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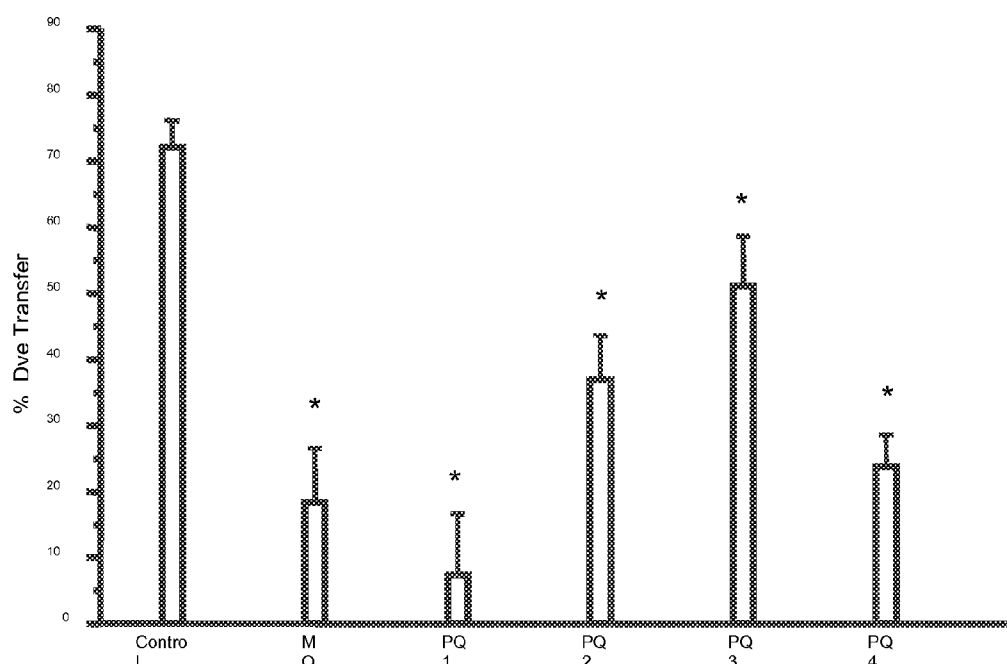
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(54) Title: COMPOUNDS AFFECTING GAP JUNCTION ACTIVITY



(57) Abstract: This invention relates to novel quinoline compounds which affect gap junction activity. Also provided are methods of using such compounds and compositions containing the compounds to treat gap junction disorders.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

COMPOUNDS AFFECTING GAP JUNCTION ACTIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. provisional application Serial Number
5 60/745,909, filed April 28, 2006 and U.S. provisional application Serial Number
60/910,593 filed April 6, 2007 which are hereby incorporated by reference to the
extent not inconsistent with the disclosure herewith.

REFERENCE TO FEDERAL FUNDING

[0002] This invention was sponsored, at least in part, by grant Nos. RO1 EY13421,
10 P20RR017686, and CA86842 from the National Institutes of Health. The US
government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] Malaria and other parasitic diseases are serious public health problems.
The known antimalarial agent, mefloquine, and its analogs have serious side effects
15 including depression, psychotic episodes, and suicidal episodes. Because of these
serious side effects, there have been attempts to formulate alternative antiparasitic
compounds. Several approaches have attempted to use a quinoline structure and
the primaquine branched 8-amino(1-alkyl)amino group. US patent 4980360
discloses quinoline compounds having the primaquine 8-amino-1-methylbutylamino
20 group which are reported for malaria treatment. US patents 4431807 and 4617394
disclose quinoline compounds having a primaquine-like branched 8-aminoalkylamino
group which are reported for treatment of malaria. US patent 7145014 discloses
quinoline compounds having an 8-[(4-amino-1-methylbutyl(amino) group. US patent
6376511 discloses certain branched 8-aminoquinolines for the treatment of parasitic
25 infections. US patent 6479660 discloses methods of making certain antimalarial
drugs. Other quinolines designed for the treatment of malaria are described in
LaMontagne, J. Med. Chem. 1989, 32, 1728-1732.

[0004] Until recently, there was little information on the link between the structure
of these antimalarial drugs and their detrimental psychological properties. It was

discovered recently that mefloquine inhibits gap junctions, such as connexins 50 (Cx50). Gap junctions are the electrical synapses in neurons and responsible for neural transmission. They are non-specific membrane channels between cells and allow the passage of small molecules and ions from one cell to the next. Most

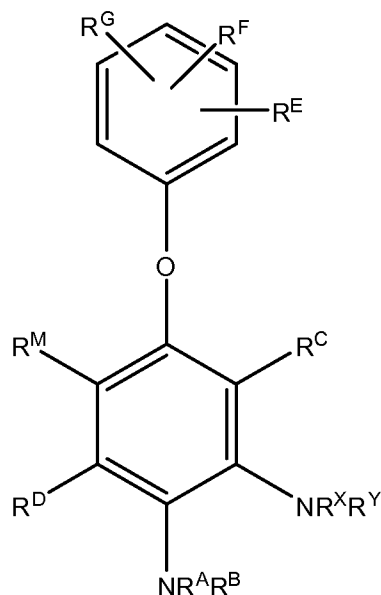
5 tissues are coupled together by gap junctions that vary in type from cell to cell. Gap junctions are made up of hexamers of gap junction proteins, called connexins, abbreviated as Cx and named for their approximate size. Each hexamer hemichannel docks to another on an adjacent cell to form a complete channel or connexon. Connexin mutations have led to a number of human diseases, such as
10 neurosensory autosomal recessive deafness, and the development of cardiovascular abnormalities.

[0005] The disruption of gap junctions produces serious neurological effects and is seen in neurodegenerative diseases such as Parkinson's disease and psychological diseases. Gap junction inhibitors are used to treat ischemic injury, such as stroke or
15 during retinal surgery. Most, if not all, tumor cells have dysfunctional gap junctional intercellular communication (GJIC). Restoring GJIC is linked to drug sensitivity and reduction of tumorigenicity. The information regarding gap junction activity should be useful in development of new compounds that can be used to restore gap junction communication, or inhibit gap junction communication, depending on the disruption.

20 New compounds that affect gap junctions are needed.

SUMMARY OF THE INVENTION

[0006] Provided are quinoline-based compounds. In one embodiment, provided are compounds having the following structure:



where R^X and R^Y are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy and nitrogen containing groups including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine;

R^C , R^D and R^M are independently selected from the group consisting of: hydrogen, halogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy, $RS-$, $RS(O)-$, and RSO_2- , where the optional substituents are independently halogen, $-S-$, $-S(O)-$, or $-SO_2-$;

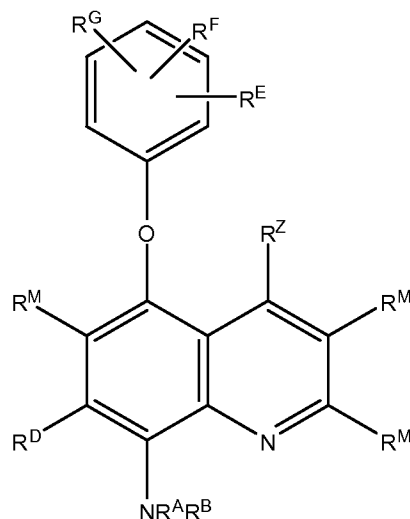
R^E , R^F , and R^G are independently hydrogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy group, oxygen, halogen, CF_3 , R_2N , $RS-$, $RS(O)-$, and RSO_2- , where the optional substituents are independently halogen, alcohol, CF_3 , R_2N , $-S-$, $-S(O)-$, and $-SO_2-$, and where any R^E , R^F , or R^G which is not hydrogen may be positioned in the ortho-, meta- or para-position;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is

an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen and nitrogen containing groups including $-NH^2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine;

- 5 or both R^X and R^Y and R^C are optionally substituted carbon ring atoms which form a six membered ring where the optional substituents are hydroxyl, halogen, C1-C6 alkyl, ketone, ether, nitro, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, wherein when R^X , R^Y and R^C form a ring structure, R^A and R^B are not both oxygen or hydrogen.
- 10 [0007] Specific groups of alkyl are C1-C6, C7-C12 and all individual values and subgroups therein. "Aryl" takes its usual meaning known in the art and is generally a group containing an aromatic ring. When the substituent "R" is used herein without further specificity, it is understood that any group that may be structurally and synthetically possible, including those groups identified as R^C or R^E above, may be
- 15 used, as known by one of ordinary skill in the art. Compounds previously described with an enabling disclosure are not intended to be claimed as compounds, but are intended to be included in methods of treatment described and claimed here to the fullest extent possible.

[0008] In one embodiment, provided are compounds having the following structure:

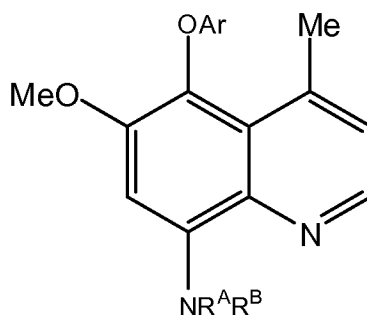


where R^Z , R^M and R^D are independently selected from the group consisting of:
hydrogen, halogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight
chain or branched alkyl or alkoxy, nitro, amine, alkylsulfanyl, alkylsulfinyl,
5 alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional
substituents are independently halogen nitro, amine, alkylsulfanyl, alkylsulfinyl,
alkylsulfonyl, arylsulfanyl, arylsulfinyl, or arylsulfonyl;

R^E , R^F , and R^G are independently hydrogen, aldehyde, ketone, alcohol, optionally
substituted C1-C6 straight chain or branched alkyl or alkoxy group, oxygen, halogen,
10 CF_3 , R_2N , $RS-$, $RS(O)-$, and RSO_2- , where the optional substituents are
independently halogen, alcohol, CF_3 , R_2N , $-S-$, $-S(O)-$, and $-SO_2-$ and where any R^E ,
 R^F , or R^G which is not hydrogen may be positioned in the ortho-, meta- or para-
position;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the
15 group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is
an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$;
and Y is selected from the group consisting of: hydrogen, optionally substituted C1-
C6 straight chain or branched alkyl or alkoxy, and nitrogen containing groups
including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine,
20 pyrazine, and pyridazine.

[0009] In one embodiment, provided are compounds having the formula:



where Ar is o-, m- or p- $C_6H_4CF_3$;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the
25 group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is

an integer from 0-2; X is independently -O-, -C(=O)-, -C(=NH)-, -S-, -S(=O)-, -SO₂-; and Y is selected from the group consisting of: hydrogen, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy, and nitrogen containing groups including -NH₂; -NR₂, where R is C1-C6 alkyl; -NO₂; -CN; phthalimide; imidazole; pyrazole; pyridine; pyrimidine; pyrazine; and pyridazine.

[0010] As used herein, an "alkoxy group" is a carbon chain where one or more carbon chain atoms is replaced with oxygen. An oxygen may be the first atom in the chain (i.e., the atom which bonds to the remainder of the molecule), or may be somewhere else in the chain.

[0011] In one embodiment, R^A is hydrogen and R^B is not hydrogen. In one embodiment, R^Z is C1-C6 alkyl. In one embodiment, R^Z is methyl. In one embodiment, R^E and R^G are hydrogen and R^F is trifluoromethyl. In one embodiment, R^E and R^G are hydrogen and R^F is m-trifluoromethyl. In one embodiment, R^M is methoxy. In one embodiment, R^M is C1-C3 alkoxy. In one embodiment, R^A is hydrogen, and R^B is a nitrogen-containing substituent.

[0012] A nitrogen-containing substituent is any substituent which contains nitrogen, including a nitro- or amino-containing group such as -NO₂; -NH₂; -CN; a ring-containing substituent such as imidazole, phthalimido, pyridine, pyrimidine, pyrazine, pyridazine -and NR₂, where one or more of the R's is not hydrogen such as a C1-C6 straight chain alkyl or alkoxy chain terminated by a nitrogen-containing substituent, such as those described here.

[0013] In the compounds of the invention, all possible combinations of variables are intended to be disclosed to the same extent as if they were each individually drawn.

[0014] Also provided is compound PQ1. Also provided is compound PQ2. Also provided is compound PQ3. Also provided is compound PQ4. Also provided is compound PQ5. Also provided is compound PQ6. Also provided is compound PQ7. Also provided is compound PQ8. Also provided is compound 13. Also provided is compound 14. Also provided is compound 15. Also provided is compound 17. Also provided is compound 18.

[0015] All substituents in the compounds of the invention may be optionally substituted with suitable substituents as known in the art. For example, in an alkyl chain, one or more of the carbons in the chain may be substituted with oxygen. Also, in an alkyl chain, one or more of the hydrogens may be substituted with a halogen, a C1-C6 optionally substituted straight chain or branched alkyl or alkoxy group, and so on. Also, any ring substituent may be positioned at the ortho-, meta- or para-position, as permitted by the other ring substituents, even if all locations are not specifically mentioned.

[0016] Also provided are methods for treating a gap junction disorder comprising administering an effective amount of a compound having a formula included herein to a patient. The patient may be a mammal, such as a mouse, rabbit, dog, cat, cow, pig, monkey, or human. The compound used to treat a gap junction disorder may be combined with a pharmaceutically acceptable carrier or other pharmaceutically acceptable additives, as known in the art.

[0017] As used herein, a "gap junction disorder" is a disorder or effect of a treatment of a disorder which results in the abnormal activity of gap junctions. Examples of gap junction disorders that are caused by Cx50 are cataracts, retinitis pigmentosa, SCA14, a type of neurodegenerative disorder known also as apinocerebellar ataxia. There is also a genetic link for Cx50 to certain forms of schizophrenia. The most common form of deafness is caused by mutations in the gap junction protein Cx26, but this protein is not acted upon by mefloquine drugs. Therefore, the current compounds and methods are especially important since they can treat gap junction disorders which are not treated by currently known drugs. Other examples of gap junction disorders include cancer, where the decreased gap junction activity of cancerous cells allows abnormal cell proliferation and growth. Another example of a gap junction disorder is treatment of several parasitic diseases with current drugs which inhibit the gap junction activity and cause undesirable neurological effects. Gap junction disorders can be treated by administering an effective amount of a compound or composition of the invention which affects the gap junction activity. As used herein, "treating" a gap junction disorder only means the gap junction activity is altered so as to cause a reduced effect of the gap junction disorder, not necessarily that the gap junction disorder is completely resolved.

Treating a gap junction disorder includes increasing the gap junction activity of cells, decreasing the inhibition of gap junction activity as compared to other compounds, and other effects which are easily understood by one of ordinary skill in the art using the disclosure herewith.

- 5 [0018] As used herein, "parasite" means any organism which may infect any other organism and cause a deleterious effect. Parasite includes the organisms which cause malaria, Trypanosomal brucei, bird flu, and dengue virus.

- [0019] Compounds which are specifically claimed are not intended to include compounds known in the art. Methods for using compounds of the invention may
10 include compounds known in the art which are not known to be useful in the methods of the invention.

- [0020] Compounds of the invention which are useful in providing improvement in gap junction disorders can be determined by routine testing which can be readily performed by one of ordinary skill in the art using methods known in the art and the
15 guidance provided here.

BRIEF DESCRIPTION OF THE FIGURES

- [0021] Figure 1 shows inhibition of T. brucei growth using PQ1, PQ2, PQ3 and PQ4.
- 20 [0022] Figure 2 shows the results of dye transfer/scrape loading experiments for R28 cells.
- [0023] Figure 3 shows MDA-MB-453 and T47D colony growth for PQ1-5 and DMSO, as well as a control.
- [0024] Figure 4 shows gap junction activity using flow cytometry of dye coupling in
25 human breast cancer cell lines.
- [0025] Figure 5 is a MTT assay showing the effect of PQ1 compound on the cell viability in T47D cells.

[0026] Figure 6 shows PQ1 on the activation of caspase 3 in T47D cells.

[0027] Figure 7 shows colony growth of PQ1 in T47D cells.

[0028] Figure 8 shows colony growth of PQ1 in MDA-MB-453 cells.

[0029] Figure 9 shows results from scrape load dye/transfer with T47D cells

5 [0030] Figure 10 shows the effect of PQ1 on gap junction activity in SW620 colon cancer cells.

[0031] Figure 11 shows the effect of PQ1 on the expression of PKC γ .

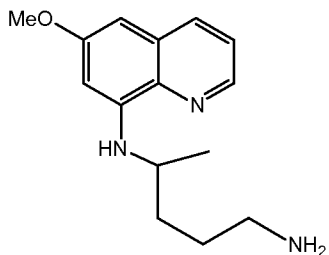
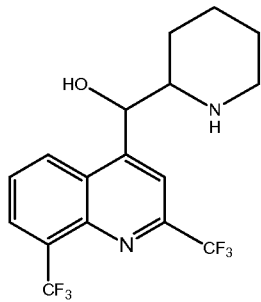
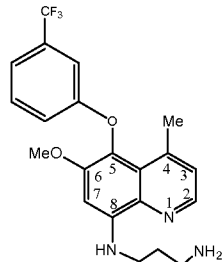
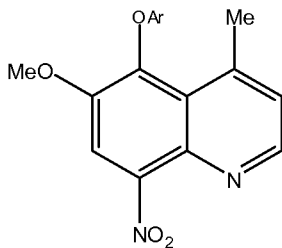
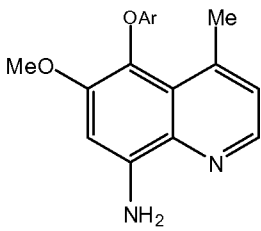
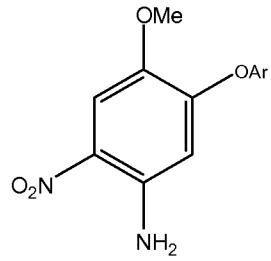
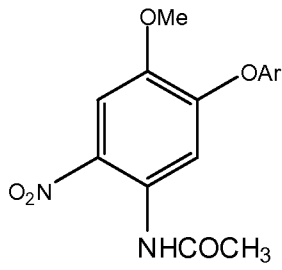
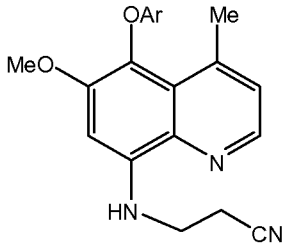
[0032] Figure 12 shows the effect of PQ1 on colony growth of SW620 colon cancer cells with various concentrations of PQ1.

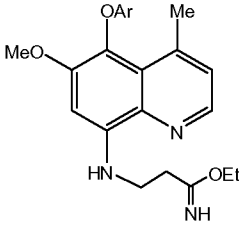
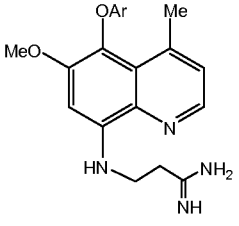
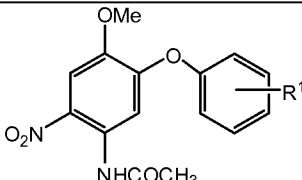
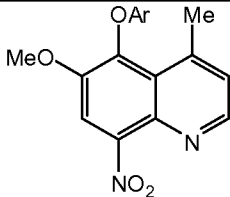
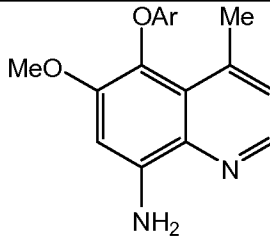
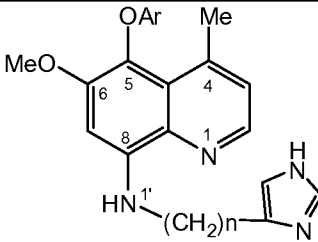
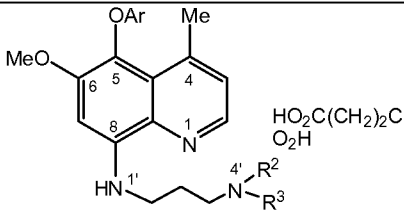
10 [0033] Figure 13 shows the effect of PQ1 on colony growth of SW620 colon cancer cells with 10uM PQ1 and other compounds.

DETAILED DESCRIPTION OF THE INVENTION

15 [0034] The following description provides nonlimiting examples of some embodiments of the invention.

[0035] Some specific compounds discussed here are shown below.

primaquine 	mefloquine 	
PQ1 	PQ2  OAr = m-CF₃C₆H₄	PQ3  OAr = m-CF₃C₆H₄
PQ4  OAr = m-CF₃C₆H₄	PQ5  OAr = m-CF₃C₆H₄	PQ6  OAr = m-CF₃C₆H₄

PQ7  OAr = <i>m</i>-CF₃C₆H₄	PQ8  OAr = <i>m</i>-CF₃C₆H₄	 13 : R¹ = OR; alkyl; NR₂ R is alkyl or phenyl
 14 : Ar = <i>o</i>-, <i>m</i>-, or <i>p</i>-R¹C₆H₄	 15 : Ar = <i>o</i>-, <i>m</i>-, or <i>p</i>-R¹C₆H₄	 HO₂C(CH₂)₂CO₂H 17 : Ar = <i>o</i>-, <i>m</i>-, or <i>p</i>-R¹C₆H₄ n = 1 - 2
 18 : Ar = <i>m</i>-CF₃C₆H₄; R³ = H or Me; R² = Me, Et, Pr; R², R³ = (CH₂)₄		

[0036] All compounds disclosed herein include pharmaceutically acceptable salts, and pharmaceutically acceptable carriers.

[0037] One method for administering these compounds comprises the use of pharmaceutically acceptable salts, including those formed from the compounds of the invention and acids such as hydrochloric, hydrobromic, succinic, sulfamic, sulfuric, phosphoric, citric, tartaric, methanesulfonic, isethionic, aceturic, malic,

fumaric, beta-resorcylic, or pamoic acid. Such salts may be administered orally in the form of tablets, capsules, or dragees when admixed with solid excipients such as lactose, sucrose, starch, microcrystalline cellulose, magnesium stearate or talc, nanogel which is any of a number of PEG-PEI (polyethylene glycol-

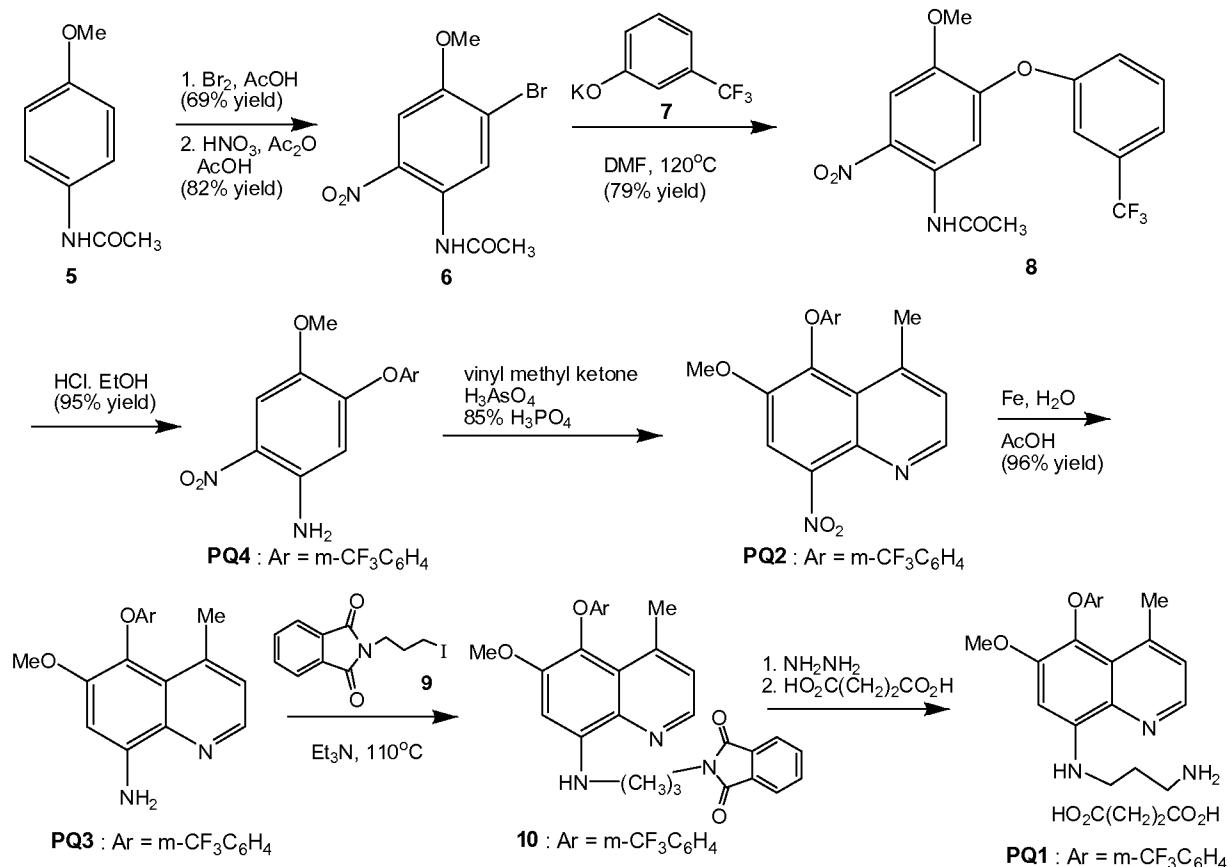
polyethyleneimine) compounds. The foregoing compositions are preferred means for oral administration over the use of flavored syrups or tinctures containing the compound. Under certain circumstances, parenteral administration may be indicated, employing an aqueous solution or an oleaginous formulation of the agent. Aqueous solutions can be prepared in water, physiological saline, Ringer's solution, or the like, either with or without buffers. Oleaginous formulations may be made in natural oils (such as, peanut oil or olive oil), or in benzyl benzoate, for example.

Experiment One: Synthesis of Primaquine Analogs

[0038] Primaquine is a radical curative and causal prophylactic antimalarial agent, however, its toxicity has limited its usage as antimalarial agent. A class of primaquine analogs were synthesized and the synthetic scheme is depicted in Scheme 1. However, in the construction of **PQ2** from **PQ4**, the ring closing reaction is not as straightforward as that reported. The synthetic procedure (*vide infra*) was modified and used to obtain a good yield of **PQ2**, which is needed for the synthesis of **PQ3** and **PQ1**.

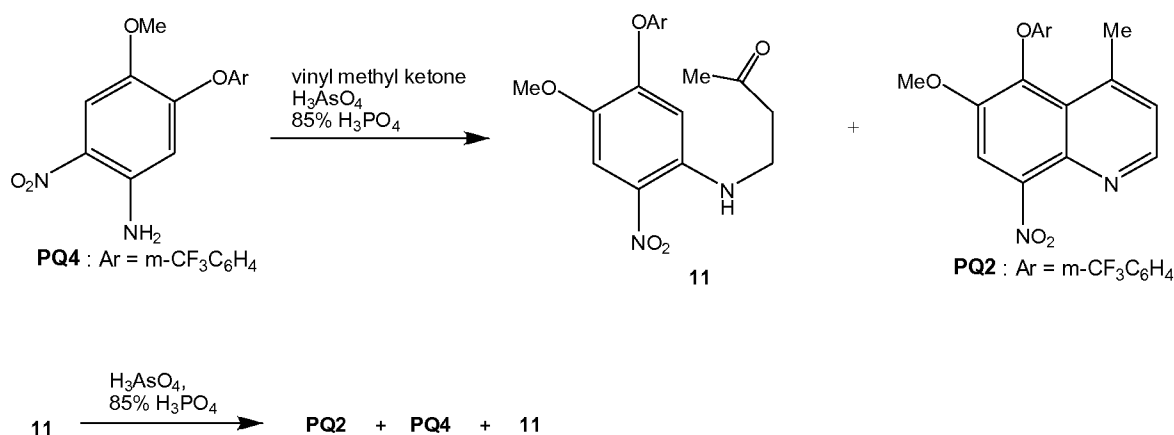
[0039] A reported synthesis (Lauer, W. M.; Rondestvedt, C.; Arnold, R. T.; Drake, N. L.; van Hook, J.; Tinker, J. Some derivatives of 8-aminoquinoline. *J. Am. Chem. Soc.* **1946**, 48, 1546-1548) of a key intermediate, 2-bromo-4-acetamino-5-nitroanisole (**6**) was followed. Hence, 4-acetaminoanisole (**5**) was brominated with bromine in acetic acid (69% yield) followed by nitration with nitric acid in acetic anhydride and acetic acid (82% yield). Displacement of bromide **6** with potassium 3-trifluoromethylphenoxide (**7**) in N,N-dimethylformamide (DMF) at 120°C gave 4-acetamino-5-nitro-2-(3-trifluoromethylphenyloxy)anisole (**8**). Removal of the acetyl protecting group of **8** with hydrochloric acid in ethanol afforded a 95% yield of 4-amino-5-nitro-2-(3-trifluoromethylphenyloxy)anisole (**PQ4**).

Scheme 1



[0040] The ring closing reaction of **PQ4** to **PQ2** with vinyl methyl ketone is complex. When **PQ4** was heated with vinyl methyl ketone, arsenic acid and phosphoric acid at 100°C after 20 minutes, a mixture of **PQ2**, compound **11**, and **PQ4** in a ratio of 1:2:1 was obtained (Scheme 2). Longer reaction times resulted in decomposition of the products, and the use of excess of vinyl methyl ketone did not improve the yield. Compounds **PQ2**, **11**, and **PQ4** were separated by silica gel column chromatography. Treatment of compound **11** with arsenic acid and phosphoric acid at 100°C for 20 minutes provided a mixture of **PQ2**, **11**, and **PQ4** in a ratio of 1:1:2. Likely, compound **11** underwent two reactions, cyclization to give compound **11** and reversed Michael reaction to give compound **PQ4**.

Scheme 2

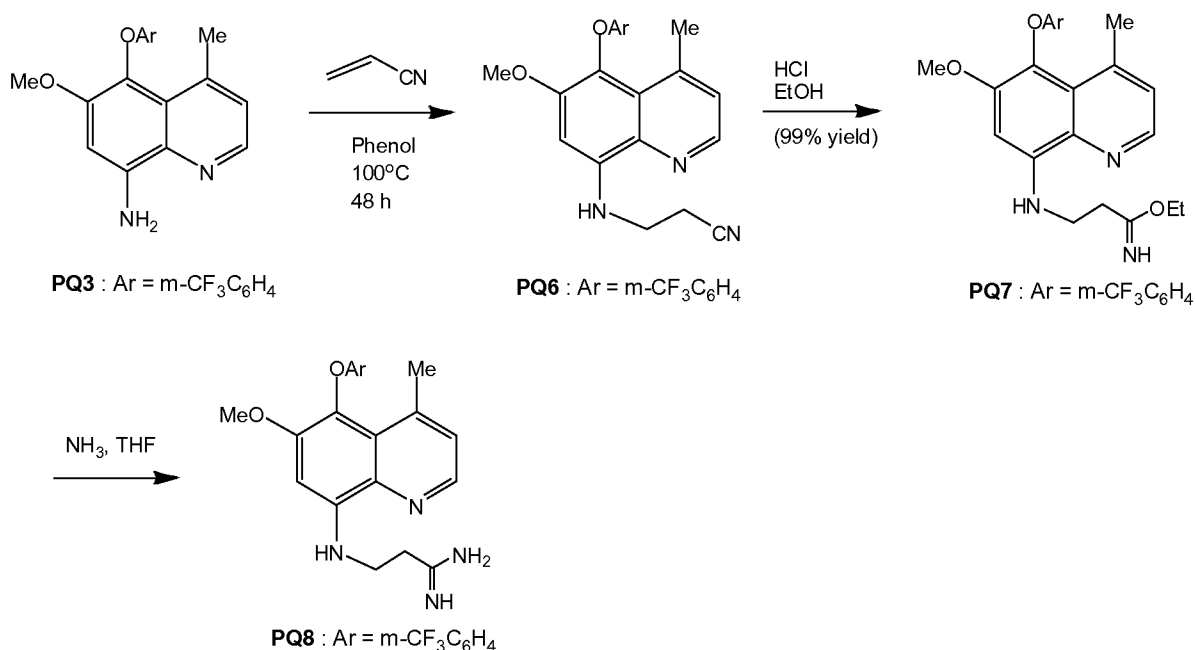


[0041] The synthesis of primaquine analogs was accomplished and previously unreported compounds including **PQ1**, were prepared. Hence, reduction of **PQ2** with Fe in water and acetic acid afforded **PQ3**, which was alkylated with *N*-(3-iodopropyl)-phthalimide (**9**) to give compound **10**. Removal of the phthalimide function of compound **10** with hydrazine followed by treatment with succinic acid furnished **PQ1**, which is soluble in water.

Other PQ1 Analogs:

[0042] A number of further analogs of **PQ1** were synthesized and some of these are depicted in Scheme 3. Michael addition of **PQ3** with acrylonitrile in phenol (used as solvent) at 100°C for 48 h afforded **PQ6**, which was converted into amidate **PQ7** upon treatment with HCl in ethanol and benzene. **PQ7** is transformed into imidate **PQ8** with ammonia in THF in a sealed tube.

Scheme 3

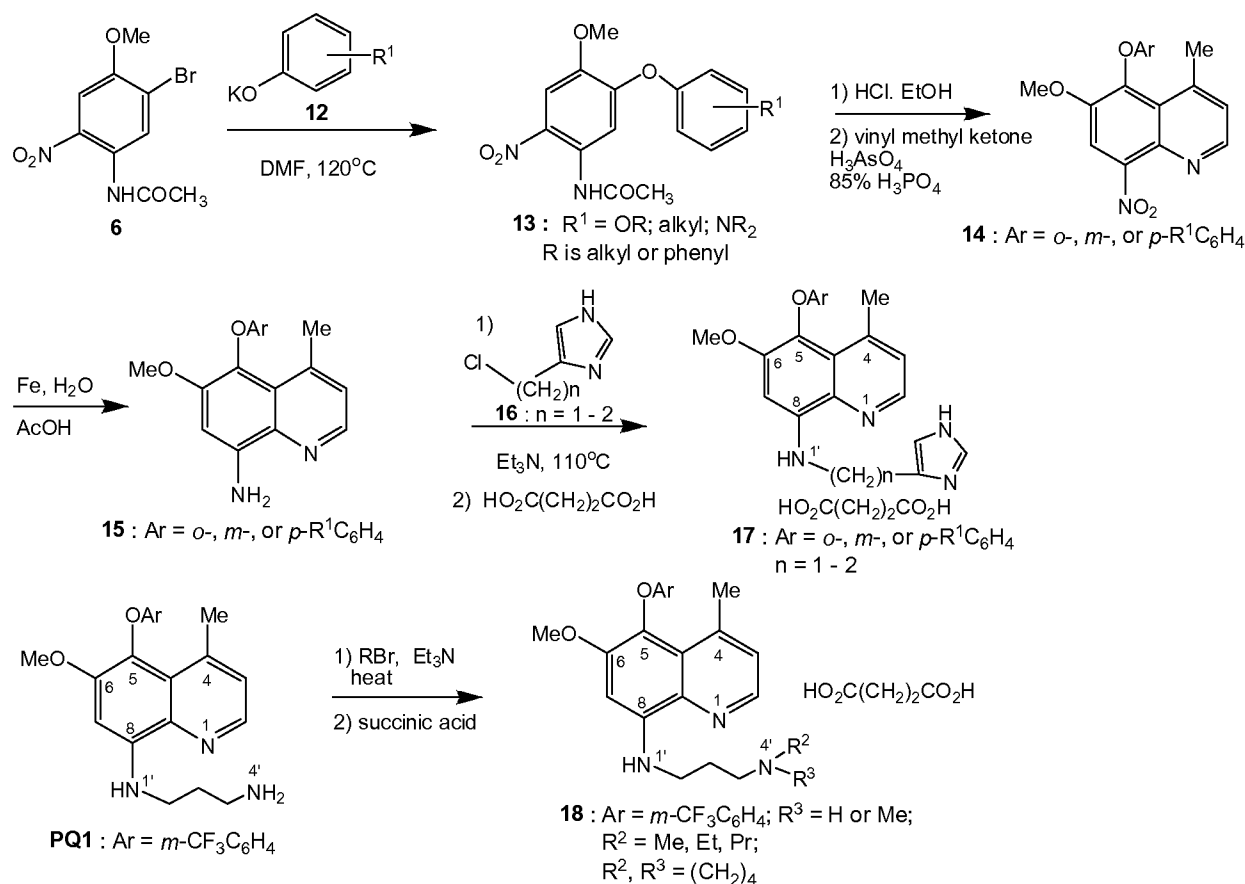


[0043] Similarly, other **PQ** analogs such as compounds **17** and **18** are synthesized (Scheme 4). Modification of the CF₃ and 4'-NH₃⁺ moieties of **PQ1** can readily be carried out using methods known to one of ordinary skill in the art. Compound **6** can be treated with various substituted phenoxides **12** containing electron donating (R¹ = *ortho*-, *meta*-, or *para*-OR, alkyl, and NR₂) and withdrawing (R¹ = CO₂R) groups, to give a series of compounds **13**. Removal of the acetamide protecting group with HCl in ethanol followed by ring closing with vinyl methyl ketone and reduction of the nitro function furnish quinolines **15**. Alkylation of the C8-amino function of compound **15** with 4-(chloromethyl)imidazole or 4-(chloroethyl)imidazole gives **PQ** analogs **17**. Five different OAr groups used in the preparations of compounds **15** provide ten analogs **17**, ie., a small library of **PQ** analogs is obtained. Beside imidazole analogs **16**, 4-(chloromethyl)pyrazole, and 3-(chloromethyl)pyrazole can also be used.

Heterocycles such as imidazole and pyrazole decrease the basicity of N4' of **PQ1**. *N*(4')-monosubstituted **PQ1** analogs, compounds **18**, are prepared from the alkylations of **PQ1** (absence of succinic acid) with various alkyl bromides and triethylamine followed by salt-formation with succinic acid. The alkylamine nitrogen(4') of **PQ1** is more basic (pK_b ~ 3.2) than that of arylamine nitrogen(1') (pK_b ~ 4.6), hence, alkylations take place on 4'-nitrogen. These modifications and others

are readily carried out by one of ordinary skill in the art without undue experimentation using the methods described herein.

Scheme 4



5

Synthetic procedures

[0044] General procedure: 4-Acetaminoanisole, vinyl methyl ketone, 3-(trifluoromethyl)phenol, and arsenic acid were obtained from Aldrich Chemical Co. Potassium *t*-butoxide was prepared by treating *t*-butanol with potassium metal at 85°C followed by evaporating excess of *t*-butanol under vacuum.

10

[0045] 2-Bromo-4-acetaminoanisole. To a solution of 10.0 g (60.5 mmol) of 4-acetaminoanisole (**5**) in 80 mL of acetic acid, was added dropwise 11.6 g (72.7 mmol) of bromine and maintaining internal temperature of the reaction below 50°C. After stirring for 1 h, the reaction mixture was poured into 400 mL of ice-water containing 1.2 g of NaHSO₃. The mixture was stirred until yellow-red color

15

disappeared and left at room temperature for overnight. The solid (product) was filtered, dried under vacuum at 70°C, and crystallized from ethanol to give 10.19 g (69% yield). ¹H NMR (CDCl₃) δ 7.67 (d, J = 2.5 Hz, 1 H), 7.44 (dd, J = 9, 2.5 Hz, 1 H), 7.1 (bs, 1 H, NH), 6.85 (d, J = 9 Hz, 1 H), 3.87 (s, 3 H, OMe), 2.16 (s, 3 H, CH₃).

- 5 [0046] 2-Bromo-4-acetamino-5-nitroanisole (6). To a cold (5°C) solution of 3.36 g (13.8 mmol) of 2-Bromo-4-acetaminoanisole in 5 mL of acetic anhydride and 10 mL of acetic acid, was added dropwise 0.9 mL (13.8 mmol) of concentrate nitric acid. The resulting solution was stirred at 5°C for 3 h, poured into 90 mL of water, stirred, and filtered to collect the solid (the desired product). The solid was washed twice
10 with cold water, dried under vacuo at 100°C, crystallized from chloroform to give 3.26 g (82% yield) of pure compound **6**. ¹H NMR (CDCl₃) δ 10.2 (s, 1 H, NH), 9.09 (s, 1 H), 7.67 (s, 1 H), 3.95 (s, 3 H, OMe), 2.28 (s, 3 H, CH₃).

- [0047] Potassium 3-(trifluoromethyl)phenoxide (7). A mixture of 1.94 g (12 mmol) of 3-(trifluoromethyl)phenol and 1.344 g (12 mmol) of potassium t-butoxide was
15 stirred under argon for 30 min. A solution was resulted, and the by-product, t-butanol, was removed under vacuum and heat to give a white solid.

- [0048] 4-Acetamino-5-nitro-2-(3-trifluoromethylphenyloxy)anisole (8). To the above solid **7** under argon, was added a solution of 3.0 g (10.4 mmol) of bromide **6** in 20 mL of DMF via cannula. The resulting solution was stirred at 120°C for 1 day. TLC
20 and NMR indicated the presence of a small amount of starting material **6**. The reaction solution was cooled to room temperature and 2 mmol of compound **7** was added. The solution was stirred at 120°C for 12 hour, diluted with 200 mL of ice and water, and the solid was collected by filtration, washed with water, dried under vacuum, and crystallized from ethanol to give 3.04 g (79% yield) of compound **8**. ¹H
25 NMR (CDCl₃) δ 10.4 (s, 1 H, NH), 8.39 (s, 1 H), 7.83 (s, 1 H), 7.5 (m, 2 H), 7.3 (m, 2 H), 3.93 (s, 3 H, OMe), 2.23 (s, 3 H, CH₃). ¹³C NMR (CDCl₃) δ 174, 155.7, 153.9, 142.4, 141.6, 130.9, 123.0, 121.7, 121.6, (116.9, 116.8, 116.7, 116.6 q for CF₃), 108.7, 106.6, 56.7, 21.8.

- [0049] 4-Amino-5-nitro-2-(3-trifluoromethylphenyloxy)anisole (PQ4). A solution of
30 2.0 g (5.3 mmol) of compound **8** in 4 mL of concentrate HCl and 30 mL of ethanol was heated under argon at reflux for 2 hours. The solution was cooled to room

temperature, poured into 200 mL of ice and water, and the orange solid was collected by filtration. The solid was washed with water twice and crystallized from ethanol to give 1.69 g (95% yield) of **PQ4**. ^1H NMR (CDCl_3) δ 7.70 (s, 1 H), 7.5 (m, 2 H), 7.32 (m, 1 H), 7.20 (m, 2 H), 6.15 (s, 1 H), 3.87 (s, 3 H, OMe).

5 [0050] 6-Methoxy-4-methyl-8-nitro-5-(3-trifluoromethylphenoxy)quinoline (**PQ2**) and 4-{N-[4-methoxy-2-nitro-5-(trifluoromethylphenoxy)]amino}2-butanone (**11**). To a flask equipped with a condenser and a thermometer, 2.0 g (6.0 mmol) of compound **PQ4**, 1.7 g (12 mmol) of H_3AsO_4 , and 15 mL of 85% H_3PO_4 were added. The mixture was stirred and heated to 100°C (bath temperature is 110°C), and 0.7
10 mL of vinyl methyl ketone was added dropwise via syringe. The orange color of the solution turned to red color immediately. After stirring at 100°C for 20 min, the reaction solution was cooled to room temperature, poured into ice and water, and the solid (HCl salt of the product) was collected by filtration. The solid was added to an aqueous NH_4OH - NaOH solution, stirred for 10 min, and the red solid was collected
15 by filtration. The solid was washed with water twice and dried under vacuum to give a crude product. The above filtrates were combined, neutralized with NH_4OH - NaOH , and extracted with chloroform three times. The combined extract was washed with brine, dried (MgSO_4), concentrated, and combined with the above crude product. The combined crude product was separated by silica gel column chromatography
20 using a mixture of hexane, diethyl ether, and dichloromethane as solvents to give 0.576 g (25% yield) of **PQ2**, 1.214 g (50% yield) of compound **11**, and 0.50 g (25% recovery) of **PQ4**. Compound **PQ2**: ^1H NMR (CDCl_3) δ 8.78 (d, $J = 4$ Hz, 1 H), 7.88 (s, 1 H), 7.40 (m, 2 H), 7.27 (m, 1 H), 7.11 (s, 1 H), 6.93 (d, $J = 7$ Hz, 1 H), 3.87 (s, 3 H, OMe), 2.74 (s, 3 H, Me). ^{13}C NMR (CDCl_3) δ 158.0, 151.0, 148.3, 146.9, 143.8,
25 139.9, 136.3, 132.9, 132.3, 130.6, 126.0, 125.2, 121.1, (119.7, 119.6, 119.5, 119.4 for CF_3), 118.3, 112.5, 111.8, 57.3, 23.4.

[0051] Compound **11**: ^1H NMR (CDCl_3) δ 8.18 (s, 1 H), 7.78 (s, 1 H), 7.50 (m, 2 H), 7.30 (m, 2 H), 6.32 (s, 1 H), 3.87 (s, 3 H, OMe), 3.40 (t, $J = 7$ Hz, 2 H), 2.75 (t, $J = 7$ Hz, 2 H), 2.17 (s, 3 H, Me). ^{13}C NMR (CDCl_3) δ 205.0, 151.0, 142.5, 141.6,
30 130.9, 122.2, 121.3, 121.2, 116.0, 111.8, 109.8, 103.0, 56.7, 42.6, 37.7, 30.5.

[0052] 8-Amino-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)quinoline (PQ3). To a solution of 0.20 g (0.53 mmol) of **PQ2** in 0.1 mL of acetic acid and 10 mL of water, was added 0.18 g (3.2 mmol) of iron. The mixture was stirred and heated at 100°C for 2 h, cooled to room temperature, extracted with dichloromethane three times. The combined extract was washed with aqueous NaHCO₃, brine, dried (MgSO₄), concentrated, and column chromatographed on silica gel using a mixture of hexane, dichloromethane, and diethyl ether as eluent to give 0.18 g (96% yield) of **PQ3**. ¹H NMR (CDCl₃) δ 8.46 (d, J = 4 Hz, 1 H), 7.34 (t, J = 8 Hz, 1 H), 7.22 (d, J = 8 Hz, 1 H), 7.15 (m, 2 H), 6.93 (d, J = 8 Hz, 1 H), 6.79 (s, 1 H), 5.15 (bs, 2 H, NH₂), 3.80 (s, 3 H, OMe), 2.63 (s, 3 H, Me). ¹³C NMR (CDCl₃) δ 150.5, 145.6, 143.7, 142.8, 130.2, 125.1, 124.6, 118.3, 118.26, 118.2, (112.3, 112.2, 112.1, 112.0) (q, CF₃), 97.9, 56.6, 23.3.

[0053] 6-Methoxy-8-(3-phthalimidopropylamino)-4-methyl-5-(3-trifluoromethylphenoxy)quinoline (**10**). A solution of 2.0 g (5.7 mmol) of **PQ3**, 1.81 g (5.7 mmol) of 3-iodopropylphthalimide (**9**), and 0.58 g (5.7 mmol) of triethylamine in 15 mL of 2-ethoxyethanol was stirred under argon at 110°C for 12 hours. The reaction mixture was cooled to room temperature, diluted with chloroform, and washed with water and brine. The organic layer was dried (K₂CO₃), concentrated to give compound **10** along with 1,3-diphthalimidopropane. The mixture was used in the next step without purification. Pure compound **10** can be obtained by dissolving the crude product in ether and HCl, and orange-red HCl salt was precipitated out. It was filtered and the solid was crystallized from ethanol to give pure **10**.

[0054] 6-Methoxy-8-[(3-aminopropyl)amino]-4-methyl-5-(3-trifluoromethylphenoxy)quinoline succinic acid (**PQ1**). A solution of the above phthalimide **10** in 100 mL of 65% hydrazine and 100 mL of ethanol was refluxed under argon for 3 hours. After cooling to room temperature, the solution was diluted with 10% aqueous KOH solution and extracted with dichloromethane three times. The combined extract was washed with brine, dried (K₂CO₃), concentrated, and column chromatographed on silica gel using dichloromethane and methanol as eluents to give 1.86 g (80% yield) of 6-methoxy-8-[(3-aminopropyl)amino]-4-methyl-5-(3-trifluoromethylphenoxy)quinoline. ¹H NMR (CDCl₃) δ 8.40 (d, J = 4 Hz, 1 H), 7.34 (t, J = 8 Hz, 1 H), 7.21 (d, J = 8 Hz, 1 H), 7.06 (m, 2 H), 6.93 (d, J = 8 Hz, 1 H),

6.48 (s, 1 H), 6.4 (bs, 1 H, NH), 3.83 (s, 3 H, OMe), 3.42 (t, $J = 8$ Hz, 2 H), 2.99 (t, $J = 8$ Hz, 2 H), 2.62 (s, 3 H, Me), 1.98 (pentet, $J = 8$ Hz, 2 H), 1.80 (bs, 2 H, NH_2). ^{13}C NMR (CDCl_3) δ 160.0, 151.1, 145.0, 143.5, 142.7, 130.2, 125.2, 118.4, 118.2, 112.2, 107.5, 92.9, 56.8, 41.6, 40.4, 33.1, 23.4. The succinic acid salt was prepared by
5 treating 0.457 g (1.12 mmol) of the quinoline with 0.134 g (1.13 mmol) of succinic acid in 10 mL of methanol. The resulting solution was concentrated to dryness, crystallized from diethyl ether to give a quantitative yield of the succinic acid salt **PQ1**.

[0055] 6-Methoxy-8-[(2-cyanoethyl)amino]-4-methyl-5-(3-trifluoromethylphenoxy)quinoline (**PQ6**). A solution of 0.25 g (0.72 mmol) of **PQ3**,
10 38 mg (0.72 mmol) of acrylonitrile, and 3 g of phenol (Klenke, B.; Stewart, M.; Barrett, M. P.; Brun, R.; Gilbert, I. H. Synthesis and biological evaluation of s-triazine substituted polyamines as potential new anti-trypanosomal drugs. J. Med. Chem. 2001, 44, 3440-3452.)¹² was heated under argon at 100°C for 48 h. The solution
15 was cooled to room temperature, diluted with methylene chloride, washed with 1 N NaOH solution, and brine. The methylene chloride layer was dried (MgSO_4), concentrated, and column chromatographed on silica gel to give 0.203 g (70% yield) of **PQ6**. ^1H NMR (CDCl_3) δ 8.43 (d, $J = 4.5$ Hz, 1 H), 7.35 (t, $J = 9$ Hz, 1 H), 7.23 (d, $J = 7$ Hz, 1 H), 7.12 (d, $J = 4.5$ Hz, 1 H), 7.07 (s, 1 H), 6.94 (d, $J = 8$ Hz, 1 H), 6.72 (t,
20 $J = 6$ Hz, 1 H), 6.52 (s, 1 H), 3.84 (s, 3 H, OMe), 3.77 (q, $J = 7$ Hz, 2 H, CH_2N), 2.81 (t, $J = 7$ Hz, 2 H, CH_2CN), 2.63 (s, 3 H, CH_3).

[0056] 6-Methoxy-8-[(2-(ethoxycarboimino)ethyl)amino]-4-methyl-5-(3-trifluoromethylphenoxy)quinoline (**PQ7**). To a cold (0 °C) solution of 63 mg (0.16 mmol) of **PQ6** in 2 mL of ethanol and 2 mL of benzene, HCl gas was bubbled in via a
25 tubing. The reaction vessel was sealed and stirred at room temperature for 3 days, cooled to 0°C, and diluted with 25 mL of ether. The precipitated solid was collected by filtration and dried to give 70 mg (99% yield) of **PQ7**. ^1H NMR (CDCl_3) δ 8.5 (bs, 1 H), 7.40 (m, 2 H), 7.30 (d, $J = 7$ Hz, 1 H), 7.06 (s, 1 H), 6.90 (d, $J = 8$ Hz, 1 H), 6.83 (s, 1 H), 4.20 (q, $J = 7$ Hz, 2 H, OCH_2), 3.90 (s, 3 H, OMe), 3.71 (bs, 2 H, CH_2N),
30 3.05 (bs, 2 H, CH_2CN), 2.88 (s, 3 H, CH_3), 1.30 (t, $J = 7$ Hz, 3 H, CH_3).

[0057] 6-Methoxy-8-[(2-(amidinoylethyl)amino]-4-methyl-5-(3-trifluoromethylphenoxy)quinoline (PQ8). A solution of **PQ7** and NH_3 (excess) in ethanol was heated at 50°C in a sealed tube to give **PQ8**.

Experiment Two: Antiparasitic Activities

5 [0058] The antiparasitic activities of exemplary compounds described here was studied using the anti-trypanosomal activity protocol described in: Rapp M, et al. J Med Chem 2006; 49:2096-102. The IC_{50} values of **PQ1**, **PQ2**, **PQ3** and **PQ4** against *Trypanosoma brucei* (*T. brucei*) are 340 ng/mL, 2,498 ng/mL, 1,083 ng/mL and 25 $\mu\text{g/mL}$. Figure 1 summarizes the compound inhibition of *T. brucei* growth. It is seen
10 that compounds of the invention are useful in inhibiting *T. brucei* growth.

Methods used for antiparasitic activities

[0059] Plasmepsin assay: The substrate used for the plasmepsin assay (BACHEM) is a synthetic peptide (Dabcyl-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-Edans) designed to mimic the cleavage site present in hemoglobin. The kinetic constants for
15 the substrate are $K_m = 0.78 \text{ s}^{-1}$ and $K_{\text{cat}} = 0.10 \mu\text{M}$ for *P. falciparum* plasmepsin, and $K_m = 0.69 \text{ s}^{-1}$ and $K_{\text{cat}} = 0.16 \mu\text{M}$ for *P. vivax* plasmepsin. The substrate is conjugated with the fluorescent donor EDANS and the quencher DABCYL. Fluorescence is only detectable when the EDANS group is separated from the DABCYL group by cleavage of the substrate. (Unger, V. M.; Kumar, N. M.; Gilula, N.
20 B.; Yeager, M. Three-dimensional structure of a recombinant gap junction membrane channel. *Science*, **1999**, 283, 1176-1180; Fleishman, S. J.; Unger, V. M.; Ben-Tal, N. Transmembrane protein structures without X-rays. *Trends in Biochem. Sci.* **2006**, 31, 106-113).

[0060] An automated plasmepsin assay protocol was developed that allowed
25 screening of a large number of compounds within a short period of time. Compounds were manually added to 96-well plates followed by the addition of assay buffer (15 mM NaCl, 100 mM Formate, pH 4.4) using an automated dilutor (BioMec 2000 from BECKMAN). After thorough mixing and dilution, the contents of the plates were transferred to test plates and plasmepsin enzyme solution was added with the
30 dilutor. After a ten-minute incubation at 37 °C, background fluorescence was

measured with a fluorescence plate reader (WALLAC Victor2). Finally, the substrate was added (final concentration of 10 μ M) and the reaction mixture was incubated for 30 minutes at 37 °C followed by fluorescence detection. Each compound was tested in this prescreen in triplicate at the concentration of 10 μ g/ml. Compounds that
5 reduced the activity of plasmepsin by 50% or more at this concentration were selected for a second screen to determine IC₅₀ values. The best inhibitors were assayed using a range of inhibitor concentrations and substrate concentrations to determine K_i values (ENZYME KINETICS from TRINITY SOFTWARE).

[0061] Culturing of Parasites. The bloodstream form of *Trypanosoma brucei* 427
10 strain was maintained under the standard cell culture conditions (37 °C, 5% CO₂). The parasites were grown in complete HMI-9 medium containing 10% FBS, 10% Serum Plus and 1× Penicillin/Streptomycin. (Angew. Chem. Int. Ed. Engl. 2003, 42 , 5274-5293).

[0062] Luciferase Assay. Luciferase assay was used to measure ATP-
15 bioluminescence in *T. brucei* cultured in 96-well plates at 37° C for 48 hours. Parasites were diluted to 1.0 x10⁵ cells/mL in complete HMI-9 medium. One hundred microliters (100 μ L) of the diluted parasites were aliquoted into sterile 96-well flat white opaque culture plates (Greiner). Each compound was serially diluted from 10 μ M to 0.1 μ M in DMSO and then mixed in the appropriate wells containing parasites.
20 The treated parasites were then incubated for 48 hours at 37° C with 5% CO₂ before monitoring viability. To measure the viability of the parasites after treatment with each compound, the parasites were lysed in the wells by adding 100 μ L of CellTiter-Glo™ (Promega). After lysis, the ATP bioluminescence of the 96-well plates was measured with a SpectraFluor Plus multidetection plate reader (Tecan).

25 [0063] Malaria parasite Plasmodium falciparum: Culturing of *P. falciparum* was carried out as described in: Trager, W., et al: Exp. Parasitol. 50: 83-89, 1980; and Zhang P et al. J Biomed Sci 2002;9:34-40.

[0064] *P. falciparum* parasites were cultured by standard methods in RPMI medium supplemented with 10% serum or AlbuMAX I serum substitute (Gibco BRL)
30 and a 2% hematocrit of human erythrocytes. Parasite synchrony was maintained by serial treatments with sorbitol. Two strains are used, 3D7 and W2; the former being

susceptible to drugs, while the latter is resistant to chloroquine, sulfadoxine, pyrimethamine, and quinine. Other strains can be used, as well.

[0065] The isotopic method was followed with modifications. Two hundred microliters of the suspension of infected erythrocytes was distributed in triplicate in 96-well tissue culture plates that were either drug-free or pre-coated with test compounds. The parasites were incubated at 37°C in 5% CO₂. [³H]hypoxanthine (specific activity, 16.3 Ci/mmol; 1 µCi/well) was added after the first 18 h of incubation to assess parasite growth. The incorporation of [³H]hypoxanthine has been established as an accurate and reliable means of determining in vitro parasite growth. After an additional 24 h of incubation, the plates were frozen to terminate the assays. The plates were thawed to lyse the infected erythrocytes, and the contents of each well were collected on glass-fiber filter papers, washed, and dried with a cell harvester. The filter disks were transferred into scintillation tubes, and 2 ml of scintillation cocktail was added. The incorporation of [³H]hypoxanthine was quantitated with a liquid scintillation counter.

Experiment Three: Studies of the Inhibitory Activities of Gap Junctions

[0066] Gap junctions provide a major cell-to-cell communication role in electrical synapses in neural systems (van Riemsdijk, M. M.; Sturkenboom, M. C. J. M.; Ditters, J. M.; Ligthelm, R. J.; Overbosch, D.; Stricker, B. H. Ch. *Clin. Pharmacol. & Therapeutics*, **2002**, 72, 294-301). Gap junctions have been called both “Good Samaritans” and “Executioners” (Cruikshank, S. J.; Hopperstad, M.; Younger, M.; Connors, B. W.; Spray, D. C.; Srinivas, M. *PNAS* **2004**, 101, 12364-12369), terms which refer to their ability to pass both necessary metabolites and apoptotic signals from one cell to another. The passage of apoptotic signals through open gap junctions has been linked to acute oxidative stress-induced neural cell death (Loewenstein, W. R. *Physiol. Rev.* **1981**, 61, 829; White, T. W.; Deans, M. R.; Kelsell, D. P.; Paul, D. L. *Nature*, **1998**, 394, 630). The “Bystander Effect” occurs when a dying adjacent cell delivers a cellular apoptotic signal such as low ATP or high Ca⁺² to an adjacent cell through uncontrolled open gap junctions. The process is well documented in cerebral ischemia, where the expansion of the ischemic infarct results from the transfer of ions and second messengers through astrocytes and other gap junction-containing neural cells. Inhibition of gap junctions can prevent

neural cell death when used acutely (Britz-Cunningham, S. H.; Shah, M. M.; Zuppan, C. W.; Fletcher, W. H. *N. Engl. J. Med.* **1995**, 332, 1323; Sosinsky, G. E.; Nicholson, B. J. Structural organization of gap junction channels. *Biochim. Biophys. Acta*, **2005**, 1711, 99-125). It is apparent that proper control of gap junctions is
5 required for neural health and that improper control would lead to neural toxicity. However, maintaining gap junctions in an open or closed state can both be neurotoxic. While closure of gap junctions would prevent the passage of apoptotic signals under acute stress, open gap junctions are required for the day-to-day control of neural cell signaling. Thus, long-term use of gap junction inhibitors would
10 be neurotoxic.

[0067] A gap junction channel is composed of two anchored connexon hexamers of gap junction proteins (Cx's) which assemble into large and more functional cell surface aggregates of up to 10,000 hexamers connecting two adjacent cells and allowing passage of up molecules of up to 1000 daltons. Some Cx's, such as Cx50,
15 also form hemichannels which communicate directly with the outside environment of the cell (Veenstra, R. D. *Recent Res. Devel. Biophys.* **2003**, 2, 65-94; Beyer, E. C.; Paul, D. L.; Goodenough, D. A. Connexin family of gap junction proteins. *J. Membrane Biol.* **1990**, 116, 187-194; Makowski, L.; Caspar, D. L. D.; Phillips, W. C.; Goodenough, D. A. *J. Cell Biol.* **1977**, 74, 629-645; Bruzzone, R.; White, T. W.;
20 Paul, D. L. *Eur. J. Biochem.* **1996**, 238, 1; Unger, V. M.; Kumar, N. M.; Gilula, N. B.; Yeager, M. *Science*, **1999**, 283, 1176-1180). Assembly and disassembly occurs in about 2 hr, and, can be stimulated by stress signals, pH, and phosphorylation by protein kinases such as PKC γ (Fleishman, S. J.; Unger, V. M.; Ben-Tal, N. *Trends in Biochem. Sci.* **2006**, 31, 106-113).

[0068] In order to study gap junctions, several inhibitors have been used including 18- α -glyrrhetic acid (AGA), which inhibits gap junction synthesis, and mefloquine, which preferentially blocks Cx50 gap junction activity. Mefloquine and related drugs have been used extensively by the military as anti-malarial prophylactics. However, their side effects include depression, psychotic episodes, suicide, and general
30 neurotoxicity.

[0069] To determine the effects of the compounds of the invention on gap junctions, human R28 cells and lucifer yellow dye and rhodamine dextran were used

to study gap junction. (Das, S.; Lauer, J.; Barnett, M.; Lin, D.; Akoev, V.; Battina, S.; Hua, D. H.; Takemoto, D. J. PKC γ phosphorylates connexin50 on serine-430. A presentation at 2006 ARVO Annual Meeting, April 30 – May 4, 2006, Fort Lauderdale, Florida). Figure 2 shows R28 cells grown to 90% confluency on coverslips. The cells were treated with different compounds as shown @ 10 μ M for 40 minutes. A mixture of lucifer yellow (LY) and rhodamine dextran (RD) was added to the cells at the center of the coverslip. Two cuts across the coverslip were made to form a transient tear in the plasma membranes of the cells to permit dye transfer through gap junctions. Cells were incubated with the dye for 20 minutes, then fixed and examined by fluorescent microscopy. For quantitative analysis the extent of dye transfer was estimated by counting the number of LY and RD labeled cells in the microscopic field and graphed. Three primaquine (PQ) analogs and mefloquine (MQ) blocked dye transfer significantly in comparison to the untreated control cells. However, **PQ1** and MQ were the most potent blocker of the gap junction passage of the lucifer yellow dye as there was hardly any passage of the dye in cells treated with these two anti-malarial drugs. **PQ3** shows inhibitory activity of about 20% as compared with the control. **PQ3** is useful as a drug having reduced gap junction inhibitory activity. The gap junction activity of other compounds of the invention may be tested as described herein without the use of undue experimentation.

Methods used in the gap junction studies

[0071] Cell Culture: Murine hippocampal HT22 cells are cultured in DMEM (high glucose), supplemented with 10% fetal bovine serum and 50 μ g/ml gentamicin, 0.05 units/ml penicillin, 50 μ g/ml streptomycin, pH 7.4, at 37° C in an atmosphere of 95% air and 5% CO₂. HT22 cells have endogenous Cx50 as their major gap junction protein. This is a common neural form of gap junction.

[0072] Gap junction Activity Assay: Gap junction activity is measured by the scrape load/dye transfer assay. HT22 cells are grown to 80% confluency then rinsed in fresh media and, in some cases, exposed to 100 μ M hydrogen peroxide as an oxidative stress or 500 μ M to induce apoptosis. After treatments, cells are rinsed in PBS then 2.5 μ l of both 1% Lucifer Yellow and 0.75% rhodamine dextrin are added to the center of the coverslips and two cuts are made crossing each other and passing through the dye. After 1 min the cells are rinsed in PBS and the dye allowed

to pass from cell to cell for an additional 10 min. Cells are then fixed in 2.5% paraformaldehyde and further rinsed in PBS. Dye transfer is evaluated by counting the number of cells taking up the Lucifer Yellow with damaged cells subtracted (those labeled with rhodamine dextrin), using a Nikon C1 confocal microscope. Four points are counted for each slide from 6 experiments and expressed as mean +/- S.E., $p < 0.05$ as significant.

[0073] Western blot and immunoprecipitations: In some cases apoptosis is determined by measurement of active caspase-3 using specific active caspase-3 antisera. Cells are collected and lysed on ice with cell lysis buffer: 20 mM Tris-HCl, pH 7.8.5, 0.5 mM EDTA, 0.5 mM EGTA, 0.5% Triton X-100, 0.1% protease inhibitor cocktail, 5mM NaFl, and 2 mM PMSF. After centrifugation at 12,000 x g for 20 min, the supernatants are collected and used as whole cell extracts. Western blots, immunoprecipitation of caspase-3 and scanning and quantitation of blots are performed as previously reported (Lin, et al, J. Biol. Chem., (2005), 280:13682-13693.).

[0074] Caspase-3 Assays: Apoptosis is measured by quantitation of active caspase-3, a common protein which is activated during apoptosis in neural cells. In some cases the active caspase-3 protein is measured on Western blots using mouse anti-active caspase-3 which is commercially available. For caspase-3 immunocytology, HT22 cells are grown in Delta T dishes to 50% confluency and treated with either primaquine analogs or hydrogen peroxide and varying concentration doses for varying times. Cells are then fixed with paraformaldehyde and treated with anti-active caspase-3 primary antisera and Alexa fluor 568 secondary antisera. Cells are examined under a confocal microscope and number of labeled cells/50 cells counted and expressed as per cent of cells in apoptosis.

Experiment Four: Anti-tumor Studies

[0075] Cancer in part is caused by the disruption in cell's homeostasis, affecting its ability to respond to extracellular signals, triggering some intracellular events which affect the gap junctional intercellular communication (GJIC). Gap junctions (GJ) are dynamic intercellular plasma membrane channels allowing the passage of small molecules (< 1 KD) between adjacent cells. They are formed by the interaction of

two hemichannels (connexon) in the adjacent cells, which, in turn are made of six subunits of connexin (Cx) proteins. The structure of connexins includes four hydrophobic membrane-spanning domains, two extracellular loops, one cytoplasmic loop, and an amino and carboxyl terminus in the cytoplasm. GJ are involved in tissue homeostasis, cell proliferation and differentiation, and the regulation of embryonic development and growth. Presently, 21 connexin genes have been identified in mammalian cells and three connexins, Cx43, Cx32 and Cx26 have been detected in normal breast tissue. Cx43 is the predominant form and detected mostly in myoepithelial cells. Cancer cells have reduced or altered GJIC capacity. Restoring GJIC is linked to drug sensitivity and reduction of tumorigenicity. Increasing gap junction activity or enhancing GJIC in tumor cells provides the targets to enhance anti-neoplastic therapies.

[0076] Compounds of the invention were studied by computational docking with the gap junction proteins (connexins) using Autodock computational docking software (Goodsell, D.S. and Olson, A.J. 1990. Automated docking of substrates to proteins by simulated annealing. *Proteins: Structure, Function, and Genetics* 8: 195-202; Morris, G.M., Goodsell, D.S., Huey, R. and Olson, A.J., 1996. Distributed automated docking of flexible ligands to proteins: parallel applications of AutoDock 2.4. *J. Computer-Aided Molecular Design* 10: 293-304; Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J. 1998. Automated docking using Lamarckian genetic algorithm and an empirical binding free energy function. *J. Comp. Chem.* 19: 1639-1662). Compounds were bound to the inert core of the hexameric hemichannel of gap junctions. The efficacy of the compounds of an embodiment of the invention was tested by determining their effect on GJIC in human breast cancer cells as an exemplary system.

[0077] Out of PQ1-5 compounds tested, PQ1 shows the highest affinity to bind with the connexins. Scrape load dye/transfer technique is used to measure the GJIC. Previous studies show that restoration of GJIC prevents cancer formation. Cancer formation is checked by preventing cell proliferation and growth thereby leading to cell death. Cancer cells are characterized by abnormal cell growth and proliferation than normal cells.

[0078] Cancer cells do not undergo apoptosis at the same rate as normal cells. Apoptosis is programmed cell death causing cell shrinkage, blebbing from the surface, condensation of the chromatin, and changes in cell surface molecules to ensure the phagocytosis of the apoptotic cells. Many cancer therapeutic agents exert their effect through initiation of apoptosis. Apoptosis is induced via stimulation of different cell surface receptors along with caspase activation. Signals leading to the activation of intracellular cysteine proteases play a pivotal role in the execution of apoptosis.

[0079] Figure 3 shows MDA-MB-453 and T47D colony growth for PQ1-5 and DMSO, as well as a control. Figure 3(A) MDA-MB-453 and (B) T47D human breast cancer cells were treated with 10 μ M of various quinolines for 7 days. Base agar plates were prepared containing 0.8% agar and 0.4% agar in Ham's F12. Cells (5×10^4 cells/33 mm well) were suspended in 100 μ l of Ham's F12 with 0.4% agar and plated. These plates were maintained at 37°C for 7 days and examined for the presence of colonies. Individual colonies of 40 μ m or greater were examined. The results demonstrated that **PQ1** alone showed approximately 80-95% growth attenuation in both breast cancer cell lines compared to control. Additionally, **PQ5** completely inhibited MDA-MB-453 colony growth. **PQ3** and **PQ4** have an inhibitory effect on T47D colony growth. However, **PQ3** caused a 2.3-fold increase in colony growth in MDA-MB-453 cells and **PQ2** caused a 2.6-fold increase in T47D colony growth compared to DMSO. Figures 7 and 8 show MDA-MB-453 and T47D colony growth for various concentrations of PQ1 and SA tested for 7 days. The procedures are generally as described above. The cell colony growth decreased using 10 and 100 nM PQ1 for both cell types.

[0080] Figure 4 shows that PQ1 has a tremendous effect on the GJIC in cells tested. The effect of **PQ1** on these cancer cells showed a 20-50% increase in GJIC, suggesting the gain of GJIC in cancer cells plays a critical role in the anti-tumor treatment (Figure 4). Figure 4(A) MDA-MB-453 and (B) T47D cells were treated with low (500nM) and high (10 μ M) concentrations of PQs for 24 hours. Monolayers of acceptor cell population were grown on 25-cm flasks in the presence of Vibrant DiD. Vibrant DiD in acceptor cells is not able to pass through the gap junctions because of its hydrophobic property. Single cell suspensions of donor treated cells were

prepared, labeled with 0.05 $\mu\text{g/ml}$ calcein-AM, incubated at 37C for 5 minutes. Quantification of dye coupling between the donor and acceptor cells was assessed by dual-excitation flow cytometry. The results demonstrated that 500nM of **PQ1** caused a 1.5-fold increase of gap junction activity compared to control; however, **PQ2** has no significant effect on gap junction activity in MDA-MB-453 cells. Cells treated with 10 μM **PQ3** have approximately 40-50% decrease in gap junction activity compared control or DMSO. Thus, these data provide evidence that increased or decreased gap junction directly affects cancer colony growth.

[0081] Saez *et al.* demonstrated that increased in GJIC is directly related to the anti-tumor effect in human mammary cancer cell line (JCB 89, 2003). However, **PQ3** has an opposite effect on mammary cancer cell lines. **PQ3** alone exhibited approximately 2.3-fold increase in a colony growth assay and caused a 50% decrease in GJIC. A summary of PQ- mediated GJIC activity and colony growth in breast and colon cancer cells is presented in Tables 1, 2, and 3, as well as figures 10-13. In Figure 10, SW620 cells were dosed for 24 hours with PQ1 at 100, 200, 300, and 500 nM. The gap junction activity was measured as a percent of control, as previously described. It was seen that all concentrations on PQ1 increased the gap junction activity in SW620 colon cancer cells as compared to the control. In Figure 11, the effect of PQ1 on the expression of PKC γ was studied at concentrations of 100, 200, 300 and 500 nM PQ1 for 24 hours. It is seen in Figure 11 that PQ1 has no effect on the expression of PKC γ at the levels tested. In Figures 12 and 13, the effect of PQ1 on the colony growth of SW620 colon cancer cells was measured with 1-100 nM PQ1 (Figure 12) and 10 μM PQ1 (Figure 13). It can be seen that PQ1 has no effect on the colony growth in SW620 at the levels tested in Figure 12, but completely inhibits SW620 at 10 μM . Figure 13 also shows the colony growth effect of PQ2-PQ5.

[0082] In the presence of 200 and 500 nM of PQ1 for 40 minutes, a significant increase in the gap junctional activity in T47D cells was seen (Figure 9). In the experiments for Figure 9, T47D cells were dosed with 100, 200 and 400 nM of PQ1 for 40 minutes. Lucifer yellow, a gap junctional permeable dye, was used to measure the GJIC. Rhodamine dextran was used as a control. The dotted line in Figure 9 indicates the line of cut. This increase could be due to the interaction

between the PQ1 and the connexin proteins which leads to the opening of the gap junction channel. PQ1 exerted no effect on the cell morphology of T47D cells (data not shown).

[0083] PQ1 showed a 40% decrease in the cell proliferation in T47D cells. This effect was seen at 200 nM of PQ1 for 24 hours (Figure 5). There was no effect seen on the cell proliferation in transformed human mammary epithelial cells (HMEC, data not shown). This suggests that PQ1 affects the cell viability of the cancerous cells without affecting the growth of normal cells.

[0084] The expression of caspases in the presence of PQ1 in T47D cells was studied. A significant 50% increase in the expression of active caspase 3 at 200 and 500 nM of PQ1 in T47D cells was found (Figure 6).

Methods used in tumor studies

[0085] Scrape load dye transfer technique (SL/DT): Lucifer yellow is an intensely fluorescent 4-aminophthalimide dye having low molecular weight (Mr 457.2) and passes from one cell to another through gap junctions only. Rhodamine dextran is a high molecular weight polymer (Mr 10,000) used as a control as passes only through injured plasma membrane. Cells were grown to 90 % confluency on cover slips, dosed with PQ 1 for 40 minutes. The 2.5 μ l of 1% (w/v) Lucifer yellow and .75% (w/v) of Rhodamine dextran was mixed and added in the center of the coverslip. Two cuts crossing each other in the center of the coverslip were made. After three minutes, cells were washed with PBS and incubated at 37°C in tissue culture media for 20 minutes. Cells were fixed and mounted on a slide, sealed and visualized under a fluorescence microscope at 10X objective.

[0086] MTT assay: This assay is done to measure cell proliferation and viability. Yellow MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple crystals, formazan in the mitochondria of living cells. A solubilization solution is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be measured by a spectrophotometer. T47 D cells (20,000) were grown in 96-well plate and dosed with 10, 100, 200, 500 nM of PQ1 compound in 100 μ l of complete media for 24 hours. MTT (20 μ l) was added to the cells and purple formazan crystals were observed after

90 minutes. A solubilization solution (0.35 N HCl) was added to dissolve the formazan and the absorbance was measured at 595 nm wavelength with a background wavelength 690 nm by a spectrophotometer.

5 [0087] Cell Morphology: T47D cells (1000 cells/ml) were seeded in a six-well plate for 24 hours. Next day, cells were dosed with varying concentrations of PQ1 for 24 hours. Cells were observed under a microscope before and after the experiment. No effect seen on the morphology of cells in the presence of PQ1.

10 [0088] Colony Growth Assay: T47D human breast cancer cells were treated with PQ1. Base agar plates were prepared containing 0.8 % agar and 0.4 % agar in Ham's F12. Cells (5 X 10⁴ cells/ 33 mm² well) were suspended in 100 µl of Ham's F12 with 0.4 % agar and plated. These plates were maintained at 37°C for 7 days and examined for the presence of colonies. Individual colonies of 40 µm or greater were examined.

15 [0089] Western Blot analysis: Cells were grown in RPMI media until they were 90% confluent in 75 cm² flasks. Cells were dosed with 200, 500 nM of PQ1 for 24 hours. Cells were harvested with lysis buffer and centrifugated. Cell lysate was run on a 12% SDSpolyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membrane. Membrane was incubated with Caspase Antibody. Western blots were detected by enhanced chemiluminescence detection reagents.

20 [0090] As will be appreciated by one of ordinary skill in the art, the compounds and methods described here can be used for the inhibition of growth of other types of cancer, such as human prostate cancer, glioma, pancreatic cancer and other types of cancer.

25 [0091] The compounds used can be used to study drug-protein interactions, and can be used as known in the art to study in vivo effects, such as by using mouse, human and other animal studies.

Table 1: The effect of PQ compounds on gap junction intercellular communication in breast cancer cell lines

Compounds					
Cell Line	PQ1	PQ2	PQ3	PQ4	PQ5
MDA-MB-231	Increase (500nM)	Decrease	No effect	Decrease	No effect
MDA-MB-453		No effect	Decrease	No effect	Decrease
MCF-7		Decrease	Decrease	No effect	No effect
ZR75		No effect	No effect	No effect	No effect
T47D		Increase (500nM)			

Table 2: The effect of PQ compounds on breast cancer cell colony growth

Compounds					
Cell Line	PQ1	PQ2	PQ3	PQ4	PQ5
MDA-MB-453	Decrease	No effect	Increase	No effect	Decrease
T47D	Decrease	Increase	Decrease	Decrease	No effect

Table 3: The effect of PQ compounds on gap junction intercellular communication in colon cancer cell lines

Compounds					
Cell Line	PQ1	PQ2	PQ3	PQ4	PQ5
HT29	No effect	Increase	Increase	Increase	Increase
SW620	Increase (300nM)	Increase (6uM)	Increase (500nM)	No effect	Decrease (300nM)

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[0092] All references throughout this specification, for example patent documents including issued or granted patents or equivalents; patent application publications;
15 and non-patent literature documents or other source material; are hereby incorporated by reference herein in their entirety, as though individually incorporated by reference, to the extent each reference is at least partially not inconsistent with the disclosure in this application (for example, a reference that is partially inconsistent is incorporated by reference except for the partially inconsistent
20 portion of the reference).

[0093] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. References cited herein are incorporated by reference herein in their entirety to indicate the state of the art, in some cases as of their filing date, and it is intended
25 that this information can be employed herein, if needed, to exclude (for example, to disclaim) specific embodiments that are in the prior art. For example, when a compound is claimed, it should be understood that compounds known in the prior art, including certain compounds disclosed in the references disclosed herein (particularly in referenced patent documents), are not intended to be included in the
30 claim.

[0094] When a group of substituents is disclosed herein, it is understood that all individual members of those groups and all subgroups, including any isomers and

enantiomers of the group members, and classes of compounds that can be formed using the substituents are disclosed separately. When a compound is claimed, it should be understood that compounds known in the art including the compounds disclosed in the references disclosed herein are not intended to be included. When
5 a Markush group or other grouping is used herein, all individual members of the group and all combinations and subcombinations possible of the group are intended to be individually included in the disclosure.

[0095] Every formulation or combination of components described or exemplified can be used to practice the invention, unless otherwise stated. Specific names of
10 compounds are intended to be exemplary, as it is known that one of ordinary skill in the art can name the same compounds differently. When a compound is described herein such that a particular isomer or enantiomer of the compound is not specified, for example, in a formula or in a chemical name, that description is intended to include each isomers and enantiomer of the compound described individual or in any
15 combination. One of ordinary skill in the art will appreciate that methods, starting materials, and synthetic methods other than those specifically exemplified can be employed in the practice of the invention without resort to undue experimentation. All art-known functional equivalents, of any such methods, starting materials, and synthetic methods are intended to be included in this invention.. Whenever a range
20 is given in the specification, for example, a temperature range, a time range, or a composition range, all intermediate ranges and subranges, as well as all individual values included in the ranges given are intended to be included in the disclosure.

[0096] As used herein, "comprising" is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude
25 additional, unrecited elements or method steps. As used herein, "consisting of" excludes any element, step, or ingredient not specified in the claim element. As used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim. Any recitation herein of the term "comprising", particularly in a description of components of a
30 composition or in a description of elements of a device, is understood to encompass those compositions and methods consisting essentially of and consisting of the recited components or elements. The invention illustratively described herein suitably

may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein.

[0097] The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0098] In general the terms and phrases used herein have their art-recognized meaning, which can be found by reference to standard texts, journal references and contexts known to those skilled in the art. The following definitions are provided to clarify their specific use in the context of the invention.

[0099] One skilled in the art readily appreciates that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent in the present invention. The methods, components, materials and dimensions described herein as currently representative of preferred embodiments are provided as examples and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention will occur to those skilled in the art, are included within the scope of the claims. Although the description herein contains certain specific information and examples, these should not be construed as limiting the scope of the invention, but as merely providing illustrations of some of the embodiments of the invention. Thus, additional embodiments are within the scope of the invention and within the claims.

[00100] The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition (see e.g. Fingl et. al., in The Pharmacological Basis of Therapeutics, 1975, Ch. 1 p. 1). It should be noted that the

attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions, or to other undesired effects. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and response of the individual patient. A program comparable to that discussed above also may be used in veterinary medicine.

[00101] Depending on the specific conditions being treated and the targeting method selected, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in Alfonso and Gennaro (1995). Suitable routes may include, for example, oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, or intramedullary injections, as well as intrathecal, intravenous, or intraperitoneal injections.

[00102] For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[00103] Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular those formulated as solutions, may be administered parenterally, such as by intravenous injection. Appropriate compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be

formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

[00104] Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above. Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

[00105] Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[00106] In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions, including those formulated for delayed release or only to be released when the pharmaceutical reaches the small or large intestine.

[00107] The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[00108] Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or

synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes.

Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran.

Optionally, the suspension may also contain suitable stabilizers or agents which

5 increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

[00109] Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to

10 obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating
15 agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

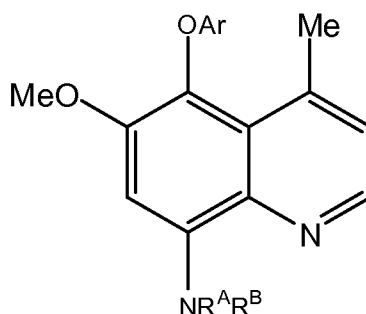
[00110] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide,
20 lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

[00111] Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a
25 plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers
30 may be added.

CLAIMS

We claim:

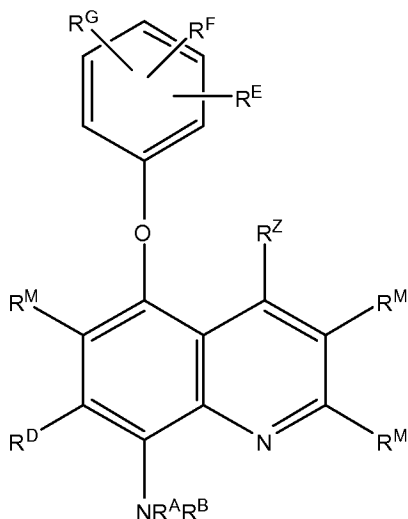
1. A compound having the following formula:



where Ar is o-, m- or p- C₆H₄CF₃;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, -(CH₂)_n-(X)_m-Y, where n is an integer from 0-6; m is an integer from 0-2; X is independently -O-, -C(=O)-, -C(=NH)-, -S-, -S(=O)-, -SO₂-; and Y is selected from the group consisting of: hydrogen, C1-C6 alkyl groups and nitrogen containing groups comprising -NH₂; -NR₂, where R is C1-C6 alkyl; -NO₂; -CN; phthalimide; imidazole; pyrazole; pyridine; pyrimidine; pyrazine; and pyridazine.

2. A compound having the formula:



where R^Z, R^M and R^D are independently selected from the group consisting of: hydrogen, halogen, aldehyde, ketone, alcohol, optionally substituted C1-C6

straight chain or branched alkyl or alkoxy, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl;

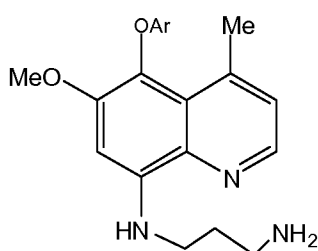
R^E , R^F , and R^G are independently hydrogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy group, oxygen, halogen nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, alcohol, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, and where any R^E , R^F , or R^G which is not hydrogen may be positioned in the ortho-, meta- or para-position;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy, and nitrogen containing groups including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine and pharmaceutically acceptable salts thereof.

3. The compound of claim 2, wherein R^E and R^F are hydrogen, and R^G is o-, p-, or m- CF_3 .
4. The compound of claim 3, wherein R^M is OMe.
5. The compound of claim 2, wherein all R^M s are hydrogen.
6. The compound of claim 1 or 2, wherein R^A and R^B are both hydrogen or both oxygen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, and $-SO_2-$; and Y is selected from the group consisting of: hydrogen, C1-C6 alkyl groups and nitrogen containing groups including $-NH_2$; $-NR_2$, where R is C1-C6 alkyl; -

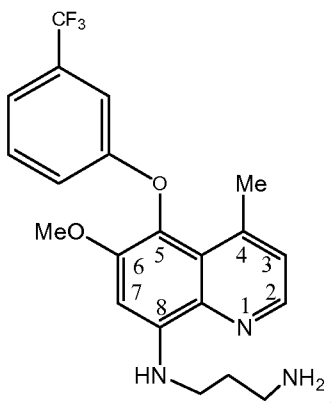
NO₂; -CN; phthalimide; imidazole; pyrazole; pyridine; pyrimidine; pyrazine; and pyridazine.

7. The compound of claim 1 or 2, wherein R^A is hydrogen and R^B is -(CH₂)_n-(X)_m-Y, where m is 0, and Y is selected from the group consisting of -NH₂; -NR₂, where R is C1-C6 alkyl; -NO₂; -CN; phthalimide; imidazole; and pyrazole.
8. The compound of claim 7, wherein R^A is hydrogen and R^B is -(CH₂)_n-(X)_m-Y, where m is 0, and Y is a nitrogen-containing group.
9. The compound of claim 1 or 2, having formula:

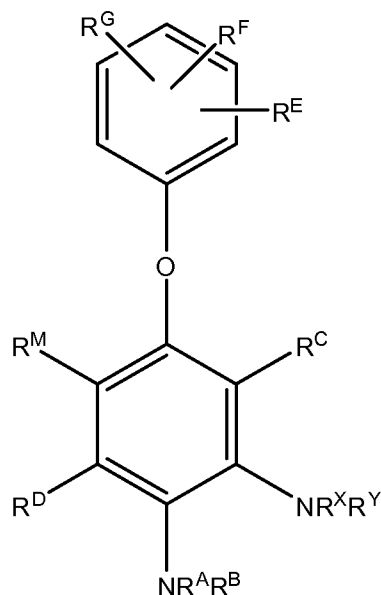


where Ar is o-, p-, or m- CF₃.

10. The compound of claim 1 or 2, having formula:



11. The compound of claim 1 or 2, further comprising a pharmaceutically acceptable carrier.
12. A method for treating a gap junction disorder, comprising administering an effective amount of a compound of formula:



where R^X and R^Y are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen and nitrogen containing groups including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine;

R^C , R^D and R^M are independently selected from the group consisting of: hydrogen, halogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl;

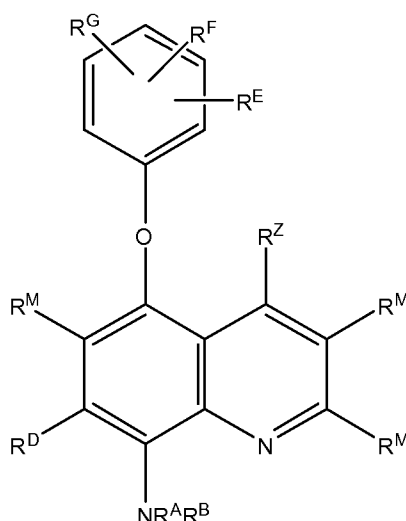
R^E , R^F , and R^G are independently hydrogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy group, oxygen, halogen, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, alcohol, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, and where any R^E , R^F , or R^G which is not hydrogen may be positioned in the ortho-, meta- or para-position;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen and nitrogen containing groups including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine;

or both R^X and R^Y and R^C are optionally substituted carbon ring atoms which form a six membered ring where the optional substituents are hydroxyl, halogen, and C1-C6 alkyl, wherein when R^X , R^Y and R^C form a ring structure, R^A and R^B are not both oxygen or hydrogen.

and a pharmaceutically acceptable carrier to a patient.

13. The method of claim 12, wherein the compound has the formula:

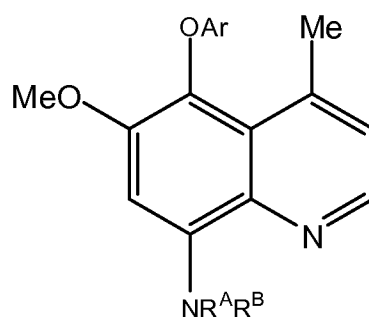


where R^Z , R^M and R^D are independently selected from the group consisting of: hydrogen, halogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, or arylsulfonyl;

R^E , R^F , and R^G are independently hydrogen, aldehyde, ketone, alcohol, optionally substituted C1-C6 straight chain or branched alkyl or alkoxy group, oxygen, halogen, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, where the optional substituents are independently halogen, alcohol, nitro, amine, alkylsulfanyl, alkylsulfinyl, alkylsulfonyl, arylsulfanyl, arylsulfinyl, and arylsulfonyl, and where any R^E , R^F , or R^G which is not hydrogen may be positioned in the ortho-, meta- or para-position;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen and nitrogen containing groups including $-NH_2$, $-NO_2$, $-CN$, phthalimide, imidazole, pyrazole, pyridine, pyrimidine, pyrazine, and pyridazine; and pharmaceutically acceptable salts thereof.

14. The method of claim 12, wherein the compound has the formula:



where Ar is o-, m- or p- $C_6H_4CF_3$;

R^A and R^B are both oxygen or both hydrogen, or are independently selected from the group consisting of: hydrogen, $-(CH_2)_n-(X)_m-Y$, where n is an integer from 0-6; m is an integer from 0-2; X is independently $-O-$, $-C(=O)-$, $-C(=NH)-$, $-S-$, $-S(=O)-$, $-SO_2-$; and Y is selected from the group consisting of: hydrogen, C1-C6 alkyl groups and nitrogen containing groups comprising $-NH_2$; $-NR_2$, where R is C1-C6 alkyl; $-NO_2$; $-CN$; phthalimide; imidazole; pyrazole; pyridine; pyrimidine; pyrazine; and pyridazine.

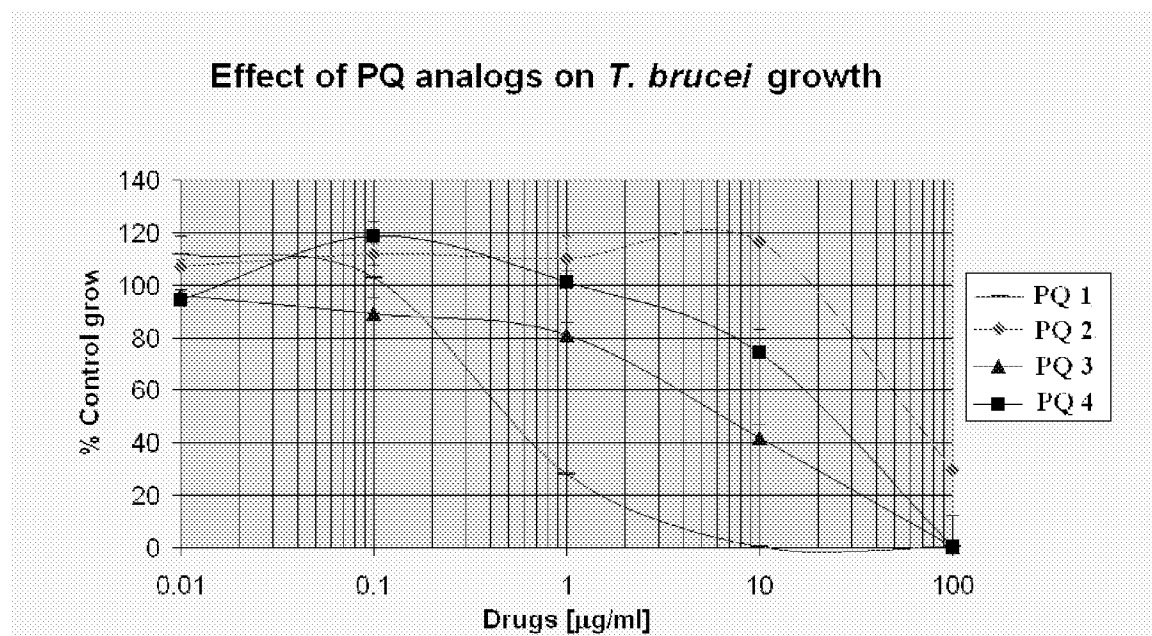


FIG. 1

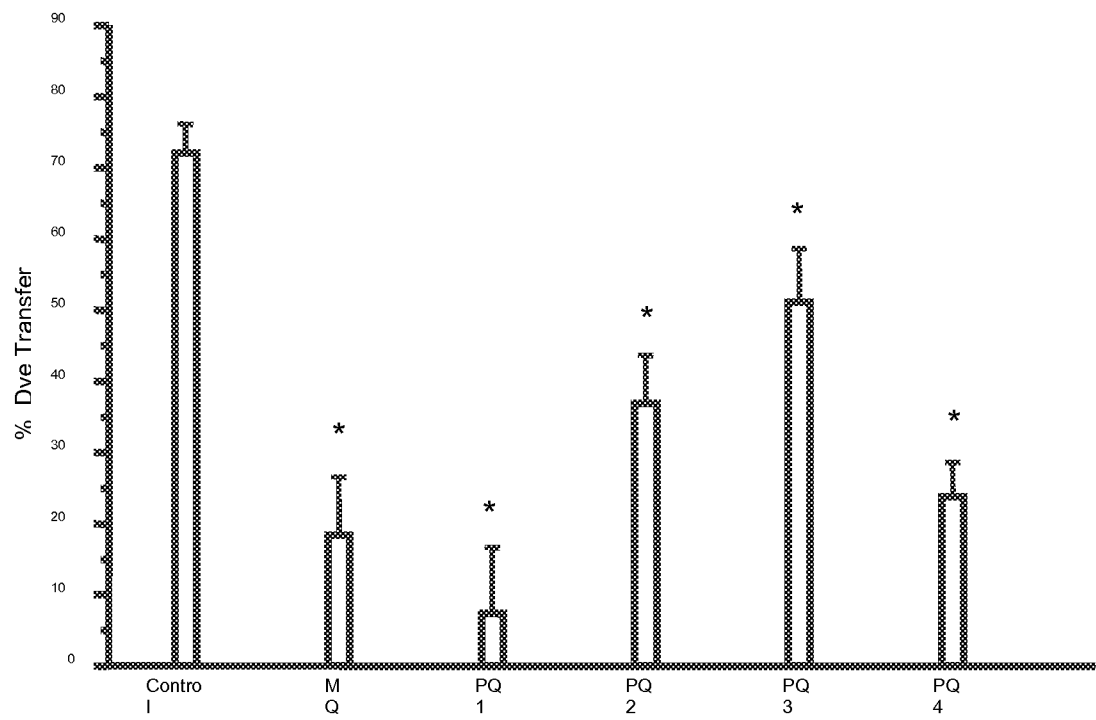
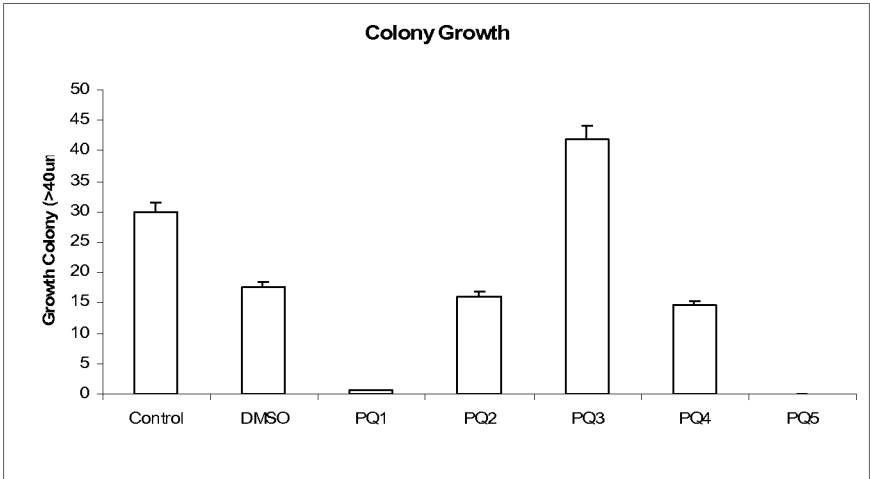


FIG. 2

A. MDA-MB-453 cells



B. T47D cells

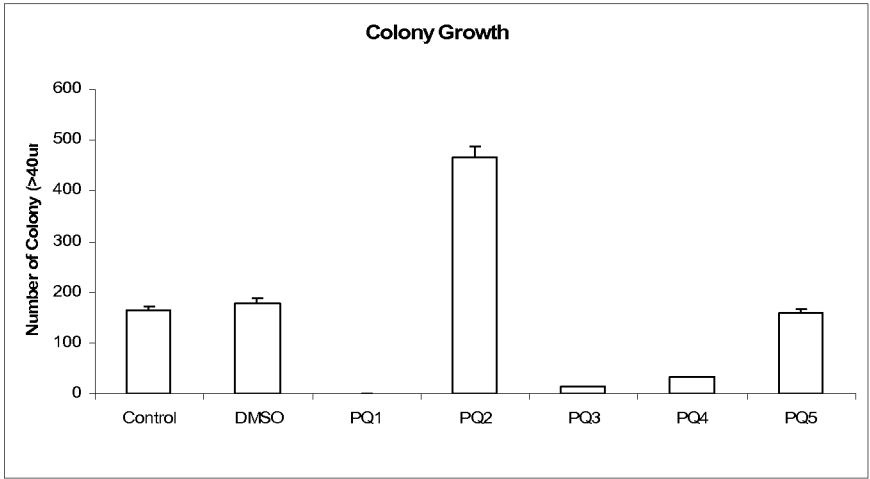
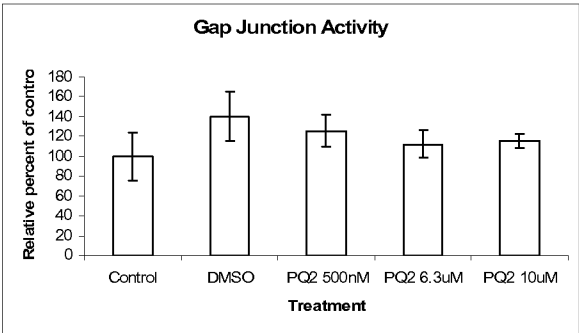
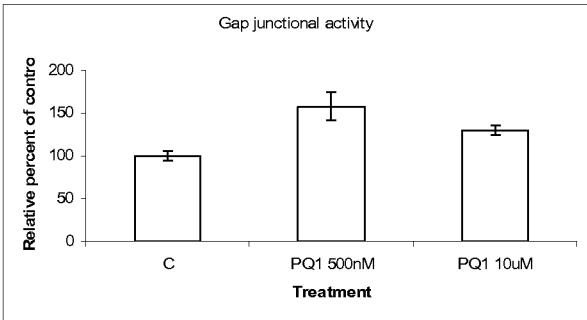


FIG. 3

A. MDA-MB-453 Cells



B. T47D Cells

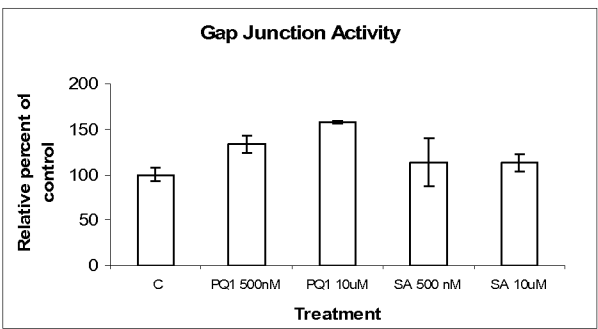
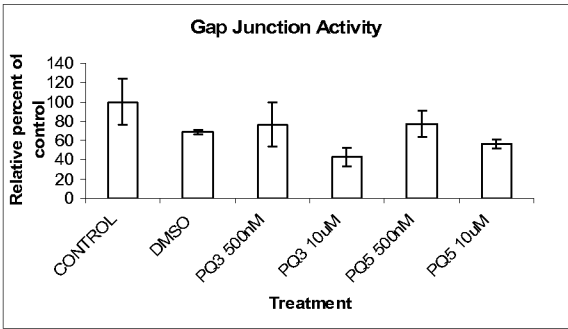
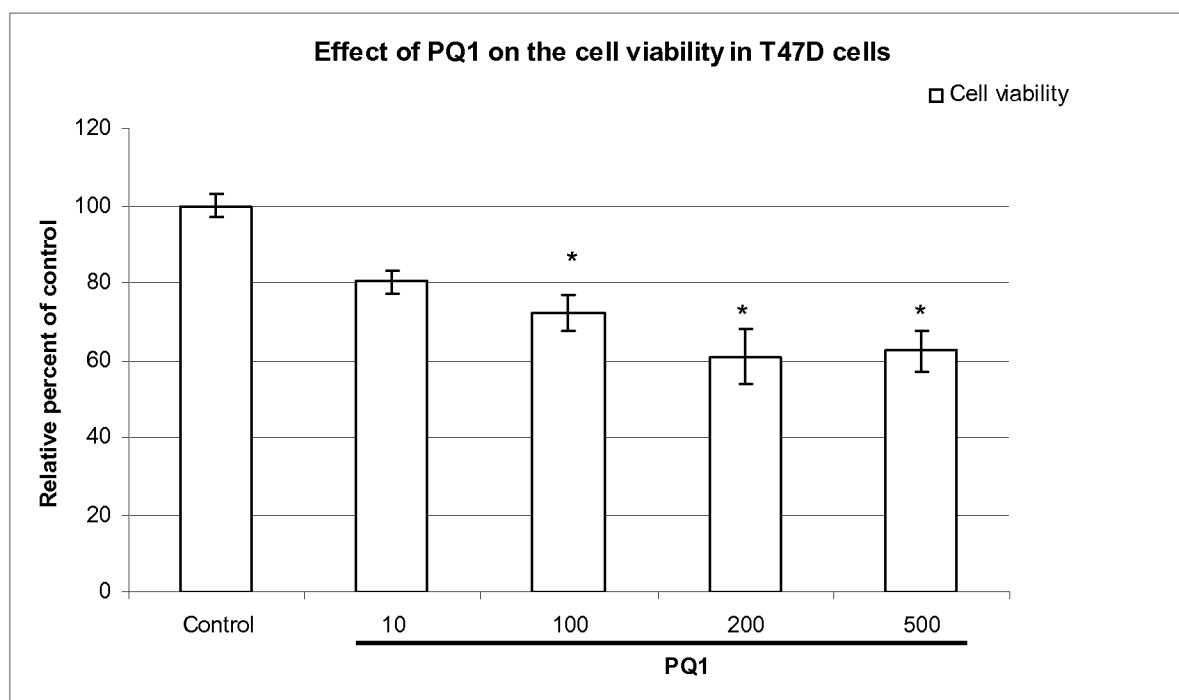


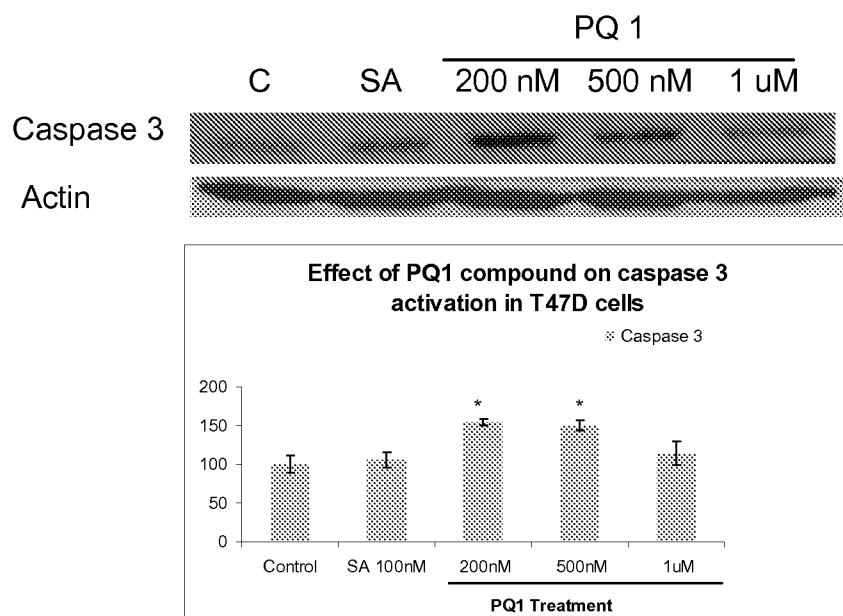
FIG. 4



**p < 0.05*

FIG. 5

Effect of PQ1 compound on the activation of Caspase 3 in T47D cells



* $p < 0.05$

FIG. 6

Colony Growth of PQ1 in T47D Cells

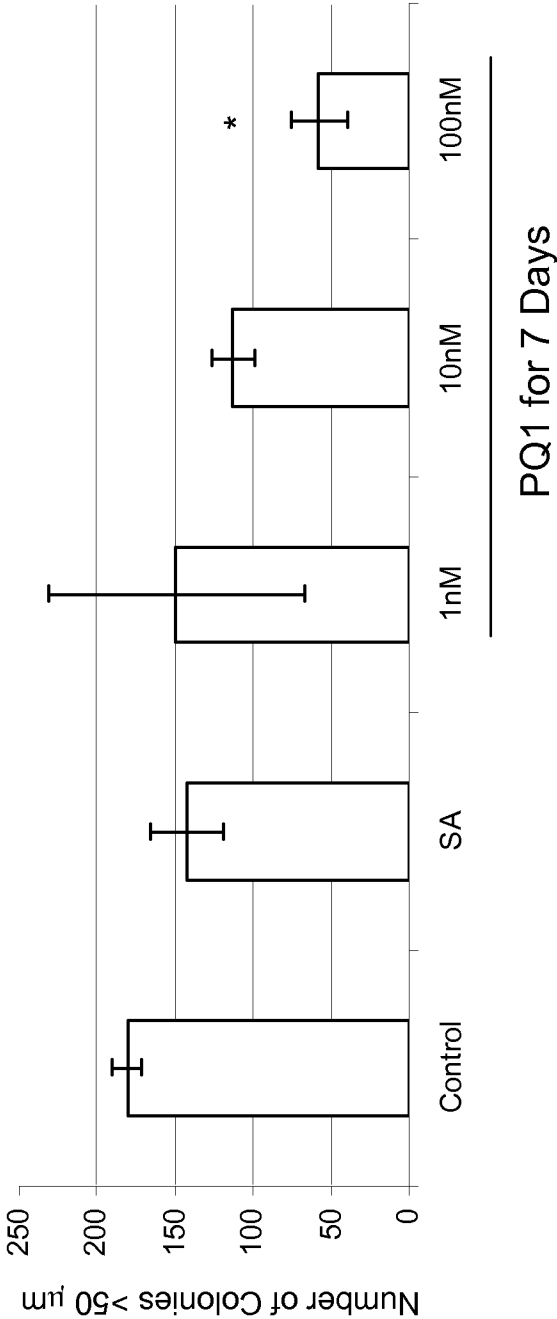


FIG. 7

Colony Growth of PQ1 in MDA-MB-453

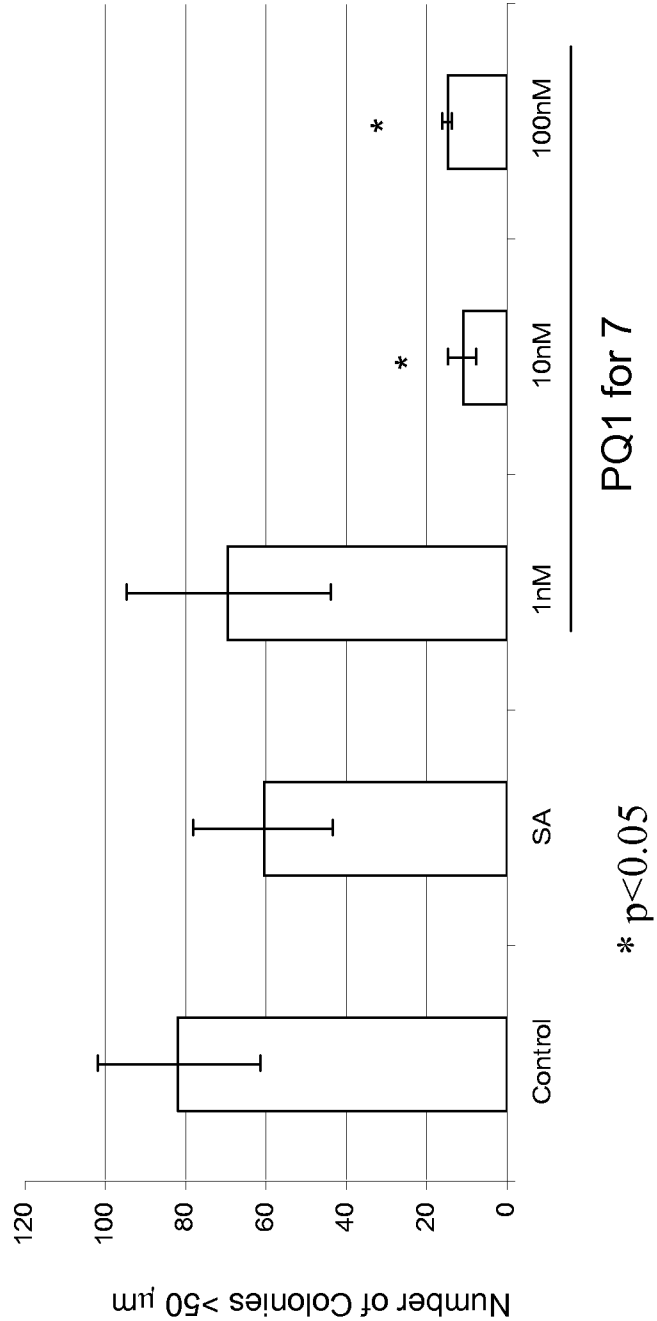


FIG. 8

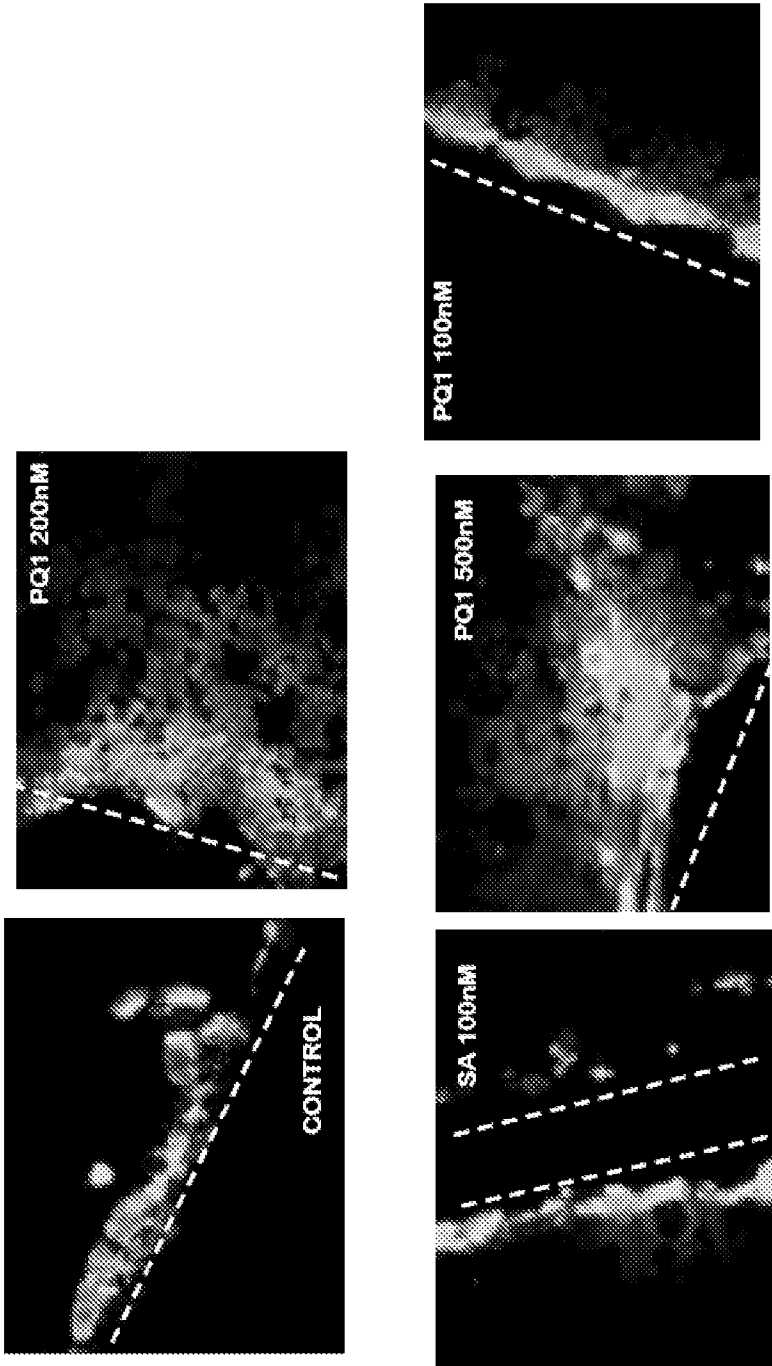


FIG. 9

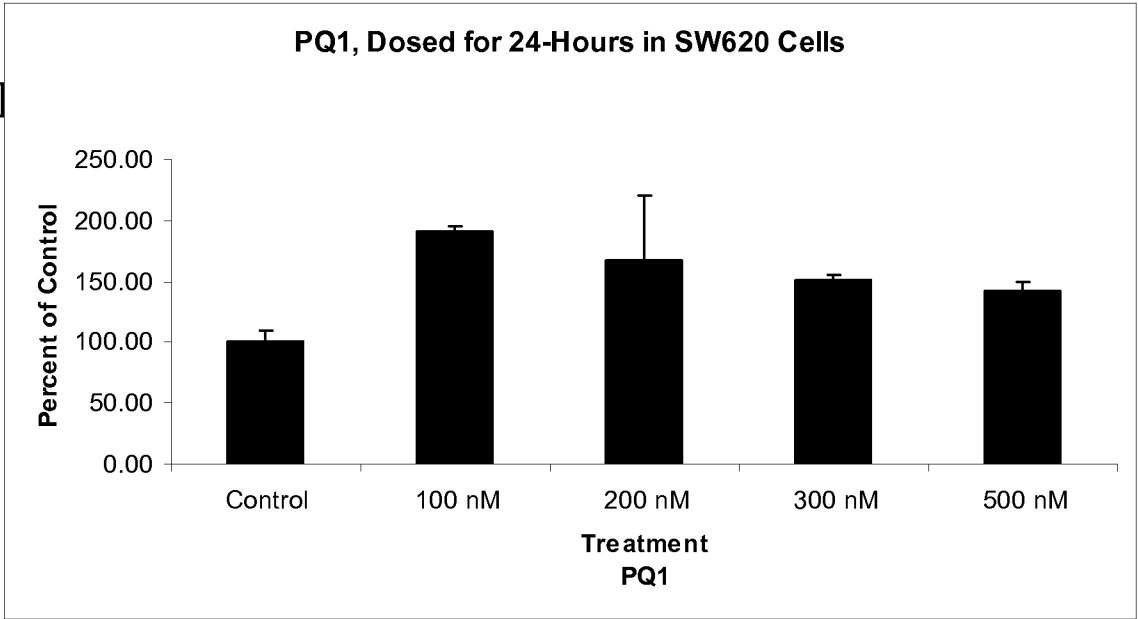


FIG. 10

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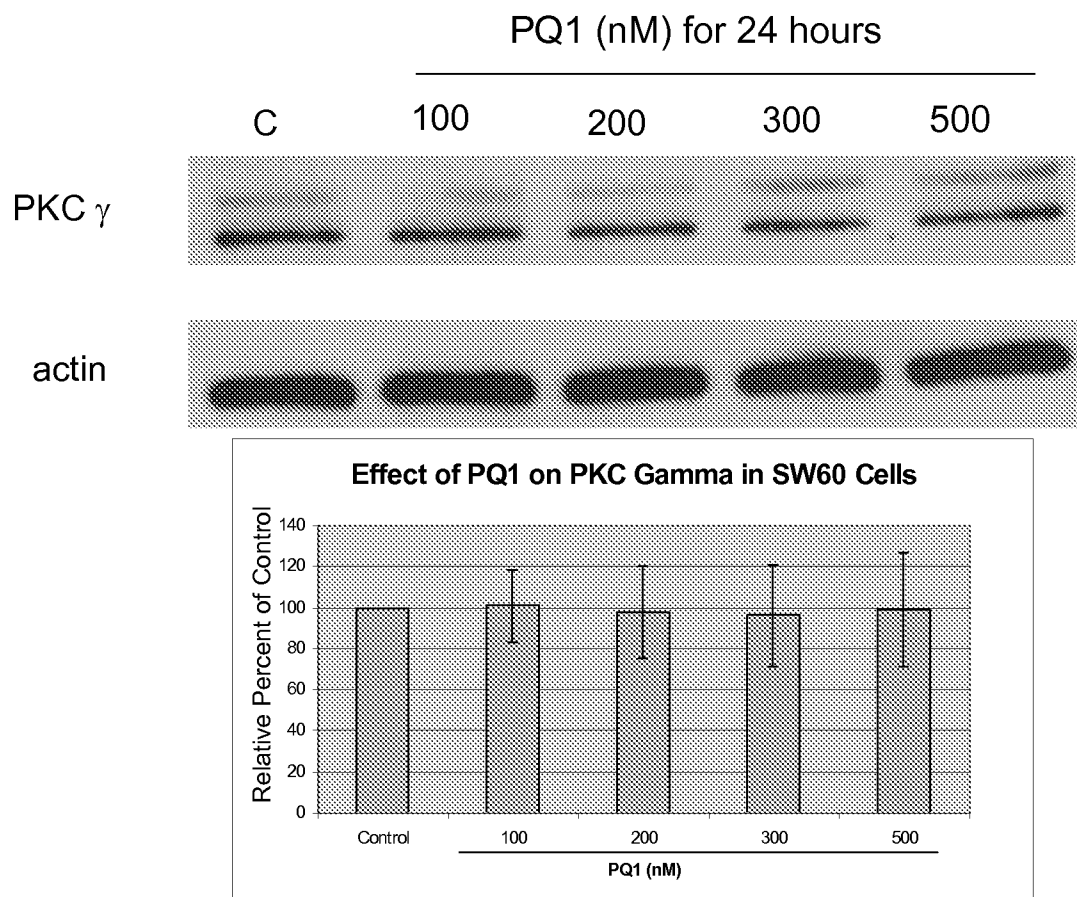
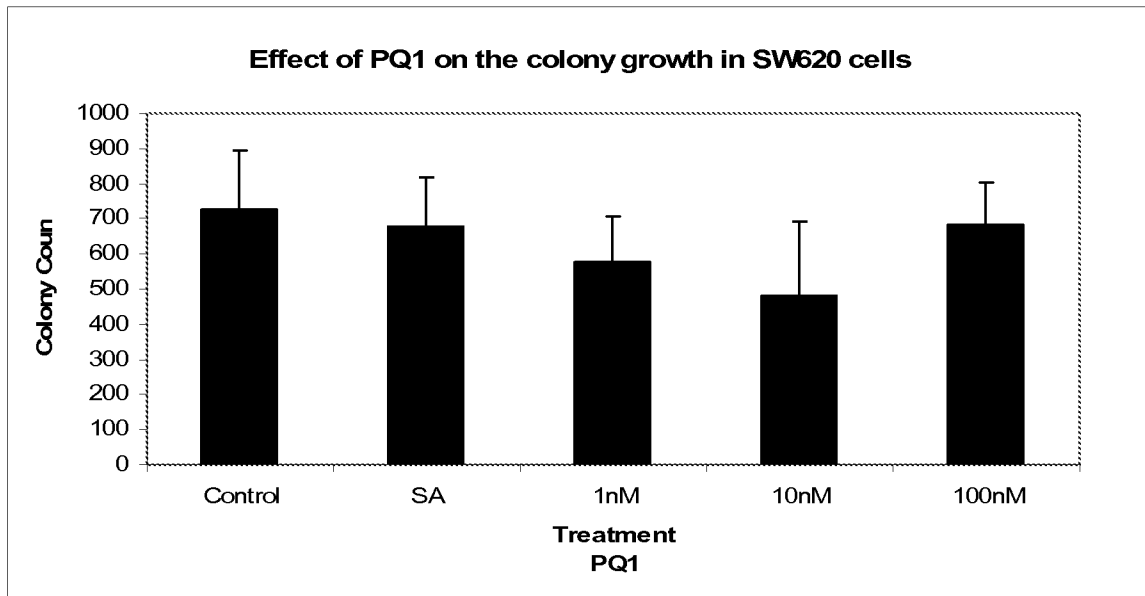


FIG. 11

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**FIG. 12**

13 / 13

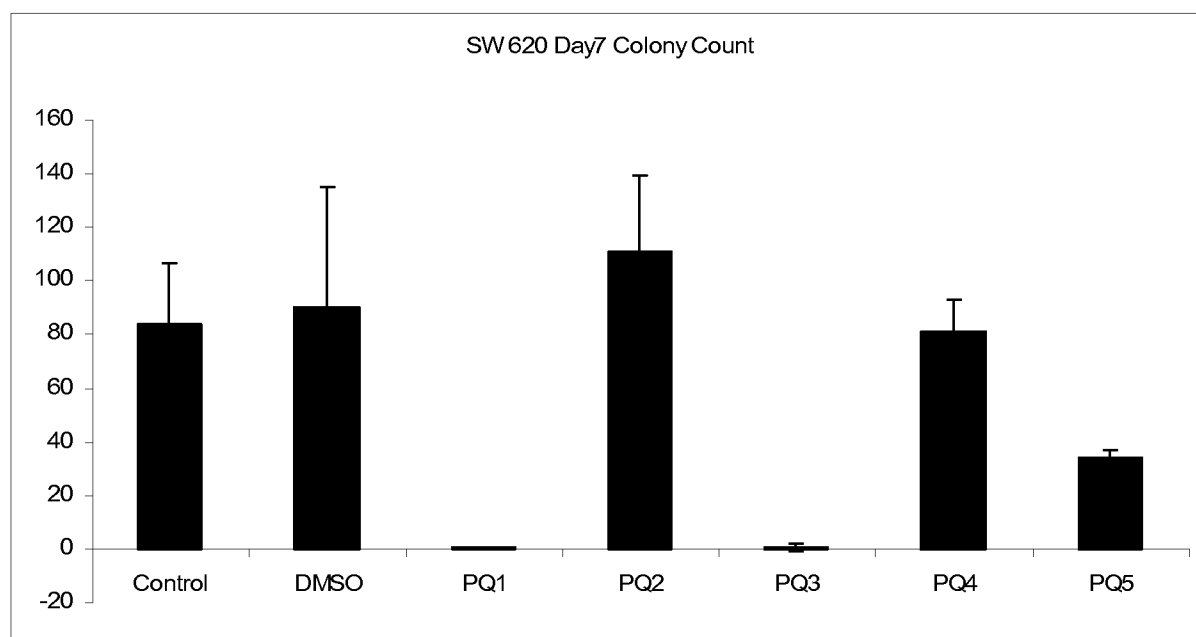


FIG. 13