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(71) Applicant *(for all designated States except US)*: **OHIO UNIVERSITY** [US/US]; TECHNOLOGY TRANSFER OFFICE, Unit 14, 340 West State Street, Athens, OH 45701 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BERGMEIER, Stephen, C.** [US/US]; 345 Carroll Road, Athens, OH 45701-3328 (US). **EVANS, Susan, C.**; 4 Coventry Lane, Athens, OH 45701-3716 (US).

(74) Agent: **DOBREA, Diane**; CALFEE, HALTER & GRISWOLD LLP, 1400 McDonlad Investment Center, 800 Superior Avenue, Cleveland, OH 44114-2688 (US).

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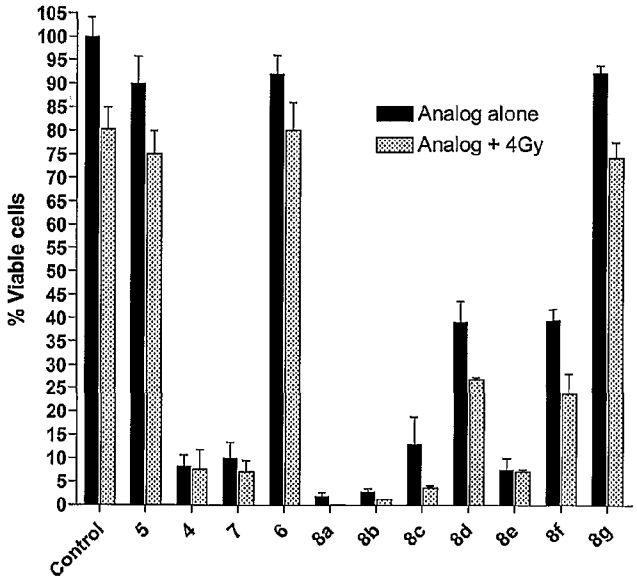

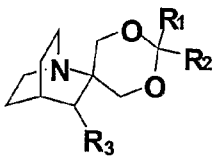

I

(57) Abstract: New compounds of formula (I): wherein R_1 and R_2 may be the same or different and are selected from H, halo, alkyl, cycloalkyl, haloalkyl, aryl, haloaryl, alkylaryl, alkoxyaryl, hydroxyl, acetate, ketal, alkoxy, and combinations thereof, and combinations thereof, or R_1 and R_2 may form a ring, as with an alkyl, alkenyl, substituted alkyl or substituted alkenyl bridge, and R_3 is O or hydroxyl. Also encompassed are derivatives, metabolites, and prodrugs thereof. Also provided are methods for preparing the quinuclidinone analogs disclosed herein. Further provided are methods of treating, preventing or delaying the onset of a cancer in a subject in need of such treatment by administering a an effective amount of a compound of formula (I), or a derivative, metabolite or prodrug thereof to a subject diagnosed with cancer or at risk of developing cancer.

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Quinuclidinone Derivatives as Anticancer Agents

Cross-Reference to Related Applications

[0001] This application claims priority to, and any other benefit of, US Provisional Patent Application No. 60/738,673 filed November 21, 2005, the entirety of which is incorporated herein, by reference.

Background of the Invention

[0002] There has been an effort to develop small molecules that specifically trigger cancer cells to undergo apoptosis or stop proliferation. Most chemotherapeutic drugs harm both normal and cancer cells. The goal, however, is to create tumor-specific compounds. The tumor suppressor p53 plays a prominent role in inducing apoptosis and cell cycle arrest. Compounds that could increase p53 activity would be very beneficial to cancer therapy. On the other hand, over 50% of human cancers have mutations in p53, therefore agents that do not rely on p53 activity are also appealing.

[0003] Some chemotherapeutic drugs destroy tumor cells directly by their own cytotoxic action, but radiotherapy may enhance the effects. Many studies show the advantages of combining radiotherapy with chemotherapy, both in terms of better local control of cancer and metastatis prevention.

[0004] A large variety of chemotherapeutic agents have been developed and use clinically. A significant problem with most chemotherapeutic agents is the development of resistance and non-specific cytotoxicity. There is a great deal of structural diversity amongst these anticancer agents, ranging from small organometallic compounds (*e.g.* cisplatin) to large aklaloids (*e.g.*CC-1065). Chemotherapeutic agents largely act through one of three mechanisms of action at the molecular level (antimetabolites, DNA interaction, and tubulin targeting).

[0005] Antimetabolites are commonly used chemotherapeutic agents.. Typical drugs include methotrexate, and 5-fluoracil (5-FU). In additiona to these well-known agents, a more recent example of an antimetabolite is gemcitabine. Antimetabolites interfere with the formation or use of a normal cellular metabolite. Most antimetabolites interfere with the synthesis of DNA and consequently many of the antimetabolites bear a strong structural similarity to components

of DNA (*e.g.*, 5-FU) or critical cofactors (*e.g.* methotrexate). Antimetabolites are often associated with significant toxicity due to interruption of normal cellular pathways.

[0006] Small molecules that interact with DNA are another widely used class of anticancer drugs. These include DNA alkylating agents and intercalators that exert their selective cytotoxicity by targeting the difference in growth rate between cancer cells and normal cells. One of the earliest examples of an anticancer drugs are the nitrogen mustards: alkylating agents that crosslink DNA. A classic nitrogen mustard is cyclosphamide. These agents tend to target guanine rich sequences. Other alkylating agents include aziridines and nitrosoureas. A particularly interesting class of alkylating agent that has some sequence specificity are the CC-1065 analogs. CC-1065 is an antibiotic isolated from a streptomycin species and binds to the minor groove of DNA to 5'-Pu-N-T-T-A or 5'-A-A-A-A-A sequences.

[0007] The anthraquinones are one of the best known examples of intercalating agents. The planar ring system of these molecules is inserted perpendicular to the long axis of the double helix. This leads to multiple mechanisms of DNA damage, including inhibition of topoisomerases. Topoisomerase inhibitors are an efficient group of new drugs that induce apoptosis and are used to treat cancer. The main pathways leading from topoisomerase-mediated DNA damage to cell death involve activation of caspases in the cytoplasm by proapoptotic molecules released from mitochondria. In some cells, the apoptotic response also involves the cancer receptor Fas (APO-1/CD95). The activation of these apoptotic signaling pathways is tightly controlled by upstream regulatory pathways that respond to DNA lesions induced by topoisomerase inhibitors in cells undergoing apoptosis. These include the proapoptotic Chk2, c-Abl and SAPK/JNK pathways, the survival PI(3)kinase-Akt-dependent pathway and the transcription factors p53 and NF-kappaB. Initiation of cellular responses to DNA lesions induced by topoisomerase inhibitors is ensured by the protein kinases DNA-PK, ATM and ATR, which bind to DNA breaks. It is believed that topoisomerase I inhibitors stabilize a DNA/topoisomerase I complex and interact with replication machinery to cause cell death. Many of these topoisomerase I inhibitors also act as radiosensitizers by producing enzyme-mediated DNA damage.

[0008] Etoposide was the first anticancer drug to be demonstrated to exert its antineoplastic effect though inhibition of topoisomerase II. It induces massive apoptosis in germ-cell malignancies, lung cancer, non-Hodgkin's lymphoma, leukemia, Kaposi's sarcoma, neuroblastoma, and soft-tissue sarcomas through activation of caspase-3, -8 and -9 and endonucleases.

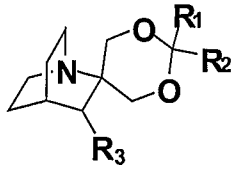
[0009] More recently, anticancer agents that target tubulin have been developed. Probably the most well-known is paclitaxel. This compound promotes microtubule assembly, which causes arrest of the cell cycle at the G2/M transition and ultimately leads to apoptosis. A new class of microtubule drugs is exemplified by epothilone B.

[0010] There has been an effort to develop small molecules that restore function to p53, a tumor suppressor gene frequently mutated in a wide variety of cancers. Several examples of such small molecules have been reported. Certain pyrimidine dervitives have been reported to both stabilize wild-type p53, as well as restore function to mutant p53. 2-Methoxyestradiol facilitates wild-type p53-mediated apoptosis. More recently a series of α-methylene ketones were identified as having good anti-proliferative activity against p53 mutant cell types. APB-14 is also reported to induce apoptosis in a number of cell types. This small molecule also restores the active conformation of mutant p53. Some reports suggested induced conformational change in p53, though no structural activity studies have been reported.

[0011] Given the toxicity, development of resistance, and lack of broad spectrum treatments, there is a continuing need for the development of new chemotherapeutic agents for the treatment of cancer. A need exists for new compounds to target existing cancer which will improve therapeutic modalities. These new anticancer agents should have minimal cytotoxic effect on normal cells.

Summary of the invention

[0012] Provided are new chemotherapeutic agents based on quinuclidinone analogs. One particular set of new chemotherapeutic agents is that of formula I:



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wherein R_1 and R_2 may be the same or different and are selected from the group consisting of H, halo, alkyl, cycloalkyl, haloalkyl, aryl, haloaryl, alkylaryl, alkoxyaryl, hydroxyl, acetate, ketal, alkoxy, and combinations thereof; and R_3 is selected from the group consisting of O and

hydroxyl. In some embodiments, R_1 and R_2 may form a ring, as with an alkyl, alkenyl, substituted alkyl or substituted alkenyl bridge. Also encompassed are derivatives, metabolites, and prodrugs thereof.

[0013] Further provided are methods for preparing the compounds disclosed herein. Further provided are methods of treating, preventing or delaying the onset of a cancer in a subject in need of such treatment by administering a an effective amount of a compound of formula I, or a derivative, metabolite or prodrug thereof to a subject diagnosed with cancer or at risk of developing cancer. The method of treatment may be used in conjunction with another treatment method, such as radiation therapy.

Brief Description of the Drawings

[0014] Figure 1 shows four small molecules reported to restore function to p53.

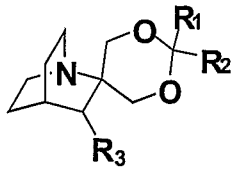
[0015] Figure 2 shows the effect of quinuclidinone derivatives on cell viability of H1299 cells in the presence and absence of 4Gy of gamma radiation. Each data point is an average of three independent experiments and expressed as M±SD. All experiments were carried with a 100 μM concentration of quinuclidinone analogs.

[0016] Figure 3 shows dose response curves of 8a and 8b in H1299 cells alone or in the presence of 4 Gy gamma radiation. Each data point is an average of three independent experiments and expressed as M±SD.

Detailed Description of the Invention

[0017] We have synthesized and tested novel quinuclidinone analogs to assay the effects on H1299 lung cancer cell lines alone or with gamma radiation. We have found two series of quinuclidinone analogs that act as anticancer agents. Of these, four interesting analogs significantly decreased cell viability in H1299 lung cancer cell lines. Two derivatives decreased cell proliferation in a dose-dependent fashion alone or in the presence of gamma radiation. Radiosensitization increased when derivative treatment preceded radiation treatment for both derivatives. These preliminary studies show an evidence for both additive and synergistic cytotoxicity for treatment of lung cancer by these novel quinuclidinone analogs.

[0018] Provided are new chemotherapeutic agents based on quinuclidinone analogs. One particular set of new chemotherapeutic agents is that of formula I:



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wherein R_1 and R_2 may be the same or different and are selected from the group consisting of H, halo, alkyl, cycloalkyl, haloalkyl, aryl, haloaryl, alkylaryl, alkoxyaryl, hydroxyl, acetate, ketal, alkoxy, and combinations thereof; and R_3 is selected from the group consisting of O and hydroxyl. In some embodiments, R_1 and R_2 may form a ring, as with an alkyl, alkenyl, substituted alkyl or substituted alkenyl bridge. Also encompassed are derivatives, metabolites, and prodrugs thereof.

[0019] In one particular embodiment, R_1 and R_2 are hydroxyl, and R_3 is a O. In another embodiment, R_1, R_2 and R_3 are all hydroxyl. In another embodiment, R_1 and R_2 are both alkyl. In another embodiment, R_1 is H, and R_2 is phenyl or substituted phenyl. The phenyl may be substituted with such groups as alkyl, alkoxyl, halo, and so forth. In some embodiments, either R_1 or R_2 or R_1 and R_2 are a phenyl connected by an alkyl group, such as $-(CH_2)_nPh$, wherein n = 1 to 4. In still other embodiments, R_1 and R_2 together may form a ring, wherein the ring may be an alkyl or aryl ring. When an alkyl ring, R_1 and R_2 are $-CH_2(CH_2)_nCH_2-$, wherein n = 1 to 5.

[0020] The compounds described herein are useful in the treatment of unwanted rapidly proliferating cells, such as cancer cells. The compounds preferably induce apoptosis in the unwanted rapidly dividing cells. The compounds may restore function to mutant p53 or may induce apoptosis by a p53-independent mechanism. The compounds described herein may also be used as part of a combination therapy, for example, a subject being treated with the compounds described herein may be simultaneously treated with radiation therapy to further decrease survival of unwanted cells.

[0021] Tumors are complex collections of cells that respond to therapies differently depending on the organ site and type of cells. Chemotherapeutic agents are developed through empirical screens to identify compounds or molecules that kill cancer cells. Most cytotoxic agents used to treat cancer act by inducing apoptosis.[1,2]

[0022] The combination of radiotherapy and chemotherapy is a unique and effective therapy. The role of radiotherapy is to control the primary tumor, while chemotherapy can be used to diminish distant metastases.[3,4] Some chemotherapeutic drugs destroy tumor cells directly by their own cytotoxic action but may also enhance the effects of radiotherapy. Many studies show the advantages of combining radiotherapy with chemotherapy, both in terms of better local control of cancer and metastasis prevention.[5-7]

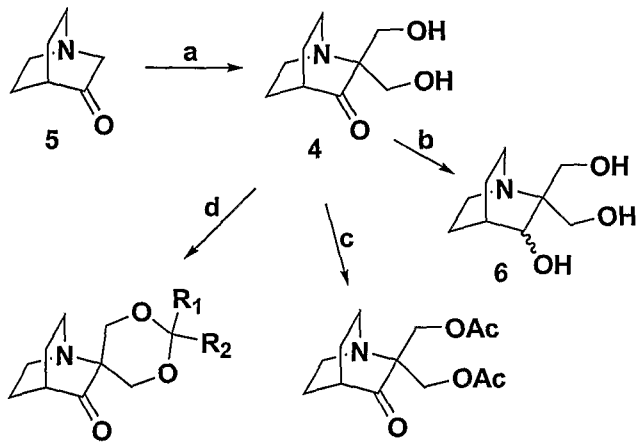
[0023] Given the toxicity, development of resistance, and lack of broad spectrum treatments, there is a continuing need for the development of new chemotherapeutic agents for the treatment of cancer. There has been an effort to develop small molecules that restore function to mutant p53, a tumor suppressor gene frequently mutated in a wide variety of cancers. Several examples of such small molecules have been reported. The pyrimidine derivative **1** is reported to both stabilize wild-type p53, as well as to restore function to mutant p53.[8-10] 2-Methoxyestradiol (**2**) facilitates wild-type p53-mediated apoptosis.[11] More recently, a series of α-methylene ketones (e.g. **3**) were identified as having good anti-proliferative activity against p53 mutant cell types.[12]

[0024] The quinuclidinone derivative **4** is also reported to induce apoptosis in a number of cell types.[13,14] This quinuclidinone also restores the active conformation of mutant p53. Original reports suggested **4** induced conformational change in p53. However, our study has indicated that this compound decreases cell proliferation and induces apoptosis in a p53-independent mechanism. The quinuclidinone **4** was identified through the screening of a library of low molecular weight molecules.[14] Consequently no structural activity studies have been reported for this intriguing molecule.

[0025] The quinuclidine ring system itself is a common structural element of a number of pharmacologically active small molecules, especially cholinergic ligands. In general, these ligands lack the 3-keto group seen in **4**.[15,16] This lack of a carbonyl group at C-3 has significant implications for the basicity of the tertiary amine. The pKa of the conjugate acid of quinuclidine is 11.4 while that of the conjugate acid of quinuclidinone is only 7.5.[17] These combined differences may account for the differences in activity between the two classes of compounds.

[0026] Given the need to develop new chemotherapeutic agents with an improved safety profile, analogs of molecules such as **4** should provide an excellent entry into novel anticancer agents. These molecules should have a selective mode of action, they are structurally unique and yet have a great deal of known chemistry upon which to prepare analogs.

[0027] We were particularly interested in molecule **4** as a starting point for the synthesis of novel anticancer agents. Given the simple structure of **4**, a large number of analogs could reasonably be proposed. In an initial series of compounds, we chose to ask the following questions: (1) is the diol necessary or could other functional groups suffice i.e. is a H-bond acceptor or H-bond donor (or both) necessary for activity, (2) is the carbonyl needed, and how might that influence the pKa of the amine.



Scheme 1. (a) 34% aq. HCHO, K_2CO_3, 50ºC, 45%. (b) $LiAlH_4$ (500 mol%), 0 ºC, 60%. (c) AcCl, DMAP, pyridine, 96%. (d) **8a**, R_1 = H, R_2 = Ph, PhCHO, *p*TSA, Δ, 23%; **8b**, R_1 = R_2 = CH_3, $(H_3C)_2C(OCH_3)_2$, pTSA, Δ, 33%; **8c**, R_1 = R_2 = $-CH_2(CH_2)_3CH_2-$, cyclohexanone, $BF_3{\bullet}OEt_2$, $HC(OEt)_3$, 20%; **8d**, R_1 = H, R_2 = cC_6H_{11}, $cC_6H_{11}CHO$, pTSA, Δ, 34%; **8e**, R_1 = H, R_2 = CH_2CH_2Ph, $PhCH_2CH_2CHO$, $BF_3{\bullet}OEt_2$, 31%; **8f**, R_1 = H, R_2 = 3,4-Cl_2Ph, 3,4-Cl_2PhCHO, $BF_3{\bullet}OEt_2$, 36%; **8g**, R_1 = H, R_2 = 4-(OMe)Ph, 4-(OMe)PhCHO, $BF_3{\bullet}OEt_2$, 21%.

[0028] The commercially available quinuclidinone **5** was converted to the diol **4** by two successive aldol condensations.[18] With key diol in hand, three modifications to this structure were carried out. The ketone was reduced to the triol **6**, using $LiAlH_4$. The diol was converted to the diacetate **7** in excellent yield using excess acetyl chloride. The diol was also converted to a series of acetals by condensations with the appropriate carbonyl compound. The yields for the acetal formation ranged from 20-40%. Significantly, both of the initial acetals prepared showed similar or improved activities relative to the parent compound **4**. We thus chose to prepare an additional set of acetals (**8c – 8f**) using the same methods.[19]

[0029] Cell viability was measured via the methyl tetrazolium (MTT) bromide mitochondrial activity assay described previously.[20] The synthesized compounds decreased cell survival and cell survival was further decreased if given in conjunction with gamma radiation (Figure 2).

[0030] As a control, we tested the starting quinuclidinone **5** in the MTT assay. The assay was performed as follows. Cell culture and drug treatment: H1299 cells that have a deletion of the p53 gene were derived from a human large cell lung carcinoma. Cells were maintained in Dulbecco's modified essential media (DMEM, Gibco) supplemented with 10% Fetal bovine serum (FBS), 100 Units/mL penicillin and 100 mg/mL streptomycin at 37°C in a 5% CO2 atmosphere. Cell proliferation (MTT assay): 4000-5000 cells/well in 100 mL of medium were seeded in a 96-well plate for 24 h prior to drug treatment. The media was then changed to media with derivative compounds and cells were treated with either gamma or UV radiation. At the end of incubation (24 h), 10 mL of 5 mg/mL MTT reagent (ATCC) was added to each well for 4 h. After incubation, 100 mL of detergent reagent was added to each well to dissolve the formazan crystals. The absorbance was determined at 570 nm. Assays were performed in triplicate and standard error determined. IC50 values were obtained by averaging values generated from non-linear regression analyses (Prism, GraphPad, San Diego, CA) of individual concentration-response curves.

[0031] As expected, this compound showed no activity. The initial lead compound **4** showed excellent activity with less than 10% of the cells remaining viable. Compound **6**, in which the carbonyl group has been reduced, showed complete loss of activity. There are two possibilities to account for this observation. The first is that the hydroxyl, while able to engage in similar types of non-covalent contacts, is no longer in an appropriate position. Alternatively, the lower basicity of the quinuclidinones (as compared to quinuclidine) is important for activity.

[0032] The diacetate **7** showed similar activity to the lead compound **4** indicating that the free hydroxyls were not necessary. As part of our study to determine the importance of the hydroxyls, we initially prepared two acetals (**8a**, $R_1 = H$, $R_2 = Ph$, and **8b**, $R_1 = R_2 = CH_3$). Both of these compounds showed improved activity relative to the parent diol **4**. Based upon these results we prepared five additional acetals as analogs of the benzylidine (**8a**) and acetonide (**8b**) derivatives. The acetal derived from cyclohexanone (**8c**) showed decreased activity relative to both the parent acetonide (**8b**) as well as the diol. This may indicate a lack of tolerance to steric bulk around the acetal ring. We prepared the acetal of cyclohexane carboxaldehyde (**8d**) in order to determine if the aromatic ring of **8a** was essential. We observed a lowering of activity relative to **8a**. Compound **8e** was prepared using 3-phenylpropionaldehyde. Our intention was to determine if the aromatic ring could be moved away from the acetal and yet still retain activity, clearly this compound is somewhat less potent than the benzylidine **8a**, but still as potent as the diol. Compounds **8f** and **8g** were prepared to explore the effects of substitution on

the aromatic ring. The 3,4-dichloro derivative lost some potency, but the 4-methoxy derivative lost almost all activity.

[0033] The compounds that showed the greatest effect on cell viability, **8a** and **8b**, were chosen for further analysis. Concentrations of **8a** and **8b**, ranging from 20-1000 μM, were prepared and dissolved in complete Dulbecco's modified essential media. H1299 cells treated with the compound alone, or in combination with 4 Gy of gamma radiation (IR) were assayed for cell survival. **8a** and **8b** decreased the percent of cell survival in a dose-dependent fashion both in the absence and presence of gamma radiation (Figure 3). The GI_{50} for all compounds were relatively similar. Compounds **8a** and **8b** alone had GI_{50}s of 17.26 μM and 19.21 μM. In the presence of gamma radiation, the GI_{50}s decreased with compound **8b**+4Gy being slightly more potent (4.74 μM) than **8a**+4Gy (4.49 μM). Similar results were obtained from dose response curves of the same concentrations of **8a** and **8b** in the presence of ultraviolet radiation (10 J/m^2) (data not shown).

[0034] Gamma irradiation (IR) is a local treatment, focusing on a specific area of the body, while chemotherapy is systemic, attacking cancer by treating the whole body. There continues to exist a need for new anticancer agents with improved safety profiles, specifically those that have a reduced liability for resistance and unwanted side effects. In this study we synthesized new acetals and di-acetates that were potent in decreasing cell viability. These analogs will enable us to examine important structural determinants of small molecule macromolecular interactions for anticancer activity. The discovery of a class of highly selective and potent quinuclidinone will lead to development of new anticancer drugs.

[0035] Table 1 shows the effect of the provided compounds (4 mM) on cell viability of H1299 null for p53 and transfected wild-type p53 cells, with and without gamma radiation:

Table 1

Compound	Cmpd Alone (H1229 cells)	Comp + 4 Gy gamma radiation (H1229 cells)	Cmpd Alone (H1229-WT p53 cells)	Comp + 4 Gy gamma radiation (H1229-WT p53 cells)
Control	100	67.8±3.4	100	75±3.3
5	76.7±1.5†	68.1±1.7	13.3±0.7	5±0.7
4	5.4±1.4	4.0±4.1	10.0±4.1	3.8±1.3
7	12.0±0.8	9.5±1.2	8.3±1.0	8.3±1.3
6	93.0±1.7	64.6±6.6	6.5±0.8	9.5±0.6
8a	4.6±0.2	3.2±1.2	10.5±0.8	9.6±3.3
8b	8.3±0.4	6.9±0.8	13.8±2.3	4.0±0.7
8c	13.0±5.8	3.7±0.5	7.5±1.6	7.5±0.7
8d	39.2±4.4	26.8±0.4	60.0±1.6	26.6±5.0
8e	7.6±2.3	7.1±0.5	28.3±3.0	28.2±5.0
8f	39.5±2.3	23.6±4.2	28.3±3.0	28.2±5.0
8g	92.2±1.5	74.1±3.3	90.9±6.3	84.4±5.0

†Percent cell viability normalized to untreated control.

[0036] By "treating" is meant curing, ameliorating or tempering the severity of the cancer or the symptoms associated therewith. The terms "treating," "treatment," and "therapy" as used herein refer to curative therapy, prophylactic therapy, and preventative therapy.

[0037] "Preventing" or "prevention" means preventing the occurrence of the cancer, or tempering the severity of the cancer if it is develops subsequent to the administration of the instant compositions. This preventing the onset of a clinically evident unwanted cell proliferation altogether or preventing the onset of a preclinically evident stage of unwanted rapid cell proliferation in individuals at risk. Also intended to be encompassed by this definition is the prevention of metastatis of malignant cells or to arrest or reverse the progression of malignant cells. This includes prophylactic treatment of those at risk of developing precancers and cancers.

[0038] The terms "therapeutically effective" and "pharmacologically effective" are intended to qualify the amount of each agent which will achieve the goal of improvement in disease severity and the frequency of incidence over treatment of each agent by itself, while avoiding adverse side effects typically associated with alternative therapies.

[0039] The term "subject" for purposes of treatment includes any human or animal subject having a neoplasia, such as cancer or precancer. For methods of prevention the subject is any human or animal subject, and preferably is a human subject who is at risk of developing a cancer. The subject may be at risk due to exposure to carcinogenic agents, being genetically predisposed to disorders characterized by unwanted, rapid cell proliferation, and so on. Besides

being useful for human treatment, the compounds of the present invention are also useful for veterinary treatment of mammals, including companion animals and farm animals, such as, but not limited to dogs, cats, horses, cows, sheep, and pigs. Preferably, subject means a human.

[0040] The term "derivative" is intended to encompass compounds which are structurally related to the quinuclidinone analogs described herein or which possess the substantially equivalent activity to the parent quinuclidinone analogs, as measured by the derivative's ability to inhibit cell viability as measured via the methyl tetrazolium (MTT) mitochondrial activity assay described herein. By way of example, such compounds may include, but are not limited to, esters and prodrugs thereof. Such compounds may be formed *in vivo*, such as by metabolic mechanisms.

[0041] Where the term alkyl is used, either alone or with other terms, such as haloalkyl or alkylaryl, it includes C_1 to C_{10} linear or branched alkyl radicals, examples include methyl, ethyl, propyl, isopropyl, butyl, *tert*-butyl, and so forth. The term "haloalkyl" includes C_1 to C_{10} linear or branched alkyl radicals substituted with one or more halo radicals. Some examples of haloalkyl radicals include trifluoromethyl, 1,2-dichloroethyl, 3-bromopropyl, and so forth. The term "halo" includes radicals selected from F, Cl, Br, and I.

[0042] The term aryl, used alone or in combination with other terms such as alkylaryl, haloaryl, or haloalkylaryl, includes such aromatic radicals as phenyl, biphenyl, and benzyl, as well as fused aryl radicals such as naphthyl, anthryl, phenanthrenyl, fluorenyl, and indenyl on so forth. The term "aryl" also encompasses "heteroaryls," which are aryls that have carbon and one or more heteroatoms, such as O, N, or S in the aromatic ring. Examples of heteroaryls include indolyl, pyrrolyl, and so on. "Alkylaryl" or "arylalkyl" refers to alkyl-substituted aryl groups such as butylphenyl, propylphenyl, ethylphenyl, methylphenyl, 3,5-dimethylphenyl, *tert*-butylphenyl and so forth.

[0043] The agents of the present invention may be administered orally, intravenously, intranasally, rectally, or by any means which delivers an effective amount of the active agent to the tissue or site to be treated. It will be appreciated that different dosages may be required for treating different disorders. An effective amount of an agent is that amount which causes a statistically significant decrease in neoplastic cell count, growth, or size. Neoplastic disorders responsive to the agents of the present invention include, but are not limited to, breast cancer.

[0044] The dosage form and amount can be readily established by reference to known treatment or prophylactic regiments. The amount of therapeutically active compound that is administered

and the dosage regimen for treating a disease condition with the compounds and/or compositions of this invention depends on a variety of factors, including the age, weight, sex, and medical condition of the subject, the severity of the disease, the route and frequency of administration, the particular compound employed, the location of the unwanted proliferating cells, as well as the pharmacokinetic properties of the individual treated, and thus may vary widely. The dosage will generally be lower if the compounds are administered locally rather than systemically, and for prevention rather than for treatment. Such treatments may be administered as often as necessary and for the period of time judged necessary by the treating physician. One of skill in the art will appreciate that the dosage regime or therapeutically effective amount of the inhibitor to be administrated may need to be optimized for each individual. The pharmaceutical compositions may contain active ingredient in the range of about 0.1 to 2000 mg, preferably in the range of about 0.5 to 500 mg and most preferably between about 1 and 200 mg. A daily dose of about 0.01 to 100 mg/kg body weight, preferably between about 0.1 and about 50 mg/kg body weight, may be appropriate. The daily dose can be administered in one to four doses per day.

[0045] The active agents may be administered along with a pharmaceutical carrier and/or diluent. The agents of the present invention may also be administered in combination with other agents, for example, in association with other chemotherapeutic or immunostimulating drugs or therapeutic agents. Examples of pharmaceutical carriers or diluents useful in the present invention include any physiological buffered medium, *i.e.*, about pH 7.0 to 7.4 comprising a suitable water soluble organic carrier. Suitable water soluble organic carriers include, but are not limited to corn oil, dimethylsulfoxide, gelatin capsules, etc.

[0046] Also included in the family of quinuclidinone analogs are the pharmaceutically acceptable salts thereof. The phrase "pharmaceutically acceptable salts" connotes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Also included in the family of heteroaryl-containing isoflavone compounds are esters thereof. Esters of the heteroaryl-containing isoflavone compounds may be prepared by conventional methods known to those skilled in the art.

[0047] Suitable pharmaceutically acceptable acid addition salts of quinuclidinone analogs may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric, and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic,

heterocyclic, carboxylic, and sulfonic classes of organic acids, examples of which include formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucoronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, ambonic, pamoic, methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, algenic, β-hydroxybutyric, galactaric, and galacturonic acids.

[0048] Suitable pharmaceutically acceptable base addition salts of quinuclidinone analogs include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium, and zinc. Alternatively, organic salts made from N,N′-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procaine may be used form base addition salts of the heteroaryl-containing isoflavone compounds. All of these salts may be prepared by conventional means from the corresponding heteroaryl-containing isoflavone compounds by reacting, for example, the appropriate acid or base with the heteroaryl-containing isoflavone compounds.

[0049] The phrase "adjunct therapy" (or "combination therapy"), in defining use of one or more quinuclidinone analogs of the present invention and one or more other pharmaceutical agent, is intended to embrace administration of each agent in a sequential manner in a regimen that will provide beneficial effects of the drug combination, and is intended as well to embrace co-administration of these agents in a substantially simultaneous manner, such as in a single formulation having a fixed ratio of these active agents, or in multiple, separate formulations for each agent.

[0050] There are large numbers of antineoplastic agents available in commercial use, in clinical evaluation and in pre-clinical development, which could be selected for treatment of cancers or other neoplasias by combination drug chemotherapy. Such antineoplastic agents fall into several major categories, namely, antibiotic-type agents, alkylating agents, antimetabolite agents, hormonal agents, immunological agents, interferon-type agents and a category of miscellaneous agents. Alternatively, other anti-neoplastic agents, such as metallomatrix proteases inhibitors may be used. Suitable agents which may be used in combination therapy will be recognized by those of skill in the art.

[0051] For oral administration, the pharmaceutical composition may be in the form of, for example, a tablet, capsule, suspension or liquid. The pharmaceutical composition is preferably made in the form of a dosage unit containing a particular amount of the active ingredient.

Examples of such dosage units are capsules, tablets, powders, granules or a suspension, with conventional additives such as lactose, mannitol, corn starch or potato starch; with binders such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators such as corn starch, potato starch or sodium carboxymethyl-cellulose; and with lubricants such as talc or magnesium stearate. The active ingredient may also be administered by injection as a composition wherein, for example, saline, dextrose or water may be used as a suitable carrier.

[0052] For intravenous, intramuscular, subcutaneous, or intraperitoneal administration, the compound may be combined with a sterile aqueous solution which is preferably isotonic with the blood of the recipient. Such formulations may be prepared by dissolving solid active ingredient in water containing physiologically compatible substances such as sodium chloride, glycine, and the like, and having a buffered pH compatible with physiological conditions to produce an aqueous solution, and rendering said solution sterile. The formulations may be present in unit or multi-dose containers such as sealed ampoules or vials.

[0053] All documents referenced herein are incorporated by reference.

[0054] Although this invention has been described with respect to specific embodiments, the details of these embodiments are not to be construed as limitations.

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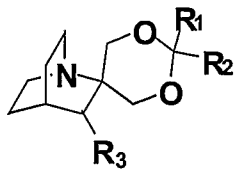
The invention claimed is:

1. A compound of formula I:



I

wherein R_1 and R_2 are the same or different and are selected from the group consisting of H, halo, alkyl, cycloalkyl, haloalkyl, aryl, haloaryl, alkylaryl, alkoxyaryl, hydroxyl, acetate, ketal, alkoxy, and combinations thereof; and
wherein R_1 and R_2 may form a ring,
wherein R_3 is selected from the group consisting of O and hydroxyl,
and derivatives, metabolites, and prodrugs thereof.

2. The compound of claim 1 wherein R_1 and R_2 are the same or different and are selected from the group consisting of H, C_1-C_4 alkyl, cycloalkyl, phenyl, phenylmethyl, phenylethyl, phenylpropyl, phenylbutyl, halophenyl, dihalophenyl, methoxyphenyl, ethoxyphenyl, propoxyphenyl, butoxyphenyl, hydroxyl, and combinations thereof; and derivatives, metabolites, and prodrugs thereof.

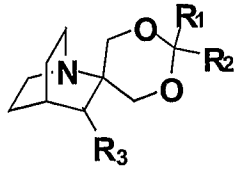
3. The compound of claim 2 wherein R_1 and R_2 are selected from the group consisting of H, methyl, cyclohexyl, phenyl, phenylethyl, dichlorophenyl, methoxylphenyl, and combinations thereof; and derivatives, metabolites, and prodrugs thereof.

4. The compound of claim 3 wherein R_1 is H and R_2 is phenyl, and derivatives, metabolites, and prodrugs thereof.

5. The compound of claim 3 wherein R_1 and R_2 are methyl, and derivatives, metabolites, and prodrugs thereof.

6. The compound of claim 1 wherein R_1 and R_2 form a ring, the ring selected from the group consisting of alkyl, alkenyl, aryl, substituted alkyl, substituted alkenyl, substituted aryl, heteroalkyl, hetertoalkenyl, and heteroaryl, and derivatives, metabolites, and prodrugs thereof.

7. A method of treating, preventing or delaying the onset of a cancer in a subject in need of such treatment, the method comprising administering a therapeutically effective amount of a compound of formula I



I

wherein R_1 and R_2 are the same or different and are selected from the group consisting of H, halo, alkyl, cycloalkyl, haloalkyl, aryl, haloaryl, alkylaryl, alkoxyaryl, hydroxyl, acetate, ketal, alkoxy, and combinations thereof; and

wherein R_1 and R_2 may form a ring,

wherein R_3 is selected from the group consisting of O and hydroxyl,

and derivatives, metabolites, and prodrugs thereof,

to a subject in need of such treatment.

8. The method of claim 7 wherein R_1 and R_2 are the same or different and are selected from the group consisting of H, C_1-C_4 alkyl, cycloalkyl, phenyl, phenylmethyl, phenylethyl, phenylpropyl, phenylbutyl, halophenyl, dihalophenyl, methoxyphenyl, ethoxyphenyl, propoxyphenyl, butoxyphenyl, hydroxyl, and combinations thereof

and derivatives, metabolites, and prodrugs thereof.

9. The method of claim 8 wherein R_1 and R_2 are selected from the group consisting of H, methyl, cyclohexyl, phenyl, phenylethyl, dichlorophenyl, methoxylphenyl, and combinations thereof; and derivatives, metabolites, and prodrugs thereof.

10. The method of claim 7 wherein R_1 and R_2 form a ring, the ring selected from the group consisting of alkyl, alkenyl, aryl, substituted alkyl, substituted alkenyl, substituted aryl, heteroalkyl, hetertoalkenyl, and heteroaryl; and derivatives, metabolites, and prodrugs thereof.

11. The method of claim 7 wherein the subject is a human subject.

12. The method of claim 11 wherein the compound of formula I is administered to the subject as part of a combination therapy with radiation therapy.

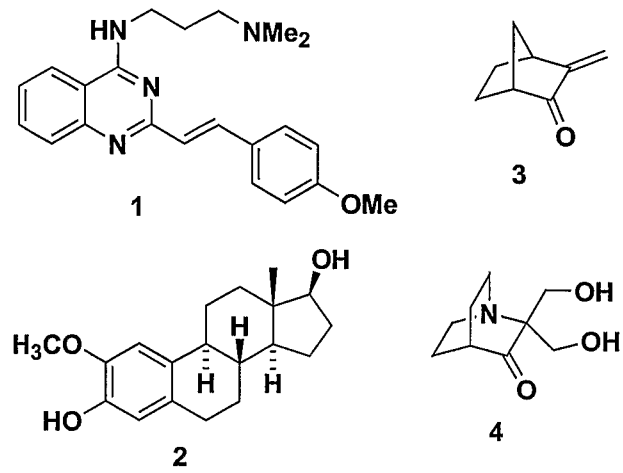


Figure 1

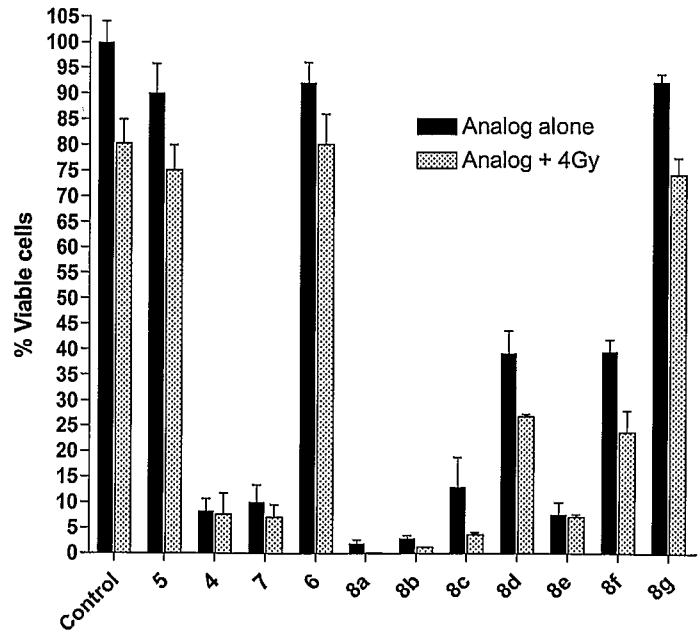


Figure 2

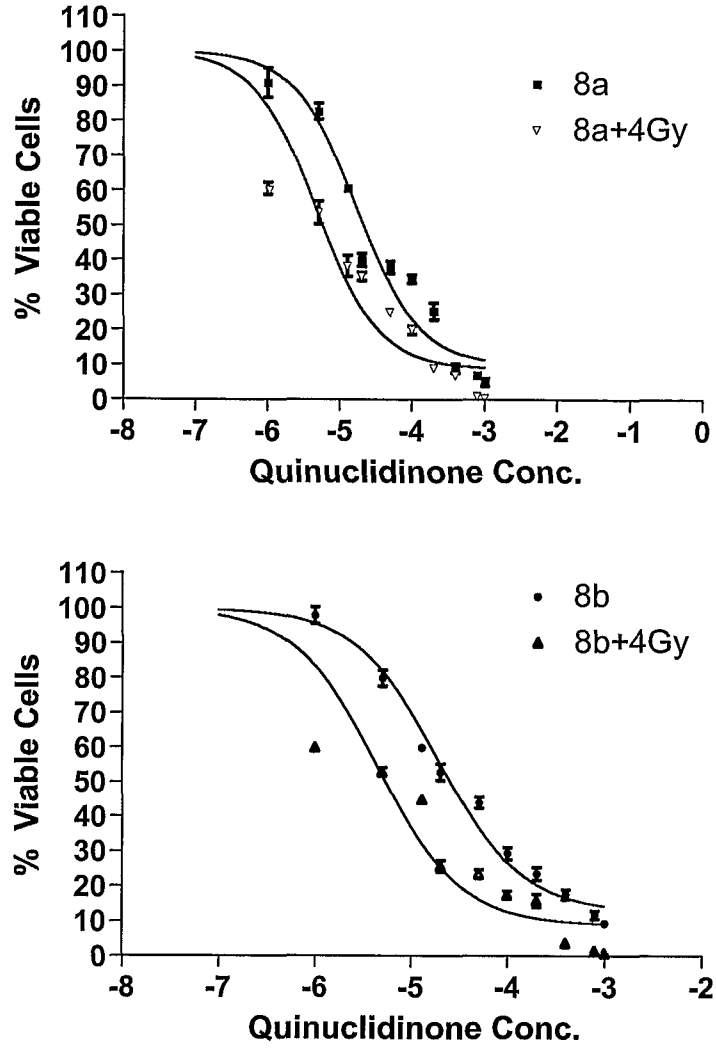

% Viable Cells
110
100
90
80
70
60
50
40
30
20
10
0
-8
-7
-6
-5
-4
-3
-2
-1
0
Quinuclidinone Conc.
8a
8a+4Gy
% Viable Cells
110
100
90
80
70
60
50
40
30
20
10
0
-8
-7
-6
-5
-4
-3
-2
Quinuclidinone Conc.
8b
8b+4Gy

Figure 3