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(54) Title: NOVEL METHOD OF USE OF MULTIDRUG RESISTANCE MODULATORS

(57) Abstract: A method of suppressing multidrug resistance in a mammal by administering an effective amount of a 10,11-methanodibenzosuberane derivative or a pharmaceutically acceptable salt thereof.

NOVEL METHOD OF USE OF MULTIDRUG RESISTANCE MODULATORS

Multidrug resistance is a major obstacle to the successful treatment of cancer. Tumors may be drug-resistant due to the overexpression of P-glycoprotein (Pgp, ABCB1) or multidrug resistance associated protein (MRP) (see e.g. Barrand, M. A.; Bagrij, T.; Neo, S.-Y., *Gen. Pharmac.* **1997,** 28, 639). Both are members of the ATP-binding cassette (ABC) transporter superfamily that is likely to contain several hundred members (see e.g. Ling, V., *Cancer Chemother. Pharmacol.* **1997,** 40, S3). They are membrane-bound glycoproteins of molecular masses of 170 kDa for Pgp with 12 transmembrane-spanning domains (TMs) and 190 kDa for MRP with as many as17 TMs (see e.g. Stride, B. D.; Cole, S. P. C.; Deeley, R. G., *J Biol. Chem.* **1999,** 274, 22877 and Hipfner, D. R.; Almquist, K. C.; Leslie, E. M.; Gerlach, J. H.; Grant, C. E.; Deeley, R. G.; Cole, S. P. C., *J Biol Chem* **1997,** 272, 23623). Although the transporters show 50% identity, overexpression of either transport protein confers resistance to a wide array of structurally diverse oncolytics that includes Vinca alkaloids, anthracyclines, taxanes and podophyllotoxins (see e.g. Bosch, I.; Croop, J. M., *Cytotechnology* **1998,** 27, 1; Cole, S. P. C.; Sparks, K. E.; Fraser, K.; Loe, D. W.; Grant, C. E.; Wilson, G. M.; Deeley, R. G., *Cancer Res.* **1994,** 54, 5902; and Grant, C. E.; Valdimarsson, G.; Hipfner, D. R.; Almquist, K. C.; Cole, S. P. C.; Deeley, R. G., *Cancer Res.* **1994,** 54, 357).

One approach to overcome resistance by these mechanisms is to develop modulators, compounds that are non-cytotoxic that inhibit their function. When a modulator is present in the growth medium, the sensitivity of the resistant cells to the oncolytic can be enhanced by inhibiting the efflux mechanism so that the cells accumulate more drug thereby becoming more drug sensitive. Tsuruo was first to show that the sensitivity of Pgp-expressing multidrug-resistant cells was enhanced by the presence of the calcium-channel blocker, verapamil. Subsequently, cyclosporin A was also identified as a Pgp modulator. Later, other analogs were developed specifically that lacked other pharmacological effects. This included development of the R-isomer of verapamil, that

lacked cardiotoxic effects and PSC-833, a non-immunosuppressive analog of cyclosporin A.

One of the most potent Pgp modulators to date is (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride (disclosed in US Patent Number 5,654,304, incorporated by reference herein), a derivative of MS-027 that contains a difluorocyclopropyl substitution in the dibenzosuberane moiety. Like other second generation modulators, this compound was developed without other pharmacological effects; the compound is highly selective; and the compound is one for which Pgp has a higher affinity.

Although it is known that certain compounds reverse multidrug resistance in tumor cells (see for example U.S. Pat. No. 5,654,304), discovery of compounds, which would prevent multidrug resistance from forming, are highly desirable.

Unexpectedly and surprisingly, we have discovered that certain 10,11-methanodibenzosuberane derivatives function to suppress the development of multidrug resistance.

An aspect of the present invention is a method of suppressing multidrug resistance in a mammal by administering an effective amount of a 10,11-methanodibenzosuberane derivative or a pharmaceutically acceptable salt thereof.

The current invention concerns the discovery of a method of suppressing the development of multidrug resistance in a mammal by administering an effective amount of a 10,11-methanodibenzosuberane derivative or a pharmaceutically acceptable salt thereof.

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The term "alkyl" refers to a fully saturated monovalent radical containing only carbon and hydrogen, and which may be a cyclic, branched or straight chain radical. This term is further exemplified by radicals such as methyl, ethyl, t-butyl, pentyl, pivalyl, heptyl and adamantyl.

The term "lower alkyl" refers to a cyclic, branched or straight chain monovalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals

as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, i-butyl (or 2-methylpropyl), cyclopropylmethyl, i-amyl, n-amyl, and hexyl.

The term "alkylene" refers to a fully saturated divalent radical containing only carbon and hydrogen, and which may be a branched or straight chain radical. This term is further exemplified by radicals such as methylene, ethylene, n-propylene, t-butylene, i-pentylene, and n-heptylene.

The term "lower alkylene" refers to a divalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals as methylene, ethylene, n-propylene, i-propylene, n-butylene, t-butylene, i-butylene (or 2-methylpropylene), isoamylene, pentylene, and n-hexylene.

The term "lower acyloxy" refers to the group --O--C(O)--R' where R' is lower alkyl.

The term "aryl" refers to a monovalent unsaturated aromatic carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl), which can optionally be mono-, di- or tri-substituted, independently, with fluoro, chloro, bromo, trifluoromethyl, cyano, nitro and/or difluoromethoxy.

The term "heteroaryl" refers to a monovalent unsaturated aromatic carbocyclic radical having at least one hetero atom, such as N, O or S, within the ring, such as quinolyl, benzofuranyl and pyridyl.

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

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances in which it does not.

A "pharmaceutically acceptable salt" may be any salt derived from an inorganic or organic acid. The term "pharmaceutically acceptable anion" refers to the anion of such acid addition salts. The salt and/or the anion are chosen not to be biologically or otherwise undesirable.

The anions are derived from inorganic acids, such as hydrochloric acid, hydrobromic acid, sulfuric acid (giving the sulfate and bisulfate salts), nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid,

glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, salicylic acid, p-toluenesulfonic acid and the like.

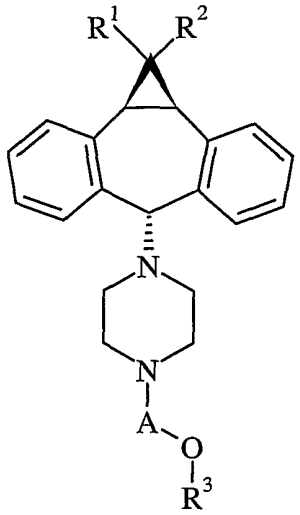
The term "suppress" as it relates to the present invention refers to inhibiting the usual growth and development of multidrug resistance in a mammal.

The term "inhibiting" as it relates to the present invention refers to prohibiting, restraining, or slowing the progression of the expression of multidrug resistance.

As used herein, the term "effective amount" as it relates to the present invention refers to an amount of a compound or drug, which is capable of performing the intended result. For example, an effective amount of (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride that is administered in an effort to suppress multidrug resistance is that amount which is required to inhibit the usual growth and development of multidrug resistance in a mammal.

Throughout this document, the person or animal to be treated will be described as a "mammal", and it will be understood that the most preferred subject is a human. The use of the present compounds in non-human animals is contemplated. It will be understood that the dosage ranges for other animals will necessarily be quite different from the doses administered to humans, and accordingly that the dosage ranges described be recalculated. For example, a small dog may be only $1/10^{th}$ of a typical human's size, and it will therefore be necessary for a much smaller dose to be used. The determination of an effective amount for a certain non-human animal is carried out in the same manner described below in the case of humans, and veterinarians are well accustomed to such determinations.

The term "10,11-methanodibenzosuberane derivatives" as it relates to the present invention refers to a class of chemical compounds of formula I:



wherein A is $-CH_2-CH_2-$, $-CH_2-CHR^a-CH_2-$, $-CH_2-CHR^a-CH_2-CH_2-$, and R^a is OH;

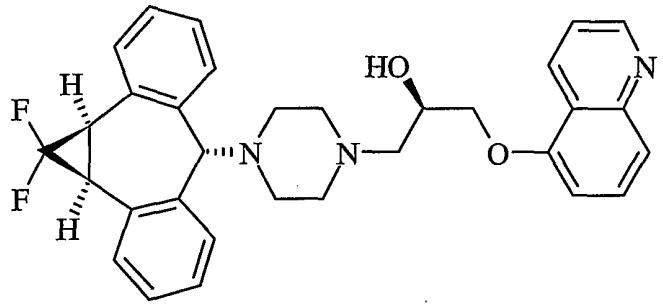
R^1 is H, F, Cl, or Br;

R^2 is H, F, Cl, or Br; and

R^3 is heteroaryl or phenyl, each optionally substituted with F, Cl, Br, CF_3, CN, NO_2, or $OCHF_2$. These compounds are disclosed in US Patent No. 5,654,304 (hereinafter referred to as '304). The skilled artisan will be knowledgeable of the methods of preparing the 10,11-methanodibenzosuberane derivatives contemplated for use in the present invention, for example see the '304 patent and PCT publication WO00/75121. Preferred compounds of the invention include *anti*-1-(10,11-dichloromethanodibenzosuber-5-yl)-4-formylpiperazine, *syn*-1-(10,11-dichloromethanodibenzosuber-5-yl)-4-formylpiperazine, *anti*-1-(10,11-dibromomethanodibenzosuber-5-yl)-4-formylpiperazine, *syn*-1-(10,11-dibromomethanodibenzosuber-5-yl)-4-formylpiperazine, *anti*-1-(10,11-methanodibenzosuber-5-yl)-4-formylpiperazinesyn-1-(10,11-methanodibenzosuber-5-yl)-4-formylpiperazine, *anti*-1-(10,11-chlorofluoromethanodibenzosuber-5-yl)-4-formylpiperazine, *syn*-1-(10,11-chlorofluoromethanodibenzosuber-5-yl)-4-formylpiperazine, *syn*-1-(10,11-difluoromethanodibenzosuber-5-yl)piperazine, *anti*-1-(10,11-dichloromethanodibenzosuber-5-yl)piperazine, *syn*-1-(10,11-dichloromethanodibenzosuber-5-yl)piperazine, *anti*-1-(10,11-dibromomethanodibenzosuber-5-yl)piperazine, *syn*-1-(10,11-dibromomethanodibenzosuber-5-yl)piperazine, *anti*-1-(10,11-methanodibenzosuber-5-

yl)piperazine, *syn*-1-(10,11-methanodibenzosuber-5-yl)piperazine, *anti*-1-(10,11-chlorofluoromethanodibenzosuber-5-yl)piperazine, *syn*-1-(10,11-chlorofluoromethanodibenzosuber-5-yl)piperazine, (2R,S)-*anti*-5-{3-(4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl] -2-hydroxypropoxy}quinoline, (2R,S)-*syn*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl] -2-hydroxypropoxy}quinoline, (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]- 2-hydroxypropoxy}quinoline, (2S)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]- 2-hydroxypropoxy}quinoline, (2R)-*syn*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2 -hydroxypropoxy}quinoline, (2S)-*syn*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2 -hydroxypropoxy}quinoline, (2R,S)-*anti*-5-{3-[4-(10,11-dichloromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline, (2R)-*anti*-5-{3-[4-(10,11-dichloromethanodibenzosuber-5-yl)piperazin-1-yl]- 2-hydroxypropoxy}quinoline, (2S)-*anti*-5-{3-[4-(10,11-dichloromethanodibenzosuber-5-yl)piperazin-1-yl]- 2-hydroxypropoxy}quinoline, (2R,S)-*anti*-5-{3-[4-(10,11-methanodibenzosuber-5-yl)piperazin-1-yl]-2-hydr oxypropoxy}quinoline, (2R,S)-*anti*-4-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}benzofurazan, (2R,S)-*syn*-4-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl] -2-hydroxypropoxy}benzofurazan, (2R,S)-*anti*-4-{3-[4-(10,11-dichloromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}benzofurazan, (2R,S)-*anti*-1-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl) piperazin-1-yl]-2-hydroxypropoxy}-2-nitrobenzene, (2R,S)-*anti*-1-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}-2-chlorobenzene, (2R,S)-*anti*-1-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}-2-difluoromethoxybenzene, (2R,S)-*anti*-3-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}pyridine, (2R,S)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-propoxy}quinoline, (2R,S)-*anti*-5-{4-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-butoxy}quinoline, (2R,S)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-propoxy}-2-nitrobenzene, (2R,S)-

anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-3-hydroxybutoxy}quinoline, and more preferably (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline and it's trihydrochloride salt, as is represented by formula II:



A further preferred embodiment of the present invention encompasses the crystal form of (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride ("Hydrate I"), see e.g. , WO00/75121.

When employed as a pharmaceutical, Hydrate I is usually administered in the form of pharmaceutical compositions. These compositions can be administered by a variety of routes including oral, rectal, transdermal, and intranasal. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise Hydrate I.

The term "substantially pure" refers to the crystal phase purity of Hydrate I. In practice we have found that small amounts of other crystalline forms do not adversely affect the advantageous properties of Hydrate I. According to the present invention substantially pure refers to Hydrate I, which is greater than 90%, preferably greater than 95% of the total crystalline material.

This invention also includes pharmaceutical compositions which contain Hydrate I (referred to as "active ingredient" herein below) associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient 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 or semi-solid, which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of

tablets, pills, powders, lozenges, sachets, cachets, emulsions, aerosols, ointments containing, for example, up to 10% by weight of the active compound, tablet, soft and hard gelatin capsules, suppositories, and sterile packaged powders.

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, sterile 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 propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions 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 compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 mg to about 30 mg, of the active ingredient. The term "unit dosage forms" 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. Preferably, Hydrate I is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

Hydrate I is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, 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, the severity of the patient's symptoms, and the like.

For preparing compositions such as tablets, the active ingredient is mixed with a pharmaceutical excipient to form a solid pre-formulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these

pre-formulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid pre-formulation is then subdivided into unit dosage forms of the type described above containing from, for example, 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 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.

Compositions for inhalation or insufflation include liquids, suspensions, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described supra. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable 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 face masks tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions 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 composition 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 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. 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.

Other suitable formulations for use in the present invention can be found in Remington's Pharmaceutical Sciences, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

As noted above, Hydrate I is suitable for use in a variety of drug delivery systems described above. Additionally, in order to enhance the in vivo serum half-life of the present compound, it may be encapsulated, introduced into the lumen of liposomes, prepared as a colloid, or other conventional techniques may be employed which provide an extended serum half-life of the compounds. A variety of methods are available for preparing liposomes, as described in, e.g., Szoka, et al., U.S. Patent Nos. 4,235,871, 4,501,728 and 4,837,028 each of which is incorporated herein by reference.

The amount of Hydrate I administered to the patient will vary depending upon the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are administered to a patient already suffering from Alzheimer's disease in an amount sufficient to at least partially arrest further onset of the symptoms of the disease and its

complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the degree or severity of the disease state in the patient, the age, weight and general condition of the patient, and the like. Preferably, for use as therapeutics, Hydrate I is administered at dosages ranging from about 1 to about 500 mg/kg/day.

Methods

Modulation of Pgp.

When the effect of (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride was examined on the multidrug resistant CEM/VLB_{100} cells, cells become more sensitive to anticancer agents, doxorubicin, etoposide, vinblastine, and paclitaxel, representing four different classes of agents.

Table 1.

Effect of (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride on the Cytotoxicity of Anticancer Agents

Cell line	Doxorubicin	Etoposide (fold-shift)	Vinblastine	Paclitaxel
CEM/VLB_{100}				
0.1 μM	13	19	440	1200
2.0 μM	11	20	450	1600
CCRF-CEM				
2.0 μM	<1	ND	<1	<1

ND, not determined.

Table 1 summarizes the enhancement of drug sensitivity as the "fold shift" in the EC_{50} of the oncolytic measured in the absence or presence of (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline

trihydrochloride. Resistant CEM/VLB_{100} cells become more sensitive to these anticancer agents in the presence of 0.1 μM or 2 μM (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride and the drug sensitive, parental cells, CCRF-CEM, are not effected by the modulator. This modulator displaces the equilibrium binding of [^{3}H]vinblastine to CEM/VLB_{100} membranes in the presence of ATP with an apparent K_i of 59 nM (Table 3). Furthermore, when the equilibrium binding of [^{3}H] (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride is measured directly the K_d is 73 nM with a B_{max} of 79 pmol/mg prot. which is very close to the B_{max} of [^{3}H]vinblastine binding measured in the absence of ATP. This suggests that (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride binds to a conformation of Pgp that does not have ATP bound. Although Pgp has a high affinity for (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride, the modulator does not serve as a substrate. [^{3}H]Vinblastine showed enhanced effluxed from drug resistant CEM/VLB_{100} cells compared to that of drug sensitive CCRF-CEM cells. In contrast, the [^{3}H](2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride was not effluxed from the cells over a 3 hour time course. Therefore, the modulator does not serve as a substrate for the Pgp pump and consequently (2R)-*anti*-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride would be expected to have a long duration of action once the modulator is associated with the cells as previously reported Dantzig, A. H.; Shepard, R. L.; Cao, J.; Law, K. L.; Ehlhardt, W. J.; Baughman, T. M.; Bumol, T.; Starling, J. J., *Cancer Res.* **1996,** 56, 4171.

Effect on cytochrome P-450.

The cytochrome P-450 (CYP) family of enzymes is involved in the oxidative metabolism of many drugs including the four classes of anticancer agents. Of the 15

members of this family, four CYP450 forms are important in the oxidative metabolism of greater than 90% of the metabolism of drugs.

Table 2.

Removal of Natural Product Oncolytics

	Anthracyclines	Podophyllotoxins	Vincas	Taxanes
Pgp	+	+	+	+
MRP1	+	+	+	
MRP2	+	+		
CYP3A	+	+	+	+
CYP2C				+

+ indicates that the protein listed on the left can use the anticancer agent as a substrate to either pump it out of the cell or metabolize it

Table 2 lists specificity of two forms important for the oxidative metabolism of natural product anticancer agents along with the specificity of the aforementioned ABC transporters. CYP3A metabolizes greater than 50% of drugs and all four classes of oncolytics that are pumped by Pgp. An overlap in substrate specificity for CYP3A and Pgp has been reported previously (Wacher, V. J.; Wu, C. Y.; Benet, L. Z., *Mol Carcinog.* 1995, 13, 129). The affinity of these CYPs for (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride using form-selective substrates of each isozyme by enzyme kinetic determinations was determined (Dantzig, A. H.; Shepard, R. L.; Law, K. L.; Tabas, L.; Pratt, S.; Gillespie, J. S.; Binkley, S. N.; Kuhfeld, M. T.; Starling, J. J.; Wrighton, S. A., *JPET* 1999, 290, 854.). The apparent K_i of (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride for CYP3A, CYP2C9, and CYP2D6 were determined to be 3.8, 12.3, and 25.3 μM, respectively. The K_i for inhibition of the equilibrium binding of [^{3}H]vinblastine to Pgp is 0.059 μM for Pgp. CYP1A2 was inhibited only slightly in the presence of up to 50 μM (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-

hydroxypropoxy}quinoline trihydrochloride. Thus, (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride is a much more potent modulator of Pgp by 60 fold or greater than of these CYP450s.

Enhanced in vivo efficacy.

(2R)-anti-5-{3-[4-(10,11-Difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride was examined for its ability to sensitize multidrug resistant tumor cells that overexpress Pgp in vivo [13, 14]. P388/ADR is a murine Pgp-expressing multidrug resistant leukemia cell line that has been extensively studied for its ability to be sensitized to anticancer agents by Pgp modulators. The coadministration of (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride i.v. enhanced survival significantly when given intravenously in combination with the oncolytics, daunorubicin, doxorubicin, or etoposide, administrated i.p. to mice implanted with P388/ADR. The coadministration of 20 mg/kg (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride and 0.3 mg/kg daunorubicin increased survival by 1.6-fold compared to daunorubicin alone. Survival was significantly enhanced with the coadministration of 20 mg/kg (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride with 4 mg/kg doxorubicin or 20 mg/kg etoposide compared to the single agent alone. This model indicates that the modulator is active when injected at a site distal to the tumor implant.

These results could be due to the ability of (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride to inhibit Pgp in the tumor cells or alternatively due to inhibition of Pgp in normal tissue. Normal tissue distribution of Pgp throughout the body, includes the adrenal gland, kidney, liver, pancreas, intestines, as well as the lungs and blood brain barrier. Inhibition of Pgp could alter the absorption and elimination of anticancer agents by the kidney and liver, for example, and would result in the anticancer agent having a

longer half life and therefore greater exposure. If this were the case, then the increased efficacy seen in vivo could be due simply to a higher exposure of the tumor to the anticancer agent. Mice were coadministered the modulator with i.p. doses of the oncolytic and the resulting plasma levels were measured over an 8 hour time course. Plasma levels were measured as the "area under the curve", AUC, of the anticancer agent for mice treated with 5 mg/kg doxorubicin or 30 mg/kg etoposide with either saline (control) or 30 mg/kg (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride or 100 mg/kg cyclosporin A. Cyclosporin A had a significant effect on the AUC of both oncolytics, while (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride had little to no effect on either drug. This indicates that the enhanced survival observed in the P388/ADR implanted mouse model is due to direct modulation of Pgp at the tumor site.

Because P388 cells grown in vivo are insensitive to treatment with paclitaxel, the efficacy of paclitaxel was examined in another tumor model, human non-small cell lung cancer. Paclitaxel was also efficacious to Pgp-expressing UCLA-P3.003VLB tumors when coadministered with (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride. The combination of 20 mg/kg paclitaxel and 30 mg/kg (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride reduced tumor mass at days 12 and 19 ($p<0.05$) without significant change in body weight or effect on the plasma levels of paclitaxel. In addition, treatment of mice bearing the parental, non-small cell lung tumor, UCLA-P3 with paclitaxel was quite effective. The group of mice that was treated with both 30 mg/kg (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride plus 10 mg/kg paclitaxel had a reduction in tumor mass for an additional 14 days as compared to the group that was treated with 10 mg/kg paclitaxel alone. Thus, the coadministration of the Pgp modulator with paclitaxel resulted in the suppression of the development of resistance. These data indicate that the administration of the 10,11-

methanodibenzosuberane derivatives will be useful at the initiation of cancer chemotherapy before the development of drug-resistant tumor cells.

We Claim:

1. A method of suppressing multidrug resistance in a mammal by administering an effective amount of a 10,11-methanodibenzosuberane derivative or a pharmaceutically acceptable salt thereof.

2. The method according to claim 1 wherein multidrug resistance is due to overexpression of Pgp.

3. The method according to Claim 1 or 2 wherein the mammal is human.

4. The method according to any one of Claims 1-3 wherein the 10,11-methanodibenzosuberane derivative is (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride.

5. A use of a 10,11-methanodibenzosuberane derivative, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the suppression of multidrug resistance.

6. The method according to claim 5 wherein multidrug resistance is due to overexpression of Pgp.

7. The method according to Claim 5 or 6 wherein the mammal is human.

8. The method according to any one of Claims 5-7 wherein the 10,11-methanodibenzosuberane derivative is (2R)-anti-5-{3-[4-(10,11-difluoromethanodibenzosuber-5-yl)piperazin-1-yl]-2-hydroxypropoxy}quinoline trihydrochloride.