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(54) Title: Anthraquinone Analogs

(57) Abstract: Anthraquinone analogs, methods for synthesizing anthraquinone analogs, and methods for inhibiting growth of one or more types of cells using anthraquinone analogs. Anthraquinone analogs can be synthesized according to methods described herein. Optionally, the synthesis methods described herein include choosing an appropriate leaving group for selectively producing 1-alkyl-1H-naphtho[2,3-d]triazole-4,9-diones or 2-alkyl-2H-naphtho[2,3-d]triazole-4,9-diones. Anthraquinone analogs can include various functional groups that affect their ability to inhibit the growth of various cell types. For example, some anthraquinone analogs disclosed herein have antimicrobial activity while seemingly similar compounds demonstrate anticancer activity but lesser antimicrobial activity.



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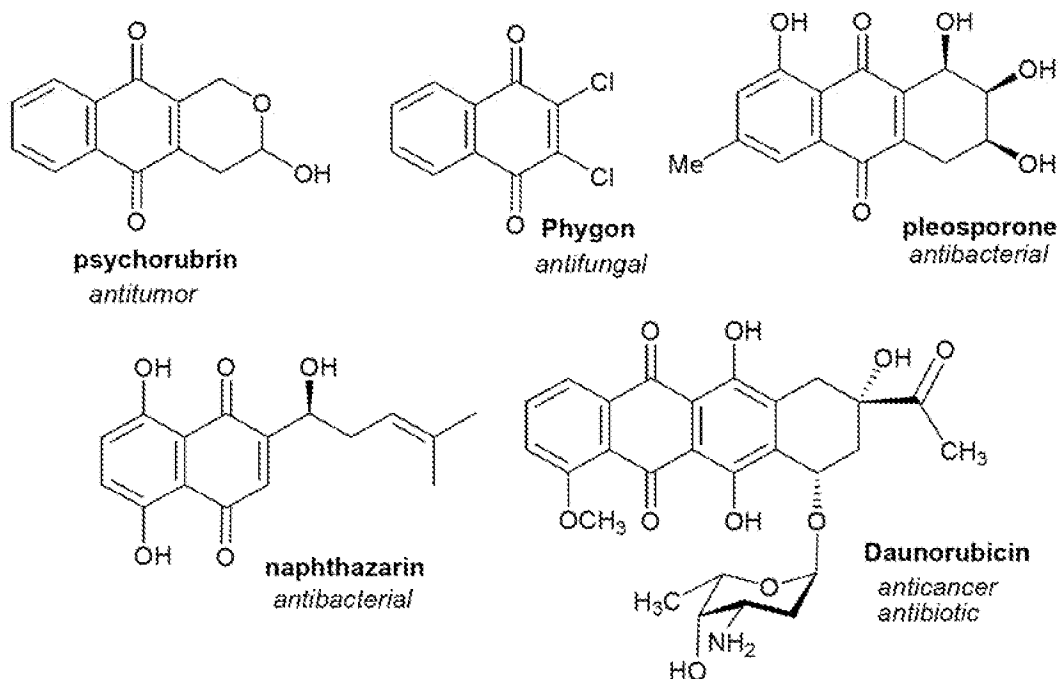
ANTHRAQUINONE ANALOGS

This application claims priority to U.S. Application 61/372,530 filed on August 11, 2010.

BACKGROUND OF THE INVENTION

[0001] Molecules containing naphthoquinone and anthraquinone scaffolds have been used to develop numerous therapeutics due to their important biological and pharmaceutical activities. For example, 1,4-naphthoquinone derivatives have been studied for their diverse biological activities including uses as antibacterial, antifungal, antimalarial, antitumor agents, or being employed as inhibitors against vitamin K dependent carboxylase, protein kinase, coenzyme Q, and even as growth stimulator for bifidobacteria. Anthraquinones, which bear the structural core of anthracycline, have also been used as antibiotics or anticancer agents. Naphthoquinone and anthraquinone are known to uncouple mitochondria oxidative phosphorylation leading to mechanistic investigations in their redox chemistry and related applications, such as the use as new material for photocells.

[0002] A number of representative naphthoquinones and anthraquinones and their associated activities are illustrated below.

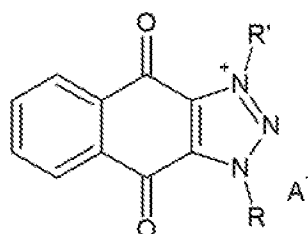


The above illustrated representative naphthoquinones and anthraquinones may be antimicrobial, anticancer, or both. A constant and persistent problem in the field of antimicrobials and anticancer agents is the emergence of resistance to known compounds. Novel antimicrobials and anticancer agents are needed to solve the problem resistance.

BRIEF SUMMARY

[0003] The present disclosure provides a solution to persistent and emerging resistance in the fields of antimicrobial and anticancer agents. Generally, the present disclosure relates to anthraquinone analogs, methods for synthesizing anthraquinone analogs, and methods for killing or inhibiting growth of one or more types of cells using anthraquinone analogs. The anthraquinone analogs described herein can be synthesized using a simplified protocol described herein. Optionally, the synthesis methods described herein may include choosing an appropriate leaving group for selectively producing 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones or 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. According to the present disclosure, anthraquinone analogs can include various functional groups that affect their ability to kill or inhibit the growth of various cell types. For example, some anthraquinone analogs disclosed herein have antimicrobial activity while seemingly similar compounds demonstrate anticancer activity but lesser antimicrobial activity.

[0004] In one embodiment, a chemical composition that includes a cationic anthraquinone analog of Formula I is disclosed.

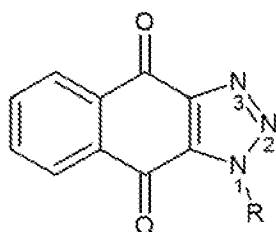


Formula I

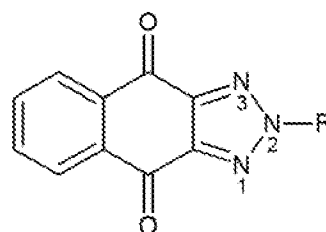
[0005] According to the present embodiment, R and R' are each one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic,

or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. According the present embodiment, sugars, saccharides, carbohydrates, and the like are specifically excluded from the possible structures for R and R'. R and R' may be the same or they may be different. A⁻ is a counter ion.

[0006] In another embodiment, a method for synthesizing an anthraquinone analog of Formula II (i.e., a 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-dione) or Formula III (i.e., a 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-dione) or a pharmaceutically acceptable salt thereof is disclosed.



Formula II

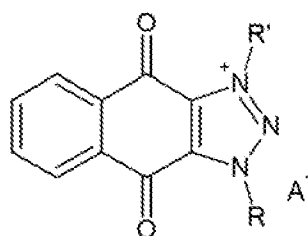


Formula III

[0007] The method includes forming a reaction mixture that includes a naphthoquinone starting material, a sodium azide compound, and an R-Z in an organic solvent (e.g., a polar organic solvent such as dimethyl formamide, DMF), and heating the reaction mixture to a temperature in a range of about 100° C to about 140° C for a period of time sufficient to yield the anthraquinone analog of Formula II or Formula III. In a subsequent step, an R' group may be added to the compound of Formula II or III to form an ionic species (e.g., a compound of Formula I).

[0008] R is selected from the group consisting of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. According to the present embodiment, sugars, saccharides, carbohydrates, and the like are specifically excluded from the possible structures for R. Z is a leaving group selected from the group consisting of chloride, iodide, bromide, tosylate, mesylate, trifluoroacetate, and combinations thereof. According to one embodiment of the present invention, Z can be selected in order to favor coupling of the R group to the 1 position (i.e., Formula II) or to the 2 position (i.e., Formula III).

[0009] In yet another embodiment, the present invention includes a method for inhibiting growth of a cell. The method includes contacting the cell with a pharmacologically effective dose of the chemical composition of a cationic anthraquinone analog of Formula I or a pharmaceutically acceptable salt thereof.



Formula I

[0010] According to the present embodiment, R and R' are each one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or

phosphorus, and combinations thereof. According the present embodiment, sugars, saccharides, carbohydrates, and the like are specifically excluded from the possible structures for R and R'. R and R' may be the same or they may be different. A⁻ is a counter ion.

[0011] Compounds of Formula I are effective against cells selected from the group consisting of cancer cells, gram-negative bacteria, gram-positive bacteria, fungi, and combinations thereof.

[0012] These and other objects and features of the present invention will become more fully apparent from the following description and appended claims, or may be learned by the practice of the invention as set forth hereinafter.

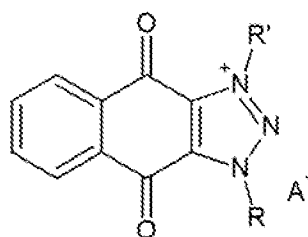
DETAILED DESCRIPTION

I. INTRODUCTION

[0013] Generally, the present disclosure relates to anthraquinone analogs, methods for synthesizing anthraquinone analogs, and methods for killing or inhibiting growth of one or more types of cells using anthraquinone analogs. The anthraquinone analogs described herein can be synthesized using a simplified protocol described herein. Optionally, the synthesis methods described herein may include choosing an appropriate leaving group for selectively producing 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones or 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. According to the present disclosure, anthraquinone analogs can include various functional groups that affect their ability to kill or inhibit the growth of various cell types. For example, some anthraquinone analogs disclosed herein have antimicrobial activity while seemingly similar compounds demonstrate anticancer activity but lesser antimicrobial activity.

II. ANTHRAQUINONE ANALOGS

[0014] In one embodiment, a chemical composition that includes a cationic anthraquinone analog of Formula I is disclosed.



Formula I

[0015] According to the present embodiment, R and R' are each a functional group selected from the group consisting of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, or cyclic, aliphatic group or an aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. According the present embodiment, sugars, saccharides, carbohydrates, and the like are specifically excluded from the possible structures for R and R'. R and R' may be the same or they may be different.

[0016] As used herein, the term "aliphatic" is meant to refer to a hydrocarbon moiety, such as an alkyl group, that can be straight or branched, saturated or unsaturated, and/or substituted or unsubstituted, which has twenty or less carbons or hetero atoms in the backbone. Additionally, an aliphatic can include 20 or less carbons or hetero atoms in the backbone. An aliphatic group may comprise moieties that are linear, branched, cyclic and/or heterocyclic, and contain functional groups such as ethers, ketones, aldehydes, carboxylates, and the like. Exemplary aliphatic groups include but are not limited to substituted and/or unsubstituted groups of methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, tridecyl, tetradecyl, pentadecyl, hexadecyl, heptadecyl, octadecyl, nonadecyl, eicosyl, alkyl groups of higher number of carbons and the like, as well as 2-methylpropyl, 2-methyl-4-ethylbutyl, 2,4-diethylpropyl, 3-propylbutyl, 2,8-dibutyldecyl, 6,6-dimethyloctyl, 6-propyl-6-butyloctyl, 2-methylbutyl, 2-methylpentyl, 3-methylpentyl, 2-ethylhexyl, and the like. The terms aliphatic or alkyl also encompasses alkenyl groups, such as vinyl, allyl, aralkyl, alkenyl, and alkynyl groups.

[0017] Substitutions within an aliphatic group can include any atom or group that can be tolerated in the aliphatic moiety, including but not limited to halogens, sulfurs, thiols, thioethers, thioesters, amines (primary, secondary, or tertiary), amides, ethers, esters, alcohols, oxygen, and the like. The aliphatic groups can by way of example also comprise modifications such as azo groups, keto groups, aldehyde groups, carbonyl groups, carboxyl groups, nitro, nitroso or nitrile groups, heterocycles such as imidazole, hydrazino or hydroxylamino groups, isocyanate or cyanate groups, and sulfur containing groups such as sulfoxide, sulfone, sulfide, and disulfide. Additionally, the substitutions can be via single, double, or triple bonds, when relevant or possible.

[0018] Further, aliphatic groups may also contain hetero substitutions, which are substitutions of carbon atoms, by hetero atoms such as, for example, nitrogen, oxygen, phosphorous, or sulfur. As such, a linker comprised of a substituted aliphatic can have a backbone comprised of carbon, nitrogen, oxygen, sulfur, phosphorous, and/or the like. Heterocyclic substitutions refer to alkyl rings having one or more hetero atoms. Examples of heterocyclic moieties include but are not limited to morpholino, imidazole, and pyrrolidino.

[0019] As used herein, the term "aromatic" is meant to refer to molecule is one in which electrons are free to cycle around circular or cyclic arrangements of atoms, which are alternately singly and doubly bonded to one another. More properly, these bonds may be seen as a hybrid of a single bond and a double bond, each bond in the ring being identical to every other.

[0020] Suitable examples of aromatic and aryl R and R' groups include, but are not limited to, benzene, phenyl, benzyl, toluene, tolyl, xylene, and the like. The aromatic group can also include hetero atoms so as to be a hetero aromatic such as pyridine,

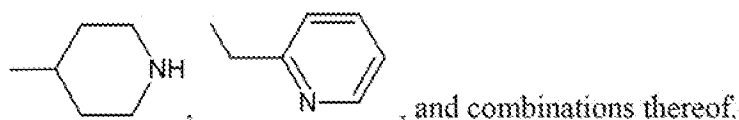
furan, tetrahydrofuran, and the like. Also, an aromatic can be a polycyclic aromatic such as naphthalene, anthracene, phenanthrene, polycyclic aromatic hydrocarbons, indole, quinoline, isoquinoline, and the like. The term "aryl" refers to any functional group or substituent derived from a simple aromatic ring, be it phenyl, thiophene, indolyl, etc. For instance, examples of C₆₋₁₄aryl groups include phenyl (C₆), indenyl (C₉), naphthyl (C₁₀), fluorenyl (C₁₃), anthracyl (C₁₄), and phenanthryl (C₁₄). In the above mentioned aryl groups, unless otherwise stated, one or more hydrogen atoms may optionally be replaced by other substituent groups. For example, aryl groups may be substituted by the following substituents groups: OH; NO₂; CN; NH₂; halogen, for example fluorine or chlorine; optionally substituted C₁₋₁₀alkyl, for example methyl, ethyl, or propyl; optionally substituted -OC₁₋₃alkyl, for example -OMe, -OEt, -COOH, -COO-C₁₋₄alkyl, for example -COOMe or -COOEt, or -CONH₂.

[0021] As can be seen, the cationic anthraquinone analog of Formula I includes a charge on the triazole ring. Even though the charge is shown on a single nitrogen, the charge is actually delocalized due to resonance of the double bonds. In order to balance the charge, the chemical composition can further include a counter ion A⁻. Suitable examples of counter ions include, but are not limited to, triflate (CF₃SO₃⁻), fluoride (F⁻), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), acetate (CH₃COO⁻), nitrite (NO₂⁻), hydrogen carbonate (HCO₃⁻), dihydrogen phosphate (H₂PO₄⁻), hydrogen sulphate (HSO₄⁻), hydroxide (OH⁻), hydrogen sulphite (HSO₃⁻), nitrate (NO₃⁻), carbonate (CO₃²⁻), sulfate (SO₄²⁻), and combinations thereof. In one embodiment, the counter ion may be a pharmaceutically acceptable counter ion such as, but not limited to, chloride or bromide. Additional counter ions that can be included in the chemical composition to

balance the charge of the cationic anthraquinone analog are known to persons having skill in the art.

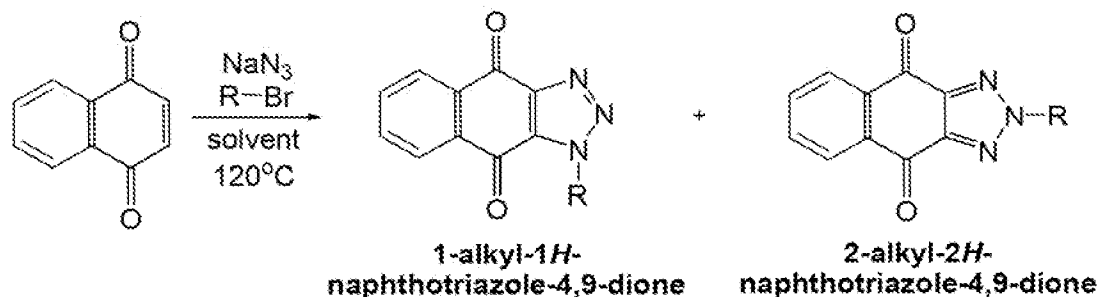
[0022] In one embodiment, R' of the cationic anthraquinone analog is an alkyl group. Suitable examples of R' alkyl groups include methyl, ethyl, propyl, butyl, pentyl, combinations thereof, and the like. In a specific example, R' is a methyl group.

[0023] In one embodiment, R' of the cationic anthraquinone analog is a methyl group and R is selected from the group consisting of CH₃, CH₂CH₃, CH₂(CH₂)₂CH₃, CH₂(CH₂)₃CH₃, CH₂(CH₂)₆CH₃, CH₂(CH₂)₁₀CH₃, CH₂(CH₂)₁₄CH₃, benzyl,

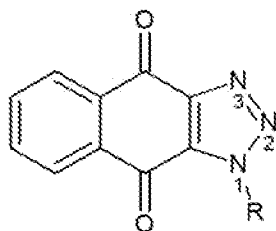


III. METHODS FOR SYNTHESIZING ANTHRAQUINONE ANALOGS

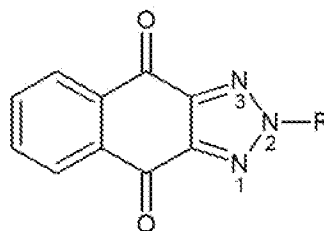
[0024] The synthesis of anthraquinone analogs generally requires multi-step processes. In contrast, the present disclosure provides for a concise one-pot divergent synthesis of 1-alkyl-1*H*- and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones, both of which can be viewed as analogs of anthraquinone fused with 1,2,3-triazole is reported herein.



[0025] In another embodiment, a method for synthesizing an anthraquinone analog of Formula II (i.e., a 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-dione) or Formula III (i.e., a 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-dione) or a pharmaceutically acceptable salt thereof is disclosed.



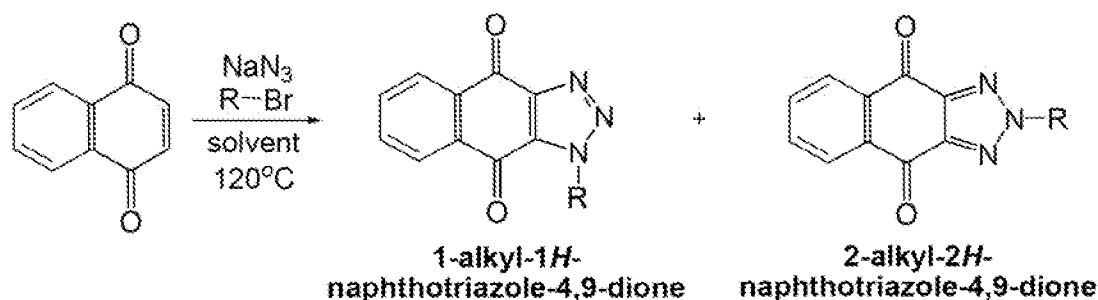
Formula II



Formula III

[0026] The method includes forming a reaction mixture that includes a naphthoquinone starting material, a sodium azide compound, and an R-Z in an organic solvent (e.g., a polar organic solvent such as dimethyl formamide, DMF), and heating the reaction mixture to a temperature in a range of about 100° C to about 140° C for a period of time sufficient to yield the anthraquinone analog of Formula II or Formula III, wherein the R group is selectively coupled to a 1 position or a 2 position on the anthraquinone analog of Formula II or Formula III.

[0027] An example of a divergent synthesis protocol for synthesizing 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones of Formula II and 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones of Formula III is illustrated below.



[0028] R is selected from the group consisting of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. Z is a leaving group selected from the group consisting of chloride, iodide, bromide, tosylate, mesylate, trifluoroacetate, and combinations thereof.

[0029] Suitable examples of organic solvents include, but are not limited to, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate, acetone, dimethylformamide (DMF), acetonitrile (MeCN), dimethyl sulfoxide (DMSO), formic acid, pentanol, n-butanol, isopropanol, n-propanol, ethanol, methanol, 1,4-dioxane, toluene, and the like. In one embodiment, the polar organic solvent is dimethyl formamide (DMF).

[0030] In one embodiment, the reaction mixture further includes an additive such as, but not limited to, a quaternary ammonium compound. Without being tied to one theory, it is believed that the quaternary ammonium compound encourages the formation of at least some of the compounds of Formula II and III by acting a phase-transfer catalyst.

[0031] Suitable examples of quaternary ammonium compounds that can be used in the methods described herein include tetrabutylammonium iodide and

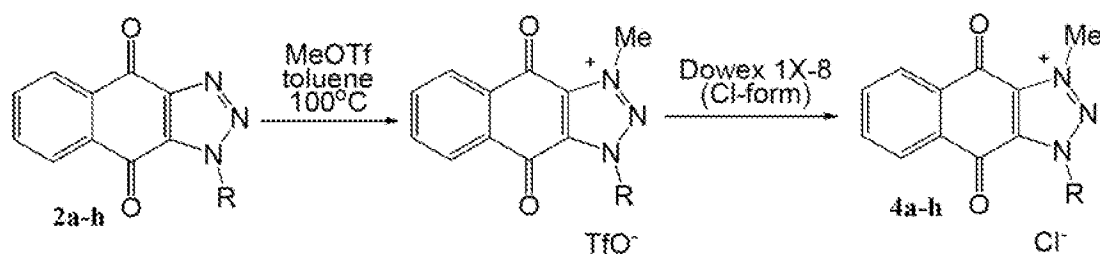
tetrabutylammonium bisulfate. Additional suitable examples of quaternary ammonium compounds include, but are not limited to, benzyltriethylammonium chloride, tetramethylammonium chloride, tetramethylammonium iodide, tetramethylammonium bromide, tetraethylammonium hydroxide, benzyltrimethylammonium hydroxide, dimethyldioctadecylammonium chloride, dodecyltrimethylammonium chloride, trimethylphenylammonium chloride, tetrabutylammonium bromide, tetrabutylammonium chloride, tetrabutylammonium hydroxide, tetrapropylammonium chloride, benzyltributylammonium chloride, and combinations thereof.

[0032] Both 1-alkyl-1*H*- and 2-alkyl-2*H*-alkylated naphtho[2,3-*d*]triazole-4,9-diones are completely insoluble in aqueous media making these two classes of compounds generally unsuitable for biological evaluation. One feasible approach for improving the solubility of these compounds is to convert them into cationic compound via alkylation of the N-3 of the triazole motif. The N-3 is labeled above as position 3 in Formulas II and III.

[0033] As such, in one embodiment, the method further includes coupling an R' group to a 3 position of the anthraquinone analog of Formula II or Formula III to form a cationic anthraquinone analog. In the case of compounds of Formula II, adding an R' to the N-3 position results in the formation of a compound of Formula I. Compounds can be alkylated by a general procedure as follows: To a solution of starting material (e.g., about 0.05 g) in toluene (e.g., about 10 mL), MeOTf (4 equiv.) was added. The reaction mixture was stirred at 100°C for 24 hours. After completion of the reaction, the solvent was removed and the crude product was loaded with a short column packed with Dowex 1X resin (Cl⁻ form). The column was eluted with MeOH (ca. 20 mL). After removal of the solvent, the product was obtained as brownish solid.

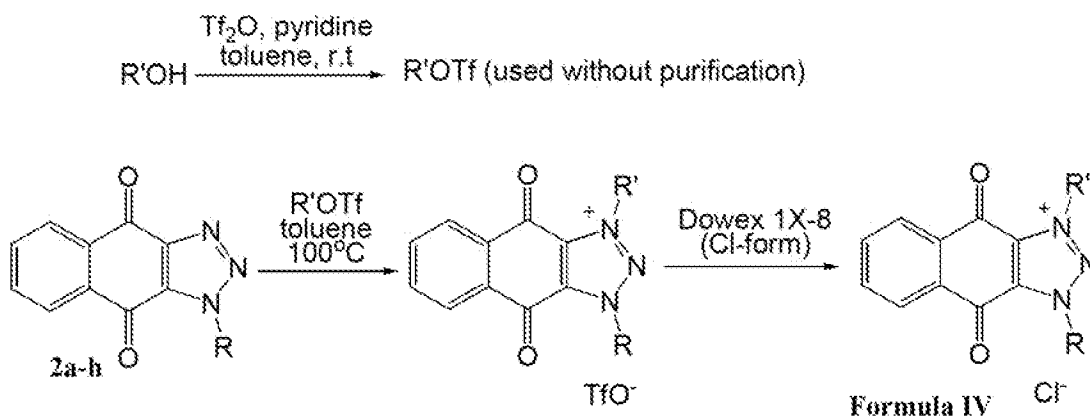
While the procedure described above relates specifically to methylation, essentially the same procedure can be used for other alkyl groups. In one embodiment, the R' group is one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. In a specific embodiment, R' is a methyl group. Results of methylation are illustrated in the table below (Table 1).

Table 1: Synthesis Scheme 1.



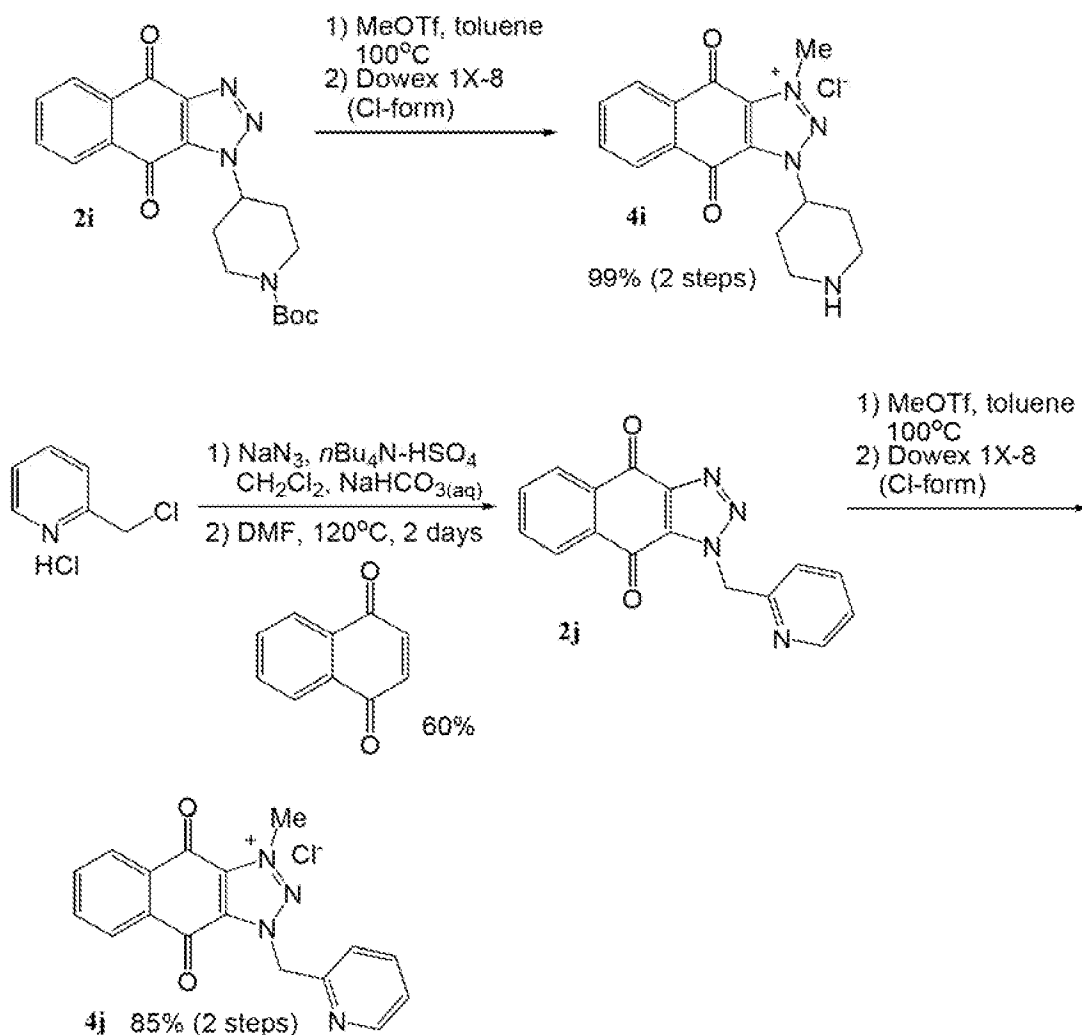
Compound	R	Product	Yield (%)
2a	CH ₃	4a	81%
2b	CH ₂ CH ₃	4b	82%
2c	CH ₂ (CH ₂) ₂ CH ₃	4c	84%
2d	CH ₂ (CH ₂) ₃ CH ₃	4d	84%
2e	CH ₂ (CH ₂) ₆ CH ₃	4e	99%
2f	CH ₂ (CH ₂) ₁₀ CH ₃	4f	88%
2g	CH ₂ (CH ₂) ₁₄ CH ₃	4g	43%
2h	Bn	4h	85%

[0034] Compounds of Formula II or Formula III can be alkylated with alkyl groups other than methyl using the general procedure illustrated below.



Two such analogs have been prepared with R'=ethyl and R'=octyl in 50% and 38% overall yields, respectively.

[0035] The synthesis of compound 4i began with a Boc-protected 1-(4-(N-tert-butoxycarbonylpiperidinyl))-1H-naphtho[2,3-d]triazole-4,9-dione (2i) (Scheme 2). During the methylation step, the by-product TfOH also triggered the deprotection of Boc group and provided the desired product following ion-exchange. The synthesis of 4j started with the synthesis of 2-picoyl azide followed by cycloaddition. The resulting cycloaddition product 2j can be converted to the corresponding cationic adduct 4j via alkylation with MeOTf.



Scheme 2

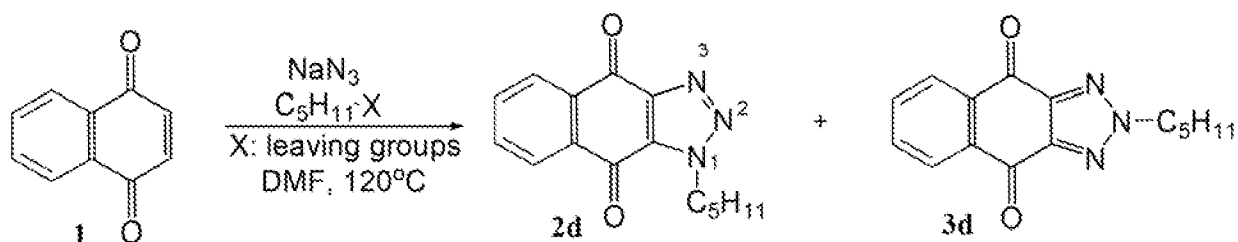
[0036] While the specific examples shown herein illustrate the cationic analogs of Formula II and Formula III having either a TfO⁻ (i.e., triflate or CF₃SO₃⁻) or Cl⁻ counter ion, one will appreciate that a number of counter ions are possible. As such, in one embodiment, the cationic anthraquinone analog includes a counter ion selected from the group consisting of triflate (CF₃SO₃⁻), fluoride (F⁻), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), acetate (CH₃COO⁻), nitrite (NO₂⁻), hydrogen carbonate (HCO₃⁻), dihydrogen

phosphate (H_2PO_4^-), hydrogen sulphate (HSO_4^-), hydroxide (OH^-), hydrogen sulphite (HSO_3^-), nitrate (NO_3^-), carbonate (CO_3^{2-}), sulfate (SO_4^{2-}), and combinations thereof.

[0037] It was found that the leaving group Z in the R-Z starting material could be selected to favor synthesis of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones or 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. In order to investigate this phenomenon, the synthesis protocol described herein was employed using pentyl group as the R group to be incorporated. Various leaving groups including bromide, chloride, tosylate (TsO), mesylate (MsO), and trifluoroacetate (CF_3CO_2), were investigated (Table 2).

[0038] From the ratio of 2d/3d determined by ^1H NMR, it is apparent that better leaving groups (Br) favor the formation of 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones while poor leaving groups (MsO and CF_3CO_2) prefer the formation of 2-alkyl-2*H*-naphtho[2,3-*d*]triazole-4,9-diones. Use of tetrabutylammonium iodide (TBAI) improved the overall yield of compounds where the leaving group is likely stabilized by the additive.

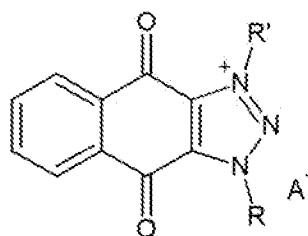
[0039] Therefore, in one embodiment the method of the present invention includes using a leaving group selected from the group consisting of chloride, iodide, or bromide in order to favor the formation of anthraquinone analogs of Formula II. In another embodiment, the method includes using a leaving group selected from the group consisting of tosylate, mesylate, or trifluoroacetate in order to favor the formation of anthraquinone analogs of Formula III.

Table 2. The ratio of 2d/3d

Leaving group (X)/Additive	1H: 2H ratio (2d/3d)	Overall yield
Pentyl bromide, TBAI	7.12 : 1.00	80%
Pentyl bromide	5.00 : 1.00	75%
Pentyl chloride, TBAI	1.27 : 1.00	76%
Pentyl chloride	1.02 : 1.00	99%
Pentyl tosylate	1.58 : 1.00	75%
Pentyl mesylate	1.17 : 1.00	29%
Pentyl trifluoroacetate	1.00 : 1.06	38%

IV. ANTIMICROBIAL AND ANTICANCER ACTIVITIES

[0040] The anthraquinone analogs described herein can be used to kill or inhibit growth of a cell, to treat cancer or an infection due to a parasite, gram-negative (G-) bacteria, a gram-positive (G+) bacteria, fungi, and combinations thereof. In this regard, the invention provides a method of inhibiting growth of a cell. The method includes contacting the cell with a pharmacologically effective dose of the chemical composition of a cationic anthraquinone analog of Formula I.



Formula I

[0041] R and R' are each one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof. R and R' may be the same or different.

[0042] A⁻ shown in Formula I is a counter ion. Suitable examples of counter ions include, but are not limited to, triflate (CF₃SO₃⁻), fluoride (F⁻), chloride (Cl⁻), bromide (Br⁻), iodide (I⁻), acetate (CH₃COO⁻), nitrite (NO₂⁻), hydrogen carbonate (HCO₃⁻), dihydrogen phosphate (H₂PO₄⁻), hydrogen sulphate (HSO₄⁻), hydroxide (OH⁻), hydrogen sulphite (HSO₃⁻), nitrate (NO₃⁻), carbonate (CO₃²⁻), sulfate (SO₄²⁻), and combinations thereof. In one embodiment, the counter ion may be a pharmaceutically acceptable counter ion such as, but not limited to, chloride or bromide.

[0043] Compounds of Formula I were tested for their activity against a variety of gram-positive (G⁺) and gram-negative (G⁻) bacteria including *Escherichia coli* (G⁻, American Type Culture Collection ("ATCC") 25922), *Staphylococcus aureus* (G⁺, ATCC 25923), *Klebsiella pneumoniae* (G⁻, ATCC 13883), *Pseudomonas aeruginosa* (G⁻, ATCC 27853), *Mycobacterium smegmatis* (G⁺, ATCC14468), Methicillin-resistant *S. aureus* (G⁺, ATCC 33591) (MRSA), vancomycin-resistant *Enterococcus faecalis*

(G+, ATCC 51299) (VRE), and *E. faecalis* (G+, ATCC 29212). Similar cationic compounds, such as benzyldimethylhexadecylammonium chloride (BDC), hexadecylpyridinium bromide (HPB), hexadecyltrimethylammonium bromide (HTB), and clinically used antibiotics were employed as controls.

[0044] The MIC was determined by a procedure as follows: A solution of selected bacteria was inoculated in Trypticase Soy broth at 35°C for 1 - 2hrs. After which, the bacteria concentration was found, and diluted with broth, if necessary, to an absorption value of 0.08 to 0.1 at 625 nm. The adjusted inoculated medium (100 μ L) was diluted with 10 mL broth, and then applied to a 96-well microtiter plate (50 μ L). A series of solutions (50 μ L each in 2-fold dilution) of the tested compounds was added to the testing wells. The 96-well plate was incubated at 35°C for 12 - 18 hrs. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of compound needed to inhibit the growth of bacteria. The MIC results are repeated at least three times.

[0045] The results of the MIC studies with each of the compounds are summarized below in Tables 3a and 3b.

Table 3a: Minimum Inhibitory Concentration (MIC)^a

Compounds	<i>E. coli</i> ^b (G-)	<i>S. aureus</i> ^c (G+)	<i>K. pneumoniae</i> ^d (G-)	<i>S. aureus</i> ^e (MRSA, G+)
Neomycin	4	1	2	125
Kanamycin	2-4	1-2	0.5-1	125-250
Vancomycin	≥ 250	1	≥ 250	0.5-1
Amikacin	1	0.5-1	1	16
BDC	ND ^j	2-4	ND	1
HPB	ND	2-4	ND	4
HTB	1	0.5-1	1	2
4a	32-64	4-8	64	4-8
4b	16-32	1-2	32-64	1-2
4c	16-32	2-4	32-64	1-2
4d	8-16	2	32	1-2
4e	8	0.032-0.064	8-16	0.5-1
4f	2-4	0.032	2-4	0.25-0.5
4g	0.5-1	0.125-0.25	2-4	0.25-0.5
4h	8-16	2	8-16	0.5-1
4i	32-64	2-4	64	2-4
4j	16-32	2-4	16-32	2-4

^a Unit: µg/mL, ^b *Escherichia coli* (ATCC 25922), ^c *Staphylococcus aureus* (ATCC 25923), ^d *Klebsiella pneumoniae* (ATCC 13883), ^e *Staphylococcus aureus* (ATCC 33591) (MRSA), ^j ND: Not Determined.

Table 3b: Minimum Inhibitory Concentration (MIC)^a

Compounds	<i>P. aeruginosa</i> ^f (G-)	<i>M. smegmatis</i> ^g (G+)	<i>E. faecalis</i> ^h (G+)	<i>E. faecalis</i> ⁱ (VRE, G+)
Neomycin	64	ND ^j	16-32	125-250
Kanamycin	125-250	1-2	ND	ND
Vancomycin	≥ 250	ND	1-2	125
Amikacin	0.25	0.25	32-64	≥ 250
BDC	ND	ND	ND	ND
HPB	ND	ND	ND	ND
HTB	16-32	1	2-4	4-8
4a	125	32-64	≥250	125-250
4b	≥250	16-32	≥250	≥250
4c	≥250	16	≥250	≥250
4d	≥250	8-16	≥250	125-250
4e	≥250	1	125-250	125-250
4f	16	1-2	4-8	4-8
4g	≥250	2-4	2-4	4-8
4h	125-250	8-16	≥250	≥250
4i	64	16	≥250	≥250
4j	≥250	16	≥250	≥250

^a Unit: µg/mL, ^f *Pseudomonas aeruginosa* (ATCC 27853), ^g *Mycobacterium smegmatis* (ATCC14468), ^h *Enterococcus faecalis* (ATCC 29212), ⁱ *E. faecalis* (ATCC51299) (VRE), ^j ND: Not Determined.

[0046] In general, these compounds are much more active against G⁺ bacteria than G⁻ bacteria. From the minimum inhibition concentration (MIC) generated from compounds with linear alkyl groups, some of the compounds are surprisingly effective against, in particular, the G⁺ bacteria. For example, against *S. aureus* and MRSA, the MIC's for 4e, 4f, and 4g are lower than all the controls employed. The MIC's of 4e and 4f are even in mid-nanomolar range. Compounds with non-linear alkyl groups, 4h (Bn), 4i (4-piperidinyl), and 4j (2-picoly) are less active than those with linear alkyl groups.

[0047] As such, in one embodiment, the method of inhibiting growth of a cell further comprises contacting a G⁺ bacteria with a compound of Formula I having an R'=methyl and an R=CH₂(CH₂)₈CH₃, wherein the pharmacologically effective dose is in a range from about 0.03 µg/ml to about 1 µg/ml. In another embodiment, the method of inhibiting growth of a cell further comprises contacting a G⁻ bacteria with a compound of Formula I having an R'=methyl and an R selected from the group consisting of CH₂(CH₂)₁₀CH₃ and CH₂(CH₂)₁₄CH₃, wherein the pharmacologically effective dose is in a range from about 0.03 µg/ml to about 4 µg/ml.

[0048] A relatively clear structure-activity relationship (SAR) can be deduced from the compounds with linear alkyl groups. The antibacterial activity slightly increases as the number of carbon of the alkyl group increases and significant antibacterial activity emerges as the chain length reaches eight carbons (octyl group, 4e), and such high activity remains even with sixteen carbons (hexadodacyl group, 4g). Nevertheless, compound 4e (C8) has different antibacterial profile as compared to compounds 4f (C12) and 4g (C16) particularly against G⁻ bacteria and *E. faecalis*. The former compound, 4e shows high antibacterial activity selectively toward G⁺ bacteria except *E. faecalis*. Compounds with shorter linear alkyl chain than 4e also display similar

antibacterial profile as 4e. The latter two compounds, 4f and 4g manifest rather broad antibacterial activity against G- and G+ strains comparable to the commonly used cationic control, HTB.

[0049] Enterococci are facultative anaerobic organisms that can thrive in both oxygen-rich and oxygen-deficient environments. The lack of activity of 4e against *E. faecalis* is particularly interesting since it implies that 4e and compounds with shorter alkyl chains (4a-d) have a different antibacterial mode of action from 4f and 4g. Commercially available cationic antibacterial agents, such as HTB, carry a lipophilic alkyl chain with length around twelve to eighteen carbons, which often lowers the solubility of these agents in aqueous media. The shorter chain length of 4e is potentially advantageous since it can be quite soluble in aqueous media unlike HTB. Finally, all the cationic compounds including HTB are not very activity against *P. aeruginosa*, which is known to exert drug resistance via lowering its membrane permeability.

[0050] Two structural motifs are expected to be responsible for the observed antibacterial activity: the cationic anthraquinone analog and the alkyl groups at the N-1 position. As mentioned previously, the neutral 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones, compound 2a-j are insoluble in aqueous media making it difficult to evaluate the antibacterial activity of these compounds. 1-alkyl-1*H*-naphtho[2,3-*d*]triazole-4,9-diones with carbohydrates attached to the N-1 position enables these compounds to have moderate to excellent solubility in aqueous media. However, these compounds are largely inactive against *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). Therefore, the cationic form of the anthraquinone analog is thought to be important for the observed antibacterial activity. The chain-length of the linear alkyl groups also

plays important role as evidenced by the observation of minimal antibacterial activity with shorter alkyl group chain lengths and an increase in antibacterial activity from C8 to C16.

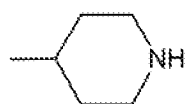
[0051] Commonly used antiseptic quaternary ammonium compounds often contain linear lipophilic alkyl chains (C12-C18). The amphiphilic property of these cationic agents allows the molecules to exert their antibacterial activity by disrupting the bacterial membrane. Since compound 4e (C8) exerts excellent antibacterial activity as compared to 4f and 4g, membrane disruption that is related to the lipophilicity of the commercially used cationic antiseptic agents may not offer a sole explanation for the observed activity of 4e. Without being tied to one theory, it is speculated that the observed antibacterial activity is a combination of the cationic nature and the alkyl group each contribute to the exceptionally high antibacterial activity of 4e via an unknown mode of action, which seems to be specific toward G⁺ bacteria. Noticeable antibacterial activities, which are also specific toward G⁺ bacteria for compounds with shorter alkyl chains (4a-d, C2-C5) implies that the cationic anthraquinone moiety plays an important role in the antibacterial activity of these compounds. As the chain length extended to C12 and C16, the increased lipophilicity of 4f and 4g begins to exert broad antibacterial activity.

[0052] The selectivity of certain embodiments disclosed herein against G⁺ bacteria may provide for additional applications. For example, it may be possible to employ compound 4e as antibacterial agent and avoid *Clostridium difficile* infection (CDI). *C. difficile* is a G⁺ anaerobic bacterium and is the most significant cause of pseudomembranous colitis, a severe infection of the colon, often appears after normal gut flora is eradicated by the use of antibiotics following surgery. Many associated

deaths have been reported, especially among the elderly. The presence of beneficial bacteria within our intestines is necessary to help the human body develop properly and to remain healthy. Since compound 4e is less active against G- bacteria, it may be possible to selectively “kill” pathogenic G+ bacteria and avoid CDI.

[0053] Some compounds of Formula I are also effective anticancer agents. Although, to a certain extent, the anticancer activity is the reverse of antibacterial activity. That is, compounds of Formula I with shorter and/or non-linear R groups were found to have greater anticancer activity, whereas these compounds showed considerably less antibacterial activity relative to longer/linear chain lengths in the study discussed herein.

[0054] As such, in one embodiment, the method of inhibiting growth of a cell further comprises contacting a cancer cell type (e.g., Leukemia, Non-Small Cell Lung Cancer, Colon Cancer, Melanoma, etc.) with a compound of Formula I having an R'=methyl and an R selected from the group consisting of CH₃, benzyl, and



, wherein the pharmacologically effective dose is in a range from about 0.1 µg/ml to about 1.0 µg/ml. Compounds of Formula I having an R'=methyl and an R selected from the group consisting of CH₂(CH₂)₆CH₃, CH₂(CH₂)₁₀CH₃ and CH₂(CH₂)₁₄CH₃ show appreciably less anticancer activity.

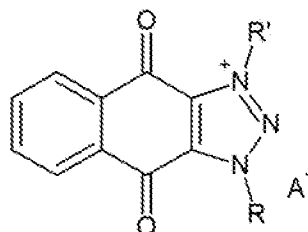
[0055] It is interesting to note that the data showing that a compound of Formula I having R and R'=methyl is not particularly effective against G+ or G- cells suggests that this compounds is not merely toxic to living cells. Rather the observed anticancer activity proceeds by an unknown mechanism that is due to a unique physiological characteristic of the cancerous cells themselves.

[0056] The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

CLAIMS

What is claimed is:

1. A chemical composition, comprising a cationic anthraquinone analog of Formula I:



Formula I

wherein:

R and R' are each one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof,

wherein R and R' may be the same or different; and

A⁻ is a counterion.

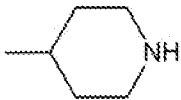
2. The chemical composition of claim 1, wherein the counter ion is selected from the group consisting of triflate (CF_3SO_3^-), fluoride (F^-), chloride (Cl^-), bromide (Br^-), iodide (I^-), acetate (CH_3COO^-), nitrite (NO_2^-), hydrogen carbonate (HCO_3^-), dihydrogen phosphate (H_2PO_4^-), hydrogen sulphate (HSO_4^-), hydroxide (OH^-), hydrogen sulphite (HSO_3^-), nitrate (NO_3^-), carbonate (CO_3^{2-}), sulfate (SO_4^{2-}), and combinations thereof.

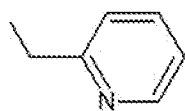
3. The chemical composition of claim 2, wherein the counter ion is chloride.

4. The chemical composition of claim 1, wherein R' is selected from the group consisting of methyl, ethyl, propyl, butyl, pentyl, and combinations thereof.

5. The chemical composition of claim 1, wherein R' is a methyl group.

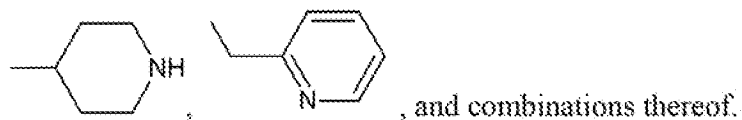
6. The chemical composition of claim 1, wherein R is a methyl group and R is selected from the group consisting of CH_3 , CH_2CH_3 , $\text{CH}_2(\text{CH}_2)_2\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_3\text{CH}_3$,

$\text{CH}_2(\text{CH}_2)_6\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3$, benzyl, ,

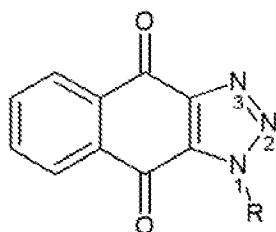


, and combinations thereof.

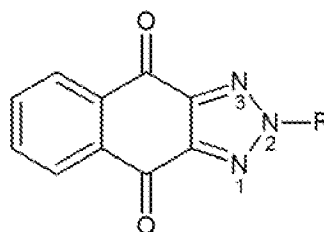
7. The chemical composition of claim 1, wherein R' is selected from the group consisting of methyl, ethyl, propyl, butyl, pentyl, and combinations thereof, and wherein R is selected from the group consisting of CH₃, CH₂CH₃, CH₂(CH₂)₂CH₃, CH₂(CH₂)₃CH₃, CH₂(CH₂)₆CH₃, CH₂(CH₂)₁₀CH₃, CH₂(CH₂)₁₄CH₃, benzyl,



8. A method for synthesizing an anthraquinone analog of Formula II or Formula III or a pharmaceutically acceptable salt thereof, the method comprising:



Formula II



Formula III

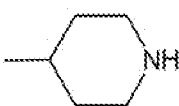
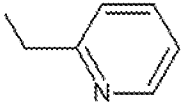
forming a reaction mixture that includes a naphthoquinone starting material, a sodium azide compound, and an R-Z in an organic solvent,

wherein Z is a leaving group selected from the group consisting of chloride, iodide, bromide, tosylate, mesylate, trifluoroacetate, and combinations thereof, and

wherein R is selected from the group consisting of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof,

heating the reaction mixture to a temperature in a range of about 80° C to about 140° C for a period of time sufficient to yield the anthraquinone analog of Formula II or Formula III, wherein the R group is selectively coupled to a 1 position or a 2 position on the anthraquinone analog of Formula II or Formula III.

9. The method of claim 8, wherein R is selected from the group consisting of CH_3 , CH_2CH_3 , $\text{CH}_2(\text{CH}_2)_2\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_3\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_6\text{CH}_3$, $\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$,

$\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3$, benzyl, , , and combinations thereof.

10. The method of claim 8, wherein the solvent is selected from the group consisting of dimethyl formamide (DMF), 1,4-dioxane and toluene.

11. The method of claim 8, wherein the reaction mixture further includes a quaternary ammonium compound selected from the group consisting of tetrabutylammonium iodide, tetrabutylammonium bisulfate, benzyltriethylammonium chloride, tetramethylammonium chloride, tetramethylammonium iodide, tetramethylammonium bromide, tetraethylammonium hydroxide, benzyltrimethylammonium hydroxide, dimethyldioctadecylammonium chloride, dodecyltrimethylammonium chloride, trimethylphenylammonium chloride, tetrabutylammonium bromide, tetrabutylammonium chloride, tetrabutylammonium hydroxide, tetrapropylammonium chloride, benzyltributylammonium chloride, and combinations thereof.

12. The method of claim 8, further comprising coupling an R' group to a 3 position of the anthraquinone analog of Formula II or Formula III to form a cationic anthraquinone analog, wherein the R' group is one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms

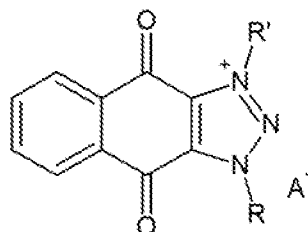
being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof.

13. The method of claim 12, wherein the cationic anthraquinone analog includes a counter ion selected from the group consisting of triflate (CF_3SO_3^-), fluoride (F^-), chloride (Cl^-), bromide (Br^-), iodide (I^-), acetate (CH_3COO^-), nitrite (NO_2^-), hydrogen carbonate (HCO_3^-), dihydrogen phosphate (H_2PO_4^-), hydrogen sulphate (HSO_4^-), hydroxide (OH^-), hydrogen sulphite (HSO_3^-), nitrate (NO_3^-), carbonate (CO_3^{2-}), sulfate (SO_4^{2-}), and combinations thereof.

14. The method of claim 8, wherein having the leaving group be one of chloride, iodide, or bromide favors formation of the anthraquinone analog of Formula II.

15. The method of claim 8, wherein having the leaving group be one of tosylate, mesylate, or trifluoroacetate favors formation of the anthraquinone analog of Formula III.

16. A method for inhibiting growth of a cell, comprising:
contacting the cell with a pharmacologically effective dose of the chemical composition of a cationic anthraquinone analog of Formula I or a pharmaceutically acceptable salt thereof.



Formula I

wherein:

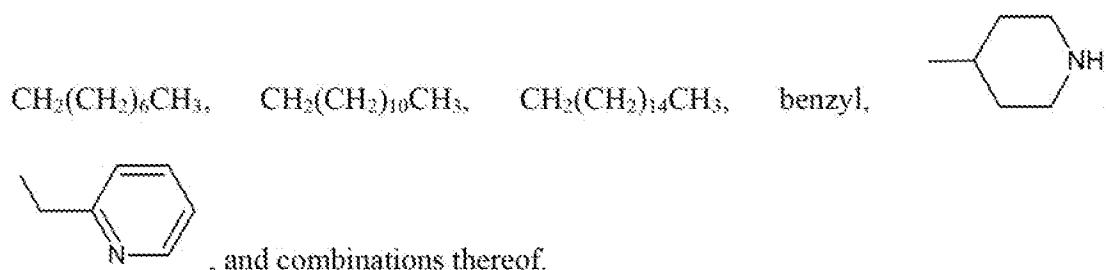
R and R' are each one of a saturated, unsaturated, substituted, unsubstituted, straight chain, branched chain, cyclic, aromatic, or an aryl group having from 1 to 20 carbon and/or hetero chain atoms, the hetero chain atoms being selected from the group consisting of oxygen, nitrogen, sulfur, or phosphorus, and combinations thereof,

wherein R and R' may be the same or different, and

wherein A⁻ is a counter ion.

17. The method of claim 16, wherein the cell is selected from the group consisting of a cancer cell, a gram-negative bacterium, a gram-positive bacterium, a fungus, and combinations thereof.

18. The method of claim 16, wherein R' is a methyl group and R is selected from the group consisting of CH₃, CH₂CH₃, CH₂(CH₂)₂CH₃, CH₂(CH₂)₃CH₃,



19. The method of claim 16, further comprising contacting a gram-positive bacteria with a compound of Formula I having an R'=methyl and an R= $\text{CH}_2(\text{CH}_2)_6\text{CH}_3$, wherein the pharmacologically effective dose is in a range from about 0.03 $\mu\text{g/ml}$ to about 1 $\mu\text{g/ml}$.

20. The method of claim 16, further comprising contacting a gram-negative bacteria with a compound of Formula I having an R'=methyl and an R selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{10}\text{CH}_3$ and $\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3$, wherein the pharmacologically effective dose is in a range from about 0.03 $\mu\text{g/ml}$ to about 8 $\mu\text{g/ml}$.

21. The method of claim 16, further comprising contacting a cancer cell with a compound of Formula I having an R'=methyl and an R selected from the group

