Abstract: ABSTRACT OF THE INVENTION The present invention provides, in one aspect, a method of inhibiting the expression of a multi-drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor. In another aspect, it provides a method of inhibiting the production of a protein expressed by a multi-drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor. In another aspect, the present invention provides a method of enhancing the effectiveness of a chemotherapeutic agent in an animal having cancer, which comprises administering to said animal an effective amount of the chemotherapeutic agent and at least one cholesterol absorption inhibitor. Further, there are provided compositions and kits for use in cancer treatment which comprise at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor.
TITLE: METHOD OF INHIBITING THE EXPRESSION OF MULTI-DRUG RESISTANCE GENES AND INHIBITING THE PRODUCTION OF PROTEINS RESULTING FROM THE EXPRESSION OF SUCH GENES THEREBY ENHANCING THE EFFECTIVENESS OF CHEMOTHERAPEUTIC AGENTS TO TREAT CANCERS

FIELD OF THE INVENTION
This present invention relates to the field of cancer treatment.

BACKGROUND OF THE INVENTION
It has been found that treatment of human malignancies with certain chemotherapeutic agents is frequently ineffective due to either endogenous or acquired tumour cell resistance. The problem is further exacerbated since many of the mechanisms of tumour cell resistance results in collateral resistance to a wide range of structurally unrelated chemotherapeutic drugs with different mechanisms of action. This condition is referred to as multi-drug resistance. This is a significant problem in cancer therapy and appears to be the main impediment to increasing cure rates with current chemotherapeutic agents.

Approximately 2.04 million new cases of cancer are reported annually in the seven major worldwide pharmaceutical marketplaces alone (U.S., Japan, Germany, Italy, France, Spain, UK)\textsuperscript{1}. Unfortunately, of these, only 5-10\% will respond successfully to chemotherapy. Approximately 40-45\%, or more than 800,000 patients annually, will develop multiple drug resistance to one or more chemotherapeutic programs. It is no exaggeration to say that the emergence of multiple drug resistance is a major obstacle to successful cancer chemotherapy worldwide. It is a problem heretofore not successfully addressed.

At the molecular and cellular level, several mechanisms likely account for multi-drug resistance. Suggested cellular mechanisms for expression of tumor cell drug resistance to various chemotherapeutic agents include: decreased drug uptake or increased drug efflux, altered redox potential, enhanced DNA repair, increased drug sequestration mechanisms or amplification of the drug-target protein.
To date, two genes encoding multidrug-resistance export proteins have been identified in the human genome. The first of these, MDR1, encodes P-glycoprotein, a 170 kDa multigene-spanning transmembrane protein belonging the ATP Binding Cassette (ABC) Transporter protein superfamily. It is suggested that one mechanism by which tumor cells acquire MDR is by over expression of P-glycoprotein (Pgp).

P-glycoprotein likely acts by rapidly pumping hydrophobic chemotherapeutic agents out of the tumor cells, thereby decreasing intracellular accumulation of certain chemotherapeutic agents below their cytostatic concentrations. One *in vitro* solution is to increase the chemo drug concentration. However, since cancer chemotherapeutic agents are already administered at their maximally tolerated range *in vivo*, increasing the doses is an unacceptable solution leading, on most cases to extreme toxicity.

P-glycoprotein is structurally similar to the cystic fibrosis transporter protein, the major histocompatibility complex-linked peptide transporter, and a non-P-glycoprotein-related multidrug resistance protein (MRP). P-glycoprotein is expressed in diverse sites including the normal human adrenal cortex, the luminal aspect of bile canaliculi and colonic epithelium, the renal tubular epithelium and the endothelial cell of the blood-brain and blood testicular barriers. The function of the P-glycoproteins at these sites is unclear but appears to function as an energy dependent pump of broad specificity possibly related to secretion of hormones and protection against toxins. Expression of the P-glycoprotein can actively efflux a large number of hydrophobic, and heterocyclic cancer chemotherapeutic agents including adriamycin (doxorubicin), colchicine, colcemid, etoposide, paclitaxel, vincristine, vinblastine as well as others.

P-glycoproteins are encoded by a highly conserved family of genes. The MDR-1 gene encodes class I P-glycoprotein that confers multidrug resistance in humans. The pgp-1 and pgp-2 genes in hamsters and the mdr-3 and MDR-1 genes in mice encode the class I and II proteins, both of which confer multidrug resistance in rodents.
Several lines of evidence support cause-effect association between increased P-glycoprotein and multidrug resistance in vitro. Structural features of the protein are characteristic of an energy-dependent efflux pump. Over expression of the protein is associated with multidrug resistance. There has also been discovered a positive correlation between the degree of expression of P-glycoprotein and a drug-resistant phenotype.

Cancers of the colon, kidney, breast, adrenal cortex and liver frequently show high levels of P-glycoprotein at diagnosis, even though the patients have not been previously treated with anti cancer drugs. In a variety of tumor types, relapse or disease progression following the initial chemotherapy regime is refractory to treatment, and frequently involves MDR and expression of elevated levels of MDR-1. Drug resistance can manifest in cells with low gene copy number and can be due to regulation at the transcriptional, translational or post-translational level. Although in vitro studies often show amplification of MDR-1 as the cause for drug resistance, elevated gene copy number is apparently rare in human tumors.

Increased levels of MDR-1 gene product or its mRNA have been associated with poor prognosis in a number of human tumor studies. Clinical studies in breast carcinoma have shown that a significant percentage of patients expressed large levels of P-glycoprotein and the low P-glycoprotein expressing patients had significantly better rates of response to chemotherapy and progression-free survival than did high expressing groups. Sixty-eight percent of the tumors in a retrospective study of colon carcinoma expressed high levels of P-glycoprotein and over expression correlated with aggressiveness measured by blood cell invasion and metastasis. In three clinical studies involving acute myelogenous leukemia, the MDR-1 negative groups all had higher rates of complete remission and longer durations of disease-free survival than the MDR-1 positive groups.

In three studies in neuroblastoma, investigators have shown that MDR-1 expression frequently occurs in non localized and post treatment tumors and correlates with poor prognosis. Likewise in rhabdomyosarcoma, P-glycoprotein levels were
correlated with increased malignant staging and the P-glycoprotein negative group had significantly better rate of response as well as longer durations of relapse-free survival and overall survival than did the P-glycoprotein positive patients. P-glycoprotein expression was also highly correlated with advanced and refractory myeloma. Together this data suggest that there is a correlation of P-glycoprotein expression with clinical prognosis in a variety of human malignancies.

Drugs that are alleged to reverse MDR have been shown to have mechanisms by decreasing drug efflux (e.g., calcium channel blockers verapamil, diltiazem and nicardipine, calmodulin inhibitors reserpine, quinidine or quineine, cyclosporin A, cyclosporin derivatives, or FK506 and rapamycin. Clinical reversal of resistance for most of these agents has been limited, mainly due to clinically toxic concentration levels that were needed to achieve a reversal of resistance.

It is clear that the expression of multiple drug resistance genes, the production of their protein products and the acquisition of a MDR phenotype significantly constrains the chemotherapeutic choices available to the clinician, and significantly worsens prognosis for the afflicted individual.

Therefore, there is a need in the art to find more effective MDR reversing and/or inhibiting agents which are non-toxic.

It is an object of the present invention to provide novel compounds which may obviate or mitigate the disadvantages of prior known compounds used to overcome MDR.

It is an object of the present invention to provide novel compositions which may obviate or mitigate the disadvantages of prior known compositions used to overcome MDR.

**SUMMARY OF THE INVENTION**
The present invention provides, in one aspect, a method of inhibiting the expression
of a multi-drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor.

The present invention provides, in another aspect, a method of enhancing the effectiveness of a chemotherapeutic agent in an animal having cancer, which comprises administering to said animal an effective amount of at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor.

The present invention provides, in yet another aspect, a method of reversing a multi-drug resistance phenotype exhibited by an animal cell which comprises exposing the cell to an effective amount of at least one cholesterol absorption inhibitor.

The present invention provides, in yet another aspect, a method of inhibiting the production of a protein expressed by a multiple drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor.

The present invention provides, in yet another aspect, a composition for use in cancer treatment which comprises at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor.

The present invention provides, in yet another aspect, a kit comprising at least two separate components:

a) a composition comprising at least one cholesterol absorption inhibitor; and

b) a composition comprising at least one chemotherapeutic agent;

along with instructions describing the administration of each composition.

The crux of the present invention is the provision and co-administration (though not necessarily concurrently or proximally consecutively) of cholesterol absorption inhibitors and chemotherapeutic agents. It has been found that cholesterol absorption inhibitors effectively inhibit the expression of multi-drug resistance genes. It is suggested that the reduction in production of proteins expressed by these genes
prevents the efflux of cancer chemotherapeutic agents from animal cells, maximizing their effectiveness. This is important due to the documented problems relating to the rendering virtually ineffective of heretofore promising chemotherapeutic agents by the multiple drug resistance mechanism.

Some of the preferred cholesterol absorption inhibitors of the present invention (those depicted below including those in formulae (i) through (iv) comprise an ascorbyl moiety. These particular compounds have numerous additional advantages. In particular, solubility in aqueous solutions such as water is improved by the ascorbyl moiety thereby allowing oral administration per se. Likewise, other modes of administration are facilitated. Accordingly, these selected compounds of the present invention can be prepared and used as such or they can be easily incorporated into pharmaceutical preparations, optionally in conjunction with the selected chemotherapeutic agent, regardless of whether such preparations are water-based. This enhanced solubility generally translates into lower administration dosages of the compounds in order to achieve the desired therapeutic effect.

These effects and other significant advantages will become apparent herein below.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The present invention is illustrated by way the following non-limiting drawings in which:

Figure 1 is a bar graph showing the level of MDR-1 expression (normalized ratio of MDR-1/GAPDH; "GAPDH" or glyceraldehyde-3-phosphate dehydrogenase) in CaCo2 cells after treatment for one week with one of the cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 2 is a graph showing the titration for primer drop of GAPDH;

Figure 3 depicts a polymerase chain reaction (1.5% agarose gel) gel electrophoresis results for MDR-1, GAPDH;
Figure 4 is a bar graph showing an MTS- and LDH-Assay of cell viability after treatment of CaCo2 cells with one of the cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 5 a bar graph showing a BCA-Assay of protein concentration after treatment of CaCo2 with one of the cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 6 a bar graph showing the level of ABCC1 (MRP-1) expression (normalized ratio of MRP-1/GAPDH) in CaCo2 cells after treatment for one week with one of the cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 7 depicts a polymerase chain reaction (1.5% agarose gel) gel electrophoresis results for MDR-1, GAPDH; RNA isolation with TRIZOL→ RT-PCR→ PCR after treatment of CaCo2 cells with liposomal formulations of one of cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 8 is a bar graph showing the level of MDR-1 expression (normalized ratio of MDR-1/GAPDH) in CaCo2 cells after treatment for one week with one of the cholesterol absorption inhibitors described herein: a liposomal formulation of an ascorbyl stanyl phosphate ester called "FM-VP4" at 2.5, 5 and 10μm as compared to a control and empty liposomes;

Figure 9 is a Western Blot analysis of P-glycoprotein in CaCo2 cells after incubation with one of cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called "FM-VP4";

Figure 10 is an electrophoretic gel profile of sample PCR products (measuring expression profile of mdr-1 gene in Caco-2 cells as an effect of a time-dependent treatment with 10μM FM-VP4);
Figure 11 is a bar graph showing the fluorescent bands of the PCR products of Figure 10 imaged under UV light (UV-Epi Chem II) and quantified with the UVP-Labworks software;

Figure 12 is a bar graph of the effect of pre-incubation with FM-VP4 for 1 week on accumulation of Rh123 in Caco-2 monolayer. Data represents the average of ± SD. *P<0.002; **P<0.0001;

Figure 13 is a bar graph showing the effect of pre-incubation with FM-VP4 for 1 week on P-gp transport of Rh123 across Caco-2 monolayer (basolateral to apical). Data represents the average of 3 ± SD. *P<0.07; **P<0.009.

PREFERRED EMBODIMENTS OF THE INVENTION
The following detailed description is provided to aid those skilled in the art in practising the present invention. However, this detailed description should not be construed so as to unduly limit the scope of the present invention. Modifications and variations to the embodiments discussed herein may be made by those with ordinary skill in the art without departing from the spirit or scope of the present invention.

As used herein, “animal” means any member of the animal kingdom, including all mammals and most preferably humans. Veterinary use is also contemplated.

As used herein, the term "compound" is interchangeable with the terms "derivative", "structure" and "analogue".

As used herein, the terms “effective” or "therapeutically effective", are intended to qualify the amount of the compound(s) or composition administered to an animal, in particular a human, in order to elicit a biological or medical response of a cell, tissue, system, animal or mammal that is being sought by the person administering the
compound(s) or composition and which amount achieves one or more of the following goals:

a) treating or alleviating a cancer;
b) preventing, treating or alleviating tumour growth;
c) inhibiting or reducing the expression of one or more multiple drug resistance genes;
d) inhibiting or reducing the production of one or more proteins expressed by multiple drug resistance genes;
e) enhancing the effectiveness of a chemotherapeutic agent in treating a cancer; and
f) sensitizing a cell to one or more chemotherapeutic agents.

As used herein, the term "ileal bile acid transporter" or "IBAT" is synonymous with apical sodium co-dependent bile acid transporter, or ASBT.

As used herein, "benzothiepine IBAT inhibitor" means an ileal bile acid transport inhibitor which comprises a therapeutic compound comprising a 2,3,4,5-tetrahydro-1-benzothiepine 1,1-dioxide structure.

As used herein, the term "a multiple drug resistance gene" or any abbreviation thereof, refers to one or more of the following genes: ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); ABCC3 (MRP-3).

As used herein, the term "prodrug" refers to compounds that are drug precursors, which, following administration to a patient, release the drug in vivo via some chemical or physiological process (for example, a prodrug, on being brought to physiological pH or through enzyme action is converted to the desired drug form).

As used herein, the term "solvate" refers to a molecular or ionic complex of molecules or ions of solvent with those of solute (for example the compounds of formulae a) to f) or prodrugs of compounds a) to f)). Non-limiting examples of useful solvents include polar, protic solvents such as water and/or alcohols (for example, methanol).
As used herein, the term "sterol" includes all sterols without limitation, for example: (from any source and in any form: α, β and γ) sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol, poriferasterol, clionasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrillaasterol, peposterol, avenasterol, isoavenasterol, fecosterol, pollinastasterol, cholesterol and all natural or synthesized forms and derivatives thereof, including isomers.

The term "stanol" refers to, for example: (from any source and in any form: α, β and γ) saturated or hydrogenated sterols including all natural or synthesized forms and derivatives thereof, and isomers, including sitostanol, campestanol, stigmastanol, brassicastanol (including dihydrobrassicastanol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrillastanol, pepostanol, avenastanol, isoavenastanol, fecostanol, and pollinastanol.

It is to be understood that modifications to the sterols and stanols i.e. to include side chains also falls within the purview of this invention. It is also to be understood that, when in doubt throughout the specification, and unless otherwise specified, the term "sterol" encompasses both sterol and stanol. The terms "phytosterol" and "phytostanol" may also be used and refer to all plant-derived sterols or stanols respectively.

The sterols and stanols for use in forming derivatives in accordance with this invention may be procured from a variety of natural sources or they may be artificially synthesized. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sunflower oil, sesame oil and fish (and other marine-source) oils. They may also be derived from yeasts and fungi, for example ergosterol. Accordingly, the present invention is not to be limited to any one source of sterols. US Patent Serial No. 4,420,427 teaches the preparation of sterols
from vegetable oil sludge using solvents such as methanol. Alternatively, phytosterols and phytostanols may be obtained from tall oil pitch or soap, by-products of forestry practises as described in US Patent Serial No.5,770,749, incorporated herein by reference. A further method of extracting sterols and stanols from tall oil pitch is described in Canadian Patent Application Serial No. 2,230,373 which was filed on February 20, 1998 (corresponding to PCT/CA99/00150 which was filed on February 19, 1999) and US Patent Application Serial No 10/060,022 which was filed on January 28, 2002 the contents of all of which are incorporated herein by reference.

Accordingly, it is to be understood that the widest possible definition is to be accorded to the terms “sterol” and “stanol” as used herein, including, but not limited to: free sterols and stanols, esterified sterols and stanols with aliphatic or aromatic acids (thereby forming aliphatic or aromatic esters, respectively), phenolic acid esters, cinnamate esters, ferulate esters, phytosterol and phytostanol glycosides and acylated glycosides or acylglycosides. Thus, the terms “sterols” and “stanols” encompasses all analogues, which may further have a double bond at the 5-position in the cyclic unit as in most natural sterols, or one or more double bonds at other positions in the rings (for example, 6, 7, 8(9), 8(14), 14 5/7) or no double bonds in the cyclic unit as in stanols. Further, there may be additional methyl groups as, for example, in $\alpha_1$-sitosterol.

Sterols are naturally occurring compounds that perform many critical cellular functions. Sterols such as campesterol, stigmasterol and beta-sitosterol in plants, ergosterol in fungi and cholesterol in animals are each primary components of cellular and sub-cellular membranes in their respective cell types. Phytosterols, in particular, have received a great deal of attention due to their ability to decrease serum cholesterol levels when fed to a number of mammalian species, including humans.

Optionally, the compounds of the present invention are formed of naturally-derived or artificially synthesized beta-sitosterol, campestanol, sitostanol, and campesterol and each of these compounds so formed is then admixed in a pharmaceutical composition prior to delivery in various ratios. In the most preferred form, the compound of the present invention comprises a chemical linkage between one or more disodium
ascorbyl phytostanyl phosphates (referred to herein as “FM-VP4”) which comprises two major components: disodium ascorbyl campestanol phosphate (“DACP”) and disodium ascorbyl sitostanyl phosphate (“DASP”).

Within the scope of the present invention, "cholesterol absorption inhibitor" refers to any compound having a negative effect on cholesterol transport, uptake or absorption, by whatever mechanism and includes any compound which inhibits bile acid reabsorption or transport.

Preferably, the cholesterol absorption inhibitor comprises one or more sterols, stanols or mixtures thereof or derivatives thereof as described herein. Without limiting the generality of the foregoing, this includes all free sterols and stanols, and all sterol and stanol aliphatic and aromatic esters, sterol and stanol phenolic acid esters, sterol and stanol cinnamate esters, sterol and stanol ferulate esters, sterol and stanol glycosides, sterol and both stanol acylated glycosides or acylglycosides.

In a most preferred form, the cholesterol absorption inhibitor comprises one or more derivatives or compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

i)

\[
R_4 \overset{\text{O}}{\underset{\text{P}}{\overset{\text{O}}{\underset{\text{R}}{\underset{\text{OH}}{\text{IV}}}}}}
\]

ii)

\[
R_4 \overset{\text{O}}{\underset{\text{C}}{\overset{\text{C}}{\underset{\text{O}}{\overset{\text{R}}{\text{IV}}}}}}
\]

iii)

\[
R_4 \overset{\text{R}}{\text{IV}}
\]

iv)
\[ R_4 \longrightarrow (CH_2)_n \biggarrow \biggarrow O \longrightarrow C \longrightarrow O \longrightarrow R \]

wherein R is a sterol or stanol moiety, R₄ is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

Preferably, the cholesterol absorption inhibitor is a disodium ascorbyl stanyl phosphate composition which comprises a mixture of disodium ascorbyl campestenanyl phosphate and disodium ascorbyl sitostanyl phosphate.

The compounds of formulae i) to iv) can be prepared by known methods, for example those described below and in PCT/CA00/00730, which was filed on June 20, 2000 and claims priority back to US Patent Application 09/339,903 filed on June 23, 1999, the entire contents of which are incorporated herein by reference.

In general, compounds of formulae i) to iv) can be prepared as follows: the selected sterol or stanol (or halophosphate, halocarbonate or halo-oxalate derivatives thereof) and ascorbic acid are mixed together under reaction conditions to permit condensation of the "acid" moiety with the "alcohol" (sterol). These conditions are the same as those used in other common esterification reactions such as the Fisher esterification process in which the acid component and the alcohol component are allowed to react directly or in the presence of a suitable acid catalyst such as mineral acid, sulfuric acid, phosphoric acid, p-toluenesulfonic acid. The organic solvents generally employed in such esterification reactions are ethers such as diethyl ether, tetrahydrofuran, or benzene, toluene or similar aromatic solvents and the temperatures can vary from room to elevated temperatures depending on the reactivity of the reactants undergoing the reaction.

In a preferred embodiment, the process to form the ester comprises "protecting" the hydroxyl groups of the ascorbic acid or derivatives thereof as esters (for example, as
acetate esters) or ethers (for example, methyl ethers) and then condensing the protected ascorbic acid with the sterol/stanol halophosphahe, halocarbonate or halo-oxalate under suitable reaction conditions. In general, such condensation reactions are conducted in an organic solvent such as diethyl ether, tetrahydrofuran, or benzene, toluene or similar aromatic solvents. Depending on the nature and reactivity of the reactants, the reaction temperatures may vary from low (-15°C) to elevated temperatures.

In more detail, the following is one preferred mode of preparing the compounds of formulae i) to iv) and in particular formula i): ascorbic acid is initially protected from decomposition by the formation of 5,6-isopropylidene-ascorbic acid. This can be achieved by mixing acetone with ascorbic acid and an acidic catalyst such as sulfuric acid or hydrochloric acid under suitable reaction conditions. Phytostanol chlorophosphate is prepared by forming a solution of phytostanol in toluene and pyridine (although other nitrogen bases such as aliphatic and aromatic amines may alternatively be used) and treating this solution with a phosphorus derivative such as phosphorus oxychloride. The residue so formed after filtration and concentration of the mother liquor is phytostanol chlorophosphate. The latter is then mixed with 5,6-isopropylidene-ascorbic acid and, after the addition of a suitable alcohol such as ethanol and HCl, concentrated. Alternatively, pyridine/THF may be added and the product concentrated. After final washing and drying, the resultant novel product a stanol-phosphate-ascorbate.

In another preferred form of the process to prepare compounds of formulae i) to iv), ascorbic acid is protected at the hydroxyl sites not as 5,6-isopropylidene-ascorbic acid but as esters (for example as acetates, phosphates and the like...). The latter may then be condensed with sterols or stanols, derivatized as described above, using known esterification methods ultimately to produce the compounds. The formation of mono and diphosphates of ascorbic acid is described thoroughly in the literature. For example, US Patent Serial No. 4,939,128 to Kato et al., the contents of which are incorporated herein by reference, teaches the formation of phosphoric acid esters of ascorbic acid. Similarly, US Patent Serial No. 4,999,437 to Dobler et al., the contents
of which are also fully incorporated herein by reference, describes the preparation of ascorbic acid 2-phosphate. In Dobler et al., the core reaction of phosphorylating ascorbic acid or ascorbic acid derivatives with POCl₃ in the presence of tertiary amines (described in German Laid Open Application DOS 2,719,303) is improved by adding to the reaction solution a magnesium compound, preferably an aqueous solution of a magnesium compound. Any of these known ascorbic acid derivatives can be used.

In more detail, the following is another preferred mode of preparing the compounds of formulae i) to iv) and in particular formula ii): prepare the "protected" ascorbic acid and follow the same process outlined in detail above; however, the phosphorus oxylchloride is replaced by oxalyl chloride thereby yielding a stanol-oxalate-ascorbate.

In a preferred form, the cholesterol absorption inhibitor of the present invention comprises one or more disodium ascorbly phytostanyl phosphates (referred to as "FM-VP4") which comprises two major components: disodium ascorbly campestanyl phosphate ("DACP") and disodium ascorbly sitostanyl phosphate ("DASP").

Alternatively, and in another preferred form of the invention, the cholesterol absorption inhibitor comprises a compound from the family of hydroxy substituted azetidinones. Most preferably this azetidinone is a hydroxy substituted azetidinone compound represented by the formula:

\[
\begin{align*}
\text{V} \\
\end{align*}
\]

or a biologically acceptable salt thereof, wherein: \(\text{Ar}_1\) and \(\text{Ar}_2\) are independently selected from the group consisting of aryl and \(\text{R}_4\) -substituted aryl;

\(\text{Ar}_3\) is aryl or \(\text{R}_5\) -substituted aryl;
X, Y and Z are independently selected from the group consisting of --CH₂ --, --CH((lower alkyl)-- and --C(dilower alkyl)--;

R and R₂ are independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₆ and --O(CO)NR₆ R₇ ;

R₁ and R₃ are independently selected from the group consisting of hydrogen, lower alkyl and aryl;

q is 0 or 1; r is 0 or 1; m, n and p are independently 0, 1, 2, 3 or 4; provided that at least one of q and r is 1, and the sum of m, n, p, q and r is 2, 3, 4, 5 or 6; and provided that when p is 0 and r is 1, the sum of m, q and n is 1, 2, 3, 4 or 5;

R₄ is 1-5 substituents independently selected from consisting of lower alkyl, --OR₆, --O(CO)R₆, --O(CH₂)₁₋₅ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₆, --NR₆ (CO)NR₆ R₇, --NR₆ SO₂ R₆, --COOR₆, --CONR₆ R₇, --COR₆, --SO₂ NR₆ R₇, --O(CHR₂)₁₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, --(lower alkyiene)COOR₆, --CH.dbd.CH--COOR₆, --CF₃, --CN, --NO₂ and halogen;

R₅ is 1-5 substituents independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₆, --O(CH₂)₁₋₅ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₆, --NR₆ (CO)NR₆ R₇, --NR₆ SO₂ R₆, --COOR₆, --CONR₆ R₇, --COR₆, --SO₂ NR₆ R₇, --O(CHR₂)₁₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, --(lower alkyiene)COOR₆ and --CH.dbd.CH--COOR₆ ;

R₆, R₇ and R₃ are independently selected from the group consisting of hydrogen, lower alkyl, aryl and aryl-substituted lower alkyl; and

R₉ is lower alkyl, aryl or aryl-substituted lower alkyl.

More preferably, in the compound of formula V, Ar₁ is phenyl or R₄ -substituted phenyl, Ar₂ is phenyl or R₄ -substituted phenyl and Ar₃ is R₅ -substituted phenyl.
In alternative embodiments, within formula V, Ar₁ is R₄ -substituted phenyl wherein R₄ is halogen; Ar₂ is R₄ -substituted phenyl wherein R₄ is halogen or --OR₆, wherein R₆ is lower alkyl or hydrogen; and Ar₃ R₆ -substituted phenyl, wherein R₆ is --OR₆, wherein R₆ is lower alkyl or hydrogen.

In alternative embodiments within formula V, wherein in the compound X, Y, and Z are each --CH₂ --; R₁ and R₃ are each hydrogen; R and R₂ are each --OR₆, wherein R₆ is hydrogen; and the sum of m, n, p, q and r is 2, 3 or 4.

In alternative embodiments within formula V, wherein in the compound, m, n and r are each zero, q is 1 and p is 2.

In alternative embodiments within formula V, wherein in the compound, p, q and n are each zero, r is 1 and m is 2 or 3.

In preferred embodiments within formula V, the compound is selected from the group consisting of:
3(R)-(3(R)-hydroxy-2-phenylethyl)-4(R)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
3(R)-(2(R)-hydroxy-2-phenylethyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
3(S)-(1(S)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
3(S)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
3(R)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
rel-3(R)-(S)-hydroxy-(2-naphthalenyl)methyl!-4(3S)-(4-methoxyphenyl)-1-phe nyl-1 -phenyl-2-azetidinone;
rel-3(R)-(R)-hydroxy-(2-naphthalenyl)methyl!-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;
3(R)-(3(R)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone ne;
3(R)-(3(S)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone ne;
4(S)-(4-hydroxyphenyl)-3(R)-(3(R)-hydroxy-3-phenylpropyl-1-(4-methoxyphenyl )-2-azetidinone;
4(S)-(4-hydroxyphenyl)-3(R)-(3(S)-hydroxy-3-phenylpropyl-1-(4-methoxyphenyl )-2-
azetidinone;
rel 3(R)→3(RS)-hydroxy-3→4-(methoxymethoxy)-phenyl!-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;
1-(4-fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl)!-4(S)-(4-hydroxyphenyl)-2-azetidinone;
1-(4-fluorophenyl)-3(R)→3(R)-(4-fluorophenyl)-3-hydroxypropyl)!-4(S)-(4-hydroxyphenyl)-2-azetidinone;
4(S)→4-(acetyloxy)phenyl!-3(R)-(3(R)-hydroxy-3-phenylpropyl!)-1-(4-methoxyphenyl)-2-azetidinone;
4(S)→4-(acetyloxy)phenyl!-3(R)-(3(S)-hydroxy-3-phenylpropyl!)-1-(4-methoxyphenyl)-2-azetidinone;
1-(4-fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl)!-4(S)→4-(phenylmethoxy)phenyl!-2-azetidinone;
3(R)→3(R)-acetyloxy)-3-phenylpropyl!-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;
3(R)→3(S)-acetyloxy)-3-phenylpropyl!-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;
3(R)→3(R)-(acetyloxy)-3-(4-fluorophenyl)propyl!-4(S)→4-(acetyloxy)phenyl!-1-(4-fluorophenyl!)-2-azetidinone;
3(R)→3(S)-(acetyloxy)-3-(4-fluorophenyl)propyl!-4(S)→4-(acetyloxy)phenyl!-1-(4-fluorophenyl!)-2-azetidinone;
3(R)→3(R)-(acetyloxy)-3-(4-chlorophenyl)propyl!-4(S)→4-(acetyloxy)phenyl!-1-(4-chlorophenyl!)-2-azetidinone;
3(R)→3(S)-(acetyloxy)-3-(4-chlorophenyl)propyl!-4(S)→4-(acetyloxy)phenyl!-1-(4-chlorophenyl!)-2-azetidinone; and
rel 1-(4-fluorophenyl!)-4(S)-(4-hydroxyphenyl!)-3(1R)-(1(R)-hydroxy-3-phenylpropyl!)-2-azetidinone.

Such hydroxy substituted azetidinones are described and claimed in US Patent Serial Nos. 5,846,966 and 5,767,115 to the Schering Corporation, the entire contents of both of which are incorporated herein by reference. In particular, 2-azetidinone derived inhibitors referred to as "Ezetimibe" and sold by Schering under the trade-mark Zetia™ are preferred for use herein.
In an alternative embodiment, the cholesterol absorption inhibitor within the scope of the present invention may be an inhibitor of bile acid transport or reabsorption, including, but not limited to ileal, apical and hepatic transport inhibitors.

There having been found a causal relationship between the recirculation of bile acids from the lumen of the intestinal tract and the reduction of serum cholesterol, ileal bile acid transport inhibitors "IBATs" are now are being extensively investigated for their role in lowering cholesterol and treating atherosclerosis. Stedronsky\textsuperscript{37} discusses the biochemistry, physiology and known active agents surrounding bile acids and cholesterol.

Some IBAT inhibitors useful in the present invention are disclosed in PCT/US95/10863, the contents of which are incorporated herein by reference. More IBAT inhibitors are described in PCT/US97/04076, herein incorporated by reference. Still further IBAT inhibitors useful in the present invention are described in U.S. application Ser. No. 08/816,065, herein incorporated by reference. More IBAT inhibitor compounds useful in the present invention are described in WO 98/40375, herein incorporated by reference. An array of additional IBAT inhibitor compounds useful in the present invention are described in U.S. Pat. No. 5,994,391, also incorporated herein by reference.

Further IBATs are disclosed by various Hoechst Aktiengesellschaft patent applications which disclose bile acid transport inhibiting compounds and are each separately listed below:

H5. EP Application No. 0 379 161.
H6. EP Application No. 0 549 967.
H7. EP Application No. 0 559 064.
H8. EP Application No. 0 563 731.
Within the scope of the present invention, other bile acid transport inhibitors include selected benzothelepines, such as those disclosed in WO 93/321146, PCT/US97/04076, PCT/US95/10863, EP 508425, FR 2661676, WO 99/35135, WO 92/18462, and U.S. Patent No. 5,994,391 (Lee et al.).

WO 92/16055 to The Wellcome Foundation Limited describes a number of suitable benzothiazepine compounds. Additional hypolipidemic benzothiazepine compounds (particularly 2,3,4,5-tetrahydrobenzo-1-thi-4-azepine compounds) are disclosed in patent application Nos. WO 96/05188, WO 96/05188 and WO 96/16051.

Further IBAT inhibitor compounds include a class of naphthalene compounds, described by T. Ichihashi et al. in J. Pharmacol. Exp. Ther., 284(1), 43-50 (1998). In this class, S-8921 (methyl 1-(3,4-dimethoxyphenyl)-3-(3-ethylvaleryl)-4-hydroxy-6,7,8-trimethoxy-2-na phthoate) is particularly useful. The structure of S-8921 is shown in formula B-20 of this publication. Further naphthalene compounds or lignin derivatives are described in PCT Patent Application No. WO 94/24087.

Other IBATs include, for example, SC-635 developed by Pharmacia Corporation, IL and Monsanto, MO; 264W94 developed by GlaxoSmithKline; S-8921 developed by Shiongi) and (3R,5R)-3-buty-3-ethyl-2,3,4,5-tetrahydro-7,8-dimethoxy-5-phenyl-1-4-benz othiazepine 1,1-dioxide.

In an alternative embodiment, the cholesterol absorption inhibitor within the scope of the present invention may be an androstanal and/or androstene derivative, wherein androstane and/or androstene are coupled with ascorbic acid and represented by one or more of the general formulae:
wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ may individually be chosen from hydrogen, OH,
carbonyl, and an ascorbyl moiety; and R₇ may be hydrogen or any halogen.

Most preferably, the ascorbyl moiety which is coupled to the compound from the androstan or androstene family is selected individually from one or more of the following structures:
wherein M+ represents any metal, alkali earth metal, or alkali metal.

The androstane/androstene compounds of formulae vi) to viii), incorporating one or more of the ascorbyl linkers of formulae ix) to xx), can be prepared by known methods, for example those described in PCT/CA03/00824, the contents of which are incorporated herein by reference or can be prepared by the methods described above for the preparation of compounds of formulae i) to iv), adjusted accordingly.

Salts
As used herein, the term "biologically acceptable salts" refers any salts that retain the desired biological and/or physiological activity of the compounds as described herein and exhibit minimal undesired toxicological effects. Accordingly, reference to all compounds herein thereby includes reference to respective acidic and/or base salts thereof, formed with inorganic and/or organic acids and bases.

Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclpentanepropionates, digluconates, dodecylsulfates, heptanoates, hexanoates, hydrochlorideshydrobromides, hydroiodides, 2-hydroethanesulfonates, lactates, maleates, methanesulfonates, 2-naphthalenesulfonates, nicotinates, nitrates, oxalates, pectinates, persulfonates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates, sulfonates, tartrates, thiocyantes, tolenesulfonates, undecanoates and the like.

Those compounds which contain an acid moiety may form salts with a variety or organic and inorganic bases.

Accordingly, the present invention encompasses not only the parent compounds comprising, for example, the selected sterol and/or stanol a but also, where possible (i.e. where the parent contains a free hydroxyl group), the present invention encompasses the biologically acceptable metal, alkali earth metal, or alkali metal salts of the disclosed compounds.

The salts, as described herein, are even more water soluble than the corresponding parent compounds and therefore their efficacy and evaluation both in vitro and in vivo may be enhanced.

Salt formation of the compounds of the present invention can be readily performed, for example, by treatment of any parent compound containing a free OH group with a
series of bases (for example, sodium methoxide or other metal alkoxides) to produce the corresponding alkali metal salts. Other metal salts of calcium, magnesium, manganese, copper, zinc, and the like can be generated by reacting the parent with suitable metal alkoxides.

With respect to the formation of these derivatives, it is to be appreciated that, while selected synthesis processes are described, there are a number of other means by which the variety of derivatives disclosed and claimed can be made. It is well within the purview of a skilled person in this chemical field, once a particular derivative is chosen, to undertake the synthesis using commonly available techniques in the art. For this reason, the complete synthesis of each and every claimed derivative is not described.

To the extent that the compounds as described herein and salts thereof may exist in their tautomeric form, all such tautomeric forms are contemplated herein as part of the present invention.

All stereoisomers of the compounds of the present invention, such as those which may exist due to asymmetric carbons on various constituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons) and diastereomeric forms, are contemplated within the scope of the present invention. Individual stereoisomers of the compounds of the present invention may, for example, be admixed as racemates or with all other, or other selected stereoisomers. The chiral centres of the compounds can have the S or R configuration as defined by the IUPAC 1974 Recommendations. Such stereoisomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention. When diastereomeric or enantomeric products are prepared, they can be separated by conventional methods, for example, chromatographic or fractional crystallization.

Isomers may include geometric isomers, for example cis-isomers or trans-isomers across a double bond. All such isomers are contemplated among the compounds useful in the present invention.
The compounds useful in the present invention also include tautomers.

While the present invention fully covers the inhibition of expression of the following multiple drug resistance genes: ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); ABCC3 (MRP-3) it has been found that the cholesterol absorption inhibitors, as described herein, are particularly useful in inhibiting the expression of MDR-1 (ABCB1), which has P-glycoprotein as its gene product. In addition, it has been found that the cholesterol absorption inhibitors, as described herein, are particularly useful in inhibiting the production of P-glycoprotein, as expressed by the MDR-1 gene.

Methods of Use

According to one aspect of the present invention, there is provided a method of inhibiting the expression of a multi-drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor as described herein.

According to another aspect of the present invention, there is provided a composition for use in cancer treatment which comprises at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor as described herein.

According to yet another aspect of the present invention there is provided a method of enhancing the effectiveness of a chemotherapeutic agent in an animal having cancer, which comprises administering to said animal an effective amount of at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor as described herein.

According to yet another aspect of the present invention there is provided a method of reversing a multi-drug resistance phenotype exhibited by an animal cell which comprises exposing the cell to an effective amount of at least one cholesterol absorption inhibitor as described herein.
According to yet another aspect of the present invention, there is provided a method of inhibiting the production of a protein expressed by a multiple drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor.

According to a further aspect of the present invention there is provided a kit comprising at least two separate components:

a) a composition comprising at least one cholesterol absorption inhibitor as described herein; and

b) a composition comprising at least one chemotherapeutic agent;

along with instructions describing the administration of each composition.

The methods, composition and kit of the present invention are suitable for use in the treatment of cancers in general including, but not limited to breast, prostate, liver, pancreatic, kidney, stomach, intestinal, colon, lung, brain, adrenal cortex, bone marrow, embryonic stem cell, parenchymatous, epithelial, lymph varieties (including Hodgkins and non-Hodgkins lymphomas), bone, testicular, cervical, uterine, esophageal, mouth, and skin.

It is suspected that marked success will be found in treating those cancers which frequently show high levels of P-glycoprotein at diagnosis, even though the patients have not been previously treated with chemotherapeutic agents (combinations of one or more anti cancer drugs). Such cancers include, but are not limited to: colon, kidney, breast, adrenal cortex and liver.

The methods, compositions and kit of the present invention are not intended to be limited to any one particular cancer chemotherapeutic agent or combination of agents. However, preferred chemotherapeutic agents include, but are not limited to, all hydrophobic, and heterocyclic cancer chemotherapeutic agents such as adriamycin (doxorubicin), phosphates, colcemid, etoposide, paclitaxel, bisantene, vincristine, and vinblastine.
The compositions of the present invention allow for a "combination therapy" wherein
the cholesterol absorption inhibitor and the selected chemotherapeutic agent are
either co-administered in a substantially simultaneous manner, for example, in a single
tablet or capsule having a fixed ratio of active ingredients or in multiple, separate
administrations for each moiety. This separate administration includes sequential
dosage forms.

For greater clarity, this "combined administration" encompasses co-administration of
these therapeutic agents in a substantially simultaneous manner, such as in a single
dosage form having a fixed ratio of active ingredients or in multiple, separate dosage
forms for each agent. In addition, such administration also encompasses use of
each type of agent in a sequential manner. In either case, the treatment regimen will
provide beneficial effects of the drug combination in achieving one or more of the
therapeutic goals outlined herein.

Accordingly, the present invention provides various means of achieving one or more of
the following therapeutic goals:
   a) treating or alleviating a cancer;
   b) preventing, treating or alleviating tumour growth;
   c) inhibiting or reducing the expression of a multi-drug resistance gene;
   d) inhibiting or reducing the production of one or more proteins expressed by
      multiple drug resistance genes;
   e) enhancing the effectiveness of a chemotherapeutic agent in treating a cancer;
      and
   f) sensitizing a cell to one or more chemotherapeutic agents;

which comprises administering to an animal or a cell derived from or within said
animal, a non-toxic and therapeutically effective amount of at least one cholesterol
absorption inhibitor as described herein and at least one cancer chemotherapeutic
agent. This invention further comprises the use of any of the disclosed compounds
and compositions for any indications described herein, more specifically, for use in
achieving one or more of the therapeutic goals as defined above.

In particular, and for greater certainty, it has been found that the co-administration (though not necessarily concurrently or proximally consecutively) of cholesterol absorption inhibitors and chemotherapeutic agents effectively inhibits the expression of the multi-drug resistance gene thereby reducing the production of its respective protein, for example, P-glycoprotein. Such reduction prevents the efflux of cancer chemotherapeutic agents from animal cells, maximizing their effectiveness.

Heretofore, there has been no recognition of the significant effect of cholesterol absorption inhibitors on multi-drug resistance. While there has been research in the area of overcoming such drug resistance, the focus has been on the modulating effects of other compounds on either P-glycoprotein inhibition or MDR expression, for example, by the design of compounds which selectively bind the MDR-1 gene. While by no means exhaustive, an overview of such research is provided below.

WO0213815 discloses a pharmaceutical composition comprising a select compound (having formula I therein described) which itself has no pharmacological activity but which allegedly enhances the bioavailability of other active ingredients by inhibiting p-glycoprotein present in the intestinal wall. The enables the absorption of active ingredients (ex anti-cancer drugs) which are otherwise poorly absorbed.

WO02034291 discloses molecules which bind to the MDR-1 gene and selectively inhibit gene expression. Delivery systems for these drugs include non-polymer systems involving lipids. Also covered is a method of inhibiting MDR gene expression which comprises contacting a nucleic encoding MDR polypeptide with a HIF-1 SUMO-1 complex blocking agent.

WO0160349 discloses a pharmacological agent comprising picolinic acid, fusaric acid and derivatives thereof. The fusaric acid component was found to be effective in controlling growth of cells with high levels P-glycoprotein. It is suggested that fusaric acid may have some role in the treatment of tumors that are resistant to
MDR-associated drugs. Note that WO0220486, also by the same inventor, discloses similar information.

WO0048571 discloses pharmaceutical compositions comprising n-benzoylstaurosporine and their use as an anti-tumour, anti-proliferative agents. WO0226208 discloses tocol-based particulate emulsions for the delivery of chemotherapeutic agents. The emulsion comprises one or more tocols, surfactants, an optional co-solvent and the chemotherapeutic agent.

US Patent Serial No. 5,639,887 describes various bicyclic amines that are effective in resensitizing multiple drug resistant cells to chemotherapeutic agents such as doxorubicin, vincristine and bisantrene. US Patent Serial No. 5,670,507 teaches a method for reversing a MDR phenotype in tumors insensitive to hydrophobic chemotherapeutic drugs due to over expression of mdr-1 which comprising administering an effective amount of a long chain amino alcohol compound selected from the two disclosed formulae.

US Patent Serial No 5,866,699 discloses synthetic oligonucleotides having a nucleotide sequence complementary to at least a portion of the MDR-1 gene, or transcripts thereof, which portion encodes a nucleoside binding site. Also disclosed is a pharmaceutical formulation containing such oligonucleotides, and methods of treating MDR cancer cells, of preventing the expression of P170 in a cell, and of preventing the induction of MDR in a cancer cell.

US Patent Serial No. 5,874,567 teaches a modified oligonucleotide between 15 and 30 nucleotides in length, inclusive, having a sequence that specifically hybridizes in a human cell with a complementary sequence of a human MDR-1 gene and allelic variants thereof to inhibit expression of a multidrug resistance phenotype exhibited by the cell. The complementary sequence is selected from the group consisting of SEQ ID Nos: 103, 104 and 105 (as described therein), the modification comprising a backbone modification selected from the group consisting of dithioate,
methylphosphonate, morpholino, polyamide, or any combination thereof.


There are additional advantages to the use of cholesterol absorption inhibitors in overcoming MDR. Some of the cholesterol absorption inhibitors as described herein (compounds depicted above having formulae i through iv) comprise an ascorbyl moiety. These particular compounds have numerous added advantages. First and foremost, solubility of the compounds is greatly enhanced, both in aqueous solutions and non-aqueous media such as oils and fats. With this greater solubility, effective therapeutic dosages and concomitantly costs, can be reduced. Secondly, these derivatives are heat stable (stable to oxidation and hydrolysis) which is essential for some processing mechanisms.

The desired effects described herein may be achieved in a number of different ways. The cholesterol absorption inhibitors and chemotherapeutic agents, separately or together, may be administered by any conventional means available for use in conjunction with pharmaceuticals i.e. with a pharmaceutically acceptable carrier. The pharmaceutical compositions can comprise from about 1% to 99% of the "active" components and preferably from about 5% to 95% of the active components.

The formulations and pharmaceutical compositions can be prepared using conventional, pharmaceutically available excipients, and additives and by conventional techniques. Such pharmaceutically acceptable excipients and additives include non-toxic compatible fillers, binders, disintegrants, buffers, preservatives, anti-oxidants, lubricants, flavourings, thickeners, colouring agents, emulsifiers and the like.

The exact amount or dose of the cholesterol absorption inhibitors which is required to
achieve the desired effects will, of course, depend on a number of factors such as the particular compound or composition chosen, the potency of the compound or composition administered, the formulation in which it is administered, the mode of administration and the age, weight, condition and response of the patient. All of these factors, among others, will be considered by the attending clinician with respect to each individual or patient.

Generally, a total daily dose the cholesterol absorption inhibitor having one of formulae i)-viii) and comprising sterols and/or stanols may be administered in a daily dosage range of from 10mg to about 20 g, more preferably 10mg to 1.5g, and most preferably up to 800 mg per day in single or multiple divided doses.

Wherein the chosen a cholesterol absorption inhibitor is a sterol or stanol, whether free or as part of a compound or derivative, it may be administered in a form comprising up to 6 grams sterols and/or stanols per day. It should be recognized that the provision of much larger daily doses of sterols, stanols and their derivatives are not harmful to the animal host, as excess will simply pass through normal excretory channels.

Wherein the cholesterol absorption inhibitor is a substituted azetidinone, it may be administered in a daily dose of from about 0.1 to about 30 mg/kg of body weight, preferably about 0.1 to about 15 mg/kg, and most preferably up to 10mg/kg per day. For an average body weight of 70 kg, the dosage level is therefore from about 5 mg to about 1000 mg of drug per day, given preferably in a single dose or 2-4 divided doses.

When the cholesterol absorption inhibitor is any compound which inhibits bile acid reabsorption, it may be administered in a dose from about 0.003mg—20mg per kilogram body weight of the individual animal. In general, a total daily dose of an IBAT inhibitor can be in the range of from about 0.01 to about 1000 mg/day, preferably from about 0.1 mg to about 50 mg/day, more preferably from about 1 to about 10 mg/day.

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When the cholesterol absorption inhibitor and chemotherapeutic agent are administered separately, the number of doses and the amount of such dosage of each component given per day may not necessarily be the same. For example, it is possible that the cholesterol absorption inhibitor may require either a greater number of administrations per day than the chemotherapeutic agent and/or may require a larger dosage.

The daily dose of these cholesterol absorption inhibitors can be administered to an individual in a single dose or in multiple doses, as required. Sustained release dosages can be used.

Use of pharmaceutically acceptable carriers to formulate the compounds and compositions herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compounds and compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds and compositions can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds and compositions of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions, comprising one or more of the compounds of the present invention, include compositions wherein the active ingredients are contained in an effective amount to achieve their intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be
used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

In a preferred form, the cholesterol absorption inhibitor is administered in the form of a liposome. Liposomes are hollow microspheres composed of one or more double lipid layers. They were first used more than 30 years ago as vehicles for various drug substances, and since then knowledge of their behavior in vitro has allowed a more rational design focused on the specific treatment of certain diseases.

Formation of liposomes occurs formed when thin lipid films are hydrated. The hydrated lipid sheets detach during agitation and self-close to form multi-lamellar vesicles.

There are a number of methods known and widely practiced in the art to prepare liposomes. Chemotherapeutic agents, such as doxorubicin, are often encapsulated in liposomes using the established methods.

Typically, 100 nm diameter liposomes are prepared by exposing chloroformic solution of various lipid mixtures to high vacuum and subsequently hydrating the resulting lipid films (DSPC/CHOL, EPC/CHOL, DSPC/PEG-PE/CHOL) with pH 4 buffers, and extruding them through polycarbonate filters, after a freezing and thawing procedure. A transmembrane pH gradient is then created by adjusting the pH of the extravesicular medium to 7.5 by addition of an alkalinization agent. The technique exploits the ability of weak bases to redistribute across membranes exhibiting pH gradients where $[\text{drug}]_{\text{in}}/[\text{drug}]_{\text{out}}=([H^+]_{\text{in}}/[H^+]_{\text{out}}).

The selected drug is then entrapped by addition of the drug solution in small aliquots to the vesicle solution, at an elevated temperature, to allow drug accumulation inside the liposomes. Trapping efficiencies are determined by separating free from liposome encapsulated drug on gel filtration columns and quantifying the two fractions for lipid and drug content by liquid scintillation counting, fluorescence spectroscopy or UV-VIS spectroscopy. These liposomes are then evaluated for size distribution (quasielastic
light scattering, scanning electron microscopy), drug uptake and release studies, stability, and in vivo tumor targeting efficiency.

One preferred, but non-limiting method for use in preparing liposomes is described in the examples below.

For greater certainty, the pharmaceutical compositions of the present invention may be manufactured in any manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include lactose, sucrose, mannitol, sorbitol, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.
Dragée cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

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

Oral liquid preparations may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

Since the present invention relates to compositions with a combination of active ingredients which may be administered together or separately, there are also provided herein kits for such purpose. A kit is contemplated wherein two separate units are combined: a pharmaceutical composition comprising at last one cholesterol absorption
inhibitor, as described herein, and a separate pharmaceutical composition comprising at least one cancer chemotherapeutic agent. The kit will preferably include directions for the administration of the separate components. This type of kit arrangement is particularly useful when separate components must be administered in different dosage forms (for example, oral vs. parenteral vs. intravenous) or are administered at different dosage intervals or are administered at different dosage amounts.

Without further elaboration, the foregoing so fully illustrates the present invention that others may, by applying current or future knowledge, adapt the same for use under the various conditions described and claimed herein.

EXAMPLES

EXAMPLE 1—Formation of one of the cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester referred to herein as "FM-VP4";

Step 1: Protection of ascorbic Acid
Oleum (24%, 8.3g) was added dropwise to acetone (50ml). Ascorbic acid (12g) was introduced to the mixture at 0°C and the reaction mixture was stirred at 0°C for 6 hours. The obtained crystals were filtered off under suction, the filtered cake was pressed to dryness and then washed with acetone (30ml). The product, 5,6-isopropylidene ascorbic acid (14g) was obtained.

Step 2: Attachment to Phytostanols
A solution of phytostanol mixture (24g) (campestanol: 36.4%; sitostanol: 62.3%) in toluene (500ml) and pyridine (25ml) was added dropwise to a mixture of phosphorous oxychloride (9ml) in toluene (200ml) at 0°C. The mixture was stirred at room temperature for 3 hours. The pyridine hydrochloride was filtered off and the mother liquor was concentrated to recover the toluene. The residue was dissolved in dry THF (100ml) and a solution of the above prepared protected ascorbic acid (14g) in dry THF (400ml) was added dropwise at 0°C. The stirring at room temperature was maintained
for 1 hr. The solution was concentrated to remove the solvent. Ethanol (400ml) and 3N HCl (200ml) were added, the mixture was heated to 50°C for 30 min and concentrated. Ethyl acetate (600ml) was added, the resultant solution was washed with water (3×300ml), dried over sodium phosphate, concentrated and the product (phytostanol-phosphate-ascorbate) was obtained as a white powder 22g.

Step 3: Conversion to Sodium Salt
The above prepared acid (17g) was dissolved in ethanol (100ml) and a solution of sodium methoxide (2.7g) in ethanol (50ml) was added at stirring and at room temperature. The stirring was maintained for 30 min. after the addition. The resultant white cake was filtered off, dried and weighed, to afford a white powder 20g (phytostanol-phosphate-ascorbate sodium).

EXAMPLE 2:-- Formation of one of the cholesterol absorption inhibitors described herein: Disodium ascorbyl phosphate ester of dehydroisoandrosterone
To a dry round bottom flask, acetone (150 ml) and L-ascorbic acid (50 g) were added at 0 °C. Acetyl chloride (7.5 ml) was added dropwise through an addition funnel in 10 minutes. The reaction mixture was stirred at 0 °C for 24 hours. The precipitate was filtered off and washed with acetone (3×20 ml). The white product, 5,6-isopropylidene ascorbic acid, was dried under vacuum for 1.5 hours to give a dry powder (52 g), yield 85%.

A dry three neck round bottom flask was fitted with a stirring bar, argon inlet and an addition funnel. A solution of dehydroisoandrosterone (1.73 g, 6 mmol) in anhydrous THF (15 ml) and pyridine (2.4 ml) was added dropwise to the mixture of anhydrous THF (12 ml) and POCl₃ (0.7 ml, 7.5 mmol) at 0 °C over a period of 10 minutes. A white precipitate formed immediately. The suspension was stirred at 0 °C for 40 minutes, and at room temperature for 1 hour and 40 minutes.

To the above suspension, a solution of 5,6-isopropylidene ascorbic acid (3.6 g, 16.67 mmol) in anhydrous pyridine (3 ml) and THF (30 ml) was added dropwise at 0 °C.
over a period of 20 minutes. The suspension was stirred at 0 °C for 30 minutes, and at room temperature for 1.5 hours. The formed pyridinium chloride was filtered out and washed with THF twice. The solvents were evaporated under reduced pressure at 40 °C to afford a residue.

The residue was dissolved in THF (40 ml), and 2N HCl (30 ml) was added in one portion. The mixture was stirred at room temperature for 8 hours. THF was evaporated under a reduced pressure. The water layer was extracted with ethyl acetate (4×50 ml). The combined ethyl acetate solution was washed with brine (100 ml), and dried over Na₂SO₄. The solvent was evaporated to give a residue. The residue was dissolved in CHCl₃, and then hexanes was added to precipitate the product. The precipitated solid was filtered out, washed with hexanes and dried under vacuum (2.43 g, crude product, yield: 77%). The purification of phosphate ester was done by reverse phase C-18 chromatography (Waters, water/methanol = 90/10 to 60/40). Pure compound 4 (Figure 1, 39 mg) was isolated from 50 mg of the crude product. The overall yield (base on dehydroisoandrosterone) was 60%.

Ascorbyl phosphate ester of dehydroisoandrosterone (0.5 g, 0.95 mmol) was dissolved in methanol (3 ml) at room temperature, and then sodium methoxide in methanol (1ml, 20%) was added. The suspension was stirred at room temperature for 30 minutes. The precipitated solid was filtered out, washed with methanol, acetone and hexanes. The mother liquor was concentrated to 2 ml, acetone was added to precipitate the product. An additional white solid was obtained. The combined solid was dried under vacuum at room temperature. Disodium ascorbyl phosphate ester of dehydroisoandrosterone (0.49 g, yield 91%) was obtained.

EXAMPLE 3-- Synthesis of another of the cholesterol absorption inhibitors described herein: Disodium Ascorbyl Phosphate Ester of 5α-Androstan-3β-ol-17-one

To a dry round bottom flask, 5α-androstan-3β-ol-17-one (1.0 g, 3.4 mmol), THF (8.6 ml) and pyridine (1.38 ml) were added. The mixture was stirred at room temperature until a clear solution was obtained. To another dry round bottom flask, THF (6.9 ml)
and POCl₃ (0.4 ml, 4.25 mmol) were added, stirred at 0 °C for 5 minutes. To this mixture, the above prepared 5α-androstan-3β-ol-17-one solution was added dropwise under argon atmosphere over a period of 10 minutes. After the addition, the white suspension was stirred at 0 °C for 35 minutes, and at room temperature for 2 hours. The reaction was stopped and the white suspension was used for the coupling reaction without filtration.

5,6-Isopropylidene ascorbic acid (2.0 g, 9.52 mmol) was dissolved in pyridine (1.71 ml) and THF (17 ml). The round bottom flask which contained previously prepared white suspension was immersed in an ice-water bath. To this mixture, the above prepared THF solution of the 5,6-isopropylidene ascorbic acid was added dropwise under stirring at 0 °C over a period of 15 minutes. After the addition, the mixture was stirred at 0 °C for 25 minutes, and at room temperature for 2 hours. The white solid of pyridinium chloride was filtered out and washed with THF (8 ml). The filtrate was concentrated to remove THF and excess pyridine to give a residue (2.38 g).

The residue was dissolved in THF (30 ml), and 1N HCl (30 ml) was added in one portion. The mixture was stirred at room temperature for 16 hours and 45 minutes. 12N HCl (4 ml) was added to the reaction mixture at room temperature. The reaction mixture was stirred at room temperature for an additional 4 hours and 45 minutes. THF was evaporated under a reduced pressure. The water layer was extracted with ethyl acetate (3×60 ml). The combined ethyl acetate solution was washed with brine (60 ml), and dried over Na₂SO₄. The extract was concentrated to about 3 ml. Hexanes (15 ml) was added to precipitate the product. The precipitated solid was filtered out, washed with hexanes and dried under a reduced pressure (1.48 g).

Ascorbyl phosphate ester of 5α-androstan-3β-ol-17-one (0.5 g, 0.95 mmol) was dissolved in methanol (3 ml) at room temperature, and then sodium methoxide in methanol (1.5 ml, 20%) was added. The suspension was stirred at room temperature for 25 minutes. The precipitated solid was filtered out, washed with methanol, acetone and hexanes. The mother liquid was concentrated to 2 ml, and then acetone was added to precipitate the product. An additional product was
obtained. The combined solid was dried under a reduced pressure at room
temperature to give disodium ascorbyl phosphate ester of 5α-androstan-3β-ol-17-
one (0.38 g). The overall yield was 57% (based on 5α-androstan-3β-ol-17-one).

EXAMPLE 4—Synthesis of another of the cholesterol absorption inhibitors described
herein: Disodium Ascorbyl Phosphate Ester of Androst-5-ene-3β,17β-diol

To a dry round bottom flask, 3β-acetoxyandrost-5-ene-17β-ol (1.0 g, 3.0 mmol),
anhydrous THF (6.3 ml) and pyridine (0.73 ml) were added. The mixture was stirred
at room temperature until a clear solution was obtained. To another dry round bottom
flask, THF (2 ml) and POCl₃ (0.35 ml, 3.22 mmol) were added, stirred at −5 °C ~ -10
°C for 5 minutes. To this mixture, the above prepared 3β-acetoxyandrost-5-ene-17β-
ol solution was added drop-wise under argon atmosphere over a period of 20
minutes. After the addition, the white suspension was stirred at room temperature for
1 hour. The mixture was concentrated to remove THF and excess POCl₃ to give a
residue.

5,6-Isopropylidene ascorbic acid (0.98 g, 4.55 mmol) was dissolved in anhydrous
pyridine (0.70 ml) and THF (6.2 ml). The residue was dissolved in dry THF (4 ml). To
this mixture, the above prepared THF solution of the 5,6-isopropylidene ascorbic acid
added dropwise under stirring at 0 °C over a period of 20 minutes. After the addition,
the mixture was stirred at room temperature for 1 hour and 25 minutes. The white
solid of pyridinium chloride was filtered out and washed with THF (6 ml). The filtrate
was concentrated to remove THF and excess pyridine to give a residue.

The residue was dissolved in a mixture of ethanol (12.5 ml) and 1N HCl (12.5 ml).
The mixture was kept stirring at 50 °C ~ 55 °C for additional 3 hours and 45 minutes
(TLC monitoring). The mixture was extracted with ethyl acetate (60 ml), washed with
10% aqueous NaCl twice (30 ml, 20 ml) and dried over Na₂SO₄ (10 g) for 1.5 hours.
After the filtration, the filtrate was concentrated to 5 ml. Hexanes (10 ml) was added
to precipitate the product. The precipitate was collected, washed with hexanes (10
ml) and dried under the reduced pressure to give a slightly yellow powder (0.95 g,
crude product, yield 60%). The pure product was obtained by preparative HPLC.

Instrument is Waters Delta Preparative 4000 HPLC system. Column is Waters Symmetry C18, 5μm, 30×100 mm. Mobile phases are 0.1% H₃PO₄ in water and acetonitrile. Water and acetonitrile are HPLC grade or equivalent.

The crude product was purified by preparative HPLC. The product was collected and evaporated on a rotary evaporator to remove acetonitrile. The water solution was extracted with ethyl acetate twice. The ethyl acetate layer was dried over Na₂SO₄, concentrated and dried under a reduced pressure to give a white powder product. This product was submitted for NMR and mass spectra. Both spectra indicated the product is ascorbyl phosphate ester of androst-5-ene-3β,17β-diol.

Preparation of disodium ascorbyl phosphate ester of androst-5-ene-3β,17β-diol (5, Figure 3) was similar to the process described in Example 3.

**EXAMPLE 5**—Synthesis of another of the cholesterol absorption inhibitors described herein: Disodium Ascorbyl Phosphate Ester of Androst-5-ene-17β-ol

To a solution of pyridine (0.41 ml) and 1,2-phenylene phosphorochloridite (0.6 ml, 5 mmol) in anhydrous THF (10 ml) at 0 °C was added dropwise dehydroisoandrosterone (1.44 g, 5 mmol) in anhydrous THF (10 ml) over a period of 10 minutes. The reaction mixture was stirred at 0 °C for 30 minutes, and at room temperature for 4 hours. The reaction was monitored with TLC (hexanes/EtOAc = 2/1). The formed pyridinium chloride was filtered off and washed with THF. The solvents were evaporated at 40 °C to give a white powder.

The crude phosphate ester was dissolved in methylene chloride (25 ml), and treated with iodine (1.27 g) for 4 hours at room temperature. The reaction mixture was diluted with methylene chloride (75 ml), washed with 1N NaOH (2×50 ml) and water (2×50 ml), and dried over Na₂SO₄. The solvent was removed, and the product (1.4 g, yield 71%) was crystallized from methylene chloride and methanol.
3β-Iodoandrost-5-ene-17-one (1.27 g, 3.19 mmol) was dissolved in glacial acetic acid (40 ml) at 50-55 °C, the activated zinc dust (2.7 g) was added in one portion. The mixture was stirred at 50 °C ~ 55 °C for 2 hours, the zinc dust was filtered out and washed with methylene chloride. The solution was diluted with methylene chloride (120 ml), washed with water (2×100 ml), 1N NaOH (2×100 ml) and water (100 ml), and dried over Na₂SO₄. The solvent was removed to afford a white powder. The white powder was dried under vacuum to give androst-5-ene-17-one (0.83 g, yield: 95%).

Androst-5-ene-17-one (0.65 g, 2.34 mmol) was dissolved in methanol (25 ml) at room temperature. The solution was cooled down to 0 °C, and NaBH₄ (50 mg) was added in one portion. The mixture was stirred at 0 °C for 3 hours, and monitored with TLC (hexanes/EtOAc = 3/1). After 3 hours, another portion of NaBH₄ (20 mg) was added, and the reaction mixture was stirred at 0 °C for additional half an hour. Aqueous NH₄Cl (5%, 25 ml) and HCl (6N, 5 ml) were added slowly at 0 °C, and stirred for 1 hour. Water (100 ml) was added to completely precipitate the product. The precipitated solid was filtered out and washed with water, and dried under vacuum. The pure product (0.62 g, yield: 95%) was obtained by column chromatography.

A solution of androst-5-ene-17β-ol (0.63 g, 2.3 mmol) in anhydrous THF (8 ml) and pyridine (1 ml) was added drop-wise to the mixture of anhydrous THF (6 ml) and POCl₃ (0.28 ml, 3 mmol) at 0 °C over a period of 5 minutes. The suspension was stirred at 0 °C for 50 minutes, and then at room temperature for one hour.

To the above suspension, a solution of 5,6-isopropylidene ascorbic acid (1.38 g) in anhydrous pyridine (1.2 ml) and THF (12 ml) was added drop-wise at 0 °C over a period of 15 minutes. The suspension was stirred for 1.5 hours at 0 °C, and then overnight at room temperature. The formed pyridine hydrochloride was filtered out and washed with THF twice. The solvents were evaporated under reduced pressure at 40 °C to afford a residue.
The residue was then dissolved in THF (35 ml), and 2N HCl (30 ml) was added as one portion. The mixture was stirred overnight at room temperature. THF was evaporated under reduced pressure. The water layer was extracted with ethyl acetate (3×100 ml). The combined ethyl acetate solution was washed with brine (100 ml), and dried over Na₂SO₄. The solvent was evaporated to give a residue. The residue was dissolved in acetone, and hexanes was added to precipitate the product. The white precipitated solid was filtered out, washed with hexanes and dried under vacuum (0.82 g, crude product, yield: 70%).

Preparation of disodium ascorbyl phosphate ester of androst-5-ene-17β-ol was similar to example 2.

**EXAMPLE 6—Synthesis of another of the cholesterol absorption inhibitors described herein: Tetra-sodium Monoascorbyl Diphosphate Ester of 3β-Acetoxyandrost-5-ene-7β,17β-diol**

To a dry round bottom flask, 3β-acetoxyandrost-5-ene-7β,17β-diol (0.5 g, 1.43 mmol), pyridine (0.83 ml) and THF (4 ml) were added. The mixture was stirred at room temperature until a clear solution was obtained. To another dry round bottom flask, THF (5 ml) and POCI₃ (0.33 ml) were added, stirred at −5 °C to 0 °C for 5 minutes. To this mixture, the above prepared 3β-acetoxyandrost-5-ene-7β,17β-diol solution was added dropwise under argon atmosphere over a period of 15 minutes. After the addition, the white suspension was stirred at room temperature for 2 hours and 45 minutes. The reaction was stopped and the white suspension was used for the coupling reaction without filtration.

5,6-Isopropylidene ascorbic acid (1.30 g, 6.02 mmol) was dissolved in pyridine (1.16 ml) and THF (5.8 ml). The round bottom flask which contained previously prepared white suspension was immersed in an ice-water bath. To this mixture, the above prepared THF solution of the 5,6-isopropylidene ascorbic acid was added dropwise under stirring at 0 °C over a period of 15 minutes. After the addition, the mixture was stirred at 0 °C for 40 min and at room temperature for 17 hours. The white solid of
pyridinium chloride was filtered out and washed with THF (5 ml). The filtrate was concentrated to remove THF and excess pyridine to give a residue (2.76 g).

The crude of this compound was dissolved in a mixture of THF (30 ml) and 1N HCl (30 ml). The mixture was kept stirring at room temperature for 3.5 hours (TLC monitoring). The second portion of 1N HCl (10 ml) were added. The mixture was stirred for an additional 18.5 hours. The THF in the reaction mixture was removed under a reduced pressure. The water suspension was extracted with ethyl acetate and n-butanol (1:1, 110 ml). The organic layer was washed with distilled water (11 ml). The organic layer was concentrated on a rotary evaporator to give a residue. This residue was washed with hexanes (2×10 ml) and dried under the reduced pressure to give a crude product (1.15 g).

Preparation of sodium salt of this compound was similar to Example 3.

EXAMPLE 7—Synthesis of another of the cholesterol absorption inhibitors described herein: Tetrasodium Diascorbyl Diphosphate Ester of Androst-5-ene-3β,17β-diol

In a dry round bottom flask, androst-5-ene-3β,17β-diol (1.5 g, 5.17 mmol) was dissolved in pyridine (3.0 ml) and THF (15 ml). Into another dry round bottom flask was added THF (20 ml) and POCl₃ (1.17 ml, 12.56 mmol). The latter was stirred at −5 °C for 5 minutes before the addition of androst-5-ene-3β,17β-diol over a period of 20 minutes. White precipitate was observed shortly after this addition and after the initial 20 minutes of reaction at −5 °C, the reaction was allowed to continue at room temperature for 2.5 hours.

The flask was then cooled to 0 °C, and a solution of 5,6-isopropylidene ascorbic acid (3.19 g, 14.78 mmol) in pyridine (3 ml) and THF (15 ml) was added drop-wise over a period of 20 minutes under vigorous stirring. The reaction was allowed to continue for another two hours. Then, the reaction mixture was filtered, and the filtrate was concentrated to a thick syrup. Heptane was added and the mixture was distilled under a reduced pressure. A solid crude was obtained.
The crude was dissolved in THF/1N HCl (1:1, 150 ml), and the hydrolysis was carried out at room temperature under vigorous stirring. After 12 hours of reaction, a TLC test indicated that the hydrolysis was complete. The THF in the reaction mixture was removed under a reduced pressure at room temperature, and n-butanol and ethyl acetate (1:1, 100 ml) was used for the extraction. The organic layer was washed with water (2×20 ml), and then concentrated to afford the crude product of diascorbyl diphosphate ester of androst-5-ene-3β,17β-diol (3.0 g).

The crude diascorbyl diphosphate ester of androst-5-ene-3β,17β-diol (400 mg) was dissolved in methanol (5 ml). To this solution was added 2 ml of sodium methoxide in methanol (20%, w/v) under magnetic stirring. White precipitate was observed upon the addition of sodium methoxide methanol solution. The suspension was stirred for half an hour before it was filtered and washed with methanol and acetone. The solid product was dried under high vacuum, and tetrasodium diascorbyl diphosphate ester of androst-5-ene-3β,17β-diol (330 mg) was obtained.

EXAMPLE 8—Measuring MDR-1 Expression in CaCo2 cells after treatment with FM-VP4 for one week

Confluent CaCo2 cells (P33-39) in T-75 flasks were treated with varying amounts of FM-VP4 (0.5um, 1.0um, 5.0um and 10.0uM) for one week. The media and treatment were changed every other day. After seven days, cells were harvested and total RNA isolated with TRIZOL™. Analysis conducted by reverse transcription→ cDNA using polymerase chain reaction.

Titration for primer drop of GAPDH procedure:
50ng cDNA (CaCo2 cells)
0.5 uM MDDR_1 forward and 0.5uM MDR-1 reverse

PCR Program: 94°C for 5 minutes, 94°C for 1 minute, 55°C for 1 minutes, 72°C for 1
minute 30 seconds, repeat from step 2 for 29 more times, 72°C for 10 minutes, 4°C.

Primer drop at cycle: 0,6,9,12,16,20,23,26,28 and 30.

Figure 1 is a graph showing these results, from which it is clear that with increasing concentrations of the cholesterol absorption inhibitor, MDR-1 gene expression is significantly reduced. Further supporting these results are shown in Figure 2, a graph showing the titration for primer drop of GAPDH and Figure 3 depicting polymerase chain reaction (1.5% agarose gel) electrophoretic plate results for MDR-1 and GAPDH.

Incubation with 10 mM FM-VP4 leads clearly to a significant downregulation of MDR-1.

EXAMPLE 9-- Measuring MRP-1 Expression in CaCo2 cells after treatment with FM-VP4 for one week
The entire protocol as provided in Example 8 with respect to the MDR-1 gene was used to confirm the effects of a cholesterol absorption inhibitor on the expression of another multiple drug resistance gene: MRP-1.

The results in Figure 6, a bar graph showing the level of ABCC1 (MRP-1) expression (normalized ratio of MRP-1/GAPDH) in CaCo2 cells after treatment for one week with FM-VP4 clearly demonstrate the inhibitory effect on gene expression.

EXAMPLE 10-- Cytotoxicity Studies: The non-toxicity of the cholesterol absorption inhibitor tested was verified by MTS, LDH and BCA assays

Caco2-cells were seeded into 48- or 96-well plates. The growth media was aspirated every 2-3 days and replaced with fresh media. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂. Cells then were treated with media, 0.1% Triton X-100 (as positive control for toxicity), FM-VP4, the selected cholesterol absorption inhibitor.
After 24 hours 50ml of each well were transferred into a new 96-well plate to perform the LDH-Assay. The MTS-Assay (CellTiter 96 AQueous One Solution kit (Promega™)) was done on the original plate.

Cell viability can be reflected by the integrity of the mitochondria. MTS-Assay is a colorimetric method for determining the number of viable cells (cytotoxicity) after treatment. When the MTS reagent (a tetrazolium salt) is applied to living cells, it is converted to a color compound (formazan) with the emission of light at 492nm. Cell viability can be reflected by the integrity of mitochondria.

After performing this assay media and solution was aspirated, cells were washed 3 times with PBS and lysed for BCA Protein Assay (PIERCE). This particular colorimetric assay measures total protein levels by identifying specific peptide bonds. The protein concentration is calculated from a calibration curve constructed with a protein standard (bovine serum albumin or “BSA”).

The LDH-assay looks at the integrity of cell membranes and can be used for cytotoxicity mediated by chemicals or other agents. Cell damage is associated with leakage of intracellular, cytoplasmic contents and lactate dehydrogenase or “LDH” (a stable cytosolic enzyme), can be used as a reported molecule for this event. LDH, released from cells into the culture medium, was measured using a kit (Cytotox96 Non-Radioactive Cytotoxicity Assay) from Promega™. This method is based on a series of linked enzyme reactions, the final reaction being the reduction of a tetrazolium salt to a coloured, insoluble, formazen product which can be measured at 492nm. Background absorbance from media alone and media including the treatment was substracted from the reading to correct the values.

Figure 4 is a bar graph of the MTS- and LDH-Assay of cell viability after treatment of CaCo2 with “FM-VP4”. Figure 5 a bar graph showing a BCA-Assay of protein concentration after treatment of CaCo2 with “FM-VP4”.
The cholesterol absorption inhibitor tested, FM-VP4, does not show any toxicity (mitochondrial activity) for a concentration range up to 100mM regardless of whether the treatment was for 24 hours (cell viability is 90.9 +/- 9.8%) or 96 hours (94.6 +/- 4.4%). At 250mM for 24 hour treatment it drops down to 59.2 +/- 8.1% and to 19.3 +/- 7.6% for 96 hours. The highest tested concentration of FM-VP4 75mM showed a cell viability of 18.5 +/- 5.8% after 24 hours and 22.1 +/- 1.6%.

These findings are consistent with the Protein concentration after treatment. It shows the same trend found with the MTS-Assay.

**EXAMPLE 11**—Western Blot analysis of P-glycoprotein in Caco2-cells after incubation with FM-VP4 (→ Protein expression)

**Experiment:**

- 10 flasks of Caco2-cells (T75)
- ~ 28 days post-seeding cells were divided into 2 groups (I and II)
- both groups received the same treatment for 1 week and then divided for analysis
- treatment group I: RNA isolation with TRIZOL → RT-PCR → PCR ⇄ mdr-1 expression
- treatment group II: Protein isolation → SDS-Page Gel → Western Blot ⇄ P-glycoprotein expression

Cells from group I were harvested and total cellular RNA was extracted with TRizOL® according the protocol. RNA was reverse transcribed into cDNA and specific primers were used to evaluate the expression level of MDR-1. Internal control was run by using GAPDH (primer drop). A sample from each PCR product was subjected to electrophoresis on a 1.5% Agarose gel (containing Ethidiumbromide). A 100 bp standard was run to identify the size of the product. The fluorescent bands were imaged under UV light (UVP-Epi Chemi II Darkroom) and quantified with the UVP-Labworks software.
Cells from group II underwent a protein extraction procedure. Concentration was measured using PIERCE BCA Protein Assay Reagent Kit. 15 µg total protein from each sample was electrophoresed on a 11% SDS Page Gel and transferred to a PVDF membrane (wet transfer). Identification of P-gp was done using the immunoblot technique. The monoclonal antibody used for P-gp was JSB-1 and the secondary was horseradish peroxide rabbit anti-mouse antibody (Jackson Immuno Research Laboratories, Inc.). After optimizing the assay the best dilutions for the antibody solution turned out to be 1:200 for the primary and 1:1000 for the secondary. The protein bands were visualized using a chemiluminescence kit from PerkinElmer and an X-Ray film was placed on the membranes. The film was exposed for about 1 minute and then developed.

Figure 9 is a Western Blot analysis of P-glycoprotein in CaCo2 cells after incubation with the selected cholesterol absorption inhibitor: "FM-VP4".

Incubation with FM-VP4 clearly leads to a down-regulation of MDR-1. In other words, a lower level of P-Glycoprotein which is encoded by mdr-1 was detectable. A seven day incubation with FM-VP4 10µM led to a down-regulation of mdr-1 (confirming earlier experiments). The level of protein also correlates with that finding. A lower level of P-Glycoprotein which is encoded by MDR-1 was detectable.

These data suggest that changes in the mdr-1 gene expression lead to correlating changes in the protein level P-gp which is encoded by mdr-1.

**EXAMPLE 12**--MDR-1 gene expression in Caco2-cells after treatment with FM-VP4 Liposomes

The objective was to confirm the influence of a cholesterol-free liposomal formulation of a cholesterol absorption inhibitor ("FM-VP4") on gene expression of a multiple drug resistance gene.
A dilution of FM-VP4 in water was applied to Caco2-cells. Of interest was the influence of liposomal FM-VP4 in cholesterol-free liposomal preparation (DMPC 100:0, ratio 10:1 DMPC:FM-VP4). FM-VP4 liposomes were utilized at three different concentrations (2.5, 5 and 10μM FM-VP4).

Procedure: Caco2-cells were seeded at 10,000 cells/cm² in T-75 flasks (Corning). The growth media was changed every other day. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂. The treatment groups contained 2.5, 5 and 10μM cholesterol-free liposomal FM-VP4 as well as empty cholesterol-free liposomes without FM-VP4. Cells were harvested after one week of treatment, total RNA isolated with TRIZOL® according to the protocol. RT-PCR followed that step and specific primers were used to evaluate the expression level of MDR-1. PCR products were resolved by electrophoresis on a 1.5% Agarose gel (containing Ethidiumbromide) and the fluorescent bands were imaged under UV light and quantified using the UVP-Labworks software.

Figure 7 depicts a polymerase chain reaction (1.5% agarose gel) electrophoresis results for MDR-1, GAPDH; RNA isolation with TRIZOL→ RT-PCR→ PCR after treatment of CaCo2 cells with liposomal formulations of one of cholesterol absorption inhibitors described herein: an ascorbyl stanyl phosphate ester called “FM-VP4;”

Figure 8 is a bar graph showing the level of MDR-1 expression (normalized ratio of MDR-1/GAPDH) in CaCo2 cells after treatment for one week with liposomal FM-VP4 at 2.5, 5 and 10μm as compared to a control and empty liposomes.

All 3 tested concentrations lead to a down-regulation of MDR-1 gene. Incubation with empty liposomes does not show any effect as hypothesized and can be used as control. The down-regulation is markedly more effective than when the cholesterol absorption inhibitor is solubilized in water.
Accordingly, MDR-1 gene expression in Caco2-cells after treatment with liposomal FM-VP4 at lower concentrations is significantly reduced and at lower concentrations than non-liposomal formulations.

**EXAMPLE 13- Preparation of Liposomes comprising cholesterol absorption inhibitor**

**General Protocol**

Making dry lipid film
2. Transferred appropriate amount of stock solution to test tubes.
3. Dried Solvent was dried under stream of nitrogen gas
down with nitrogen
4. When the lipids were almost dried down, placed it in a vacuum apparatus to get rid of the residual chloroform for at least 2 hours.
5. The dry lipid film was stored by capping it and placing it in the freezer.

Hydrating lipid
1. The water bath was set to 55°C.
2. Warmed up some HBS or selected cholesterol absorption inhibitor (ex: FM-VP4) dissolved in HBS in the water bath.
3. Added appropriate amount of HBS or FM-VP4 in HBS to the test tubes.
4. Vortexed.
5. Warmed it in the water bath.

Freeze thaw Cycles
1. Filled a Dewar with liquid Nitrogen
2. Transferred the lipid mixture into a plastic cryo tube.
3. Attached the cryo tube to a crane.
4. Freezed the sample in liquid nitrogen, and then thawed in water bath. Repeated this 5 times.
5. Stored the sample in the freezer.

Extrusion/ Adjusting size of Vesicles
1. Water bath was set to 55°C.
2. Assembled the extruder.
3. Tested the extruder with the nitrogen and then HBS.
4. Increased the pressure to 300 – 600 psi.
5. Before the sample was extruded, aliquoted 500uL, transferred it to an eppendorf, and labelled it BE.
6. Lipid suspension was forced through 2 polycarbonate filters
7. using 0.1µm filter to extrude sample to a size of 100nm
8. Extruded sample for 10 times.
9. Aliquotted 500uL, and labelled it AE.
10. After dialysis, aliquoted 500uL, and labelled it AD.
EXAMPLE 14--MDR-1 gene expression in Caco-2 cells after treatment with Cholesterol Absorption Inhibitor

Objective: To investigate the time-dependent mdr-1 gene expression in Caco-2 cells after treatment with cholesterol absorption inhibitor.

Experimental Procedures: Caco-2 cells were seeded at 10,000 cells/cm² in T-75 flasks (Corning). The growth media (Dulbecco’s minimal essential medium-DMEM) containing 10% heat-activated fetal bovine serum, 292 µg/ml glutamine, 0.1mM non-essential amino acids, 100U/ml penicillin and 100mg/ml glutamine was changed every other day. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂. Cells were treated with 10µM “FM-VP4”, as described above, solubilized in water when they reached about 95% confluency. The control group only got media.

The cells were harvested after 60, 90 minutes, 6, 24 and 48 hours. Total RNA was isolated using TRIzol Reagent, followed by reverse transcription into single-stranded cDNA. PCR reactions were performed with specific primers for mdr-1 and the housekeeping gene GAPDH as an internal control. A sample from each PCR product was subjected to electrophoresis on a 1.5% agarose gel. The fluorescent bands were visualised under UV light (UV-Epi Chemi II) and quantified with UVP-labworks software.

Figures 10 and 11: Expression profile of mdr-1 gene in Caco-2 cells was examined as an effect of a time-dependent treatment with 10µM FM-VP4. A sample from each PCR product was subjected to electrophoresis on a 1.5% agarose gel (10). The fluorescent bands were imaged under UV light (UV-Epi Chem II) and quantified with the UVP-Labworks software (11).

EXAMPLE 15--Effect of Cholesterol Absorption Inhibitors on cellular accumulation of Rhodamine 123 by Caco-2 cells
Objective: To investigate the accumulation of Rhodamine which is an index for the activity of the multidrug efflux transporter P-glycoprotein (P-gp). Lower P-gp activity leads to an accumulation of Rhodamine 123 in the cells.

Rhodamine 123 is a P-gp substrate (fluorescent dye) and has been used as a probe substrate to measure the functional activity of P-gp. The excitation wavelength is 485nm, the emission wavelength 520nm. Cells were incubated with “FM-VP4”, as described herein, and Rhodamine 123 is added to the basolateral side. After lysis of the cells the content of Rhodamine 123 was determined.

Caco-2 cells were seeded on 12mm diameter polycarbonate filter inserts (Transwell, Costar) at a density of 40,000 cells/cm². The growth media (Dulbecco’s minimal essential medium-DMEM) containing 10% heat-activated fetal bovine serum, 292 μg/ml glutamine, 0.1mM non-essential amino acids, 100U/ml penicillin and 100mg/ml glutamine was changed every other day. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂. For the treatment experiments, FM-VP4 at different concentrations were added to the media and applied onto the apical and basolateral side of the Transwell plate. Transepithelial electrical resistance (TEER) of the monolayers was measured to confirm monolayer integrity. Caco-2 cells with TEER Values above 400 Ω/cm² were then used for P-gp studies.

After one week of treatment cells were washed with HBSS (without Phenol Red) with 10mM Hepes, pH 7.4 several times and pre-incubated with FM-VP4 in HBSS and 10mM Hepes for 1 hour. TEER Values were measured to check the integrity of the monolayer. Transwells with a TEER Value below 400 Ω/cm² were excluded from the experiment. The control well contained only media, positive control for inhibition of P-gp was Verapamil. 5μM Rhodamine 123 was added to the basolateral side and after 3 hours at 37°C the medium was removed and cells were washed 3 times with ice-cold PBS. Then the membrane was cut out, transferred into a 1.5ml Eppendorf tube and 250μl of 1% Triton X-100 was added and vortexed. After 10 minutes at room temperature and a centrifugation step for 5 min to remove cell debris, 25μl (in triplicates) were transferred into a 96-well plate and the fluorescence (excitation:
485nm; emission: 530nm) was measured. Finally the protein content was determined using a BCA Protein Assay (PIERCE) and Rhodamine 123 accumulation was normalized.

Figure 12: Effect of pre-incubation with FM-VP4 for 1 week on accumulation of Rh123 in Caco-2 monolayer. Data represents the average of ± SD. *P<0.002; **P<0.0001

The positive control for P-gp inhibition with 100µM Verapamil results in an accumulation of 1604 pmol Rhodamine 123 per mg protein. Enhanced cellular accumulation of a P-gp substrate Rhodamine 123 and therefore a decreased P-gp activity using FM-VP4 concentrations above 5µM for 7 days can be observed.

**EXAMPLE 16**-Influence of Cholesterol Absorption Inhibitors on the functional activity of the multidrug efflux transporter P-glycoprotein (P-gp)

Experiments were performed to measure the activity of P-gp in Caco-2 cells after treatment with FM-VP4. In previous experiments it was confirmed that FM-VP4 decreases the gene expression of mdr-1 (which encodes for P-gp) after one week administration of FM-VP4 (10µM). The down-regulation of mdr-1 resulted in a lower protein expression level of P-gp as shown in Western Blot analysis.

Human intestinal epithelia monolayers Caco-2 cells express P-gp on their apical membranes. A good system to measure apical and basolateral transport across monolayers and to determine the permeability of a certain substance is a Transwell Plate. Cells are grown on a semi-permeable membrane which is placed between two chambers. Caco-2 cells differentiate into highly functionalized epithelial barrier. Morphological and biochemical it is similar to the small intestinal columnar epithelium. To measure drug absorption like the intestinal environment, the substance is given into the apical side and the concentration in the basolateral side is measured. This represents the intestinal absorption. Secretory transport can be determined if the substance is added onto the basolateral side and the transport into the apical
chamber is measured. A good substrate for P-gp is Rhodamine 123 and it has been used as a probe to measure the functional activity of P-gp. It is a fluorescent dye. Rhodamine 123 has a molar extinction coefficient of 85,200 M⁻¹cm⁻¹ at 511nm.

Caco-2 cells were seeded on 12mm diameter polycarbonate filter inserts (Transwell, Costar). The growth media (Dulbecco's minimal essential medium-DMEM) containing 10% heat-activated fetal bovine serum, 292 µg/ml glutamine, 0.1mM non-essential amino acids, 100U/ml penicillin and 100mg/ml glutamine was changed every other day. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂. For the treatment experiments, FM-VP4 at different concentrations was added to the media and applied onto apical and the basolateral side of the Transwell plate for one week. The Control well contained only media. A positive control for inhibition of P-gp was Verapamil (calcium channel blocker).

Transepithelial electrical resistance (TEER) of the monolayers was measured to confirm monolayer integrity. Caco-2 cells with TEER Values above 400 Ω/cm² were then used for transport studies. To measure the secretory transport (basolateral to apical) 5µM Rhodamine was added into the basolateral chamber. In a time dependent manner samples were taken from the apical side and the fluorescence was determined. Immediately the samples were replaced by fresh receiver medium (media, media plus FM-VP4 or media plus P-gp inhibitor).

In summary, and as shown in Figure 13, an effect of the cholesterol absorption inhibitor on the apical uptake of Rhodamine 123 in a Caco-2 cell Transwell system could be observed. Significant P-gp inhibition occurs at concentrations of 25µM FM-VP4 when incubated at both sides (apical as well as basolateral side).
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WE CLAIM:

1. A method of inhibiting the expression of a multi-drug resistance gene in an animal cell which comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor.

2. The method of claim 1 wherein the animal cell is a tumour cell insensitive to chemotherapeutic agents due to over-expression of a multi-drug resistance gene.

3. The method of claim 1 wherein the cholesterol absorption inhibitor is a sterol or stanol, or mixture thereof, in a natural or artificially synthesized form.

5. The method of claim 1 wherein the cholesterol absorption inhibitor is a sterol or stanol in any one of their isomeric forms.

6. The method of claim 1 wherein the cholesterol absorption inhibitor is a sterol selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol, poriferasterol, clionasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrillasterol, peposterol, avenasterol, isoavenasterol, fecosterol, and pollinastasterol.

7. The method of claim 1 wherein the cholesterol absorption inhibitor is a stanol selected from the group consisting of selected from the group consisting of sitostanol, campestanol, stigmastanol, brassicastanol (including dihydrobrassicasterol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrillastanol, pepostanol, avenastanol, isoavenastanol, fecostanol, and pollinastanol.
8. The method of claim 1 wherein the cholesterol absorption inhibitor is a sterol derivative or a stanol derivative selected from the group consisting of aliphatic esters, aromatic esters, phenolic acid esters, cinnamate esters, ferulate esters, glycosides, acylated glycosides and acylglycosides.

9. The method of claim 1 wherein the cholesterol absorption inhibitor is one or more compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

i) \[
\begin{array}{c}
  \text{R}_4 \text{--P--O--R} \\
  \text{OH}
\end{array}
\]

ii) \[
\begin{array}{c}
  \text{R}_4 \text{--C--O--R}
\end{array}
\]

iii) \[
\begin{array}{c}
  \text{R}_4 \text{--R}
\end{array}
\]

iv) \[
\begin{array}{c}
  \text{R}_4 \text{--(CH}_2)_n \text{--C--O--R}
\end{array}
\]

wherein R is a sterol or stanol moiety, R₄ is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

10. The method of claim 9 wherein the cholesterol absorption inhibitor is a \{disodium ascorbyl stanyl phosphate\} composition which comprises disodium ascorbyl campestanoyl phosphate and disodium ascorbyl sitostanyl phosphate.
11. The method of claim 1 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone.

12. The method of claim 1 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone compound represented by the formula:

```
Ar^1 - X_m - (C)_q - Y_n - (C)_p - Z_R^3
```

or a biologically acceptable salt thereof, wherein: Ar_1 and Ar_2 are independently selected from the group consisting of aryl and R_4 -substituted aryl;

Ar_3 is aryl or R_5 -substituted aryl;

X, Y and Z are independently selected from the group consisting of --CH_2 --, --CH(lower alkyl)- and --C(dilower alkyl)-;

R and R_2 are independently selected from the group consisting of --OR_6, --O(CO)R_6, --O(CO)OR_6 and --O(CO)NR_6 R_7 ;

R_1 and R_3 are independently selected from the group consisting of hydrogen, lower alkyl and aryl;

q is 0 or 1; r is 0 or 1; m, n and p are independently 0, 1, 2, 3 or 4; provided that at least one of q and r is 1, and the sum of m, n, p and r is 2, 3, 4, 5 or 6; and provided that when p is 0 and r is 1, the sum of m, q and n is 1, 2, 3, 4 or 5;

R_4 is 1-5 substituents independently selected from consisting of lower alkyl, --OR_6, --O(CO)R_6, --O(CO)OR_6, --O(CH_2)_1-5 OR_6, --O(CO)NR_6 R_7, --NR_6 R_7, --NR_6 (CO)R_7, --NR_6 (CO)OR_6, --NR_6 (CO)NR_7 R_6, --NR_6 SO_2 R_9, --COOR_6, --CONR_6 R_7, --COR_6, --
SO₂ NR₆ R₇, S(O)₂ R₉, --O(CH₂)₃₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, -(lower alkylene)COOR₆, --CH=CH--COOR₆, --CF₃, --CN, --NO₂ and halogen;

R₅ is 1-5 substituents independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₆, --O(CH₂)₁₋₅ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₆, --NR₆ (CO)NR₇ R₈, --NR₆ SO₂ R₉, --COOR₆, --CONR₆ R₇, --COR₆, --SO₂ NR₉ R₇, S(O)₂ R₉, --O(CH₂)₃₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, -(lower alkylene)COOR₆ and --CH=CH--COOR₆;

R₆, R₇ and R₈ are independently selected from the group consisting of hydrogen, lower alkyl, aryl and aryl-substituted lower alkyl; and

R₉ is lower alkyl, aryl or aryl-substituted lower alkyl.

13. The method of claim 12 wherein, in the compound, Ar₁ is phenyl or R₄ substiuted phenyl, Ar₂ is phenyl or R₄ -substituted phenyl and Ar₃ is R₅ -substituted phenyl.

14. The method of claim 12, wherein in the compound, Ar₁ is R₄ -substituted phenyl wherein R₄ is halogen; Ar₂ is R₄ -substituted phenyl wherein R₄ is halogen or --OR₆, wherein R₆ is lower alkyl or hydrogen; and Ar₃ R₅ -substituted phenyl, wherein R₅ is --OR₆ wherein R₆ is lower alkyl or hydrogen.

15. The method of claim 12 wherein in the compound X, Y, and Z are each --CH₂ --; R₁ and R₃ are each hydrogen; R and R₂ are each --OR₆, wherein R₆ is hydrogen; and the sum of m, n, p, q and r is 2, 3 or 4.

16. The method of claim 12 wherein in the compound, m, n and r are each zero, q is 1 and p is 2.

17. The method of claim 12 wherein in the compound, p, q and n are each zero, r is 1 and m is 2 or 3.

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18. The method of claim 12 wherein the compound is selected from the group consisting of

3(R)-(2(R)-hydroxy-2-phenylethyl)-4(R)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(2(R)-hydroxy-2-phenylethyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(S)-(1(S)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(S)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel-3(R)-\(\alpha\)-(S)-hydroxy-(2-naphthalenyl)methyl-4(3S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel-3(R)-\(\alpha\)-(R)-hydroxy-(2-naphthalenyl)methyl-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(3(R)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

3(R)-(3(S)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

4(S)-(4-hydroxyphenyl)-3(R)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

4(S)-(4-hydroxyphenyl)-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

rel 3(R)-\(\alpha\)-3(RS)-hydroxy-3-(4-methoxymethoxy)-phenyl-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;
1-(4-fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl)-4(S)-(sup .4 -
hydroxyphenyl)-2-azetidinone;

1-(4-fluorophenyl)-3(R)→3(R)-(4-fluorophenyl)-3-hydroxypropyl)-4(S)-(4-hy
droxyphenyl)-2-azetidinone;

4(S)→4-(acetyloxy)phenyl)-3(R)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyp henyl)-
2-azetidinone;

4(S)→4-(acetyloxy)phenyl)-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1(4-methoxyp enyl)-
2-azetidinone;

1-(sup.4 -fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl)-4(S)→4-(phen
ylmethoxy)phenyl)-2-azetidinone;

3(R)→3(R)-acetyloxy)-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetid inone;

3(R)→3(S)-acetyloxy)-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetid inone;

3(R)→3(R)-(acetyloxy)-3-(4-fluorophenyl)propyl)-4(S)→4-(acetyloxy)phenyl -1-(4-
fluorophenyl)-2-azetidinone;

3(R)→3(S)-(acetyloxy)-3-(4-fluorophenyl)propyl)-4(S)→4-(acetyloxy)phenyl -1-(4-
fluorophenyl)-2-azetidinone;

3(R)→3(R)-(acetyloxy)-3-(4-chlorophenyl)propyl)-4(S)→4-(acetyloxy)phenyl -1-(4-
chlorophenyl)-2-azetidinone;

3(R)→3(S)-(acetyloxy)-3-(4-chlorophenyl)propyl)-4(S)→4-(acetyloxy)phenyl -1-(4-
chlorophenyl)-2-azetidinone; and
rel 1-(4-fluorophenyl)-4(S)-(4-hydroxyphenyl)-3(1R)-(1(R)-hydroxy-3-phenylpropyl)-2-azetidinone.

19. The method of claim 1 wherein the cholesterol absorption inhibitor is an androstan e and/or androstene derivative, wherein androstane and/or androstene are coupled with ascorbic acid and represented by one or more of the general formulae:

![Formula VI](image1)

![Formula VII](image2)
VIII

wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ may individually be chosen from hydrogen, OH, carbonyl, and an ascorbyl moiety; and $R_7$ may be hydrogen or any halogen.

20. The method of claim 19 wherein the ascorbyl moiety which is coupled to the compound from the androstane or androstene family is selected individually from one or more of the following structures:
wherein M+ represents any metal, alkali earth metal, or alkali metal.

21. The method of claim 1 wherein the cholesterol absorption inhibitor is an inhibitor of bile acid transport or reabsorption and is selected from the group consisting of all ileal, apical and hepatic transport inhibitors.

22. A method of enhancing the effectiveness of a chemotherapeutic agent in an animal having cancer, which comprises administering to said animal an effective amount of:
   a) the chemotherapeutic agent; and
   b) at least one cholesterol absorption inhibitor.

23. The method of claim 22 wherein the cholesterol absorption inhibitor is a sterol or stanol, or mixture thereof, in a natural or artificially synthesized form.

24. The method of claim 22 wherein the cholesterol absorption inhibitor is a sterol or stanol in any one of their isomeric forms.

25. The method of claim 22 wherein the cholesterol absorption inhibitor is a sterol selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol, poriferasterol, clionasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrillasterol, peposterol, avenasterol, isoavenasterol, fecosterol, and pollinastasterol.

26. The method of claim 22 wherein the cholesterol absorption inhibitor is a stanol selected from the group consisting of selected from the group consisting of sitostanol, campestanol, stigmastanol, brassicastanol (including dihydrobrassicastanol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrillastanol, pepostanol, avenastanol, isoavenastanol, fecostanol,
and pollinastastanol.

27. The method of claim 22 wherein the cholesterol absorption inhibitor is a sterol derivative or a stanol derivative selected from the group consisting of aliphatic esters, aromatic esters, phenolic acid esters, cinnamate esters, ferulate esters, glycosides, acylated glycosides and acylglycosides.

28. The method of claim 22 wherein the cholesterol absorption inhibitor is one or more compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

i)

\[ R_4 - \overset{\text{O}}{\overset{\text{P}}{\text{O}}} - O - R \]

ii)

\[ R_4 - \overset{\text{O}}{\overset{\text{C}}{\text{C}}} - \overset{\text{O}}{\overset{\text{C}}{\text{O}}} - R \]

iii)

\[ R_4 \longrightarrow R \]

iv)

\[ R_4 - (\text{CH}_2)_n \overset{\text{O}}{\overset{\text{C}}{\text{O}}} - R \]

wherein R is a sterol or stanol moiety, R_4 is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

29. The method of claim 28 wherein the cholesterol absorption inhibitor is a disodium ascorbyl stanyl phosphate composition which comprises disodium ascorbyl...
campesterol phosphate and disodium ascorbyl sitosteryl phosphate.

30. The method of claim 22 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone.

31. The method of claim 22 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone compound represented by the formula:

\[
\begin{array}{c}
\text{Ar}^1 \to \text{X} \to \text{Y} \to \text{Z} \\
\text{R}^1 \to \text{R}^2 \\
\text{R}^3 \to \text{Ar}^3
\end{array}
\]

or a biologically acceptable salt thereof, wherein: \(\text{Ar}_1\) and \(\text{Ar}_2\) are independently selected from the group consisting of aryl and \(\text{R}_4\)-substituted aryl;

\(\text{Ar}_3\) is aryl or \(\text{R}_5\)-substituted aryl;

\(\text{X}, \text{Y}\) and \(\text{Z}\) are independently selected from the group consisting of \(-\text{CH}_2-, -\text{CH}(\text{lower alkyl})-\) and \(-\text{C}(\text{dilower alkyl})-\);

\(\text{R}\) and \(\text{R}_2\) are independently selected from the group consisting of \(-\text{OR}_6, -\text{O}(\text{CO})\text{R}_6, -\text{O}(\text{CO})\text{OR}_6\) and \(-\text{O}(\text{CO})\text{NR}_6\text{R}_7\);

\(\text{R}_1\) and \(\text{R}_3\) are independently selected from the group consisting of hydrogen, lower alkyl and aryl;

\(q\) is 0 or 1; \(r\) is 0 or 1; \(m, n, p\) are independently 0, 1, 2, 3 or 4; provided that at least one of \(q\) and \(r\) is 1, and the sum of \(m, n, p, q\) and \(r\) is 2, 3, 4, 5 or 6; and provided that when \(p\) is 0 and \(r\) is 1, the sum of \(m, q\) and \(n\) is 1, 2, 3, 4 or 5;

\(\text{R}_4\) is 1-5 substituents independently selected from consisting of lower alkyl, --
OR₆, --O(CO)R₆, --O(CO)OR₆, --O(CH₂)₁₋₆ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₇, --NR₆ (CO)NR₇ R₈, --NR₈ SO₂ R₈, --COOR₈, --CONR₈ R₇, --COR₈, --SO₂ NR₈ R₇, S(O)₀₋₂ R₈, --O(CH₂)₁₋₁₀ --COOR₈, --O(CH₂)₁₋₁₀ CONR₈ R₇, -(lower alkylene)COOR₈, --CH==CH--COOR₈, --CF₃, --CN, --NO₂ and halogen;

R₆ is 1-5 substituents independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₆, --O(CH₂)₁₋₆ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₇, --NR₆ (CO)NR₇ R₈, --NR₈ SO₂ R₈, --COOR₈, --CONR₈ R₇, --COR₈, --SO₂ NR₈ R₇, S(O)₀₋₂ R₈, --O(CH₂)₁₋₁₀ --COOR₈, --O(CH₂)₁₋₁₀ CONR₈ R₇, -(lower alkylene)COOR₈ and --CH==CH--COOR₈;

R₆, R₇ and R₈ are independently selected from the group consisting of hydrogen, lower alkyl, aryl and aryl-substituted lower alkyl; and

R₉ is lower alkyl, aryl or aryl-substituted lower alkyl.

32. The method of claim 31 wherein, in the compound, Ar₁ is phenyl or R₄ substituted phenyl, Ar₂ is phenyl or R₄ substituted phenyl and Ar₅ is R₅ substituted phenyl.

33. The method of claim 31, wherein in the compound, Ar₁ is R₄ substituted phenyl wherein R₄ is halogen; Ar₂ is R₄ substituted phenyl wherein R₄ is halogen or --OR₆, wherein R₆ is lower alkyl or hydrogen; and Ar₃ R₅ substituted phenyl, wherein R₅ is --OR₆, wherein R₆ is lower alkyl or hydrogen.

34. The method of claim 31 wherein in the compound X, Y, and Z are each --CH₂ --; R₁ and R₃ are each hydrogen; R and R₂ are each --OR₆, wherein R₆ is hydrogen; and the sum of m, n, p, q and r is 2, 3 or 4.

35. The method of claim 31 wherein in the compound, m, n and r are each zero, q is 1 and p is 2.
36. The method of claim 31 wherein in the compound, p, q and n are each zero, r is 1 and m is 2 or 3.

37. The method of claim 31 wherein the compound is selected from the group consisting of

rel 3(R)-(2(R)-hydroxy-2-phenylethyl)-4(R)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel 3(R)-(2(R)-hydroxy-2-phenylethyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(S)-(1(S)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(S)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel-3(R)-(S)-hydroxy-(2-naphthalenyl)methyl-4(3S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel-3(R)-(R)-hydroxy-(2-naphthalenyl)methyl-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(3(R)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone; 

3(R)-(3(S)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;
4(S)-(4-hydroxyphenyl)-3(R)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

4(S)-(4-hydroxyphenyl)-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

rel 3(R)→3(RS)-hydroxy-3→4-(methoxymethoxy)-phenyl!-1,4(S)-bis-(4-methoxyphene nyl)-2-azetidinone;

1-(4-fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl!-4(S)-(sup .4 -hydroxyphenyl)-2-azetidinone;

1-(4-fluorophenyl)-3(R)→3(R)-(4-fluorophenyl)-3-hydroxypropyl!-4(S)-(4-hydroxyphenyl)-2-azetidinone;

4(S)→4-(acetyloxy)phenyl!-3(S)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyp henyl)-2-azetidinone;

4(S)→4-(acetyloxy)phenyl!-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1(4-methoxyph enyl)-2-azetidinone;

1-(.sup.4 -fluorophenyl)-3(R)→3(S)-(4-fluorophenyl)-3-hydroxypropyl!-4(S)→4-(phen ylmethoxy)phenyl!-2-azetidinone;

3(R)→3(R)-acetyloxy)-3-phenylpropyl!-1,4(S)-bis-(4-methoxyphenyl)-2-azetid inone;

3(R)→3(S)-acetyloxy)-3-phenylpropyl!-1,4(S)-bis-(4-methoxyphenyl)-2-azetid inone;

3(R)→3(R)-(acetyloxy)-3-(4-fluorophenyl)propyl!-4(S)→4-(acetyloxy)phenyl! -1-(4-fluorophenyl)-2-azetidinone;
3(R)→3(S)-(acetyloxy)-3-(4-fluorophenyl)propyl-4(S)→4-(acetyloxy)phenyl-1-(4-fluorophenyl)-2-azetidinone;

3(R)→3(R)-(acetyloxy)-3-(4-chlorophenyl)propyl-4(S)→4-(acetyloxy)phenyl-1-(4-chlorophenyl)-2-azetidinone;

3(R)→3(S)-(acetyloxy)-3-(4-chlorophenyl)propyl-4(S)→4-(acetyloxy)phenyl-1-(4-chlorophenyl)-2-azetidinone; and

rel 1-(4-fluorophenyl)-4(S)-(4-hydroxyphenyl)-3(1R)-(1(R)-hydroxy-3-phenylpropyl)-2-azetidinone.

38. The method of claim 22 wherein the cholesterol absorption inhibitor is an androstane and/or androstene derivative, wherein androstane and/or androstene are coupled with ascorbic acid and represented by one or more of the general formulae:
wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ may individually be chosen from hydrogen, OH, carbonyl, and an ascorbyl moiety; and $R_7$ may be hydrogen or any halogen.

39. The method of claim 38 wherein the ascorbyl moiety which is coupled to the compound from the androstane or androstene family is selected individually from one or more of the following structures:
wherein M+ represents any metal, alkali earth metal, or alkali metal.

40. The method of claim 22 wherein the cholesterol absorption inhibitor is an inhibitor of bile acid transport or reabsorption and is selected from the group consisting of all ileal, apical and hepatic transport inhibitors.

41. A method of reversing a multi-drug resistance phenotype exhibited by an animal cell which comprises exposing the cell to an effective amount of at least one cholesterol absorption inhibitor.

42. The method of claim 41 wherein the animal cell is a tumour cell insensitive to chemotherapeutic agents due to over-expression of a multi-drug resistance gene.

43. The method of claim 41 wherein the cholesterol absorption inhibitor a sterol or stanol, or mixture thereof, in a natural or artificially synthesized form.

44. The method of claim 41 wherein the cholesterol absorption inhibitor is a sterol or stanol, or mixture thereof, in any one of their isomeric forms.

45. The method of claim 41 wherein the cholesterol absorption inhibitor is a sterol selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol,
poriferasterol, clonasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrillaasterol, peposterol, avenasterol, isoavenasterol, fecosterol, and pollinastasterol.

46. The method of claim 41 wherein the cholesterol absorption inhibitor is a stanol selected from the group consisting of selected from the group consisting of sitostanol, campestanol, stigmastanol, brassicastanol (including dihydrobrassicastanol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrillaestanol, pepostanol, avenastanol, isoavenastanol, fecostanol, and pollinastanol.

47. The method of claim 41 wherein the cholesterol absorption inhibitor is a sterol derivative or a stanol derivative selected from the group consisting of aliphatic esters, aromatic esters, phenolic acid esters, cinnamate esters, ferulate esters, glycosides, acylated glycosides and acylglycosides.

48. The method of claim 41 wherein the cholesterol absorption inhibitor is one or more compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

i) 
\[
\begin{align*}
\text{R}_4 & \longrightarrow \text{O} \longrightarrow \text{R} \\
\text{OH}
\end{align*}
\]

ii) 
\[
\begin{align*}
\text{R}_4 & \longrightarrow \text{C} \longrightarrow \text{C} \longrightarrow \text{O} \longrightarrow \text{R}
\end{align*}
\]

iii) 
\[
\text{R}_4 \longrightarrow \text{R}
\]
wherein R is a sterol or stanol moiety, R₄ is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

49. The method of claim 48 wherein the cholesterol absorption inhibitor is a disodium ascorbyl stanyl phosphate composition which comprises disodium ascorbyl campestaneryl phosphate and disodium ascorbyl sitostanyl phosphate.

50. The method of claim 41 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone.

51. The method of claim 41 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone compound represented by the formula:

or a biologically acceptable salt thereof, wherein: Ar₁ and Ar₂ are independently selected from the group consisting of aryl and R₄-substituted aryl;

Ar₃ is aryl or R₆-substituted aryl;

X, Y and Z are independently selected from the group consisting of —CH₂—, —CH(lower alkyl)— and —C(dilower alkyl)—;
R and R₂ are independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₉ and --O(CO)NR₆ R₇;

R₁ and R₃ are independently selected from the group consisting of hydrogen, lower alkyl and aryl;

q is 0 or 1; r is 0 or 1; m, n and p are independently 0, 1, 2, 3 or 4; provided that at least one of q and r is 1, and the sum of m, n, p, q and r is 2, 3, 4, 5 or 6; and provided that when p is 0 and r is 1, the sum of m, q and n is 1, 2, 3, 4 or 5;

R₄ is 1-5 substituents independently selected from consisting of lower alkyl, --OR₆, --O(CO)R₆, --O(CO)OR₉, --O(CH₂)₁₋₅ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₉, --NR₆ (CO)NR₇ R₈, --NR₆ SO₂ R₉, --S(O)₂ R₉, --O(CH₂)₁₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, --(lower alkylene)COOR₆, --CH=CH--COOR₆, --CF₃, --CN, --NO₂ and halogen;

R₅ is 1-5 substituents independently selected from the group consisting of --OR₆, --O(CO)R₆, --O(CO)OR₉, --O(CH₂)₁₋₅ OR₆, --O(CO)NR₆ R₇, --NR₆ R₇, --NR₆ (CO)R₇, --NR₆ (CO)OR₉, --NR₆ (CO)NR₇ R₈, --NR₆ SO₂ R₉, --S(O)₂ R₉, --O(CH₂)₁₋₁₀ --COOR₆, --O(CH₂)₁₋₁₀ CONR₆ R₇, --(lower alkylene)COOR₆ and --CH=CH--COOR₆;

R₆, R₇ and R₉ are independently selected from the group consisting of hydrogen, lower alkyl, aryl and aryl-substituted lower alkyl; and

R₉ is lower alkyl, aryl or aryl-substituted lower alkyl.

52. A composition for use in cancer treatment which comprises at least one chemotherapeutic agent and at least one cholesterol absorption inhibitor.

53. The composition of claim 52 wherein the cholesterol absorption inhibitor a sterol or stanol, or mixture thereof, in a natural or artificially synthesized form.
54. The composition of claim 52 wherein the cholesterol absorption inhibitor is a sterol or stanol, or mixture thereof, in any one of their isomeric forms.

55. The composition of claim 52 wherein the cholesterol absorption inhibitor is a sterol selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol, poriferasterol, clionasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrilasterol, peposterol, avenasterol, isoavenasterol, fecosterol, and pollinastasterol.

56. The composition of claim 52 wherein the cholesterol absorption inhibitor is a stanol selected from the group consisting of selected from the group consisting of sitostanol, campestanol, stigmasteranol, brassicastanol (including dihydrobrassicastanol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrilastanol, pepostanol, avenastanol, isoavenastanol, fecostanol, and pollinastastanol.

57. The composition of claim 52 wherein the cholesterol absorption inhibitor is a sterol derivative or a stanol derivative selected from the group consisting of aliphatic esters, aromatic esters, phenolic acid esters, cinnamate esters, ferulate esters, glycosides, acylated glycosides and acylglycosides.

58. The composition of claim 52 wherein the cholesterol absorption inhibitor is one or more compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

\[ R_4 - O - O - R \\
\text{OH} \]
ii) \[ R_4 - \overset{O}{\underset{O}{\underset{\text{C}}{\text{C}}} - \text{O} - R} \]

iii) \[ R_4 - R \]

iv) \[ R_4 - (\text{CH}_2)_n \overset{\text{O}}{\underset{\text{C}}{\text{O}}} - R \]

wherein R is a sterol or stanol moiety, R₄ is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

59. The composition of claim 52 wherein the cholesterol absorption inhibitor is a disodium ascorbyl stanyl phosphate composition which comprises disodium ascorbyl campestatnayl phosphate and disodium ascorbyl sitostanyl phosphate.

60. The composition of claim 52 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone.

61. A kit comprising at least two separate components:
   a) a composition comprising at least one cholesterol absorption inhibitor; and
   b) a composition comprising at least one chemotherapeutic agent;
along with instructions describing the administration of each composition.

62. A method of inhibiting the production of a protein expressed by a multiple drug resistance gene in an animal cell comprises administering to an animal an effective amount of at least one cholesterol absorption inhibitor.
63. The method of claim 62 wherein the cholesterol absorption inhibitor is a sterol or stanol, or mixture thereof, in a natural or artificially synthesized form.

64. The method of claim 62 wherein the cholesterol absorption inhibitor is a sterol or stanol in any one of their isomeric forms.

65. The method of claim 62 wherein the cholesterol absorption inhibitor is a sterol selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol (including dihydrobrassicasterol), desmosterol, chalinosterol, poriferasterol, clionasterol, ergosterol, coprosterol, codisterol, isofucosterol, fucosterol, clerosterol, nervisterol, lathosterol, stellasterol, spinasterol, chondrillasterol, peposterol, avenasterol, isoavenasterol, fecosterol, and pollinastasterol.

66. The method of claim 62 wherein the cholesterol absorption inhibitor is a stanol selected from the group consisting of sitostanol, campestanol, stigmasteranol, brassicastanol (including dihydrobrassicastanol), desmostanol, chalinostanol, poriferastanol, clionastanol, ergostanol, coprostanol, codistanol, isofucostanol, fucostanol, clerostanol, nervistanol, lathostanol, stellastanol, spinastanol, chondrillastanol, pepostanol, avenastanol, isoavenastanol, fecostanol, and pollinastanol.

67. The method of claim 62 wherein the cholesterol absorption inhibitor is a sterol derivative or a stanol derivative selected from the group consisting of aliphatic esters, aromatic esters, phenolic acid esters, cinnamate esters, ferulate esters, glycosides, acylated glycosides and acylglycosides.

68. The method of claim 62 wherein the cholesterol absorption inhibitor is one or more compounds comprising a sterol or stanol, including biologically acceptable salts thereof, having one or more of the following formulae:

i)
wherein R is a sterol or stanol moiety, R₄ is derived from ascorbic acid and n=1-5, including all biologically acceptable salts or solvates or prodrugs of at least one such compound or of the salts or of the solvates thereof.

69. The method of claim 68 wherein the cholesterol absorption inhibitor is a disodium ascorbyl stanyl phosphate composition which comprises disodium ascorbyl campestanyl phosphate and disodium ascorbyl sitostanyl phosphate.

70. The method of claim 62 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone.

71. The method of claim 62 wherein the cholesterol absorption inhibitor is a hydroxy substituted azetidinone compound represented by the formula:
or a biologically acceptable salt thereof, wherein: \(A_r_1\) and \(A_r_2\) are independently selected from the group consisting of aryl and \(R_4\)-substituted aryl;

\(A_r_3\) is aryl or \(R_5\)-substituted aryl;

\(X, Y\) and \(Z\) are independently selected from the group consisting of --CH\(_2\)--, --CH(lower alkyl)-- and --C(dilower alkyl)--;

\(R\) and \(R_2\) are independently selected from the group consisting of --OR\(_6\), --O(CO)R\(_6\), --O(CO)OR\(_9\) and --O(CO)NR\(_6\) R\(_7\);

\(R_1\) and \(R_3\) are independently selected from the group consisting of hydrogen, lower alkyl and aryl;

\(q\) is 0 or 1; \(r\) is 0 or 1; \(m, n\) and \(p\) are independently 0, 1, 2, 3 or 4; provided that at least one of \(q\) and \(r\) is 1, and the sum of \(m, n, q\) and \(r\) is 2, 3, 4, 5 or 6; and provided that when \(p\) is 0 and \(r\) is 1, the sum of \(m, q\) and \(n\) is 1, 2, 3, 4 or 5;

\(R_4\) is 1-5 substituents independently selected from consisting of lower alkyl, --OR\(_6\), --O(CO)R\(_6\), --O(CO)OR\(_9\), --O(CH\(_2\))\(_{1-5}\) OR\(_6\), --O(CO)NR\(_6\) R\(_7\), --NR\(_6\) R\(_7\), --NR\(_6\) (CO)R\(_7\), --NR\(_6\) (CO)OR\(_9\), --NR\(_6\) (CO)NR\(_7\) R\(_8\), --NR\(_6\) SO\(_2\) R\(_9\), --COOR\(_6\), --CONR\(_6\) R\(_7\), --COR\(_6\), --SO\(_2\) NR\(_6\) R\(_7\), S(O)\(_{0-2}\) R\(_9\), --O(CH\(_2\))\(_{1-10}\) --COOR\(_6\), --O(CH\(_2\))\(_{1-10}\) CONR\(_6\) R\(_7\), --(lower alkylene)COOR\(_6\), --CH=CH--COOR\(_6\), --CF\(_3\), --CN, --NO\(_2\) and halogen;

\(R_5\) is 1-5 substituents independently selected from the group consisting of --OR\(_6\), --O(CO)R\(_6\), --O(CO)OR\(_9\), --O(CH\(_2\))\(_{1-5}\) OR\(_6\), --O(CO)NR\(_6\) R\(_7\), --NR\(_6\) R\(_7\), --NR\(_6\) (CO)R\(_7\), --NR\(_6\) (CO)OR\(_9\), --NR\(_6\) (CO)NR\(_7\) R\(_8\), --NR\(_6\) SO\(_2\) R\(_9\), --COOR\(_6\), --CONR\(_6\) R\(_7\), --COR\(_6\), --SO\(_2\) NR\(_6\) R\(_7\), S(O)\(_{0-2}\) R\(_9\), --O(CH\(_2\))\(_{1-10}\) --COOR\(_6\), --O(CH\(_2\))\(_{1-10}\) CONR\(_6\) R\(_7\), --(lower alkylene)COOR\(_6\) and --CH=CH--COOR\(_6\);
R₆, R₇ and R₈ are independently selected from the group consisting of hydrogen, lower alkyl, aryl and aryl-substituted lower alkyl; and

R₈ is lower alkyl, aryl or aryl-substituted lower alkyl.

72. The method of claim 71 wherein, in the compound, Ar₁ is phenyl or R₄ -substituted phenyl, Ar₂ is phenyl or R₄ -substituted phenyl and Ar₃ is R₅ -substituted phenyl.

73. The method of claim 71, wherein in the compound, Ar₁ is R₄ -substituted phenyl wherein R₄ is halogen; Ar₂ is R₄ -substituted phenyl wherein R₄ is halogen or --OR₆, wherein R₆ is lower alkyl or hydrogen; and Ar₃ R₅ -substituted phenyl, wherein R₅ is -OR₆, wherein R₆ is lower alkyl or hydrogen.

74. The method of claim 71 wherein in the compound X, Y, and Z are each --CH₂ --; R₁ and R₃ are each hydrogen; R and R₂ are each --OR₆, wherein R₆ is hydrogen; and the sum of m, n, p, q and r is 2, 3 or 4.

75. The method of claim 71 wherein in the compound, m, n and r are each zero, q is 1 and p is 2.

76. The method of claim 71 wherein in the compound, p, q and n are each zero, r is 1 and m is 2 or 3.

77. The method of claim 71 wherein the compound is selected from the group consisting of

3(R)-(2(R)-hydroxy-2-phenylethyl)-4(R)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;

3(R)-(2(R)-hydroxy-2-phenylethyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;

3(S)-(1(S)-hydroxy-3-phenyl(propyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetid inone;
3(S)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

3(R)-(1(R)-hydroxy-3-phenylpropyl)-4(S)-(4-methoxyphenyl)-1-phenyl-2-azetidinone;

rel-3(R)\((S)\)-hydroxy-(2-naphthalenyl)methyl\(-4(3S)-(4\text{-methoxyphenyl})\)-1-phenyl-2-azetidinone;

rel-3(R)\((R)\)-hydroxy-(2-naphthalenyl)methyl\(-4(S)-(4\text{-methoxyphenyl})\)-1-phenyl-2-azetidinone;

3(R)-(3(S)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

3(R)-(3(S)-hydroxy-3-phenylpropyl)-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

4(S)-(4-hydroxyphenyl)-3(R)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

4(S)-(4-hydroxyphenyl)-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-2-azetidinone;

rel 3(R)\((R S)\)-hydroxy-3\(\rightarrow\)4-(methoxymethoxy)-phenyl\(-1,4(S)-bis-(4\text{-methoxyphe}

1-(4-fluorophenyl)-3(R)\(\rightarrow\)3(S)-(4-fluorophenyl)-3-hydroxypropyl)\(-4(S)-(\text{sup } .4 -

1-(4-fluorophenyl)-3(R)\(\rightarrow\)3(R)-(4-fluorophenyl)-3-hydroxypropyl)\(-4(S)-(4-hy

droxypyphenyl)-2-azetidinone;

4(S)\(\rightarrow\)4-(acetyloxy)phenyl\(-3(R)-(3(R)-hydroxy-3-phenylpropyl)-1-(4-methoxyphenyl)-

2-azetidinone;
4(S)-4-(acetyloxy)phenyl-3(R)-(3(S)-hydroxy-3-phenylpropyl)-1(4-methoxyph enyl)-2-azetidinone;

1-(sup.4-fluorophenyl)-3(R)-3(S)-(4-fluorophenyl)-3-hydroxypropyl)-4(S)-4-(phenylmethoxy)phenyl-2-azetidinone;

3(R)-3(R)-acetyloxy)-3-phenylpropyl-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

3(R)-3(S)-acetyloxy)-3-phenylpropyl-1,4(S)-bis-(4-methoxyphenyl)-2-azetidinone;

3(R)-3(R)-(acetyloxy)-3-(4-fluorophenyl)propyl-4(S)-4-(acetyloxy)phenyl -1-(4-fluorophenyl)-2-azetidinone;

3(R)-3(S)-(acetyloxy)-3-(4-fluorophenyl)propyl-4(S)-4-(acetyloxy)phenyl -1-(4-fluorophenyl)-2-azetidinone;

3(R)-3(R)-(acetyloxy)-3-(4-chlorophenyl)propyl-4(S)-4-(acetyloxy)phenyl -1-(4-chlorophenyl)-2-azetidinone;

3(R)-3(S)-(acetyloxy)-3-(4-chlorophenyl)propyl-4(S)-4-(acetyloxy)phenyl -1-(4-chlorophenyl)-2-azetidinone; and

rel 1-(4-fluorophenyl)-4(S)-(4-hydroxyphenyl)-3(1R)-(1(R)-hydroxy-3-phenylpropyl)-2-azetidinone.

78. The method of claim 62 wherein the cholesterol absorption inhibitor is an androstane and/or androstene derivative, wherein androstane and/or androstene are coupled with ascorbic acid and represented by one or more of the general formulae:
wherein R₁, R₂, R₃, R₄, R₅, R₆ may individually be chosen from hydrogen, OH,
carbonyl, and an ascorbyl moiety; and R₇ may be hydrogen or any halogen.

79. The method of claim 78 wherein the ascorbyl moiety which is coupled to the compound from the androstane or androstene family is selected individually from one or more of the following structures:

[Diagram IX and X]

99
wherein \( M^+ \) represents any metal, alkali earth metal, or alkali metal.

80. The method of claim 62 wherein the cholesterol absorption inhibitor is an inhibitor of bile acid transport or reabsorption and is selected from the group consisting of all ileal, apical and hepatic transport inhibitors.

81. The method of claim 1 wherein the multiple drug resistance gene is selected from one or more of the group consisting of ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); and ABCC3 (MRP-3).
82. The method of claim 22 wherein the multiple drug resistance gene is selected from one or more of the group consisting of ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); and ABCC3 (MRP-3).

83. The method of claim 41 wherein the multiple drug resistance gene is selected from one or more of the group consisting of ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); and ABCC3 (MRP-3).

84. The composition of claim 52 wherein the multiple drug resistance gene is selected from one or more of the group consisting of ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); and ABCC3 (MRP-3).

85. The method of claim 62 wherein the multiple drug resistance gene is selected from one or more of the group consisting of ABCB1 (MDR-1); ABCA2 (ABC2); ABCB2 (TAP); ABCB3 (TAP); ABCC1 (MRP-1); and ABCC3 (MRP-3).

86. The method of claim 1 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.

87. The method of claim 22 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.

88. The method of claim 41 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.

89. The composition of claim 52 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.

90. The kit of claim 61 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.
91. The method of claim 62 wherein the cholesterol absorption inhibitor is provided in the form of a liposome.
Figure 3

PCR (1.5% Agarose gel)

adding of GAPDH-primer at cycle

0  6  9  12  16  20  23  26  28  30

mdr-1

GADPH
Figure 6

MRP-1 expression in Caco2-cells

[Graph showing MRP-1 expression levels in different conditions]
Figure 5

Protein concentration after treatment
(surplus and adherent cells)

[Graph showing protein concentration against FM-VP4 concentration (µM)]
Figure 8
**Western Blot**

1°Ab: monoclonal antibody JSB-1
1:200 dilution
2°Ab: 1:1000, rabbit α-mouse
FIGURE 10
FIGURE 11