a preferred embodiment, the reactive tetracycline-based precursor compound is an arene tetracycline diazonium salt, and alternately iodo derivatized tetracycline compounds, or tetracycline compounds that possess a double bond and are reactive with boronic acid derivatives, e.g., at position 13. In one embodiment, the reactive tetracycline-based precursor compound and a transition metal catalyst form a reactive chemical intermediate useful in making novel tetracyclines, through techniques known in the art (see, for example, Hegedus,

Transition Metals in the Synthesis of Complex Organic Molecules, University Science Books, Mill Valley, CA, 1994, incorporated herein by reference). The reactive chemical intermediate are preferably formed *in situ* with the reactive organic substituent precursor.

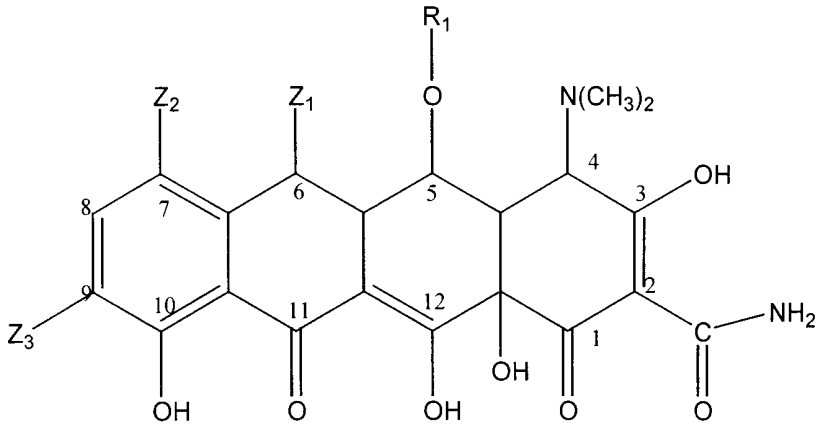
"Transition metal catalyst" is an art-recognized term which includes transition metals and catalysts comprising a transition metal, e.g., including elements 21 through 29, 39 through 47, 57 through 79, and 89 on. Exemplary transition metal catalysts include $CuCl_2$, copper (I) triflate, copper thiophene chloride, palladium (II) chloride, organopalladium catalysts such as palladium acetate, $Pd(PPh_3)_4$, $Pd(AsPh_3)_4$, $PdCl_2(PhCN)_2$, $PdCl_2$ $(Ph_3P)_2$, $Pd_2(dba)_3$-$CHCl_3$ ("dba"=dibenzylacetone); and combinations thereof. Other transition metal catalysts include those containing metals such as rhodium (e.g. rhodium (II) acetate and $Rh_6(CO)_{16}$), iron, iridium, chromium, zirconium, and nickel. A skilled artisan will be able to select the appropriate transition metal catalyst to perform the desired reaction, based on the existing literature (see, for example, Lipshutz, B.H. *Org. React.* 1992, 41:135, incorporated herein by reference.)

"Reactive organic substituent precursor" includes organic substituents having a reactive group that allows for addition to the reactive tetracycline-based precursor compound as disclosed herein. Preferably the reactive organic substituent precursor comprises at least one reactive group. In an embodiment, the reactive organic substituent precursor may include π-bonded species such as methylene compounds, aryl boronic acids, active aromatic rings and unsubstituted and substituted olefins and alkynes, nitriles, acetylenes, substituted acetylenes, arylethylenes, styrenes, conjugated dienes, isoprenes, vinyl ethers, α, β-unsaturated aldehydes and ketones, aryl vinyl and arylisoprenyl ketones, iodoalkenes and iodoarenes, quinones, α, β-unsaturated acids and their derivatives.

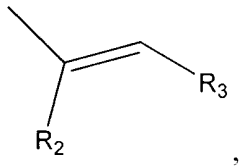
"Reactive organic substituent precursors" also include compounds (which may be formed *in situ*) which react with the reactive intermediate to form a desired tetracycline analog. For example, the reactive intermediate can be transmetallated to form a wide variety analogs through reactions with other organometal complexes such as tributyltin compounds and lithium diorganocuprates (see for example, Kalanin, *Synthesis*, 1992, 413; Sawamuru, *Chem. Rev.* 1992, 92:857; Negeishi, *Acct. Chem. Res.*, 1982, 15:340, incorporated herein by reference). Other precursors include those suitable for transition metal catalyzed reactions include

compounds with bonds which are reactive with the transition metal containing intermediates. Such precursors include, for example, compounds with halogen groups, hydroxyl groups, triflate groups, thiol groups, amino groups. Intramolecular reactions are also included wherein the reactive organic substituent precursor is bonded or associated with the reactive chemical intermediate (see Hegedus, *supra*).

Compounds of the invention include 7-substituted tetracycline analogs, 9-substituted tetracycline analogs, and 13-substituted tetracycline analogs. These compounds may be illustrated by the general formula



wherein Z_1, Z_2, and Z_3 are individually H or



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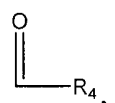
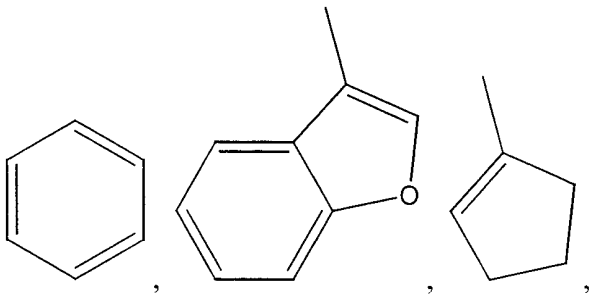
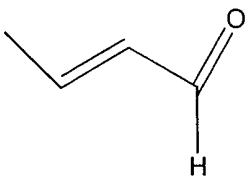
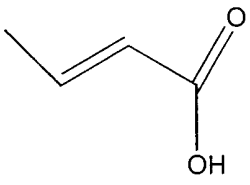
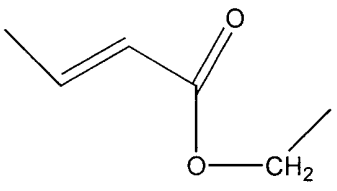
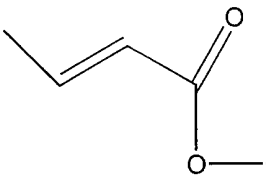
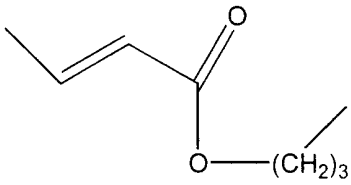
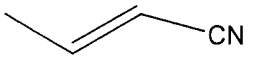
wherein R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, aryloxycarbonyl; or R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring having 5 to 15 atoms in the ring; and R_1 is H or OH.

In another embodiment R_2 is hydrogen, and R_3 is  , where R_4 is hydrogen, cyano, or a C_1-C_5 alkoxy group. In another embodiment R_1 and R_2, taken together, form a substituted

or unsubstituted carbocyclic or heterocyclic ring having 5 to 15 atoms in the ring; the ring may be a conjugated or unconjugated aromatic ring system, preferably C_5 to C_8.

Suitable substituents for Z include  , , ,  ,  ,  ,  ,  , and  .

The invention also provides in another aspect a method for preparing substituted tetracycline compounds, desirably 7, 9 or 13-substituted compounds, and, in another aspect, tetracycline compounds prepared by this method. The compounds can be prepared as generally depicted in the Schemes set forth hereinbelow. In the discussions of the Schemes, the various substituent groups are the same as defined above; "R" includes R_2 and R_3. Also, for purposes of exemplification only, doxycycline is depicted as the "base" tetracycline compound, although it will be understood that a wide variety of tetracycline compounds can be employed in the same manner. For example, the base tetracycline compound substituted at the 7-, 9- and/or 13-positions suitably may be oxytetracycline; chlortetracycline; demeclocycline; doxycycline; chelocardin; minocycline; rolitetracycline; lymecycline; sancycline; methacycline; apicycline; clomocycline; guamecycline; meglucycline; mepylcycline; penimepicycline; pipacycline; etamocycline; penimocycline, semi-synthetic intermediates thereof, and the like.

Tetracycline compounds of the present invention may readily be prepared by the methods outlined in accordance with the following schemes. Scheme I refers to the preparation of tetracycline compounds which may be prepared from a starting compound of formula 1, a

clinically useful tetracycline antibiotic named doxycycline. It has been found that 6-(substituted)-5-hydroxy-6-deoxytetracyclines (1, R_2=CH_3, doxycycline) or their mineral acid salts can be dissolved in concentrated acids, e.g., H_2SO_4 as an appropriate solvent, and reacted with nitrating reagents such as sodium or potassium nitrate to produce 7 and 9-nitro tetracycline derivatives (2, 3). These compounds are separated by a variety of techniques, with the preferred method being preparative HPLC on C_{18}-reverse phase silica gel with a binary gradient system comprising either phosphate-buffered ethylene diamine tetraacetic acid, sodium salt (EDTA) with a methanol gradient or a gradient of acetonitrile over 0.1% trifluoroacetic acid. These isolated compounds are readily reduced to the amine functional group using typical reducing reagents such as hydrogen with transition metal catalysts, platinum oxide, palladium on carbon, or similar to produce the 7-NH_2 and 9-NH_2 tetracyclines (4, 5) in good yield. Alternatively, 7-NH_2 tetracyclines (doxycycline) can be prepared (such as detailed in U. S. Patent 3,483,251, incorporated herein by reference) via a reductive alkylation of 7-(N,N dicarboxybenzyloxyhydrazino)tetracyclines.

Compounds possessing the anilino functional groups can undergo a diazotization reaction with nitrous acid (HONO) or organic agents such as butyl nitrite readily forming the diazonium salts, (such as the hydrochloride or tetrafluoroborate salts) (6, 7) in nearly quantitative yield. This reactive tetracycline-based precursor compound (6, 7) as a suitable diazonium salt form, can now chemically complex with organopalladium catalysts and species that results in carbon-carbon bond formation between the tetracycline reactant intermediate and the reactive organic substituent precursor of choice. Transition metal catalysts such as $CuCl_2$ (the Meerwin reaction) as well as palladium catalysts such as palladium chloride, palladium acetate or other catalysts mentioned above, with palladium acetate being preferred, are used to produce the 7 and 9 substituted position derivatives of tetracyclines. The reactions are typically run in polar solvents such as DMSO, water, and alcohols with trace mineral acids (HCl, 0.1%) to react with substituted or unsubstituted aromatic or heteroaromatic, alkyl, alkenyl, or alkynyl substructures producing the desired substituted compounds. Non-polar solvents may also be used in which to run the reactions.

It is known that transition metal halides, such as palladium and copper halides, react with arenediazonium salts to form complexes capable of further reactions. Transition metal halides as catalysts facilitate carbon-carbon bond formation via a radical oxidation-reduction

addition of carbon substructures (double bonds and other structures possessing π-bonds) to the electron deficient nitrogen diazonium reactive group. For example, palladium catalyzed carbon-carbon bond formation occurs readily when a suitable alkene in the reacting system forms reactive coordination complexes. This is followed by insertion into carbon sigma bonds to give a ternary complex. Catalysts such as palladium are cycled and regenerated via, for example, a β-hydride elimination, thereby forming a carbon-carbon covalent bond. Using these conditions, molecular substructures possessing a π-bond system, such as alkenes or acrylic acid esters or any one of the many other compounds possessing a double bond, are readily arylated with reactive tetracycline-based precursor compounds, e.g., tetracycline arenediazonium salts. Other transition metal catalyzed reactions such as transmetallation and insertion reactions, e.g., of carbon monoxide) are also contemplated (see, Hegedus, supra for examples of transition metal catalyzed reactions).

Homogeneous catalysis of carbon-carbon bond formation is possible using palladium complexes and suitable reactive species. Tetracyclines, e.g., doxycycline or minocycline, are used to generate the reactive diazonium functional group within the D ring while the reactive addend is available from structurally diverse chemical families.

Therefore, reactive tetracycline-based precursor compounds such as tetracycline arenediazonium salts, i.e., having reactive functional groups at, e.g., positions 7 and 9 of the tetracycline molecule similarly can be reacted with alkenes, substituted alkenes, vinyl monomers, aromatic and heteroaromatic reactive groups (unsubstituted or substituted) in the presence of the appropriate transition metal catalyst to produce 7-(substituted) and 9-(substituted) tetracyclines (8, 9, Scheme I) in good yield. 7-position substituted tetracycline 9-diazonium salts, for example (Scheme II), produced by the reaction sequence of minocycline (10) nitration to the 9-NO_2 derivative (11), followed by catalytic reduction to the 9-NH_2 derivative (12) followed by diazotisation (13), may also be reacted with double bond compounds such as olefins and reactive products and reagents producing minocycline derivatives of formula (II) (14, Scheme II).

In an embodiment, reaction products of formulas I and II may be further derivatized and reacted with reagents as depicted in Schemes III-VII, thus acting as intermediates for making other compounds not readily obtained otherwise. 9-alkenyl substituted doxycyclines (8, 9) of

formula I may undergo hydrogenation of the 9-alkenyl group with platinum or palladium catalysts on carbon under low pressure hydrogen to form the 9-alkyl derivatives of doxycycline (15, 16, Scheme III). Similarly, 9-alkenyl derivatives of minocycline (14) may also be reduced to the alkyl derivatives using catalytic hydrogenation methods as shown in Scheme IV (17).

7 or 9 derivatives of doxycycline of formula I (Schemes I and III) may also react with carboxylic acids while dissolved in strong acids such as anhydrous hydrogen fluoride or methanesulphonic acid or trifluoromethanesulfonic acid to produce the 5-ester derivatives of 7 and 9 substituted doxycyclines (18, 19, Scheme V).

7 or 9 derivatives of doxycycline of formula I (Schemes I, III and V) may also form Mannich base derivatives by the reaction of the 7 or 9 derivatives with formaldehyde and an appropriate base (pyrrolidine) to produce Mannich base addition products (20, 21, Scheme VI).

9 derivatives of minocycline of formula II (14, Scheme II) may also form Mannich base derivatives by the reaction of the 7 or 9 derivative with formaldehyde and an appropriate base (pyrrolidine) to produce the Mannich base addition products (22, Scheme VII).

Tetracycline diazonium reactive functional groups generated in Scheme I may also be reacted with carbon monoxide in alcohols in the presence of transition metal catalysts such as palladium acetate to produce 7 and 9-carboxylic acid derivatives (23, 24) in good yield which readily esterified to produce 9 position tetracycline esters (25, 26, Scheme VIII).

Minocycline diazonium reactive functional groups generated in Schemes II may also be reacted with carbon monoxide in alcohols in the presence of transition metal catalysts such as palladium acetate to produce the 9-carboxylic acid derivative (2) in good yield which is readily esterified to produce 9 position minocycline carboxylic acid esters (28, Scheme IX).

Other reactions are possible with 7 and 9 aminotetracyclines via a diazonium functional group. Tetracycline arene diazonium salts also react with active methylene compounds such as esters of acetoacetate, and derivatives thereof, active aromatic rings and unsubstituted and substituted olefins, acetylenes, substituted acetylenes, arylethylenes, styrenes, conjugated dienes, isoprenes, vinyl ethers, α , β-unsaturated aldehydes and ketones, aryl vinyl and arylisoprenyl ketones, quinones, α , β-unsaturated acids and their derivatives. All of the multiple bond compounds are readily coupled to arenediazonium salts, as well as nucleophiles.

Position 7 and the 7 and 9 reactive tetracycline-based precursor compounds (halogenated derivatives of tetracyclines as shown in Scheme X) also produce 7 and 9 derivatives of tetracyclines. Aromatic substitution reactions by iodination, bromination or chlorination to produce the 7 and 9 halogen derivatives of doxycycline (29, 30) or sancycline (31, 32) in good yield by reactions described, e.g., by Hlavka, J.J., et al., J. Am. Chem. Soc., 84, 1961, 1426-1430. Position 7 and 9 halogenated derivatives of the tetracyclines may be further coupled with iodoalkenes or iodoarenes in N-methylpyrrolidinone with transition metal catalysts such as copper thiophene chloride or others to produce the position 7 or 9 derivatives of doxycycline (33, 34) or position 7 or 9 derivatives of doxycycline (35, 36) in good yield.

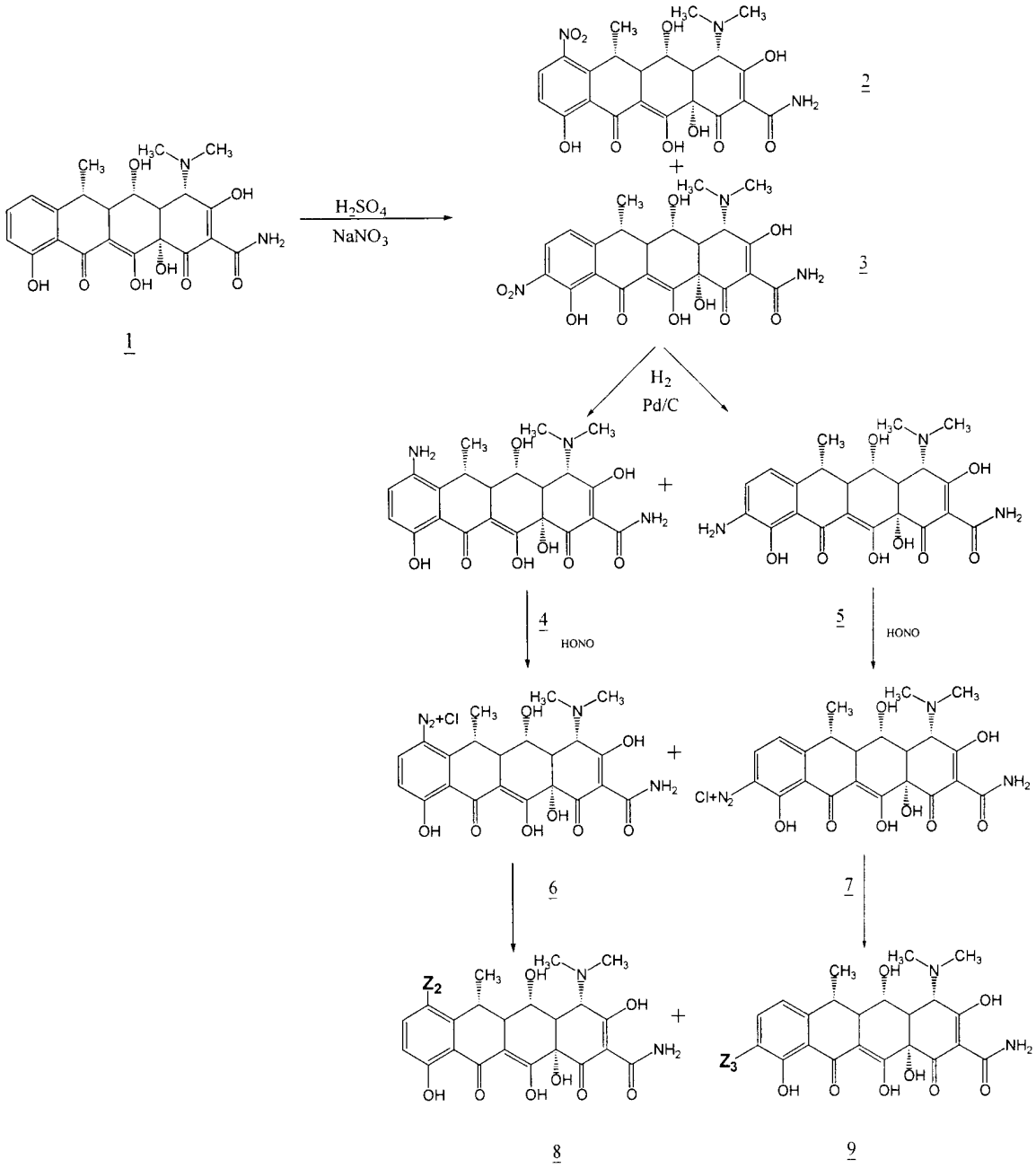
Position 13-derivatives of tetracyclines may be prepared via the reaction of phenylboronic acids with the exocyclic double bond of methacycline (37) (Scheme XI) in alcohols such as methanol, in the presence of palladium chloride or other transition metal catalysts to produce the 13-phenyl derivatives of methacycline in good yield (38).

The following synthetic schemes are illustrative of the present invention:

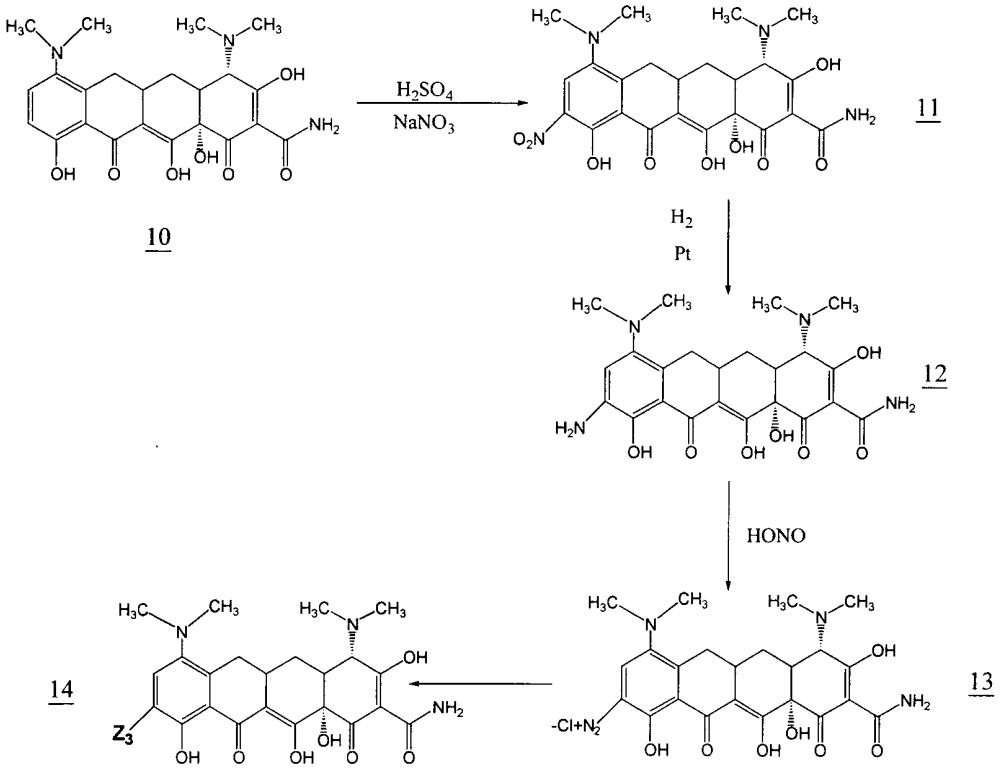
Scheme I	7-(substituted)-6-methyl-6-deoxy 5-hydroxy tetracyclines and 9-(substituted)-6-methyl-6-deoxy 5-hydroxy tetracyclines
Scheme II	9-(substituted) minocyclines
Scheme III	7-(alkyl substituted)-6-methyl-6-deoxy 5-hydroxy tetracyclines and 9-(alkyl substituted)-6-methyl-6-deoxy 5-hydroxy tetracyclines
Scheme IV	9-(alkyl substituted) minocyclines
Scheme V	7-(alkyl or aryl substituted)-6-methyl-6-deoxy 5-acyloxy tetracyclines and 9-(alkyl or aryl substituted)-6-methyl-6-deoxy 5-acyloxy tetracyclines
Scheme VI	7-(alkyl or aryl substituted)-6-methyl-6-deoxy 5-hydoxy tetracyclines and 9-(alkyl or aryl substituted)-6-methyl-6-deoxy 5-hydroxy 2-(carboxamido substituted) tetracyclines
Scheme VII	9-(alkyl substituted)- 2-(carboxamido substituted) minocyclines
Scheme VIII	7-(carboxy or carboxy ester)-6-methyl-6-deoxy 5-hydoxy tetracyclines and 9-(carboxy or carboxy ester)-6-methyl-6-deoxy 5-hydroxy 2-(carboxamido substituted) tetracyclines
Scheme IX	9-(carboxy or carboxy ester) minocyclines

Scheme X	7-(alkenyl or aryl)-6-methyl-6-deoxy 5-hydoxy tetracyclines and 9-(alkenyl or aryl)-6-methyl-6-deoxy 5-hydroxy tetracyclines, X 9-(alkenyl or aryl)-6-demethyl-6-deoxytetracyclines and 9-(alkenyl or aryl)-6-demethyl-6-deoxytetracyclines
Scheme XI	13-(substituted)-6-methylene-5-hydroxy-6-deoxytetracyclines

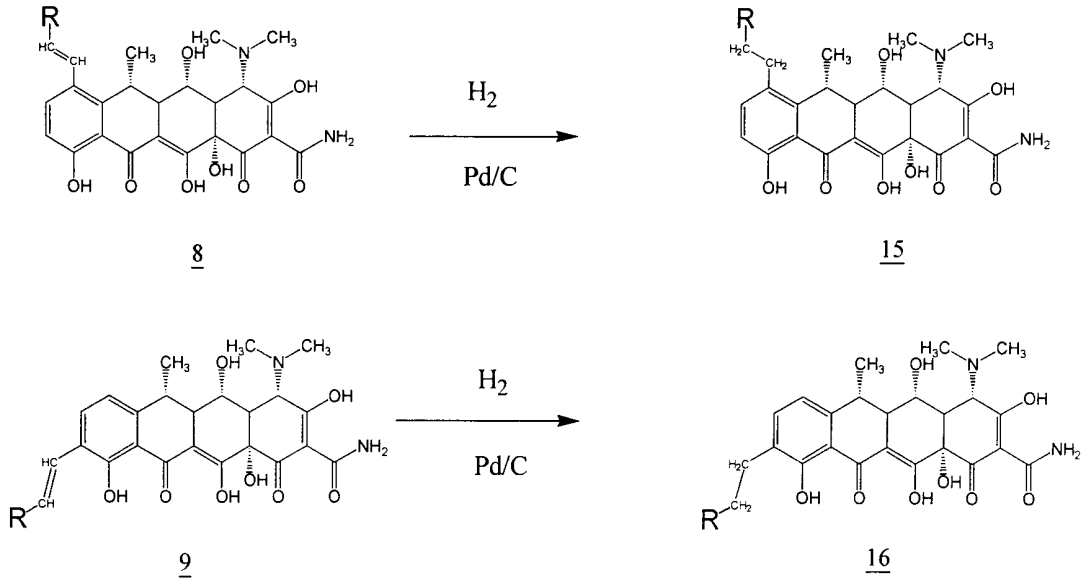
Scheme I



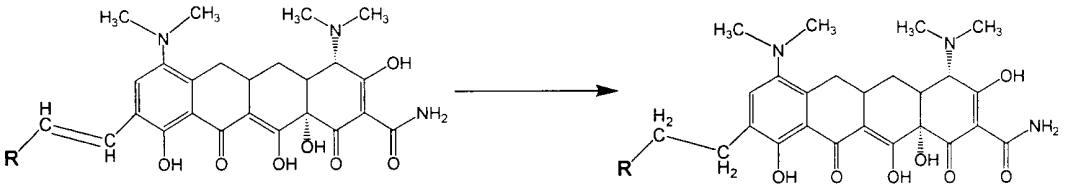
Scheme II



Scheme III

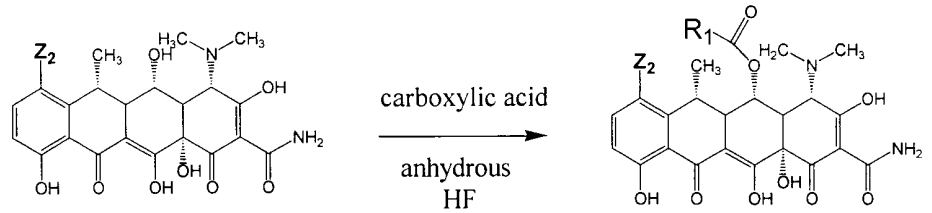


Scheme IV



14 17

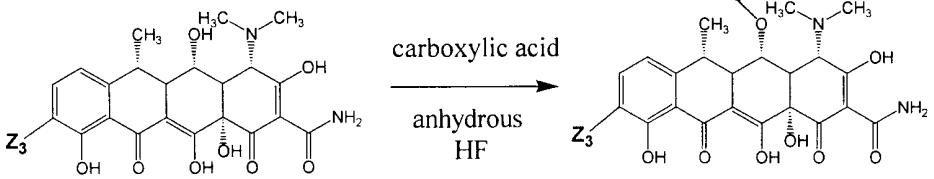
Scheme V



8 18

carboxylic acid

anhydrous HF

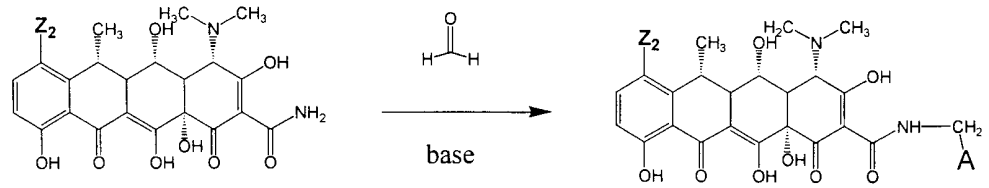


9 19

carboxylic acid

anhydrous HF

Scheme VI



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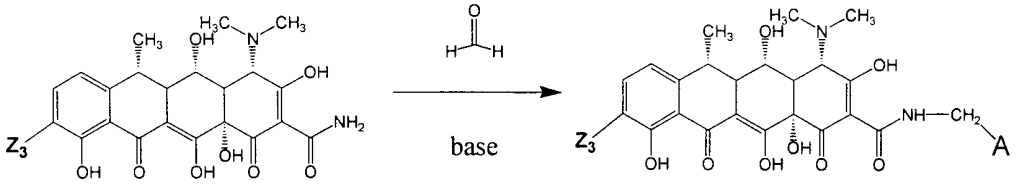
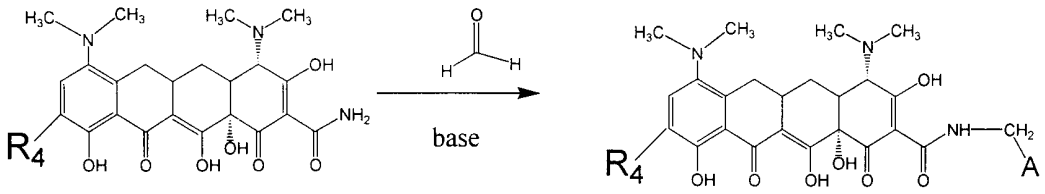


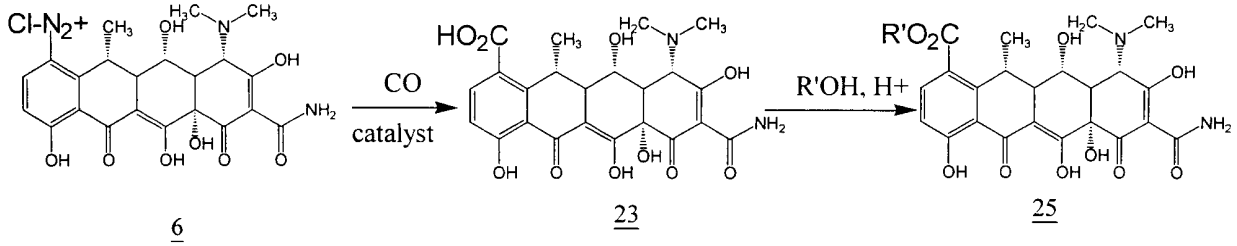
9 21

Scheme VII

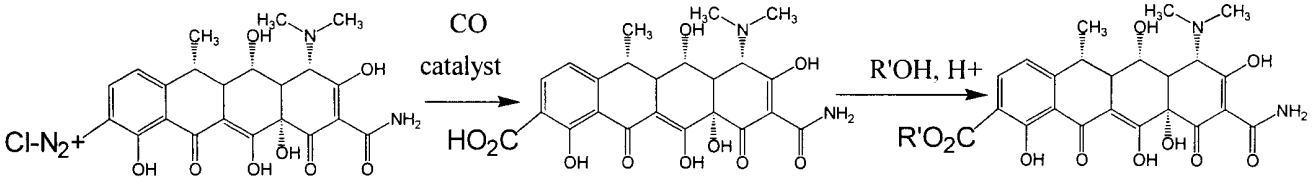


14 22

Scheme VIII

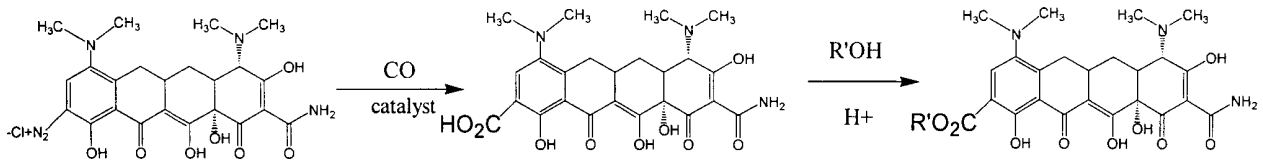


6 23 25



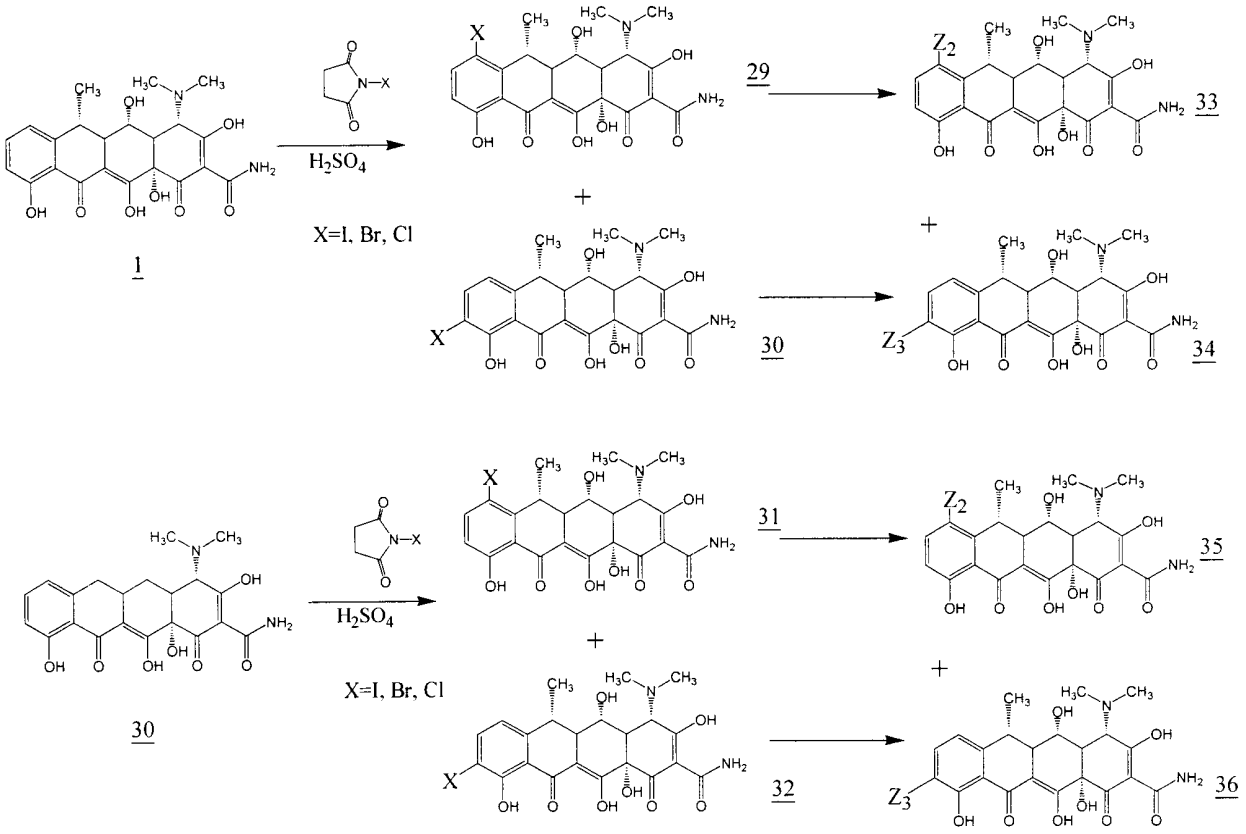
7 24 26

Scheme IX

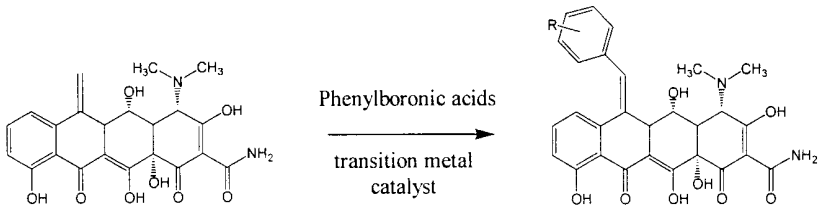


13 27 28

Scheme X



Scheme XI



Compounds of the invention are active against susceptible microorganisms such as bacteria, fungi, rickettsia, parasites and the like, and diseases associated with such microorganisms, including tetracycline-sensitive bacteria as well as tetracycline-resistant bacteria. Particularly preferred compounds of the invention exhibit 24-hr minimum inhibitory concentration (MIC) values of about 10 μg/mL or less, more preferably about 1 μg/mL or less, against tetracycline-resistant *E. coli, S. aureus* and *E. faecalis* strains such as *E. coli* pHCM1, *S. aureus* RN4250 and *E. faecalis* pMV158. Preferred compounds of the invention also include those that exhibit such MIC values against tetracycline-sensitive *E. coli, S. aureus* and *E. faecalis* strains such as *E. coli* D31m4, *S. aureus* RN450 and *E. faecalis* ATCC9790.

As discussed above, the invention provides methods of treatment against microorganism infections and associated diseases, which methods in general will comprise administration of a therapeutically effective amount of one or more compounds of the invention to a subject, which may be an animal or plant, and typically is a mammal, preferably a primate such as a human.

In therapeutic methods of the invention, one or more 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. 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.

At least many of the compounds of the invention suitably may be administered to a subject in a protonated and water-soluble form, e.g., as a pharmaceutically acceptable salt of an organic or inorganic acid, e.g., hydrochloride, sulfate, hemi-sulfate, phosphate, nitrate, acetate,

oxalate, citrate, maleate, mesylate, etc. Also, where an appropriate acidic group is present on a compound of the invention, a pharmaceutically acceptable salt of an organic or inorganic base can be employed such as an ammonium salt, or salt of an organic amine, or a salt of an alkali metal or alkaline earth metal such as a potassium, calcium or sodium salt.

Therapeutic compounds can be administered to a subject in accordance with the invention by any of a variety of routes. Topical (including transdermal, buccal or sublingual), and parenteral (including intraperitoneal, subcutaneous, intravenous, intradermal or intramuscular injection) are generally preferred.

For parenteral application, particularly suitable are solutions, preferably oily or aqueous solutions as well as suspensions, emulsions, or implants, including suppositories. Therapeutic compounds will 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.

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.

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 monolauriate 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.

In addition to treatment of humans, 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.

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.

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 0.01 to 100 milligrams per kilogram of bodyweight of recipient per day, preferably in the range of 0.1 to 50 milligrams per kilogram bodyweight of recipient per day, more preferably in the range of 1 to 20 milligrams per kilogram bodyweight 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.

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 toxicity, hypersensitivity reactions, changes in blood, and impairment of absorption through aluminum, calcium, and magnesium ions should be duly considered in the conventional manner.

BIOLOGICAL ACTIVITY

Method for *in vitro* Evaluation

Various compounds made in accordance with the invention were evaluated for anti-bacterial activity *in vitro* as follows. The minimum inhibitory concentration, the lowest concentration of drug that inhibits bacterial growth at 18 hrs at their appropriate temperature, is determined by the broth dilution method using L-broth or Mueller-Hinton broth. The Mueller-Hinton broth was cation-adjusted accordingly and all bacteriological methods were performed as was described by 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 organisms

tested represent gram-positive and gram-negative bacterial species that are susceptible to tetracyclines or are resistant to tetracyclines due to the ability to efflux tetracyclines or which confer resistance by ribosomal protection mechanisms. The clinical strains used are either susceptible to tetracyclines or are resistant to them by either drug efflux or ribosomal protection.

Table I - LEGEND FOR COMPOUNDS

Compound	Name
Doxycycline	[4S-(4a,12aα)]- 4- (dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3, 5, 10,12,12a- pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide
Minocycline	[4S-(4a,12aα)]- 4,7-Bis (dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a- tetrahydroxy -1,11-dioxo-2-naphthacenecarboxamide
A	[4S-(4a,12aα)]-9-(nitro)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (9-nitro-6-deoxy-5-hydroxy tetracycline)
B	[4S-(4a,12aα)]-9-(amino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (9-amino-6-deoxy-5-hydroxy tetracycline)
C	[4S-(4a,12aα)]-9-(diazonium)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (9-diazonium-6-deoxy-5-hydroxy tetracycline)
D	[4S-(4a,12aα)]-9-[3'-(*E*)-propenoic acid]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (9-[3'-(*E*)-ethylpropenoic acid]-6-deoxy-5-hydroxy tetracycline)
E	[4S-(4a,12aα)]-9-[3'-(*E*)-butylpropenoate]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide (9-[3'-(*E*)-butylpropenoate]-6-deoxy-5-hydroxy tetracycline)

F [4S-(4a,12aα)]-9-[3'-(E)-butylpropenoate]- 4,7-Bis (dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3 ,10,12,12a- tetrahydroxy -1,11-dioxo-2-naphthacenecarboxamide

9-[3'-(*E*)-butylpropenoate] minocycline

G [4S-(4a,12aα)]-7-[4' –Cl-phenyl)]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide

7-(4'-Cl-phenyl) sancycline

H [4S-(4a,12aα)]-7-phenyl-9-phenyl-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide

7,9-diphenyl sancycline

I [4S-(4a,12aα)]-13-(4'-methylphenyl)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide

13-(4'-methylphenyl)-6-deoxy-6-methylene-5-hydroxy tetracycline

J [4S-(4a,12aα)]-13-(3'-carboxyphenyl)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide

13-(3'-carboxyphenyl)-6-deoxy-6-methylene-5-hydroxy tetracycline

K [4S-(4a,12aα)]-13-(4'-ethoxyphenyl)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methylene-1,11-dioxo-2-naphthacenecarboxamide

13-(4'-ethoxyphenyl)-6-deoxy-6-methylene-5-hydroxy tetracycline

TABLE II

Antibacterial Activity of Transition Metal Catalyzed Derivatives of Tetracyclines

	Doxy	D	E	F	G	H	I	J	K
E.coli ML308-225 Tc^s	0.78	25	6.25	>50	>50	>50	>50	>50	12.5
E.coli D1-299 Tc^r	25	>50	>50	>50	>50	>50	>50	>50	>50
E.coli D1-209 Tc^r	50	>50	>50	>50	>50	>50	>50	>50	>50
E.coli D31m4 Tc^s	1.56	>50	3.12	3.12	0.78	1.56	6.25	>50	12.5
E.coli D 31m4 pHCM1 Tc^r	25	>50	6.25	6.25	0.78	-	25	>50	>50
S. aureus RN450 Tc^s	<0.098	3.12	0.78	1.56	≤0.098	1.56	≤0.098	0.39	0.195
S. warnerii Tc^r ATCC12715	50	>50	6.25	3.12	≤0.098	0.78	12.5	>50	12.5
S. aureus RN4250 Tc^r	25	>50	6.25	3.12	≤0.098	0.78	12.5	>50	6.25
S. aureus MRSA5 Tc^r	6.25	>50	0.39	3.12	0.195	0.78	6.25	>50	6.25
E. hirae ATCC9790 Tc^s	0.195	3.12	3.12	3.12	≤0.098	0.78	0.39	3.12	0.39
E. hirae 9790 with pMV158 Tc^r	6.25	12.5	6.25	3.12	≤0.098	0.39	3.12	>50	6.25
E. hirae 9790 with pAM211 Tc^r	6.25	>50	6.25	3.12	≤0.098	1.56	12.5	>50	3.12

Tc^s = tetracycline susceptible

Tc^r = tetracycline resistant

EXPERIMENTAL

Compounds of the invention may be prepared as presented in schemes I through IX, above, and/or as described below.

In scheme I, doxycycline is dissolved in cold concentrated sulfuric acid and an equivalent of potassium nitrate is added. The reaction temperature was maintained in the range 0 to 5°C for a period of 1 to 3 hrs, producing 7 and 9-nitro-6-substituted-5-hydroxy tetracyclines of formula IV. These intermediates, with suitable chemically reactive functionality, can be reacted with a broad range of reducing agents such as PtO_2 or hydrogen and palladium or platinum catalysts producing compounds of general formula IV. The diazonium salts of the 7 and 9 amino derivatives are produced by the action of nitrites (sodium nitrite, butyl nitrite or equivalent) and the intermediate used without further purification.

EXAMPLE 1

[4S-(4α,12aα)]-9-(nitro)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

To an ice cold solution of 1.0 g of doxycycline hydrochloride in 10 mL of concentrated sulfuric acid was added 0.231g of potassium nitrate. The reaction was stirred for 1 hr under ambient atmosphere. The mixture was then poured over 150 g of ice and the resulting solid was extracted with n-butanol and dried to afford 0.9 g of the desired product as a yellow-green solid.

MS(FAB): m/z 490 (M+H).

^{1}H NMR (CD_3OD): δ 7.50(d,1H,J=8.07Hz,H-8); 6.86(d,1H,J=8.07Hz,H-7); 4.44(bs,1H,H-4); 3.62(dd,1H, J=11.42 ; 8.35 Hz,H-5); 2.95(bs,6H, NMe_2); 2.81(d,1H,J=11.45Hz, H-4a); 2.71(dq, 1H, J=12.41; 6.5 Hz, H-6); 2.53(dd,1H, J=12.23;8.20 Hz, H-5a); 1.51(d, 3H, J=6.78 Hz, CH_3).

EXAMPLE 2

[4S-(4α,12aα)]-9-(amino)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

Into a 200 mL hydrogenation bottle is added 1.0 g of product from example 1, 40 mL of methanol, 1 mL of concentrated HCl, and 100 mg of 10% palladium on carbon. Using a hydrogenation apparatus, the mixture is subjected to 30 psi of hydrogen for 3 hrs. The catalyst is filtered off and the filtrate is dried to afford 0.9 g of the dihydrochloride as a yellow solid.

MS(FAB): m/z 460 (M+H).

^{1}H NMR (CD_3OD): d 7.54(d,1H,J=8.08Hz,H-8); 6.88(d,1H,J=8.08Hz,H-7); 5.16(dd, J=10.44;7.94 Hz, H-5); 4.44(bs,1H,H-4); 3.74(d, 1H, J=2.07 Hz, H-4); 3.04(bs,6H, NMe_2); 2.90(dd,1H,J=7.94;2.07 Hz, H-4a); 2.72(dq, 1H, J=12.31; 6.56 Hz, H-6); 2.61(dd,1H, J=12.31;10.44 Hz, H-5a); 2.54(q, 2H, J=7.48 Hz, CH_2-C); 1.44(bs, 9H, CMe_3); 1.29(d, 3H, J=6.56 Hz, CH_3); 1.20(t, 3H, J=7.48 Hz, C-CH_3).

EXAMPLE 3

[4S-(4α,12aα)]-9-(diazonium)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

A 10 mL round bottom flask was charged with 100 mg of product from example 2 and dissolved in 4 mL of 0.1 N methanolic hydrochloric acid. The solution was cooled to 0°C and 35µl of butyl nitrite was added with stirring. After 1 hr, the bright red reaction mixture was added dropwise to 100 mL of cold anhydrous diethyl ether. The product was collected by filtration, washed with ether, and dried in a vacuum dessicator to give 73 mg of the diazonium salt as an orange solid.

MS(FAB): m/z 472 (M+H).

^{1}H NMR (CD_3OD): d 7.55(d,1H,J=8.08Hz,H-8); 6.86(d,1H,J=8.08Hz,H-7); 5.13(dd, J=10.44;7.94 Hz, H-5); 4.41(bs,1H,H-4); 3.72(d, 1H, J=2.07 Hz, H-4); 3.04(bs,6H, NCH_3); 2.90(dd,1H,J=7.94;2.07 Hz, H-4a); 2.70(dq, 1H, J=12.31; 6.56 Hz, H-6); 2.61(dd,1H, J=12.31;10.44 Hz, H-5a); 2.2(m, 6H, J=7.48 Hz, Acetyl); 1.44(bs, 9H, $C(CH_3)_3$); 1.29(d, 3H, J=6.56 Hz, CH_3); 1.20(t, 3H, J=7.48 Hz, C-CH_3).

General procedure for olefination. To a solution of 0.1g of 9-diazonium compound in (wet or dry) methanol is added 0.05 equivalents of palladium acetate. The reaction mixture is stirred for 5 minutes at room temperature, and 2 equivalents of the desired olefin is added. Stirring is continued for 18 hrs under ambient atmosphere or followed by HPLC. The stirring may also be continued under N_2 atmosphere. Upon completion, the catalyst is filtered off and the filtrate dried to give the crude product. The purified product is isolated by preparative reverse-phase HPLC using methanol and phosphate buffer gradient.

EXAMPLE 4

[4S-(4α,12aα)]-9-[3'-(*E*)-propenoic acid]-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

MS(FAB): m/z 515 (M+H).

EXAMPLE 5

[4S-(4α,12aα)]-9-[1'-(*E*)-(2'-phenyl)ethenyl]4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

MS(FAB): m/z 547 (M+H).

EXAMPLE 6

[4S-(4α,12aα)]- 7-[3'-(*E*)-butylpropenoate]4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

General procedure for arylation. To a solution of 9-diazonium compound in methanol is added 0.10 equivalents of palladium acetate. The mixture is stirred at room temperature for 5 minutes, and 2 equivalents of aryl boronic acid is added. After 6 hrs, the catalyst is filtered off and the filtrate is dried down. The crude product is purified by preparative reverse-phase HPLC using a methanol phosphate buffer gradient.

General procedure for carboxylation. To a three neck round bottom flask equipped with two rubber septa, a vacuum source, and a stirbar, is added 100 mg of diazonium compound, 6.0 mg of palladium acetate, and 10 mL of anhydrous dimethylformamide. The reaction vessel is evacuated, and CO is passed through the mixture for 1 hr via a syringe. The mixture is stirred for an additional 2 hr, then the solvent removed *in vacuo* to yield the crude product. The title compound was isolated by preparative C_{18} reverse-phase HPLC by using a binary solvent gradient.

EXAMPLE 7

[4S-(4α,12aα)]-9-(carboxy)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

MS(FAB): m/z 489 (M+H).

General procedure for hydrogenation. The compound is prepared by dissolving 0.100 g of Example 4 into 10 mL of methanol, adding 0.1% concentrated HCl and 10 mg of 10% palladium on carbon. The mixture is hydrogenated in at 40 psi in a Parr apparatus for 6 hrs at room temperature and monitored by HPLC. The resulting crude product is chromatographed on C_{18} reverse-phase via semi-preparative binary solvent methods to give the desired product.

General procedure for 7 position olefination. To a solution of 0.1g of 7-diazonium compound, generated in similar methods as described in Examples 1 and 2, in wet methanol is added 0.05 equivalents of palladium acetate. The reaction mixture is stirred for 5 minutes at room temperature, and 2 equivalents of the desired olefin are added. Stirring is continued for 18 hrs under ambient atmosphere and followed by HPLC. Upon completion, the catalyst is filtered through Celite and the filtrate dried to give the crude product. The purified product is isolated by preparative reverse-phase HPLC using methanol and phosphate buffer gradient.

EXAMPLE 8

9-phenyl minocycline

[4S-(4α,12aα)]-9-(phenyl)-4,7-Bis (dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-tetrahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide

The compound was prepared using 0.100 g of 9-amino minocycline and reagents and conditions similar to those found in Example 5. The reaction was stirred overnight under a nitrogen atmosphere and the solvent removed *in vacuo* to produce 0.063 g of the crude product. Chromatography using C_{18} reverse-phase preparative methods and binary solvent systems followed by extraction of the product into butanol and evaporation of the product *in vacuo*, furnished 0.027 g of the desired product as a yellow solid.

MS(FAB): m/z 571 (M+H).

EXAMPLE 9

7-iododoxycycline

30.0 mL of concentrated sulfuric acid was added to 1.00g of doxycycline hydrochloride hemihydrate with stirring and the solution cooled to 0°C. 0.973g of N-iodosuccinimide was added portionwise to the solution over one hr and the reaction monitored by HPLC and TLC to ensure completion. The solution was poured into 250mL of ice water, extracted three times with butanol, and the solvent removed under reduced pressure. The crude residue was purified by preparative HPLC to provide 1.13g (89%) of the title compound as dark yellow crystals.

MS (FAB): m/z 587 (M+H)

^{1}H NMR (Methanol d-4, 300MHz) δ 7.94 (d, J=8.19Hz, 1H), 6.78 (d, J=8.18Hz, 1H), 4.13 (s, 1H), 3.53 (m, 3H), 2.85 (s, 7H), 2.66 (m, 4H), 2.41 (s, 1H), 1.49 (d, J=6.52Hz, 3H), 0.95 (t, J=7.27Hz, 2H).

EXAMPLES 10 and 11

7-iodosancycline and 7,9-diiodosancyline

30.0 mL of concentrated sulfuric acid was added to 1.00g of sancycline hydrochloride hemihydrate with stirring and the solution cooled to 0°C. 1.09g of N-iodosuccinimide was added portionwise to the solution over one hr and the reaction mixture monitored by HPLC and

TLC. The reaction mixture was poured into 250 mL of ice water, extracted three times with n-butanol, and the solvent removed under reduced pressure. The crude residue was purified by preparative HPLC yielding 787mg (61%) of 7-iodosancycline and 291mg (22%) of 7,9-diiodosancycline as yellow and dark yellow crystals respectively.

MS (FAB): m/z 587 (M+H) 7-iodosancycline

^{1}H NMR (Methanol d-4, 300MHz) δ 7.89 (d, J=8.86Hz, 1H), 6.67 (d, 8.87Hz, 1H), 3.56 (s, 1H), 3.03 (s, 2H), 2.84 (s, 6H), 2.46 (m, 2H), 1.63 (m, 4H) 0.95 (m, 2H).

MS (FAB): m/z 667 (M+H) 7,9-diiodosancycline

^{1}H NMR (Methanol d-4, 300MHz) δ 8.35 (s, 1H), 3.78 (s, 1H), 3.33 (s, 2H), 2.88 (s, 7H), 2.41 (m, 2H), 1.41 (m, 5H).

EXAMPLE 12 - General Coupling Procedure

7-4'-Cl-phenyl sancycline

100 mg of 7-iodosancycline or 7-iodo doxycycline (0.18 mM) and 4 mg of $Pd(OAc)_2$ is added to an argon degassed solution of methanol followed by 200 µl of 2 M Na_2CO_3. The resultant solution was stirred for 10 minutes at room temperature. 4'-Cl-phenyl boronic acid (58 mg, 0.37 mM) was dissolved in 1 mL methanol, added to the iodotetracycline and the reaction flask degassed with argon 3 times. The reaction was stirred for 15 minutes at room temperature, then heated to reflux for 18 hrs. The solution was cooled, filtered and the solvent removed under reduced pressure. The crude product was purified by C18-reverse phase chromatography to yield 23 mg of product as dark yellow crystals.

MS (FAB): m/z (M+H) 525.1852

^{1}H NMR (Methanol d_4, 300MHz) δ 7.35-7.44 (m, 4H), 7.21-7.24 (d, 1H), 6.85-6.88 (d, 1H), 3.55 (s, 1H), 2.88 (s, 6H), 2.47 (m, 2H) 1.52 (m, 2H)

EXAMPLE 13 7, 9-diphenyl sancycline

MS (FAB) m/z (M+H) 567.2545

^{1}H NMR (Methanol d_4, 300 MHz) δ 7.22-7.85 (m, 11H), 4.02 (m, 1H), 3.53 (s, 1H), 2.86 (br s, 6H), 2.41 (m, 2H), 1.52 (m, 2H)

EXAMPLE 14

7-(4-fluorophenyl)sancycline

MS (FAB): m/z 509 (M+H)

^{1}H NMR (Methanol d-4, 300MHz) δ 7.41 (d, J=8.61Hz, 1H), 7.30 (td, J=6.87, 2.16 Hz, 2H), 7.16 (td, J=6.84, 2.11Hz, 2H), 6.89 (d, J=8.59Hz, 1H) 3.56 (s, 2H), 2.91 (s, 7H), 1.52 (m, 4H), 0.95 (m, 2H).

EXAMPLE 15

7-(4-nitrophenyl)sancycline

MS (FAB): m/z 536 (M+H)

^{1}H NMR (Methanol d-4, 300MHz) δ 8.28 (d, J=8.50, 2H), 7.52 (d, J=8.52, 2H), 7.42 (d, J=8.64, 1H), 6.93 (d, J=8.65, 1H), 3.51 (s, 2H), 6.73 (s, 7H), 1.50 (m, 5H), 0.92 (m, 2H).

EXAMPLE 16

7-(2-pyridyl)doxycycline

MS (FAB): m/z 522 (M+H)

^{1}H NMR (Methanol d-4, 300MHz) δ 8.62 (s, 1H), 7.94 (m, 2H), 7.49 (m, 1H), 7.40 (m, 1H), 6.94 (m, 1H), 4.21 (s, 1H), 3.56(m, 2H), 2.91 (s, 7H), 2.70 (m, 3H), 1.038 (s, 3H), 0.92 (m, 2H).

EXAMPLE 17

7-ethylenylsancycline

MS (FAB): m/z 471 (M+H)

^{1}H NMR (Methanol d-4, 300MHz) δ 7.65 (d, J= 8.79Hz, 1H), 6.80 (d, J=8.76Hz, 1H), 5.56 (d, J=18.42Hz, 1H), 5.25 (d, J=12.15Hz, 1H), 3.84 (s, 1H), 3.19 (m, 2H), 2.98 (s, 6H), 2.82 (m , 1H), 2.32 (m, 2H), 0.92 (m, 1H).

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 this invention and are covered by the following claims. The contents of all references, issued patents, and published patent applications cited throughout this application are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. A method of preparing a substituted tetracycline compound, comprising contacting a reactive tetracycline chemical complex comprising a reactive tetracycline-based precursor compound and a transition metal catalyst forming a reactive chemical intermediate with a reactive organic substituent precursor under conditions such that a tetracycline compound substituted with said organic substituent is formed.

2. A method of preparing a substituted tetracycline compound, comprising combining a reactive tetracycline-based precursor compound and a reactive organic substituent precursor in the presence of a transition metal catalyst under conditions such that a tetracycline compound substituted with said organic substituent is formed.

3. The method of claim 1 or 2, wherein said transition metal catalyst comprises an organopalladium catalyst.

4. The method of claim 3, wherein said organopalladium catalyst comprises palladium chloride, palladium acetate, $PdCl_2(PhCN)_2$, $PdCl_2(Ph_3P)_2$, $Pd_2(dba)_3$-$CHCl_3$, or a combination thereof.

5. The method of any one of claims 1-4, wherein said transition metal catalyst comprises copper, rhodium, iron, iridium, chromium, zirconium, or nickel.

6. The method of claim 5, wherein said transition metal catalyst comprises $CuCl_2$, CuI_2, rhodium (II) acetate, $Rh_6(CO)_{16}$), or combinations thereof.

7. The method of any one of claims 1-6, wherein said reactive tetracycline-based precursor compound is oxytetracycline, chlortetracycline, demeclocycline, doxycycline, chelocardin, minocycline, roliteteracycline, lymecycline, sancycline, methacycline, apicycline,

clomocycline, guamecycline, meglucycline, mepylcycline, penimepicycline, pipacycline, etamocycline, or penimocycline based precursor compound.

8. The method of any one of claims 1-6, wherein said reactive tetracycline-based precursor compound is selected from the group consisting of reactive minocycline-based precursor compounds, reactive doxycycline-based precursor compounds, and reactive sancycline-based precursor compounds.

9. The method of any one of claims 1-8, wherein said reactive tetracycline-based precursor compound is an arenediazonium salt, iodo derivative, or a boronic acid derivative of a tetracycline compound.

10. The method of any one of claims 1-9, wherein said reactive organic substituent precursor has at least one reactive π-bond containing group.

11. The method of claim 10, wherein said reactive organic substituent precursor is alkenyl, alkynyl, or aromatic.

12. The method of claim 11, wherein said reactive organic substituent precursor is alkenyl.

13. The method of claim 12, wherein said alkenyl reactive organic substituent precursor is a vinyl monomer.

14. The method of claim 12, wherein said alkenyl reactive organic substituent precursor is substituted.

15. The method of claim 12 or 14, wherein said alkenyl reactive organic substiuent precursor is a methylenyl compound, conjugated diene, isoprene, vinyl ether, iodoalkene, or a derivative thereof.

16. The method of claim 11, wherein said aryl reactive organic substituent precursor is heteroaromatic.

17. The method of claim 11 or 16, wherein said aryl reactive organic substitutent precursor is an aryl boronic acid, iodoaryl, quinone, arylethylene, or styrene.

18. The method of claim 10, wherein said reactive organic substituent precursor comprises a carbonyl or thiocarbonyl group.

19. The method of claim 18, wherein said reactive orgainc substituent precursor is an aryl vinyl ketone, arylisoprenyl ketone, α, β-unsaturated aldehyde, α, β-unsaturated ketone, α, β-unsaturated acid, or a derivative thereof.

20. The method of claim 11, wherein said alkynyl reactive organic substituent precursor is substituted or unsubstituted acetylene.

21. A reactive tetracycline chemical complex comprising a reactive tetracycline-based precursor compound and a transition metal catalyst.

22. The reactive tetracycline chemical complex of claim 21, wherein said catalyst comprises an organopalladium catalyst.

23. The reactive tetracycline chemical complex of claim 22, wherein said organopalladium catalyst comprises palladium chloride, palladium acetate, $PdCl_2(PhCN)_2$, $PdCl_2(Ph_3P)_2$, $Pd_2(dba)_3$-$CHCl_3$, or a combination thereof.

24. The reactive tetracycline chemical complex of any one of claims 21-23, wherein said transition metal catalyst comprises copper, rhodium, iron, iridium, chromium, zirconium, or nickel.

25. The reactive tetracycline chemical complex of claim 24, wherein said transition metal catalyst comprises $CuCl_2$, CuI_2, rhodium (II) acetate, $Rh_6(CO)_{16}$), or combinations thereof.

26. The reactive tetracycline chemical complex of any one of claims 21-25, wherein said reactive tetracycline-based precursor compound is oxytetracycline, chlortetracycline, demeclocycline, doxycycline, chelocardin, minocycline, roliteteracycline, lymecycline, sancycline, methacycline, apicycline, clomocycline, guamecycline, meglucycline, mepylcycline, penimepicycline, pipacycline, etamocycline, or penimocycline based precursor compound.

27. The reactive tetracycline chemical complex of any one of claims 21-25, wherein said reactive tetracycline-based precursor compound is selected from the group consisting of reactive minocycline-based precursor compounds, reactive doxycycline-based precursor compounds, and reactive sancycline-based precursor compounds.

28. The reactive tetracycline chemical complex of any one of claims 21-27, wherein said reactive tetracycline-based precursor compound is an arenediazonium salt, iodo derivative, or a boronic acid derivative of a tetracycline compound.

29. A 7-substituted tetracycline compound, wherein the substituent at the 7 position is connected with a -C-C- linkage, and wherein said substituent comprises an aromatic or heteroaromatic moiety.

30. The 7-substituted tetracycline compound of claim 29, wherein said compound is 7-4'-Cl-phenyl sancycline.

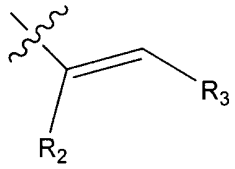
31. The 7-substituted tetracycline compound of claim 29, wherein said compound is 7-(4-fluorophenyl)sancycline.

32. The 7-substituted tetracycline compound of claim 29, wherein said compound is 7-(4-nitrophenyl)sancycline.

33. The 7-substituted tetracycline compound of claim 29, wherein said compound is 7-(2-pyridyl)doxycycline.

34. A 7-substituted tetracycline compound, wherein the substituent at the 7 position is connected with a -C-C- linkage, and wherein said substituent comprises a -C=C- bond adjacent to said -C-C- linkage.

35. The 7-substituted tetracycline compound of claim 34, wherein said substituent is of the formula



(Z)

wherein R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, aryloxycarbonyl; or R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

36. The 7-substituted tetracycline compound of claim 35, wherein R_2 is hydrogen, and R_3 is  , and R_4 is hydrogen, cyano, or a C_1-C_5 alkoxy group.

37. The 7-substituted tetracycline compound of claim 35, wherein R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

38. The 7-substituted tetracycline compound of claim 35 or 37, wherein said ring comprises 5 to 15 atoms.

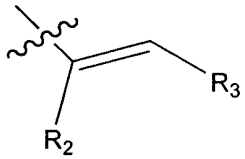
39. The 7-substituted tetracycline compound of any one of claims 35, 37 or 38, wherein said ring is a conjugated or unconjugated aromatic ring system.

40. The 7-substituted tetracycline compound of claim 35, wherein said compound is 7-ethylenylsancycline.

41. A 9-substituted tetracycline compound, wherein the substituent at the 9 position is connected with a -C-C- linkage, and wherein said substituent comprises an aromatic or heteroaromatic moiety.

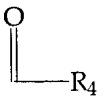
42. A 9-substituted tetracycline compound, wherein the substituent at the 9 position is connected with a -C-C- linkage, and wherein the substituent comprises a -C=C- bond adjacent to said -C-C- linkage.

43. The 9-substituted tetracycline compound of claim 42, wherein the substituent is of the formula



(Z)

wherein R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, aryloxycarbonyl; or R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

44. The 9-substituted tetracycline compound of claim 43, wherein R_2 is hydrogen, and R_3 is  , where R_4 is hydrogen, cyano, or a C_1-C_5 alkoxy group.

45. The 9-substituted tetracycline compound of claim 43, wherein R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

46. The 9-substituted tetracycline compound of claim 43 or 45, wherein said ring has from 5 to 15 atoms in the ring.

47. The 9-substituted tetracycline compound of any one of claims 43, 45, or 46, wherein said ring is a conjugated or unconjugated aromatic ring system.

48. A substituted tetracycline compound made by a method comprising contacting a reactive tetracycline chemical complex comprising a reactive tetracycline-based precursor compound and a transition metal catalyst forming a reactive chemical intermediate with a reactive organic substituent precursor under conditions such that a tetracycline compound substituted with said organic substituent is formed.

49. The substituted tetracycline compound of claim 48, wherein said reactive tetracycline-based precursor compound is substituted at positions 7, 9 or 13 with said organic substituent.

50. The substituted tetracycline compound of claim 48 or 49, wherein said catalyst comprises an organopalladium catalyst.

51. The substituted tetracycline compound of claim 50, wherein said organopalladium catalyst comprises palladium chloride, palladium acetate, $PdCl_2(PhCN)_2$, $PdCl_2(Ph_3P)_2$, $Pd_2(dba)_3$-$CHCl_3$, or a combination thereof.

52. The substituted tetracycline compound of any one of claims 48-51, wherein said transition metal catalyst comprises copper, rhodium, iron, iridium, chromium, zirconium, or nickel.

53. The substituted tetracycline compound of any one of claims 48-52, wherein said transition metal catalyst comprises $CuCl_2$, CuI_2, rhodium (II) acetate, $Rh_6(CO)_{16}$), or combinations thereof.

54. The substituted tetracycline compound of any one of claims 48-53, wherein said reactive tetracycline-based precursor compound is oxytetracycline, chlortetracycline, demeclocycline, doxycycline, chelocardin, minocycline, roliteteracycline, lymecycline, sancycline, methacycline, apicycline, clomocycline, guamecycline, meglucycline, mepylcycline, penimepicycline, pipacycline, etamocycline, or penimocycline based precursor compound.

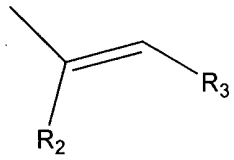
55. The substituted tetracycline compound of any one of claims 48-53, wherein said reactive tetracycline-based precursor compound is selected from the group consisting of reactive minocycline-based precursor compounds, reactive doxycycline-based precursor compounds, and reactive sancycline-based precursor compounds.

56. The substituted tetracycline compound of any one of claims 48-55, wherein said reactive tetracycline-based precursor compound is an arenediazonium salt, iodo derivative, or a boronic acid derivative of a tetracycline compound.

57. The substituted tetracycline compound of any one of claims 48-56, wherein said reactive organic substituent precursor has at least one reactive π-bond containing group.

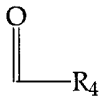
58. The substituted tetracycline compound of any one of claims 48-57, wherein said reactive organic substituent precursor is selected from the group consisting of alkenes, substituted alkenes, vinyl monomers, aromatic and heteroaromatic reactive groups.

59. The substituted tetracycline compound of any one of claims 48-58, wherein said organic substituent is of the formula



(Z)

wherein R_2 and R_3 are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, alkylcarbonyl, arylcarbonyl, alkoxycarbonyl, aryloxycarbonyl, amino, hydroxy, cyano, alkoxy, aryloxy, carboxyl, alkoxycarbonyl, aryloxycarbonyl; or R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

60. The substituted tetracycline compound of claim 59, wherein R_2 is hydrogen, and R_3 is  , where R_4 is hydrogen, cyano, or a C_1-C_5 alkoxy group.

61. The substituted tetracycline compound of claim 59, wherein R_2 and R_3, taken together, form a substituted or unsubstituted carbocyclic or heterocyclic ring.

62. The substituted tetracycline compound of claim 59 or 61, wherein said ring has from 5 to 15 atoms in the ring.

63. The substituted tetracycline compound of any one of claims 59, 61, or 62, wherein said ring is a conjugated or unconjugated aromatic ring system.

64. The substituted tetracycline compound of any one of claims 59, or 61-63, wherein said ring has 5 to 8 atoms in the ring.

INTERNATIONAL SEARCH REPORT

Interna ıl Application No
PCT/US 00/25040

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C237/26 C07C231/12 A61K31/65 A61P31/04

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 A61K

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, WPI Data, PAJ, CHEM ABS Data, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AHMAD, N. ET AL.: "Polynuclear Complexes of Bivalent Metal Ions with Antibiotic Tetracycline" JOUR. CHEM. SOC. PAK., vol. 12, no. 2, 1990, pages 168-173, XP000943839 the whole document	21-28
A	---	1-20, 29-64
P,X	KOZA, D.J.: "Synthesis of 7-Substituted Tetracyclin Derivatives" ORGANIC LETTERS, vol. 2, no. 6, 1 March 2000 (2000-03-01), pages 815-817, XP000943852 the whole document	1-64

☐ 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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 December 2000	10.01.01

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