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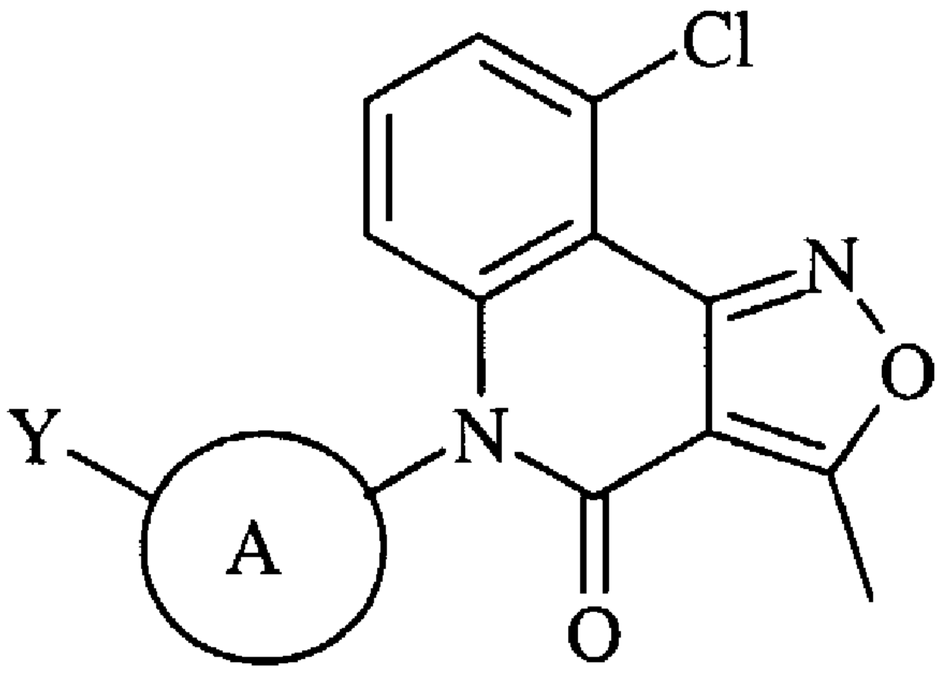

(I)

(57) Abrégé/Abstract:
The present invention relates to a compounds of formula I, wherein A is olefin, diol, or acetonide; which are useful for inhibiting resistant neoplasms where the resistance is conferred in part or in total by MRP1.

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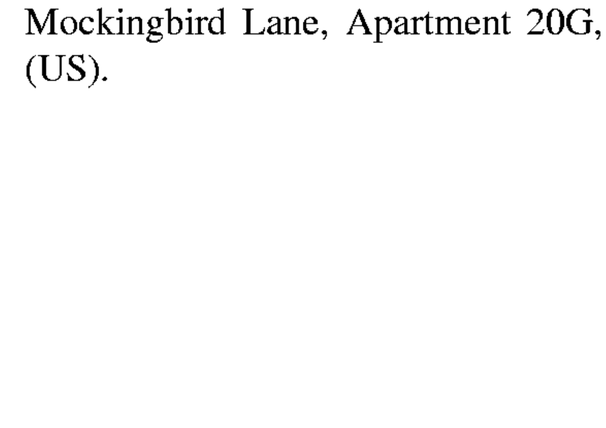


(I)

(57) Abstract: The present invention relates to a compounds of formula I, wherein A is olefin, diol, or acetonide; which are useful for inhibiting resistant neoplasms where the resistance is conferred in part or in total by MRP1.

TRICYCLIC COMPOUNDS AS MRP1-INHIBITORS

Along with surgery and radiotherapy, chemotherapy continues to be an effective therapy for many cancers. In fact, several types of cancer are now considered to be curable by chemotherapy and include Hodgkin's disease, large cell lymphoma, acute lymphocytic leukemia, testicular cancer and early stage breast cancer. Other cancers such as ovarian cancer, small cell lung and advanced breast cancer, while not yet curable, are exhibiting positive response to combination chemotherapy.

One of the most important unsolved problems in cancer treatment is drug resistance. After selection for resistance to a single cytotoxic drug, cells may become cross resistant to a whole range of drugs with different structures and cellular targets, *e.g.*, alkylating agents, antimetabolites, hormones, platinum-containing drugs, and natural products. This phenomenon is known as multidrug resistance (MDR). In some types of cells, this resistance is inherent, while in others, such as small cell lung cancer, it is usually acquired.

Such resistance is known to be multifactorial and is conferred by at least two proteins: the 170 kDa P-glycoprotein (MDR1) and the more recently identified 190 kDa multidrug resistance protein (MRP1). Although both MDR1 and MRP1 belong to the ATP-binding cassette superfamily of transport proteins, they are structurally very different molecules and share less than 15% amino acid homology. Despite the structural divergence between the two proteins, by 1994 there were no known consistent differences in the resistance patterns of MDR1 and MRP1 cell lines. However, the association, or lack thereof, of MRP1 and resistance to particular oncolytics is known. See Cole, *et. al.*, "Pharmacological Characterization of Multidrug Resistant MRP-transfected Human Tumor Cells", *Cancer Research*, 54:5902-5910, 1994. Doxorubicin, daunorubicin, epirubicin, vincristine, and etoposide are substrates of MRP1, *i.e.*, MRP1 can bind to these oncolytics and redistribute them away from their site of action, the nucleus, and out of the cell. *Id.* and Marquardt, D., and Center, M.S., *Cancer Research*, 52:3157, 1992.

Doxorubicin, daunorubicin, and epirubicin are members of the anthracycline class of oncolytics. They are isolates of various strains of *Streptomyces* and act by inhibiting nucleic acid synthesis. These agents are useful in treating neoplasms of the bone, ovaries,

bladder, thyroid, and especially the breast. They are also useful in the treatment of acute lymphoblastic and myeloblastic leukemia, Wilm's tumor, neuroblastoma, soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphomas, and bronchogenic carcinoma.

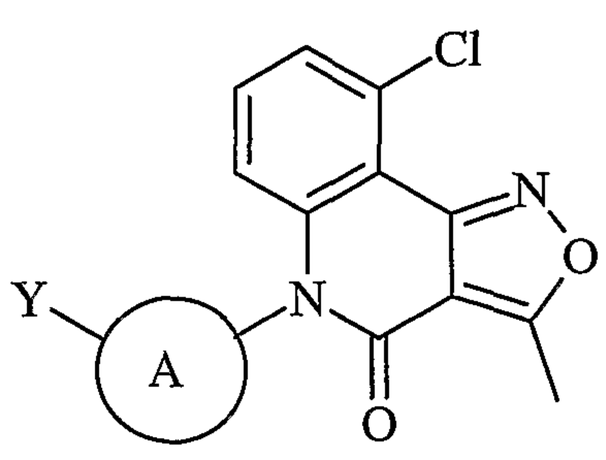
Vincristine, a member of the vinca alkaloid class of oncolytics, is an isolate of a common flowering herb, the periwinkle plant (*Vinca rosea* Linn). The mechanism of action of vincristine is still under investigation but has been related to the inhibition of microtubule formation in the mitotic spindle. Vincristine is useful in the treatment of acute leukemia, Hodgkin's disease, non-Hodgkin's malignant lymphomas, rhabdomyosarcoma, neuroblastoma, and Wilm's tumor.

Etoposide, a member of the epipodophyllotoxin class of oncolytics, is a semisynthetic derivative of podophyllotoxin. Etoposide acts as a topoisomerase inhibitor and is useful in the therapy of neoplasms of the testis, and lung.

Additionally, PCT publications WO99/51236, WO99/51228, and WO99/51227 disclose certain compounds known to be inhibitors of MRP1.

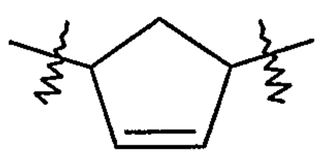
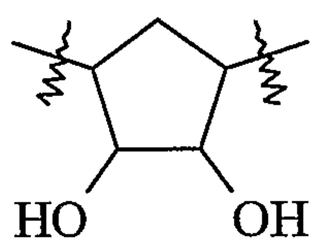
It is presently unknown what determines whether a cell line will acquire resistance via a MDR1 or MRP1 mechanism. Due to the tissue specificity of these transporters and/or in the case where one mechanism predominates or is exclusive, it would be useful to have a selective inhibitor of that one over the other. Furthermore, when administering a drug or drugs that are substrates of either protein, it would be particularly advantageous to coadminister an agent that is a selective inhibitor of that protein. It is, therefore, desirable to provide compounds, which are selective inhibitors of MDR1 or MRP1.

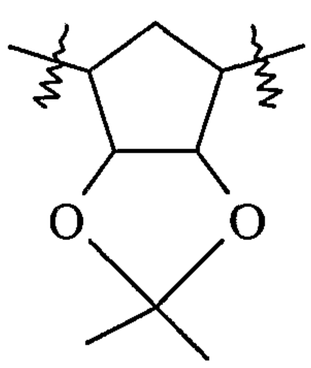
The present invention relates to a compound of formula I:



I

where:

A is  (olefin),  (diol), or

 (acetonide);

Y is –E-C(O)R^1 or -E-NR^2R^3;

E is a bond or -CH_2-;

R^1 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted heterocycle, or NR^2R^4;

R^2 is independently at each occurrence hydrogen, C_1-C_6 alkyl, (C_1-C_6 alkyl)-aryl, or aryl;

R^3 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted heterocycle, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, C(O)C(O)R^9, C(O)R^5, or R^2 and R^3, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^4 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or R^2 and R^4,

together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^5 is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, optionally substituted (C_1-C_4 alkyl)-phenoxy, $(CH_2)_tC(R^6)(R^7)N(R^6)(R^8)$, or NR^2R^4;

t is 0, 1, 2, 3, or 4;

R^6 is independently at each occurrence hydrogen or C_1-C_6 alkyl;

R^7 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^8 is independently at each occurrence hydrogen, C_1-C_6 alkyl, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, $C(O)OR^9$, $C(O)R^{10}$, or R^6 and R^8, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^9 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^{10} is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally

substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or optionally substituted (C_1-C_4 alkyl)-phenoxy; or a pharmaceutical salt thereof.

The present invention further relates to a method of inhibiting MRP1 in a mammal which comprises administering to a mammal in need thereof an effective amount of a compound of formula I, or a pharmaceutical salt or solvate thereof.

In another embodiment, the present invention relates to a method of inhibiting a resistant neoplasm, or a neoplasm susceptible to resistance in a mammal which comprises administering to a mammal in need thereof an effective amount of a compound of formula I, or a pharmaceutical salt or solvate thereof, in combination with an effective amount of an oncolytic agent.

The present invention also relates to a pharmaceutical formulation comprising a compound of formula I, or a pharmaceutical salt or solvate thereof, in combination with one or more oncolytics, pharmaceutical carriers, diluents, or excipients therefor.

The present invention relates to a product containing a compound of formula I and one or more oncolytic agents as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

In another embodiment, the present invention relates to a use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for inhibiting MRP1.

The current invention concerns the discovery that a select group of compounds, those of formula I, are selective inhibitors of multidrug resistant protein (MRP1) and are thus useful in treating MRP1 conferred multidrug resistance (MDR) in a resistant neoplasm and a neoplasm susceptible to resistance.

The terms “inhibit” as it relates to MRP1 and “inhibiting MRP1” refer to prohibiting, alleviating, ameliorating, halting, restraining, slowing or reversing the progression of, or reducing MRP1’s ability to redistribute an oncolytic away from the oncolytic’s site of action, most often the neoplasm’s nucleus, and out of the cell.

As used herein, the term "effective amount of a compound of formula I" refers to an amount of a compound of the present invention which is capable of inhibiting MRP1. The term “effective amount of an oncolytic” refers to an amount of oncolytic capable of inhibiting a neoplasm, resistant or otherwise.

The term "inhibiting a resistant neoplasm, or a neoplasm susceptible to resistance" refers to prohibiting, halting, restraining, slowing or reversing the progression of, reducing the growth of, or killing resistant neoplasms and/or neoplasms susceptible to resistance.

The term "resistant neoplasm" refers to a neoplasm, which is resistant to chemotherapy where that resistance is conferred in part, or in total, by MRP1. Such neoplasms include, but are not limited to, neoplasms of the bladder, bone, breast, lung(small-cell), testis, and thyroid and also includes more particular types of cancer such as, but not limited to, acute lymphoblastic and myeloblastic leukemia, Wilm's tumor, neuroblastoma, soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphomas, and bronchogenic carcinoma.

A neoplasm, which is "susceptible to resistance", is a neoplasm where resistance is not inherent nor currently present but can be conferred by MRP1 after chemotherapy begins. Thus, the methods of this invention encompass a prophylactic and therapeutic administration of a compound of formula I.

The term "chemotherapy" refers to the use of one or more oncolytics where at least one oncolytic is a substrate of MRP1. A "substrate of MRP1" is an oncolytic that binds to MRP1 and is redistributed away from the oncolytics site of action, (the neoplasm's nucleus) and out of the cell, thus, rendering the therapy less effective.

The terms "treat" or "treating" bear their usual meaning which includes preventing, prohibiting, alleviating, ameliorating, halting, restraining, slowing or reversing the progression, or reducing the severity of MRP1 derived drug resistance in a multidrug resistant tumor.

In the general formulae of the present document, the general chemical terms have their usual meanings. For example, the term "C_1-C_4 alkyl" refers to methyl, ethyl, propyl, isopropyl, cyclopropyl, butyl, cyclobutyl, s-butyl, and t-butyl. The term "C_1-C_6 alkyl" refers to a monovalent, straight, branched, or cyclic saturated hydrocarbon containing from 1 to 6 carbon atoms and includes C_1-C_4 alkyl groups. In addition, C_1-C_6 alkyl also includes, but is not limited to, cyclopentyl, pentyl, hexyl, cyclohexyl, and the like. The term "C_3-C_8 cycloalkyl" refers to cyclopropyl, cyclobutyl, cyclopentyl,

cyclohexyl, cycloheptyl, and cyclooctyl. The term "C_6-C_{10} bicycloalkyl" refers to bicyclo- [2.1.1]hexanyl, [2.2.1]heptanyl, [3.2.1]octanyl, [2.2.2]octanyl, [3.2.2]nonanyl, [3.3.1]nonanyl, [3.3.2]decanyl, and [4.3.1]decanyl ring system where the ring is connected to the parent molecular moiety at any point available for substitution on the ring.

The terms "C_1-C_4 alkoxy" and "C_1-C_6 alkoxy" refer to moieties of the formula O-(C_1-C_4 alkyl) and O-(C_1-C_6 alkyl) respectively.

The term "optionally substituted C_3-C_8 cycloalkyl" refers to a C_3-C_8 cycloalkyl optionally substituted once with a phenyl, substituted phenyl, hydroxy, or C_1- C_4 alkoxy.

The term "halo" or "halide" refers to fluoro, chloro, bromo, and iodo.

The term "aryl" refers to phenyl, benzyl, and naphthyl.

The term "optionally substituted (C_1-C_4 alkyl)-phenoxy" refers to unsubstituted or substituted phenoxy linked through an optionally substituted C_1-C_4 alkyl.

The terms "optionally substituted C_1-C_4 alkyl" and "optionally substituted C_1-C_6 alkyl" refers to a C_1-C_4 alkyl or C_1-C_6 alkyl, respectively, unsubstituted or substituted from 1 to 3 times with halo, C_1-C_4 alkanol, NH_2, or hydroxy.

The term "optionally substituted (C_1-C_4 alkyl)-heterocycle" refers to optionally substituted heterocycle linked through an optionally substituted C_1-C_4 alkyl.

The term "N-heterocycle" refers to a nitrogen containing heterocycle linked through a nitrogen atom.

The term "optionally substituted (C_1-C_4 alkyl)-aryl" refers to optionally substituted aryl linked through an optionally substituted C_1-C_4 alkyl.

The term "optionally substituted (C_1-C_4 alkyl)-CO-aryl" refers to an optionally substituted aryl linked through a carbonyl and an optionally substituted C_1-C_4 alkyl.

The terms "optionally substituted aryl" refers to an aryl group optionally substituted from 1 to 5 times independently with C_1-C_6 alkyl, halo, hydroxy, trifluoromethyl, C_1-C_6 alkoxy, benzyloxy, or trifluoromethoxy.

The terms "optionally substituted phenyl" refers to a phenyl group optionally substituted from 1 to 5 times independently with C_1-C_6 alkyl, halo, hydroxy, trifluoromethyl, C_1-C_6 alkoxy, benzyloxy, or trifluoromethoxy.

The terms "optionally substituted C_6-C_{10} bicycloalkyl" refers to a C_6-C_{10} bicycloalkyl group optionally substituted from 1 to 5 times independently with C_1-C_6 alkyl, halo, hydroxy, trifluoromethyl, C_1-C_6 alkoxy, benzyloxy, or trifluoromethoxy.

The term "heterocycle" is taken to mean stable aromatic and non-aromatic 5- and 6-membered rings containing from 1 to 3 heteroatoms selected from the group consisting of nitrogen, oxygen and sulfur, said rings being optionally benzofused. Non-aromatic rings include, for example, pyrrolidinyl, piperidinyl, piperazinyl, tetrahydrofuryl, oxazolidinyl, dioxanyl, pyranyl, and the like. Benzofused non-aromatic rings include indolinyl, 1,2,3,4-tetrahydroquinolinyl, 1,2,3,4-tetrahydroisoquinolinyl and the like. Aromatic rings include furyl, thienyl, pyridinyl, pyrrolyl, N-methylpyrrolyl, oxazolyl, isoxazolyl, pyrazolyl, imidazolyl, triazolyl, oxadiazolyl, thiadiazolyl, thiazolyl, pyrimidinyl, pyrazinyl, pyridazinyl, and the like. Benzofused aromatic rings include isoquinolinyl, benzoxazolyl, benzthiazolyl, quinolinyl, benzofuranyl, thionaphthyl, indolyl and the like.

The term "optionally substituted heterocycle" refers to a heterocycle ring optionally substituted 1 or 2 times independently with a C_1-C_6 alkyl, halo, benzyl, phenyl, trifluoromethyl, or an oxo group.

The term "optionally substituted N-heterocycle" refers to a heterocycle ring, linked through the nitrogen atom, optionally substituted 1 or 2 times independently with a C_1-C_6 alkyl, halo, benzyl, phenyl, trifluoromethyl, or an oxo group.

The term "protecting group" refers to an amino protecting group or a hydroxy protecting group. The species of protecting group will be evident from whether the "Pg" group is attached to a nitrogen atom (amino protecting group) or attached to an oxygen atom (hydroxy protecting group).

The term "amino protecting group" as used in this specification refers to a substituent(s) of the amino group commonly employed to block or protect the amino functionality while reacting other functional groups on the compound. Examples of such amino-protecting groups include the formyl group, the trityl group, the phthalimido

group, the acetyl group, the trichloroacetyl group, the chloroacetyl, bromoacetyl, and iodoacetyl groups, urethane-type blocking groups such as benzyloxycarbonyl, 9-fluorenylmethoxycarbonyl ("FMOC"), and the like; and like amino protecting groups. The species of amino protecting group employed is not critical so long as the derivatized amino group is stable to the condition of subsequent reaction(s) on other positions of the molecule and can be removed at the appropriate point without disrupting the remainder of the molecule. Similar amino protecting groups used in the cephalosporin, penicillin, and peptide arts are also embraced by the above terms. Further examples of groups referred to by the above terms are described by T.W. Greene, "Protective Groups in Organic Synthesis", John Wiley and Sons, New York, N.Y., 1991, Chapter 7 hereafter referred to as "Greene". A preferred amino protecting group is t-butyloxycarbonyl.

The term "hydroxy protecting group" denotes a group understood by one skilled in the organic chemical arts of the type described in Chapter 2 of Greene. Representative hydroxy protecting groups include, for example, ether groups including methyl and substituted methyl ether groups such as methyl ether, methoxymethyl ether, methylthiomethyl ether, *tert*-buylthiomethyl ether, (phenyldimethylsilyl)methoxy-methyl ether, benzyloxymethyl ether, *p*-methoxybenzyloxy-methyl ether, and *tert*-butoxymethyl ether; substituted ethyl ether groups such as ethoxyethyl ether, 1-(2-chloroethoxy)-ethyl ether, 2,2,2-trichloroethoxymethyl ether, and 2-(trimethylsilyl)ethyl ether; isopropyl ether groups; phenyl and substituted phenyl ether groups such as phenyl ether, *p*-chlorophenyl ether, *p*-methoxyphenyl ether, and 2,4-dinitrophenyl ether; benzyl and substituted benzyl ether groups such as benzyl ether, *p*-methoxybenzyl ether, *o*-nitrobenzyl ether, and 2,6-dichlorobenzyl ether; and alkylsilyl ether groups such as trimethyl- triethyl- and triisopropylsilyl ethers, mixed alkylsilyl ether groups such as dimethylisopropylsilyl ether, and diethylisopropylsilyl ether; and ester protecting groups such as formate ester, benzylformate ester, mono- di- and trichloroacetate esters, phenoxyacetate ester, and *p*-chlorophenoxyacetate and the like. The species of hydroxy protecting group employed is not critical so long as the derivatized hydroxy group is stable to the conditions of subsequent reaction(s) on other positions of the intermediate molecule and can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other hydroxy protecting group(s).

The term "carbonyl activating group" refers to a substituent of a carbonyl that renders that carbonyl prone to nucleophilic addition. Suitable activating groups are those that have a net electron withdrawing effect on the carbonyl. Such groups include, but are not limited to, alkoxy, aryloxy, nitrogen containing aromatic heterocycles, or amino groups such as oxybenzotriazole, imidazolyl, nitrophenoxy, pentachlorophenoxy, N-oxysuccinimide, N,N'-dicyclohexylisoure-O-yl, N-hydroxy-N-methoxyamino, and the like; acetates, formates, sulfonates such as methanesulfonate, ethanesulfonate, benzenesulfonate, or p-toluenylsulfonate, and the like; and halides especially chloride, bromide, or iodide.

In general, the term "pharmaceutical" when used as an adjective means substantially non-toxic to living organisms. For example, the term "pharmaceutical salt" as used herein, refers to salts of the compounds of formula I which are substantially non-toxic to living organisms. See, *e.g.*, Berge, S.M, Bighley, L.D., and Monkhouse, D.C., "Pharmaceutical Salts", *J. Pharm. Sci.*, 66:1, 1977. Typical pharmaceutical salts include those salts prepared by reaction of the compounds of formula I with an inorganic or organic acid or base. Such salts are known as acid addition or base addition salts respectively. These pharmaceutical salts frequently have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Examples of pharmaceutical acid addition salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, γ-hydroxybutyrate, glycollate, tartrate, methanesulfonate, ethanesulfonate, propanesulfonate, naphthalene-1-sulfonate, napththalene-2-sulfonate, mandelate, and the like of a compound of formula I.

Examples of pharmaceutical base addition salts are the ammonium, lithium, potassium, sodium, calcium, magnesium, methylamino, diethylamino, ethylene diamino, cyclohexylamino, and ethanolamino salts, and the like of a compound of formula I.

The term "solvate" represents an aggregate that comprises one or more molecules of the solute, such as a formula I compound, with one or more molecules of solvent. The compounds of the present invention may, depending upon their structure and manner of synthesis and isolation, exist as a pharmaceutically acceptable solvate. These solvates include water, methanol, and ethanol. Solvated forms of the compounds of the present invention represent a further embodiment of the present invention.

The term "suitable solvent" refers to a solvent that is inert to the ongoing reaction and sufficiently solubilizes the reactants to effect the desired reaction. Examples of suitable solvents include but are not limited to, dichloromethane, chloroform, 1,2-dichloroethane, diethyl ether, acetonitrile, ethyl acetate, 1,3-dimethyl-2-imidazolidinone, tetrahydrofuran, dimethylformamide, toluene, chlorobenzene, dimethylsulfoxide, mixtures thereof, and the like.

The term "carbonyl activating reagent" refers to a reagent that converts the carbonyl of a carboxylic acid group to one that is more prone to nucleophilic addition and includes, but is not limited to, such reagents as those found in "The Peptides", Gross and Meienhofer, Eds., Academic Press (1979), Ch. 2 and M. Bodanszky, "Principles of Peptide Synthesis", 2nd Ed., Springer-Verlag Berlin Heidelberg, 1993, hereafter referred to as "The Peptides" and "Peptide Synthesis" respectively. Specifically, carbonyl activating reagents include nucleophilic sources of a halogen such as, thionyl bromide, thionyl chloride, oxalyl chloride, and the like; alcohols such as nitrophenol, pentachlorophenol, and the like; amines such as N-hydroxy-N-methoxyamine and the like; acid halides such as acetic, formic, methanesulfonic, ethanesulfonic, benzenesulfonic, or p-tolylsulfonic acid halide, and the like; and compounds such as 1,1'-carbonyldiimidazole, benzotriazole, imidazole, N-hydroxysuccinimide, dicyclohexylcarbodiimide, and the like.

The term "suitable thermodynamic base" refers to a base which acts as a proton trap for any protons which may be produced as a byproduct of the desired reaction or to a base which provides a reversible deprotonation of an acidic substrate and is reactive

enough to effect the desired reaction without significantly effecting any undesired reactions. Examples of thermodynamic bases include, but are not limited to, carbonates, bicarbonates, and hydroxides (*e.g.*, lithium, sodium, or potassium carbonate, bicarbonate, or hydroxide), tri-(C_1-C_4 alkyl)amines, or aromatic nitrogen containing heterocycles (*e.g.*, pyridine).

The term "hydroxy protecting group" denotes a group understood by one skilled in the organic chemical arts of the type described in Chapter 2 of T.W. Greene, "Protective Groups in Organic Synthesis", John Wiley and Sons, New York, N.Y., 1991, hereafter referred to as "*Greene*". Representative hydroxy protecting groups include, for example, dihydropyran, ether groups including methyl and substituted methyl ether groups such as methyl ether, methoxymethyl ether, methylthiomethyl ether, *tert*-buylthiomethyl ether, (phenyldimethylsilyl)methoxy-methyl ether, benzyloxymethyl ether, *p*-methoxybenzyloxy-methyl ether, and *tert*-butoxymethyl ether; substituted ethyl ether groups such as ethoxyethyl ether, 1-(2-chloroethoxy)-ethyl ether, 2,2,2-trichloroethoxymethyl ether, and 2-(trimethylsilyl)ethyl ether; isopropyl ether groups; phenyl and substituted phenyl ether groups such as phenyl ether, *p*-chlorophenyl ether, *p*-methoxyphenyl ether, and 2,4-dinitrophenyl ether; benzyl and substituted benzyl ether groups such as benzyl ether, *p*-methoxybenzyl ether, *o*-nitrobenzyl ether, and 2,6-dichlorobenzyl ether; and alkylsilyl ether groups such as trimethyl- triethyl- and triisopropylsilyl ethers, mixed alkylsilyl ether groups such as dimethylisopropylsilyl ether, and diethylisopropylsilyl ether; and ester protecting groups such as formate ester, benzylformate ester, mono-, di-, and trichloroacetate esters, phenoxyacetate ester, and *p*-chlorophenoxyacetate and the like. Further examples of groups referred to by the above terms are described by "*Greene*". The species of hydroxy protecting group employed is not critical so long as the derivatized hydroxy group is stable to the conditions of subsequent reaction(s) on other positions of the intermediate molecule and can be selectively removed at the appropriate point without disrupting the remainder of the molecule including any other hydroxy protecting group(s).

While all of the compounds of the present invention are useful, certain of the compounds are particularly interesting and are preferred. The following listing sets out several groups of preferred compounds, formulations, and methods. It will be understood

that each of the listings may be combined with other listings to create additional groups of preferred embodiments.

1) A is the olefin;

2) A is the diol;

3) A is the acetonide;

4) A is *trans* substituted;

5) A is *cis* substituted;

6) E is a bond;

7) Y is –E-NR^2R^3;

8) When Y is –E-NR^2R^3, R^3 is hydrogen;

9) When Y is –E-NR^2R^3, R^3 is aryl;

10) When Y is –E-NR^2R^3, R^3 is optionally substituted aryl;

11) When Y is –E-NR^2R^3, R^3 is $C(O)R^5$;

12) When Y is –E-NR^2R^3, R^3 is $C(O)R^5$, R^5 is C_1-C_6 alkyl;

13) When Y is –E-NR^2R^3, R^3 is $C(O)R^5$, R^5 is optionally substituted (C_1-C_4 alkyl)-aryl;

14) When Y is –E-NR^2R^3, R^3 is $C(O)R^5$, R^5 is optionally substituted aryl;

15) When Y is –E-NR^2R3, R^3 is $C(O)R^5$, R^5 is $(CH_2)_tC(R^6)(R^7)N(R^6)(R^8)$;

16) The compounds of the examples;

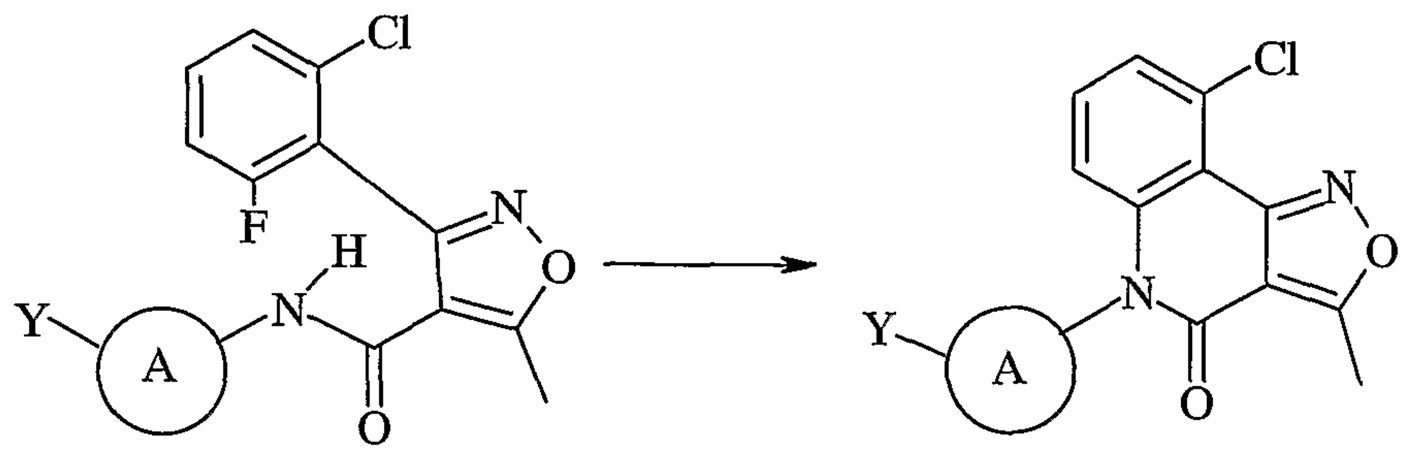
17) The compound is a pharmaceutical salt; and

18) The compound is the hydrochloride salt.

The compounds of the present invention can be prepared by a variety of procedures, some of which are illustrated in the Schemes below. The particular order of steps required to produce the compounds of formula I is dependent upon the particular compound being synthesized, the starting compound, and the relative lability of the substituted moieties.

Compounds of formula I(a) may be prepared from compounds of formula II as illustrated in Scheme 1 below where Y and A are as described *supra*.

Scheme 1



Compounds of formula I may be prepared by dissolving or suspending a compound of formula II in a suitable solvent, preferably dimethylformamide, and adding a suitable base, including potassium methoxide, potassium *tert*-butoxide, potassium carbonate, sodium hexamethyldisilazane, and preferably potassium hexamethyldisilazane. The base is typically employed in an one to one ratio. However, as the skilled artisan would appreciate, a slight molar excess, usually in about a 1.1 to about a 3 fold molar excess relative to the compound of formula II, is acceptable.

The reactants are typically combined at a temperature from about 0°C to about 100°C, preferably from about 50°C to about 60°C. The reactants are preferably combined at room temperature and the resulting solution is typically mixed for from about 5 minutes to about 18 hours, preferably from about 3 hours to about 6 hours.

Any protecting groups remaining in the cyclized compound of formula I may be removed as taught in Greene to provide the compounds of formula I. Preferred choices of protecting groups and methods for their removal may be found in the Preparations and Examples sections below.

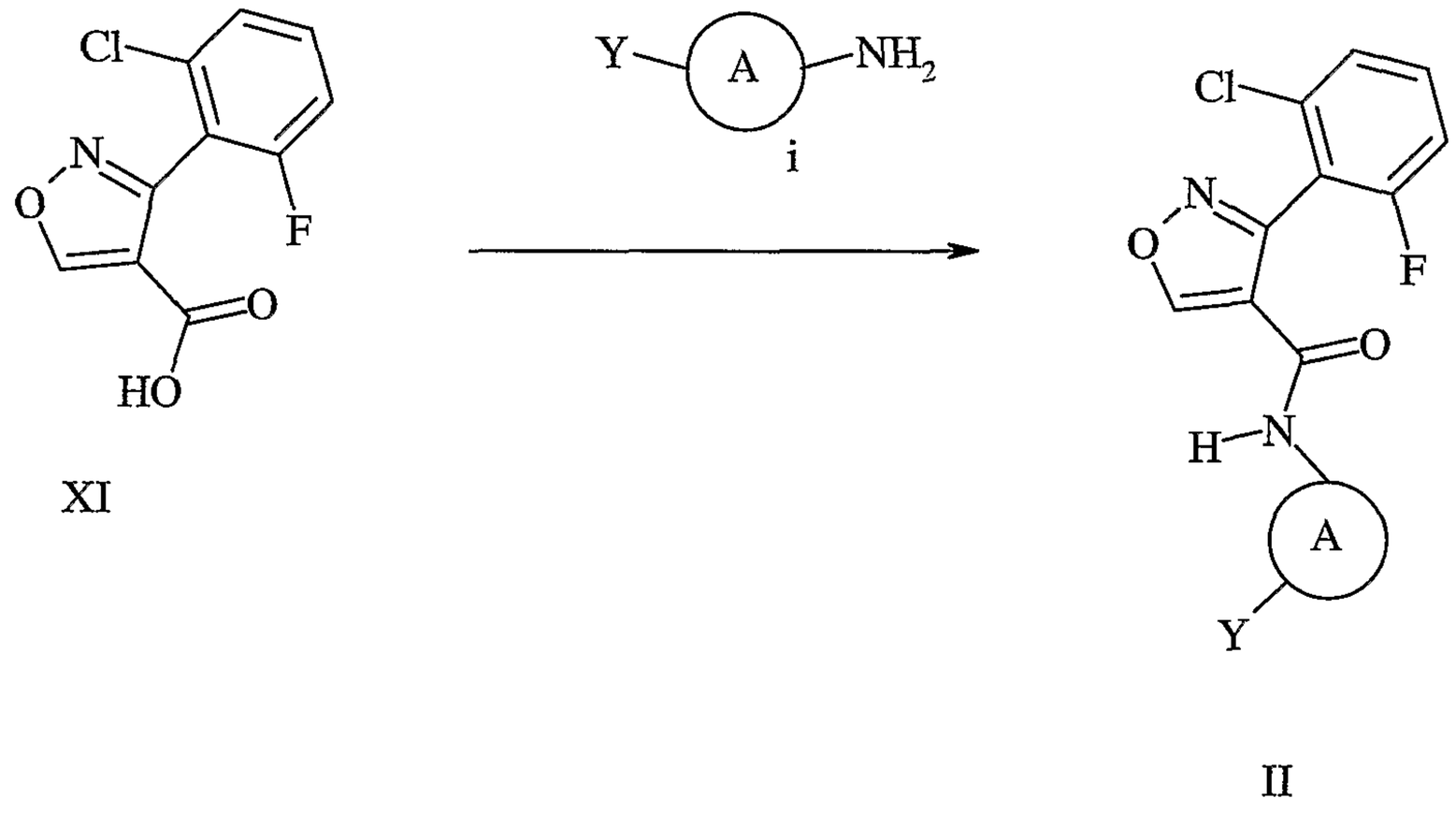
Compounds of formula I may also be prepared by palladium chemistry methods as generally described in Preparations 3, 6 and 8. The salt form of the tricycle (see Route 1 *infra* for synthesis of the tricyclic salt) is added to an appropriate ester derivative of the

olefin form of A in the presence of an appropriate catalyst, as is illustrated in Preparation 3. This alcohol is further converted to the ester by methods well known in the art, see for example preparation 4. The ester is then converted to the protected amide by methods will known in the art, see for example preparation 5. The amide may be further converted to compounds of formula I by general organic chemistry techniques, see Examples 1-7.

The starting materials and compounds of the present invention may be obtained by a number of routes. For example, compounds of formula II may be prepared according to the routes shown in Schemes 2-5.

Where Y and A are as described *supra*, compounds of formula II may be prepared according to Scheme 2.

Scheme 2



Compounds of formula XI may be converted to the corresponding acid halide by methods well known to one skilled in the art. Compounds of formula II may be prepared by dissolving or suspending an acid halide of a compound of formula XI in a suitable solvent and adding a compound of formula i in a suitable solvent. Triethylamine or dimethylformamide are convenient solvents and are typically preferred for the compound of formula XI. A 1:1 mixture of DMF and dichloromethane is a convenient solvent

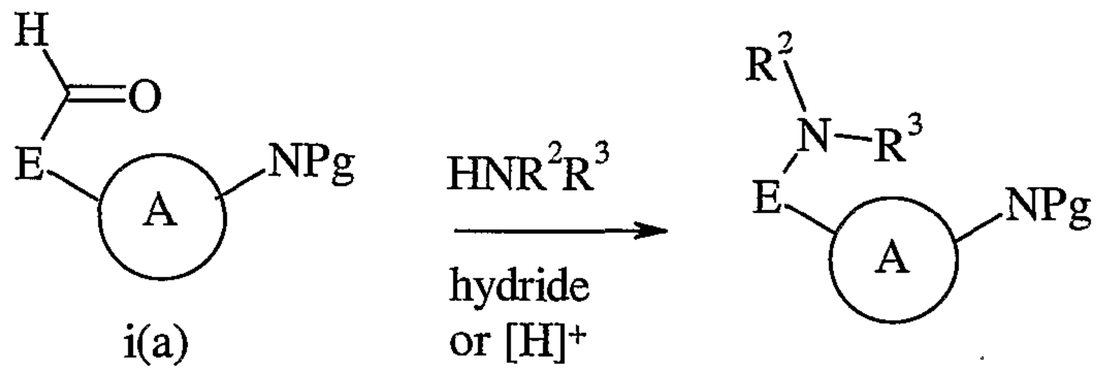
mixture and is typically preferred for the amine of formula i. This amide forming reaction is also preferably run in the presence of 4-dimethylaminopyridine (DMAP).

DMAP is employed in a catalytic fashion. For example, about 5 molar percent to about 15 molar percent, relative to the compound of formula i, is typically employed. A 10 molar percent is usually preferred.

Compounds of formula i; wherein the amino groups are protected; are used to prepare compounds of formula i(e). These compounds are well known in the art and to the extent not commercially available, are readily synthesized by standard procedures commonly employed in the art.

Compounds of formula i(b) where Y is -E-NR^2R^3 and A, E, R^2, and R^3 are as described *supra* and Pg is an amino protecting group can be prepared by reductive animation as illustrated in Scheme 3 from compounds of formula i(a) wherein Y is –E-$C(O)R^1$ and R^1 is hydrogen.

Scheme 3

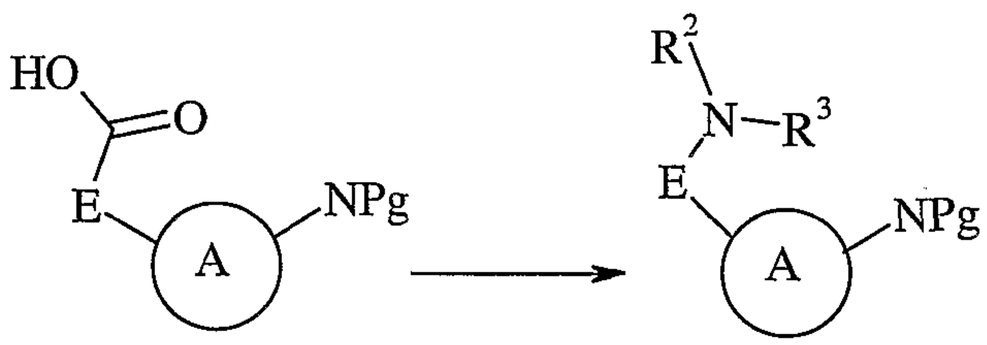


i(a) i(b)

Compounds of formula i(b) may be prepared from compounds of formula i(a) in a manner similar to that as taught in the Larock, "Comprehensive Organic Transformations", pg. 421-430, VCH Publishers, New York, N.Y., 1989, hereafter referred to as "Larock".

Additionally, the skilled artisan will appreciate that the compounds of formula i(b) may also be prepared from compounds of i(c) as is shown in Scheme 4.

Scheme 4

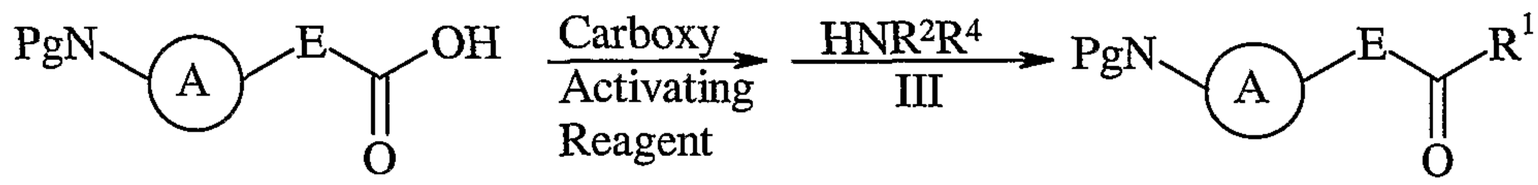


i(c) i(b)

Compounds of formula i(b) may be prepared by reductive alkylation from compounds of formula i(c) in a manner similar to that as taught in the Larock reference at pages 434-435. Compounds of formulas i(a) and i(c) are well known in the art and to the extent not commercially available, are readily synthesized by standard procedures commonly employed in the art.

Compounds of formula i(d) where Y is $C(O)R^1$ and R^1 is NR^2R^4 may be prepared from compounds of formula i(e) as illustrated in Scheme 5 below where E, A, and Pg are as described *supra.*

Scheme 5



i(e) i(d)

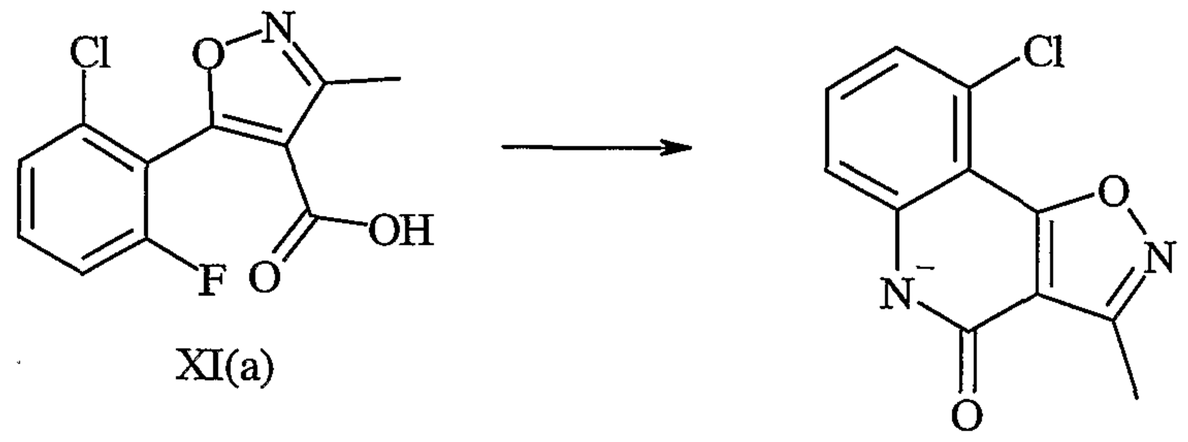
Compounds of formula i(e) may be converted to other compounds of the invention via solution or solid phase synthetic techniques. For example, acids of formula i(e) may be treated with activating agents to form the activated carboxylic acid derivatives of formula i(e) by methods well known in the chemical arts. See, *e.g.*, The Peptides, Peptide Synthesis and the Examples and Preparations sections below.

Generally, preparation of compounds of formula i(d) where R^1 is NR^2R^4 is performed in a manner similar to that as taught in the Larock reference at pages 972-976. Specifically, such compounds of formula i(d) may be prepared by dissolving or suspending a compound of the activated carboxylic acid derivatives of formula i(e) in a suitable solvent, optionally in the presence of a suitable base, and adding an amine of formula III. Typically a preferred and convenient solvent is dichloromethane. Preferred bases are triethylamine and piperidinylmethylpolystyrene resin. The amine is typically employed in molar excess. For example, about a 1.5 to about a 3 molar excess, relative to the compound of the activated carboxylic acid derivatives of formula i(e) is usually employed. About 1.8 to about 2.2 fold molar excess is typically preferred. The reaction is usually performed in a temperature range of about 0°C to about the reflux temperature of the solvent for from 10 minutes to 18 hours. Preferably, the reaction is performed at about 15°C to about 40°C for from 5 minutes to about 2.5 hours.

Furthermore, the transformations described in Schemes 2 - 5 may be performed after the cyclization described in Scheme 1 to provide the compounds of formula I with a fully elaborated R substituent.

Scheme 6 describes generically the tricyclic salt formation of 5H-isoxazolo[4,5-c]quinolin-4-one from the carboxylic acid compounds, represented by the compound of formula XI(a). Compounds of formula XI(a) are commercially available and may be prepared by common synthetic techniques, see e.g. WO99/51227.

Scheme 6



XI(a)

Additionally, compounds of formula XI(a) may be prepared in a manner similar to that described in the literature, for example, see Chen YP, et. al, *Heterocycles*, **1995**, *41*, 175, and Chantegrel B, et. al, *J. Org. Chem.*, **1984**, *49*, 4419-4424.

Compounds of formula XI(a) may be converted to the tricyclic salt used in the palladium chemistry method described *supra*, by converting the compound of formula XI(a) to the corresponding carboxamide, then cyclizing the piperidine ring as generally described in Scheme 1.

The pharmaceutical salts of the invention are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid or base. The reactants are generally combined in a mutual solvent such as diethylether, tetrahydrofuran, methanol, ethanol, isopropanol, benzene, and the like for acid addition salts, or water, an alcohol or a chlorinated solvent such as dichloromethane for base addition salts. The salts normally precipitate out of solution within about one hour to about ten days and can be isolated by filtration or other conventional methods.

Acids commonly employed to form pharmaceutical acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as *p*-toluenesulfonic, methanesulfonic acid, ethanesulfonic acid, oxalic acid, *p*-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, tartaric acid, benzoic acid, acetic acid, and the like. Preferred pharmaceutical acid addition salts are those formed with mineral acids such as hydrochloric acid, hydrobromic acid, and sulfuric acid, and those formed with organic acids such as maleic acid, tartaric acid, and methanesulfonic acid.

Bases commonly employed to form pharmaceutical base addition salts are inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

It should be recognized that the particular counterion forming a part of any salt of this invention is not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

The optimal time for performing the reactions of Schemes 1 – 6 can be determined by monitoring the progress of the reaction via conventional chromatographic techniques. Furthermore, it is preferred to conduct the reactions of the invention under an inert atmosphere, such as, for example, argon, or, particularly, nitrogen. Choice of solvent is generally not critical so long as the solvent employed is inert to the ongoing reaction and sufficiently solubilizes the reactants to effect the desired reaction. The compounds are preferably isolated and purified before their use in subsequent reactions. Some compounds may crystallize out of the reaction solution during their formation and then collected by filtration, or the reaction solvent may be removed by extraction, evaporation, or decantation. The intermediates and final products of formula I may be further purified, if desired by common techniques such as recrystallization or chromatography over solid supports such as silica gel or alumina.

The skilled artisan will appreciate that not all substituents are compatible with all reaction conditions. These compounds may be protected or modified at a convenient point in the synthesis by methods well known in the art. For example, the skilled artisan would appreciate that when A is in the diol or acetonide form, the conversion from the cyclopentenyl to these forms would be done in the last step.

The following Preparations and Examples are provided to better elucidate the practice of the present invention and should not be interpreted in any way as to limit the scope of same. Those skilled in the art will recognize that various modifications may be made while not departing from the spirit and scope of the invention. All publications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. The terms and abbreviations used in the instant Preparations and Examples have their normal meanings unless otherwise designated. For example "°C", "N", "mmol", "g", "mL", "M", "HPLC", "IR", "MS(FD)", "MS(IS)", "MS(FIA)", "MS(FAB)", "MS(EI)", "UV", and "^{1}H NMR", refer to degrees Celsius, normal or normality, millimole or millimoles, gram or grams, milliliter or milliliters,

molar or molarity, high performance liquid chromatography, infra red spectrometry, field desorption mass spectrometry, ion spray mass spectrometry, flow injection analysis mass spectrometry, fast atom bombardment mass spectrometry, electron impact mass spectrometry, ultraviolet spectrometry, and proton nuclear magnetic resonance spectrometry respectively. In addition, the absorption maxima listed for the IR spectra are only those of interest and not all of the maxima observed.

Preparations

Preparation 1

9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one potassium salt

To 3-(6-chloro-2-fluorophenyl)-5-methylisoxazole-4-carboxamide (2.51 g, 9.86 mmol) in 25 mL anhydrous DMF at –20°C was added 0.5 M KHMDS in toluene (23.7 mL, 11.8 mmol). After 20 minutes the cold bath was removed and the mixture was stirred for an additional 40 minutes. A yellow precipitate formed. After filtration 1.78 g (66%) of the title compound were obtained.

Preparation 2

4-(*t*-Butyldimethylsilanyloxy)cyclopent-2-enyloxy acetate

To a stirred solution of 4-hydroxy-cyclopent-2-enyloxy acetate (4.1 g, 29 mmol) in CH_2Cl_2 (35 mL) and 2,6-lutidine (8.2 mL, 67 mmol) was added *t*-butyldimethylsilyl trifluoromethanesulfonate (10 mL, 44 mmol) at 0-5°C. The reaction mixture was stirred at r.t. for 1 hour. It was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, 5:1 hexanes:diethylether) gave desired product (5.1 g, 69%). ^1H NMR ($CDCl_3$, 400 MHz) δ; 5.88 (m, 1H); 5.84 (m,1H); 5.42 (t, 1H); 4.64 (t,1H); 2.80 (m,1H); 2.02 (s, 3H); 1.59 (m, 1H); 0.83 (s,9H); 0.04 (s, 6H).

Preparation 3

5-[4-(*t*-Butyldimethylsilanyloxy)cyclopent-2-en-1-yl]-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one

To a stirred solution of 4-(*t*-butyldimethylsilanyloxy)-cyclopent-2-enyloxy acetate (3.4 g, 13.3 mmol) in DMF (140 mL) was added tetrakis(triphenylphosphine) palladium (1.54 g, 1.3 mmol) and 9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one potassium salt (4.4 g, 16.2 mmol). The reaction mixture was stirred at 55°C for 6 hours. It was diluted with ethyl acetate, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave desired product (5.7 g, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ; 7.83 (m, 1H); 7.22 (m, 2H); 6.50 (t, 1H); 5.84 (m, 2H); 4.85 (t, 1H); 2.82 (s, 3H); 2.78 (m, 1H); 2.02 (m, 1H); 0.81 (s, 9H); 0.02 (s, 3H); 0.00 (s, 3H).

Preparation 4

9-chloro-5-(4-hydroxy-cyclopent-2-enyl]-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one

To a stirred solution of 5-[4-(*t*-butyl-dimethylsilanyl-oxy)cyclopent-2-en-1-yl]-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (3.4 g, 7.9 mmol) in THF (60 mL) was added HF.pyridine/pyridine/THF (1:2:8; 50 mL). The mixture was stirred at r.t. for 6 hours. Solid $NaHCO_3$ (6 g) and ethyl acetate (60 mL) were added. Aqueous $NaHCO_3$ was then added until the reaction was quenched. The layers were separated and the organic fraction was washed (brine, water, aqueous $CuSO_4$), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave desired product (2.2 g, 88%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ; 7.59 (d, 1H); 7.37 (t, 1H); 7.30 (d,1H); 6.15 (m, 1H); 5.91(m, 1H); 5.78 (broad, 1H); 4.82 (broad, 1H); 2.81 (s, 3H); 2.82 (m, 1H); 2.18 (m,1H).

Preparation 5

Carbonic acid 4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-c]quinolin-5-yl-cyclopent-2-enyl ester ethyl ester

To a stirred solution of 9-chloro-5-(4-hydroxy-cyclopent-2-enyl]-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (430 mg, 1.37 mmol) in CH_2Cl_2 (10 mL) was added pyridine (0.55 mg, 6.85 mmol) and ethyl chloroformate (0.16 mL, 1.64 mmol) and stirred at r.t. for 5.5 hour. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, 5:1 hexanes/ethyl acetate) gave desired product (0.47 g, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ; 7.62 (d, 1H); 7.37 (t, 1H); 7.34 (d, 1H); 6.52 (t, 1H); 6.20 (m, 1H); 6.09 (m, 1H); 5.73 (t, 1H); 4.19 (q, 2H); 2.98 (m, 1H); 2.88 (s, 3H); 2.28 (m, 1H); 1.26 (t, 3H).

Preparation 6

5-(4-aminocyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one

To a stirred solution of carbonic acid 4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-*c*]quinolin-5-yl-cyclopent-2-enyl ester ethyl ester (2 g, 5.1 mmol) in THF (30 mL) was added tetrakis(triphenylphosphine) palladium (1.2 g, 1.0 mmol) and a solution of potassium di-*t*-butyl iminodicarboxylate in THF (75 mL, 0.2 M, 15 mmol). The reaction mixture was stirred at 55°C for 6 hours. It was then diluted with ethyl acetate, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave a mixture of the coupling product and di-*tert*-butyl iminodicarboxylate, which was treated with trifluoroacetic acid (5 mL). The mixture was stirred 6 hours at room temperature. It was then concentrated, dissolved in water, treated with NaOH solution (5N), extracted with EtOAc, dried over Na_2SO_4, concentrated, and gave the desired product (0.62 g, 39%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ; 7.72 (d, 1H); 7.35 (t, 1H); 7.26 (d, 1H); 6.19 (t, 1H); 5.90 (m, 2H); 4.10 (m, 1H); 2.88 (s, 3H); 2.80 (m, 1H); 1.90 (m, 1H).

Preparation 7

N-[4-(Hydroxymethyl)cyclopent-2-enyl](*t*-butoxy)carboxamide

4-*t*-Butoxycarbonylaminocyclopent-2-enecarboxylic acid (1 g, 4.4 mmol) was dissolved in THF (10 mL) and borane-THF (1.5 mL, 1M solution) was added drop-wise at 0 ^{0}C and stirred overnight at rt. The reaction mixture was poured into ice-cold water (10 mL) and extracted with ethyl acetate (2 x 50mL). The ethyl acetate extract was washed with brine, dried over sodium sulfate, filtered and evaporated to yield N-[4-(hydroxy-methyl)cyclopent-2-enyl](*t*-butoxy)-carboxamide (655 mg, 69%). ESMS: 214 $(M+1)^+$, 236 $(M+23)^+$, 248 $(M+35)^-$, 272 $(M+59)^-$. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H), 2.16 (s, 1H), 2.44-2.52 (m, 2H), 2.82-2.83 (m, 1H), 3.54-3.66 (m, 2H), 5.74-5.81 (m, 2H).

Preparation 8

(1S,4R)-4-(9-chloro-3-methyl-4-oxo(5-hydroisoxazolo[4,3-c]quinolin-5-yl))cyclopent-2-enyl acetate

A solution of N-[4-(hydroxymethyl)cyclopent-2-enyl](*t*-butoxy)carboxamide (1.58 g, 5.00 mmol), pyridine (1.21 ml, 15.0 mmol), acetic anhydride (0.94 ml, 10.0 mmol) in CH_2Cl_2 (20 ml) was stirred for 17 h. The mixture was diluted with CH_2Cl_2, extracted with 1N HCl, H_2O, and brine, and dried ($MgSO_4$). Column chromatography (silica gel, hexanes:EtOAc gradient) gave the title compound (1.63 g, 91%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (dd, 1H, J = 1.5, 7.8 Hz), 7.36-7.29 (m, 2H), 6.46 (br S, 1H), 6.16 (m, 1H), 6.04 (m, 1H), 5.77 (m, 1H), 2.97 (ddd, 8.3, 8.8, 14.2 Hz), 2.89 (s, 3H), 2.18 (ddd, 1H, J = 7.3, 7.3, 12.2 Hz), 2.08 (s, 3H) ppm.

Preparation 9

Dimethyl 2-[(1S,4R)-4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo-[4,3-c]quinolin-5-yl)cyclopent-2-enyl]propane-1,3-dioate

To a solution of (1S,4R)-4-(9-chloro-3-methyl-4-oxo(5-hydroisoxazolo[4,3-c]quinolin-5-yl))cyclopent-2-enyl acetate (1.55 g, 4.33 mmol) in THF (N_2) was added

$Pd(PPh_3)_4$ (500 mg, 0.43 mmol) under a positive flow of N_2 followed by a solution of sodium dimethyl malonate (43.3 ml at 0.2 M in THF, 8.66 mmol). The mixture was heated to 50-55° C for 6 h then cooled to room temperature. Dilution with EtOAc, extraction with H_2O and brine, drying ($MgSO_4$), and column chromatography (silica gel, hexanes:ethyl acetate gradient) gave the title compound (1.38 g, 74%). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.70 (d, 1H, J = 8.3 Hz), 7.35 (t, 1H, J = 8.1 Hz), 7.29 (d, 1H, J = 7.8 Hz), 6.35 (br s, 1H), 5.98 (m, 1H), 5.90 (m, 1H), 3.75 (s, 3H), 3.73 (m, 1H), 3.70 (s, 3H), 3.60 (d, 1H, J = 8.8 Hz), 3.50 (m, 1H), 2.87 (s, 3H), 2.60 (ddd, 1H, J = 8.3, 8.3, 12.7 Hz), 2.03 (ddd, J = 9.3, 9.3, 13.2 Hz) ppm.

Preparation 10

Methyl 2-[(1R,4R)-4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-c]quinolin-5-yl))cyclopent-2-enyl]acetate

A solution of dimethyl 2-[(1S,4R)-4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo-[4,3-c]quinolin-5-yl)cyclopent-2-enyl]propane-1,3-dioate (1.00 g, 2.33 mmol), LiCl (0.200 g, 4.66 mmol), and H_2O (0.084 ml, 4.7 mmol) in DMSO (15 ml) was lowered into an oil bath at 180°C and stirred for 4 h. The reaction was cooled to room temperature, diluted with EtOAc (50 ml), extracted with H_2O and brine, dried ($MgSO_4$), and chromatographed (silica gel, hexanes:EtOAc gradient) to give the title compound (450 mg, 52%). Mass spectrum (ES+) (m/z) 373.0 [M+1].

Preparation 11

N-[4-(toluene-4-sulfonoxymethyl)cyclopent-2-enyl](*t*-butoxy)carboxamide

N-[4-(Hydroxymethyl)cyclopent-2-enyl](*t*-butoxy)-carboxamide (655 mg, 3 mmol) was dissolved in methylene chloride (20 mL) and p-toluenesulfonyl chloride (131 mg, 0.69 mmol), triethyl amine (268 μL, 1.86 mmol) and DMAP (10 mg) were added and stirred overnight. The methylene chloride solution was washed with 1M HCl (2x10 mL), water (2x10 mL), brine (2x10 mL), dried over sodium sulfate, filtered and evaporated to

yield N-[4-(Toluene-4-sulfonoxymethyl)-cyclopent-2-enyl](*t*-butoxy)carboxamide(1.0 g, 90%).

ESMS: 368 $(M+1)^+$, 426 $(M+59)^-$. ^{1}H NMR ($CDCl_3$): δ 1.40 (s, 9H), 2.05 (s, 1H), 2.44 (s, 3H), 2.44-2.50 (m, 2H), 2.89 (br s, 1H), 3.91-3.96 (m, 2H), 4.10-4.15 (m, 1H), 5.65-5.77 (2d, 2H), 7.33-7.35 (d, 2H), 7.75-7.78 (d, 2H).

Preparation 12

N-[4-(azidomethyl)cyclopent-2-enyl](*t*-butoxy)carboxamide

To a solution of N-[4-(toluene-4-sulfonoxymethyl)-cyclopent-2-enyl](*t*-butoxy)carboxamide (1g, 2.7 mmol) dissolved in DMF (10 mL) sodium azide was added and stirred at 80 °C overnight. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (2 x 25 mL), brine (2x25 mL), dried over sodium sulfate, filtered and evaporated to yield N-[4-(azidomethyl)cyclopent-2-enyl](*t*-butoxy)carboxamide. IR (chloroform):ν 2096.6 cm^{-1} (azide). ^{1}H NMR ($CDCl_3$): δ 1.27-1.30 (m, 2H), 1.43 (s, 9H), 2.51-2.56 (m, 2H), 3.27-3.35 (m, 2H), 5.76 (s, 2H).

Preparation 13

4-Azidomethyl-cyclopent-2-enylamine

N-[4-(azidomethyl)cyclopent-2-enyl](*t*-butoxy)-carboxamide (10 g, 42 mmol) was dissolved in TFA reagent (9.25 mL TFA, 0.25 mL anisole, 0.25 mL triisopropylsilane and 0.25 mL water)and DCM (25 mL), and stirred for 30 min at rt. The reaction mixture was concentrated and the residue filtered through SCX column, eluted with ammonia (2 M solution in methanol), and evaporated to yield the title compound (4.6 g, 80%). ESMS: 139 $(M+1)^+$. ^1H NMR ($CDCl_3$): δ 1.40-1.55 (m, 1H), 2.45-2.60 (m, 1H), 2.90-3.00 (m, 1H), 3.40 (d, 2H), 4.10-4.20 (m, 1H), 5.90 (s, 2H).

Preparation 14

N-[4-(azidomethyl)cyclopent-2-enyl][5-methyl-3-(6-chloro-2-fluorophenyl)isoxazol-4-yl]carboxamide

A mixture of 4-azidomethyl-cyclopent-2-enylamine (4.6 g, 33 mmol), 2-chloro-6-fluorophenylisoxazoyl chloride (13.5 g, 49 mmol), triethyl amine (5 mL) and DMAP (100 mg) dissolved in DCM (200 mL) was stirred overnight at r.t. The reaction mixture was washed with HCl (1M, 2x10 mL), water (2x10 mL), brine (2x10 mL), dried over sodium sulfate, filtered, and evaporated to yield N-[4-(azidomethyl)-cyclopent-2-enyl][5-methyl-3-(6-chloro-2-fluorophenyl)isoxazol-4-yl]carboxamide (10 g, crude, 83%). ESMS: 376 $(M+1)^+$. ^1H NMR($CDCl_3$): δ 2.40-2,70 (m, 2H), 2.80 (d, 2H), 2.80-3.50 (m, 2H), 5.00 (br s, 1H), 5.40-5.90 (m, 2H), 7.05-7.50 (m, 3H).

Examples

Example 1

N-{4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]benzamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (28.7 mg, 0.091 mmol) in CH_2Cl_2 (2 mL) was added benzoyl chloride (0.013 mL, 0.11 mmol) and triethyl amine (0.1 mL) and stirred at r.t. for 6 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave title compound (21 mg, 55%). Mass Spectrum (FIA) (m/z) 420.2 (M+1). ^1H NMR($CDCl_3$): δ 7.83 (d, 2H); 7.38-7.61 (m, 6H); 6.08 (m, 1H); 5.96 (m, 1H); 5.58 (s, 1H); 5.40 (t, 1H); 3.02 (m, 2H); 2.18 (m, 2H).

Example 2

1-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]3-phenyl- urea

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (50 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) was added isocyanatobenzene (0.018 mL, 0.18 mmol) and DMAP (catalytic amount) and stirred at r.t. for 2 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (35 mg, 50%). Mass Spectrum (FIA) (m/z) 433.3 (M-1). Anal. Calc. For $C_{23}H_{19}ClN_4O_3$; Theoretical: C 63.52, H 4.40, N 12.88%; Found: C 63.82, H 4.45, N 12.49%.

Example 3

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-oxo-2-(3,4,5-trimethoxy-phenyl)-acetamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (50 mg, 0.16 mmol) in CH_2Cl_2 (2 mL) was added oxo-(3,4,5-trimethoxy-phenyl)-acetyl chloride (80 mg, 0.33 mmol, prepared from its acid and oxalyl chloride) and DMAP (catalytic amount) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (33 mg, 38%). Mass Spectrum (FIA) (m/z) 538.4 (M+1). ^1H NMR($CDCl_3$): δ 7.80 (d, 1H); 7.50 (t, 1H); 7.35 (d, 1H); 6.00 (m, 2H); 5.40 (m, 1H); 5.21 (m, 1H); 3.80-3.90 (m, 1H); 2.95 (s, 3H); 2.30 (m, 1H).

Example 4

1-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]3-(3,4,5-trimethoxy-phenyl)-urea

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (69 mg, 0.22 mmol) in CH_2Cl_2 (5 mL) was added 3,4,5-trimethoxy-isocyanato benzene (55 mg, 0.26 mmol) and DMAP (catalytic amount) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (116 mg, 100%). Mass Spectrum (FIA) (m/z) 425.3 (M+1) ^1H NMR($CDCl_3$): δ 7.45 (d, 1H); 7.40 (t, 1H); 7.33 (d, 1H); 6.58 (m, 3H); 5.95 (m, 1H); 5.83 (m, 1H); 5.18 (s, 1H); 3.78 (s, 9H); 2.98 (m, 2H); 2.05 (m, 2H).

Example 5

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-phenyl)acetamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (47.6 mg, 0.15 mmol) in CH_2Cl_2 (2 mL) was added phenyl acetic acid (27 mg, 0.20 mmol), Et_3N (0.052 mL, 0.38 mmol) and EDCI (38 mg, 0.20 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (36 mg, 56%). Mass Spectrum (FIA) (m/z) 434.4 (M+1). Anal. Calc. For $C_{24}H_{20}ClN_3O_3$

Theoretical: C 66.44, H 4.65, N 9.68%.

Found: C 66.18, H 4.71, N 9.32%.

Example 6

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-(2-methoxy-phenyl)-acetamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (97 mg, 0.31 mmol) in CH_2Cl_2 (4 mL) was added 2-methoxy phenyl acetic acid (66.3 mg, 0.40 mmol), Et_3N (0.11 mL, 0.40 mmol) and EDCI (77 mg, 0.40 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (59 mg, 41%). Mass Spectrum (FIA) (m/z) 464.2 (M+1) Anal. Calc. For $C_{25}H_{22}ClN_3O_4$

Theoretical: C 64.73, H 4.78, N 9.06%.

Found: C 64.78, H 4.91, N 8.83%.

Example 7

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-(3,4,5-trimethoxy-phenyl)-acetamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (47 mg, 0.15 mmol) in CH_2Cl_2 (2.5 mL) was added 3,4,5-trimethoxy phenyl acetic acid (45 mg, 0.20 mmol), Et_3N (0.053 mL, 0.38 mmol) and EDCI (38 mg, 0.20 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (23 mg, 29%). Mass Spectrum (FIA) (m/z) 522.2 (M-1). ^{1}H NMR($CDCl_3$): δ 7.43 (d, 1H); 7.37 (m, 2H); 6.42 (s, 2H); 5.88 (m, 2H); 5.18 (m, 1H); 3.78 (s, 9H); 3.47 (s, 2H); 2.90 (m, 2H); 1.98 (m, 2H).

Example 8

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-(3-fluoro-phenyl)-acetamide

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (16 mg, 0.05 mmol) in CH_2Cl_2 (3 mL) was added 3-fluoro phenyl acetic acid (1.9 mg, 0.01 mmol), Et_3N (0.003 mL, 0.03 mmol) and EDCI (2.4 mg, 0.01 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (17 mg, 74%). Mass Spectrum (FIA) (m/z) 452.0 (M+1). ^{1}H NMR($CDCl_3$): δ 7.25-7.42 (m, 4H); 7.00 (m, 3H); 5.82 (m, 2H); 5.20 (t, 1H); 3.58 (s, 2H); 2.90 (m, 2H); 1.89 (m, 2H).

Example 9

{[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enylcarbamoyl]-phenyl-methyl}-carbamic acid *t*-butyl ester

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (38 mg, 0.12 mmol) in CH_2Cl_2 (2.5 mL) was added *D-boc*-phenyl glycine (39 mg, 0.16 mmol), Et_3N (0.033 mL, 0.24 mmol), DMAP (catalytic amount), HOBT (16 mg, 0.12 mmol) and EDCI (31 mg, 0.16 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (56 mg, 85%). Mass Spectrum (FD) (m/z) 549 (M^+)
^{1}H NMR($CDCl_3$): δ 7.48 (m, 8H); 5.59 (m, 1H); 5.57 (m, 1H); 5.52 (s, 1H); 5.10 (m, 2H); 2.85 (s, 3H); 2.05 (tt, 1H); 1.49 (s, 9H).

Example 10

{[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enylcarbamoyl]-phenyl-methyl}-carbamic acid *t*-butyl ester

To a stirred solution of 5-(4-amino-cyclopent-2-enyl)-9-chloro-3-methyl-*5H*-isoxazolo[4,3-*c*]quinolin-4-one (38 mg, 0.12 mmol) in CH_2Cl_2 (2.5 mL) was added *L-boc*-phenyl glycine (39 mg, 0.16 mmol), Et_3N (0.033 mL, 0.24 mmol), DMAP (catalytic amount), HOBT (16 mg, 0.12 mmol) and EDCI (31 mg, 0.16 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (53 mg, 81%). Mass Spectrum (FIA) (m/z) 449 (M-Boc)
^{1}H NMR($CDCl_3$): δ 7.75-7.4 (m, 8H); 5.90-6.00 (m, 2H); 5.51 (s, 1H); 5.53 (m, 2H); 2.75 (m, 4H); 1.91 (tt, 1H); 1.49 (s, 9H).

Example 11

N-[4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-*c*]quinolin-5-yl)2,3-dihydroxy-cyclopentyl]-2-(3,4,5-trimethoxy-phenyl)-acetamide

To a stirred solution of N-[4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopent-2-enyl]2-(3-fluoro-phenyl)-acetamide (54 mg, 0.10 mmol) in acetone (8 mL) and THF (3 mL) was added NMO (43 mg, 0.32 mmol, 50% aqueous solution) and OsO_4 (catalytic amount) and stirred at r.t. for 5 hours. The mixture was diluted with EtOAc, quenched with aqueous $Na_2S_2O_8$ and separated. The organic layer was washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (29 mg, 52%). Mass Spectrum (FIA) (m/z) 558.2 (M+1) ^{1}H NMR($CDCl_3$): δ 7.51 (d, 1H); 7.40 (t, 1H); 7.32 (d, 1H); 6.91 (d, 1H); 6.43 (s, 2H); 4.80 (m, 1H); 4.72 (t, 1H); 4.30 (m, 1H); 4.24 (t, 1H); 3.80 (s, 6H); 3.78 (s, 3H); 3.52 (s, 2H); 2.80 (s, 3H); 2.49 (m, 1H); 2.08 (m, 1H).

Example 12

N-[6-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-*c*]quinolin-5-yl)2,3-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-4-yl]-2-(3,4,5-trimethoxy-phenyl)-acetamide

To a stirred solution of N-[4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-*c*]quinolin-5-yl)2,3-dihydroxy-cyclopentyl]-2-(3,4,5-trimethoxy-phenyl)-acetamide (114 mg, 0.20 mmol) in CH_2Cl_2 (1 mL) was added 2,2-dimethoxy propane (0.25 mL, 2 mmol) and PPTS (25 mg, 1 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (83 mg, 69%). Mass Spectrum (FIA) (m/z) 598.2 (M+1) Anal. Calc. For $C_{30}H_{32}ClN_3O_8$

Theoretical: C 60.25, H 5.39, N 7.03%.

Found: C 60.42, H 5.51, N 7.14%.

Example 13

N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-2,3-dihydroxy-cyclopentyl]-2-(3-fluoro-phenyl)-acetamide

To a stirred solution of N-[3-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-cyclopentonyl-2(3-fluoro-phenyl-methyl}-acetamide (182 mg, 0.40 mmol) in acetone (5 mL) and THF (20 mL) was added NMO (173 mg, 1.30 mmol, 50% aqueous solution) and OsO_4 (catalytic amount) and stirred at r.t. 72 hours. The mixture was diluted with EtOAc, quenched with aqueous $Na_2S_2O_8$ and separated. The organic layer was washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (170 mg, 88%).

Mass Spectrum (FIA) (m/z) 486.1 (M+1).

^{1}H NMR($CDCl_3$): δ 7.55 (d, 1H); 7.42 (t, 2H); 7.33 (d, 1H); 7.23 (d, 1H); 7.02 (dd, 1H); 6.93 (t, 1H); 6.81 (d, 1H); 4.80 (m, 1H); 4.70 (s, 1H); 4.25 (n, 2H); 4.10 (s, 1H); 3.60 (s, 2H); 2.92 (s, 3H); 2.50 (m, 1H); 2.08 (m, 1H).

Example 14

N-[6-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-2,3-dimethyl-tetrahydro-cyclopenta[1,3]dioxol-4-yl]-2-(3-fluoro-phenyl)-acetamide

To a stirred solution of N-[4-(9-chloro-3-methyl-4-oxo-*5H*-isoxazolo[4,3-*c*]quinolin-5-yl)-2,3-dihydroxy-cyclopentyl]-2-(3-fluoro-phenyl)-acetamide (69 mg, 0.14 mmol) in CH_2Cl_2 (1 mL) was added 2,2-dimethoxy propane (0.18 mL, 1.42 mmol) and PPTS (18 mg, 0.07 mmol) and stirred at r.t. for 18 hours. The mixture was diluted with CH_2Cl_2, washed (brine), dried (Na_2SO_4), filtered and concentrated. Column chromatography (silica gel, hexanes/ethyl acetate, gradient) gave the title compound (50 mg, 65%).

Mass Spectrum (FIA) (m/z) 548.1 (M+Na).

^{1}H NMR($CDCl_3$): δ 7.43 (t, 2H); 7.37 (d, 1H); 7.31 (d, 1H); 7.11 (tt, 1H); 6.80-6.96 (m, 3H); 5.04 (d, 1H); 4.71 (m, 2H); 4.62 (m, 1H); 3.43 (s, 2H); 2.95 (m, 1H); 2.80 (s, 3H); 1.81 (tt, 1H); 1.55 (s, 3H); 1.48 (s, 3H).

Example 15

2-[(1R,4R)-4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-c]quinolin-5-yl)cyclopent-2-enyl]-N-(3,4,5-trimethoxyphenyl)acetamide

A solution of methyl 2-[(1R,4R)-4-(9-chloro-3-methyl-4-oxo-5*H*-isoxazolo[4,3-c]quinolin-5-yl))cyclopent-2-enyl]acetate (0.200 g, 0.54 mmol) and NaOH (2 ml at 2 N, 4.00 mmol) in dioxane (5 ml)was heated to 50°C for 2h. Upon cooling to room temperature the volatiles were removed under reduced pressure. The residue was dissolved up in H_2O (10 ml), acidified with 1 N HCl until a pH of 2 and extracted with EtOAc. The organic fractions were washed (H_2O and brine) and dried ($MgSO_4$) to give the crude acid after removal of the solvent. The crude acid, 3,4,5-trimethoxyaniline (148 mg, 0.81 mmol), and EDCI (210 mg, 1.10 mmol)were dissolved in CH_2Cl_2 (5 ml) followed by the addition of DMAP (6 mg, 0.05 mmol). The mixture was stirred for 18 h. Dilution with CH_2Cl_2 followed by direct application of the mixture to a silica gel column and elution (hexanes:EtOAc) gave the title compound (173 mg, 61%).

Mass spectrum (ES-) (m/z) 522.3 [M-1].

The compounds of the invention are inhibitors of MRP1. Thus, the compounds of the invention may be used to inhibit any neoplasm having intrinsic and/or acquired resistance, conferred in part or in total by MRP1, to an oncolytic or oncolytics. In other words, treatment of such a neoplasm with an effective amount of a compound of this invention will cause the neoplasm to be more sensitive to chemotherapy that was previously rendered less efficacious by MRP1.

Vincristine, epirubicin, daunorubicin, doxorubicin, and etoposide are oncolytics that are substrates of MRP1. See Cole, *et. al.*, "Pharmacological Characterization of Multidrug Resistant MRP-transfected Human Tumor Cells", *Cancer Research*, 54:5902-5910, 1994. Since MRP1 is ubiquitous in mammals, particularly humans, Nooter, K, *et.*

al., "Expression of the Multidrug Resistance-Associated Protein (MRP) Gene in Human Cancers", *Clin. Can. Res.*, 1:1301-1310, (1995), chemotherapy whose goal is to inhibit a neoplasm employing any of those agents has the potential to be rendered less efficacious by MRP1. Thus, neoplasms of the bladder, bone, breast, lung(small-cell), testis, and thyroid and more specific types of cancer such as acute lymphoblastic and myeloblastic leukemia, Wilm's tumor, neuroblastoma, soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphomas, and bronchogenic carcinoma may be inhibited with a combination of one or more of the above oncolytics and a compound of this invention.

The biological activity of the compounds of the present invention was evaluated employing an initial screening assay, which rapidly and accurately measured the activity of the tested compound in inhibiting MRP1 or MDR1. Assays useful for evaluating this reversing capability are well known in the art. See, *e.g.*, T. McGrath, *et al.*, *Biochemical Pharmacology*, 38:3611, 1989; D. Marquardt and M.S. Center, *Cancer Research*, 52:3157, 1992; D. Marquardt, *et al.*, *Cancer Research*, 50:1426, 1990; and Cole, *et. al.*, *Cancer Research*, 54: 5902-5910, 1994.

Assay for Reversal of MRP1-Mediated Doxorubicin Resistance and MDR1-Mediated Vincristine Resistance: HL60/ADR and HL60/VCR are continuous cell lines, which were selected for doxorubicin and vincristine resistance respectively by culturing HL60, a human acute myeloblastic leukemia cell line, in increasing concentrations of doxorubicin or vincristine until a highly resistant variant was attained.

HL60/ADR and HL60/VCR cells were grown in RPMI 1640 (Gibco) containing 10% fetal bovine serum (FBS) and 250 μg/ml GENTAMICIN™ (Sigma). Cells were harvested; washed twice with assay medium (same as culture media); counted; and diluted to 2 x 10^5 cells/ml in assay medium. Fifty microliters of cells were aliquoted into wells of a 96 well tissue culture plate. One column of each 96 well plate served as a negative control and received assay medium containing no cells.

Test compounds and reference compounds were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mM. Samples were diluted to 20 μM in assay medium and 25 μl of each test compound was added to 6 wells. Assay standards were run in quadruplicate. Twenty-five microliters of 0.4% DMSO was added to four wells as a

solvent control. Assay media was added to all wells to achieve a final volume of 100 μl per well.

The plates were incubated at 37°C for 72 hours in a humidified incubator with a 5% carbon dioxide atmosphere. Cell viability and vitality was measured by oxidation of a tetrazolium salt using standard conditions. The plates were incubated for 3 hours at 37°C. Absorbance was determined at 490 nm using a microtitre plate reader.

The ability of a test compound to reverse the resistance of HL60/ADR and HL60/VCR cells to doxorubicin was determined by comparison of the absorbance of the wells containing a test compound in addition to the oncolytic (doxorubicin) with the absorbance of wells containing the oncolytic without a test compound. Controls were used to eliminate background and to ensure the results were not artifactual. The results of the assay are expressed as percent inhibition of cell growth. The oncolytic alone at the tested concentration does not usually inhibit the growth of HL60/ADR or HL60/VCR cells.

Representative compounds of formula I demonstrated a significant effect in reversing the MRP1 multiple drug resistance. Many of the compounds showed very significant enhancement of activity in combination with the oncolytic agent as opposed to the oncolytic agent alone. In addition, a large majority of the compounds tested displayed a significant degree of selective inhibition of the HL60/ADR cell line over the HL60/VCR cell line.

When administering an oncolytic in practicing the methods of this invention, the amount of oncolytic employed will be variable. It should be understood that the amount of the oncolytic actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual oncolytic administered, the age, weight, and response of the individual patient (mammal), and the severity of the patient's symptoms. Of course, the amount of oncolytic administered should be decided and closely monitored by that patient's physician. After deciding on the oncolytic or oncolytics to employ, "The Physician's Desk Reference®", published by Medical Economics Company at Montvale, NJ 07645-1742, is a helpful resource to the physician in deciding on amounts of the oncolytic to administer and is updated annually.

Preferred formulations, and the methods of this invention employing those formulations, are those which do not contain an oncolytic. Thus, it is preferred to administer the compounds of this invention separately from the oncolytic. The oncolytics mentioned in this specification are commercially available and may be purchased in pre-formulated forms suitable for the methods of this invention.

The compounds of formula I alone, or optionally in combination with an oncolytic, are usually administered in the form of pharmaceutical formulations. These formulations can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. Such formulations are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound of formula I.

The present invention also includes methods employing pharmaceutical formulations, which contain, as the active ingredient, the compounds of formula I, and optionally an oncolytic, associated with pharmaceutical carriers. In making the formulations of the present invention the active ingredient(s) is usually mixed with an excipient, diluted by an excipient, or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the formulations can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing for example up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound(s) to provide the appropriate particle size prior to combining with the other ingredients. If the active compound(s) is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound(s) is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, *e.g.*, about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium

silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxybenzoates; sweetening agents; and flavoring agents. The formulations of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The formulations are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of each active ingredient. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient.

The compounds of formula I are effective over a wide dosage range. For example, dosages per day normally fall within the range of about 0.5 to about 30 mg/kg of body weight. In the treatment of adult humans, the range of about 1 to about 15 mg/kg/day, in single or divided dose, is especially preferred. However, it will be understood that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered, the age, weight, and response of the individual patient, and the severity of the patient's symptoms, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. In some instances dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several smaller doses for administration throughout the day.

For preparing solid formulations such as tablets the principal active ingredient(s) is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient(s) is dispersed evenly throughout the formulation so that the formulation may

be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by enteric layer, which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The novel formulations which are liquid forms may be incorporated for administration orally or by injection and include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Formulations for inhalation or insufflation include solutions and suspensions in pharmaceutical, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid formulations may contain suitable pharmaceutical excipients as described *supra*. Preferably the formulations are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutical solvents may be nebulized by use of inert gases. Nebulized solutions may be breathed directly from the nebulizing device or the nebulizing device may be attached to a facemask, tent, or intermittent positive pressure-breathing machine. Solution, suspension, or powder formulations may be administered, preferably orally or nasally, from devices, which deliver the formulation in an appropriate manner.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used

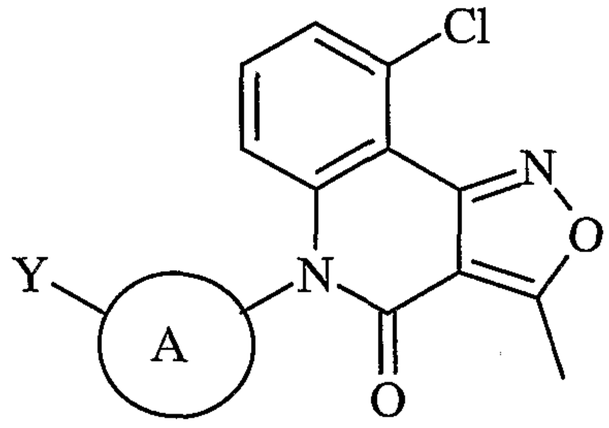
to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, *e.g.*, U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical formulation to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system, used for the transport of biological factors to specific anatomical regions of the body, is described in U.S. Patent 5,011,472, issued April 30, 1991, which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs or prodrugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions, which can transiently open the blood-brain barrier.

We Claim:

1. A compound of formula I:



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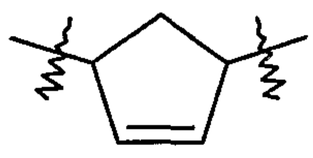
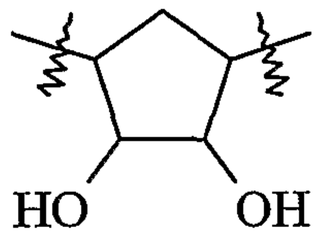
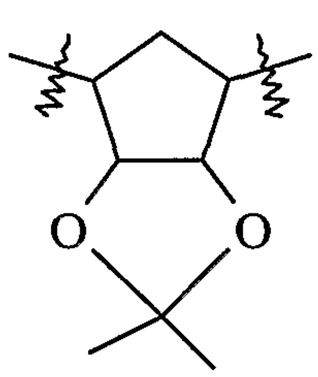
where:

A is  (olefin),  (diol), or  (acetonide);

Y is –E-C(O)R^1 or -E-NR^2R^3;

E is a bond or -CH_2-;

R^1 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted heterocycle, or NR^2R^4;

R^2 is independently at each occurrence hydrogen, C_1-C_6 alkyl, (C_1-C_6 alkyl)-aryl, or aryl;

R^3 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted heterocycle, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally

substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, C(O)C(O)R^9, C(O)R^5, or R^2 and R^3, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^4 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or R^2 and R^4, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^5 is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, optionally substituted (C_1-C_4 alkyl)-phenoxy, $(CH_2)_tC(R^6)(R^7)N(R^6)(R^8)$, or NR^2R^4;

t is 0, 1, 2, 3, or 4;

R^6 is independently at each occurrence hydrogen or C_1-C_6 alkyl;

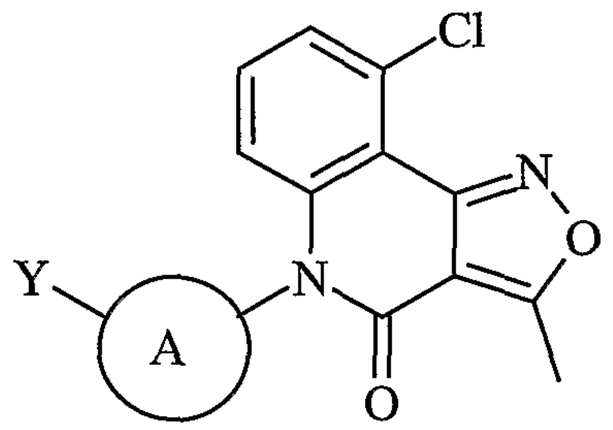
R^7 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^8 is independently at each occurrence hydrogen, C_1-C_6 alkyl, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, C(O)OR^9, C(O)R^{10}, or R^6 and R^8, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^9 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^{10} is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or optionally substituted (C_1-C_4 alkyl)-phenoxy; or a pharmaceutical salt thereof.

2. A method of inhibiting MRP1 in a mammal which comprises administering to a mammal in need thereof an effective amount of a compound of formula I:



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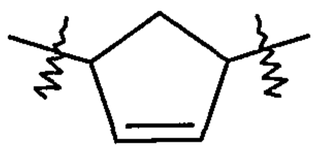
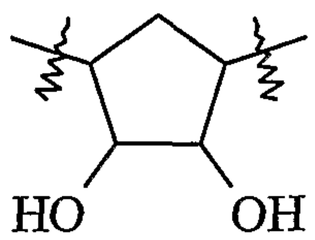
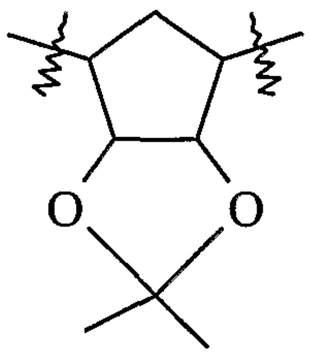
where:

A is  (olefin),  (diol), or

 (acetonide);

Y is –E-C(O)R^1 or -E-NR^2R^3;

E is a bond or -CH_2-;

R^1 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted heterocycle, or NR^2R^4;

R^2 is independently at each occurrence hydrogen, C_1-C_6 alkyl, (C_1-C_6 alkyl)-aryl, or aryl;

R^3 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted heterocycle, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, C(O)C(O)R^9, C(O)R^5, or R^2 and R^3, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^4 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or R^2 and R^4, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^5 is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, optionally substituted (C_1-C_4 alkyl)-phenoxy, $(CH_2)_tC(R^6)(R^7)N(R^6)(R^8)$, or NR^2R^4;

t is 0, 1, 2, 3, or 4;

R^6 is independently at each occurrence hydrogen or C_1-C_6 alkyl;

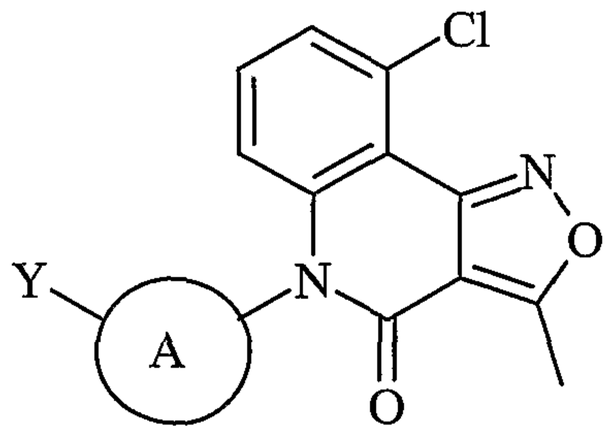
R^7 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^8 is independently at each occurrence hydrogen, C_1-C_6 alkyl, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, $C(O)OR^9$, $C(O)R^{10}$, or R^6 and R^8, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^9 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^{10} is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or optionally substituted (C_1-C_4 alkyl)-phenoxy; or a pharmaceutical salt thereof.

3. A method of inhibiting a resistant neoplasm, or a neoplasm susceptible to resistance, in a mammal which comprises administering to a mammal in need thereof an effective amount of a compound of formula I:



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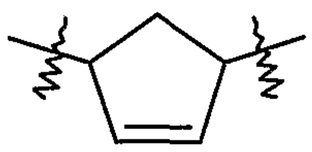
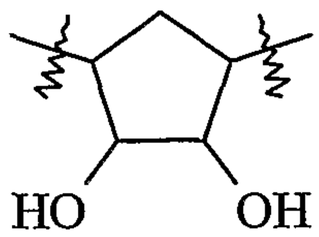
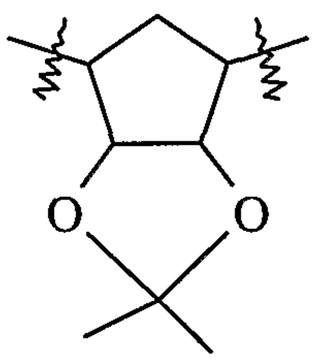
where:

A is  (olefin),  (diol), or



(acetonide);

Y is –E-C(O)R^1 or -E-NR^2R^3;

E is a bond or -CH_2-;

R^1 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted heterocycle, or NR^2R^4;

R^2 is independently at each occurrence hydrogen, C_1-C_6 alkyl, (C_1-C_6 alkyl)-aryl, or aryl;

R^3 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted heterocycle, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, C(O)C(O)R^9, C(O)R^5, or R^2 and R^3, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^4 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_1-C_4 alkoxy, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or R^2 and R^4, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^5 is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, optionally substituted (C_1-C_4 alkyl)-phenoxy, $(CH_2)_tC(R^6)(R^7)N(R^6)(R^8)$, or NR^2R^4;

t is 0, 1, 2, 3, or 4;

R^6 is independently at each occurrence hydrogen or C_1-C_6 alkyl;

R^7 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^8 is independently at each occurrence hydrogen, C_1-C_6 alkyl, optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_6-C_{10} bicycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, $C(O)OR^9$, $C(O)R^{10}$, or R^6 and R^8, together with the nitrogen to which they are attached, combine to form an optionally substituted N-heterocycle;

R^9 is independently at each occurrence hydrogen, C_1-C_6 alkyl, C_3-C_8 cycloalkyl, optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, or optionally substituted heterocycle;

R^{10} is independently at each occurrence C_1-C_6 alkyl, C_1-C_6 alkoxy, (C_1-C_4 alkoxy)-aryl, (C_1-C_4 alkoxy)-heterocycle, (C_1-C_4 alkoxy)-$SiCH_3$, optionally substituted (C_1-C_4 alkyl)-(C_3-C_8 cycloalkyl), optionally substituted (C_1-C_4 alkyl)-aryl, optionally substituted aryl, diphenylmethyl, optionally substituted (C_1-C_4 alkyl)-CO-aryl, optionally substituted (C_1-C_4 alkyl)-heterocycle, optionally substituted heterocycle, or optionally substituted (C_1-C_4 alkyl)-phenoxy; or a pharmaceutical salt thereof;

in combination with an effective amount of one or more oncolytic agents.

4. The method according to Claim 3 where the mammal is a human.

5. The method according to Claim 4 where the oncolytic(s) is selected from: doxorubicin, daunorubicin, epirubicin, vincristine, and etoposide.

6. The method according to Claim 4 where the neoplasm is of the Wilm's type, bladder, bone, breast, lung(small-cell), testis, or thyroid or the neoplasm is associated with acute lymphoblastic and myeloblastic leukemia, neuroblastoma, soft tissue sarcoma, Hodgkin's and non-Hodgkin's lymphomas, and bronchogenic carcinoma.

7. A pharmaceutical formulation comprising a compound of formula I as defined in Claim 1 in admixture with one or more pharmaceutical carriers, diluents, or excipients therefor.

8. A pharmaceutical formulation according to Claim 7 which comprises one or more oncolytic agents.

9. A product containing a compound of formula I as defined in Claim 1 and one or more oncolytic agents as a combined preparation for simultaneous, separate or sequential use in cancer therapy.

10. The formulation according to Claim 8 or a product of Claim 9 where the oncolytic(s) is selected from: doxorubicin, daunorubicin, epirubicin, vincristine, and etoposide.

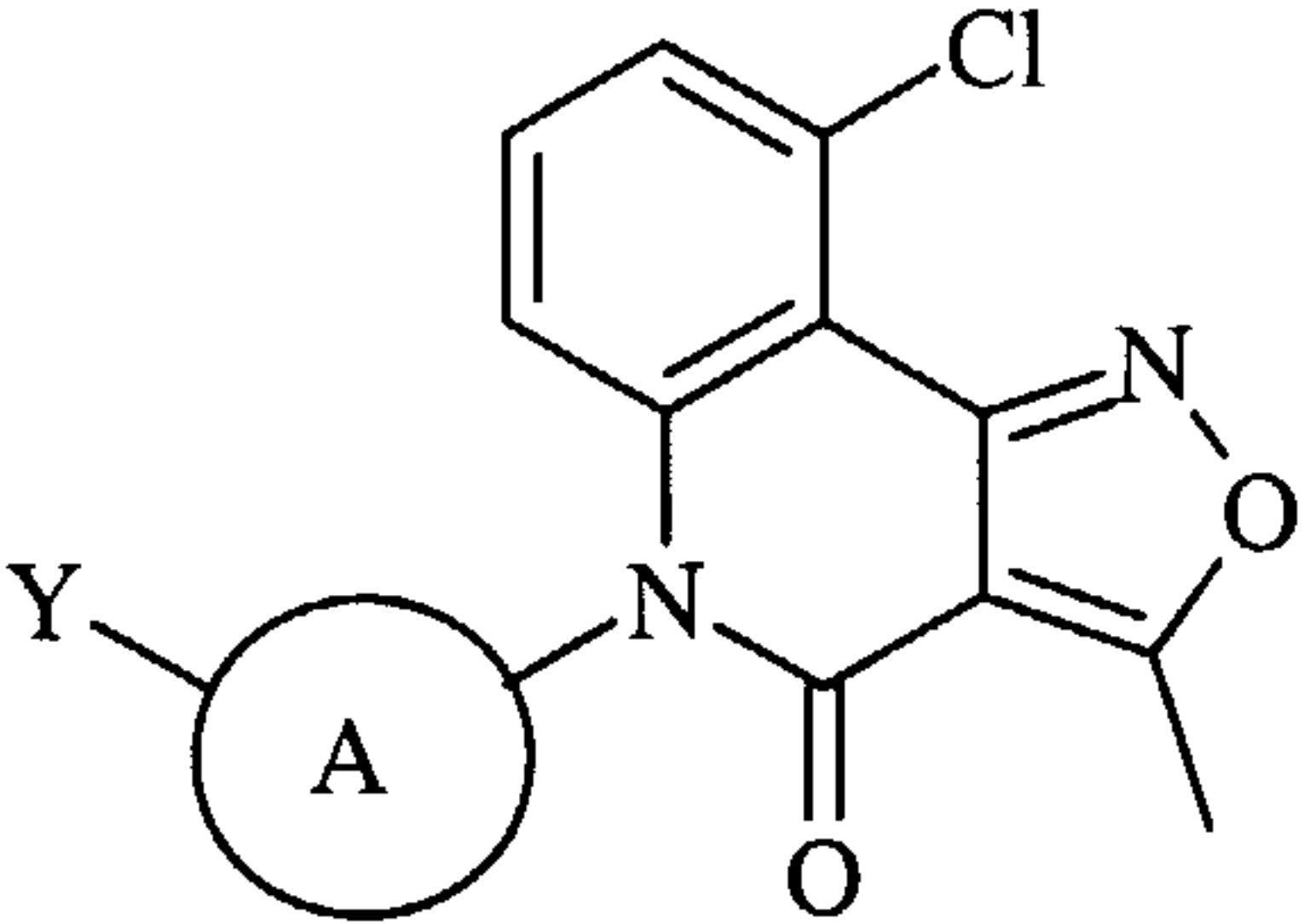
7. A compound according to claim 1 for use in the treatment of the human or animal body by therapy.

8. Use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for inhibiting MRP1.

9. Use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for inhibiting a resistant neoplasm, or a neoplasm susceptible to resistance, which inhibition comprises administering an effective amount of said compound of formula I in combination with an oncolytic agent.

10. Use according to claim 13 wherein the compound of formula I and the oncolytic agent are administered simultaneously, separately or sequentially.

11. Use according to claim 13 or 14 wherein the one or more oncolytic agents are selected from doxorubicin, daunorubicin, epirubicin, vincristine, and etoposide.



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