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(54) Title: A METHOD OF TREATMENT AND AGENTS USEFUL FOR SAME

(57) **Abstract:** The present invention relates generally to the field of chemotherapy in animal, mammalian and avian subjects. More particularly, the present invention contemplates methods and agents for treating a condition in a subject with a chemotherapeutic agent while reducing the risk of development of resistance to the chemotherapeutic agent and/or overcoming inherent or acquired resistance to the agent. The present invention particularly provides for the use of an agent which inhibits or down-regulates transporter protein-mediated resistance to a chemotherapeutic agent. The agent is proposed to be used in combination therapy with a chemotherapeutic agent to reduce the risk of development of resistance to the chemotherapeutic agent and/or to overcome inherent or acquired resistance to the agent. The present invention also provides pharmaceutical compositions comprising the transporter protein inhibitor alone or in combination with a chemotherapeutic agent.

A METHOD OF TREATMENT AND AGENTS USEFUL FOR SAME

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The present invention relates generally to the field of chemotherapy in animal, mammalian and avian subjects. More particularly, the present invention contemplates methods and agents for treating a condition in a subject with a chemotherapeutic agent while reducing the risk of development of resistance to the chemotherapeutic agent and/or overcoming inherent or acquired resistance to the agent. The present invention particularly provides for the use of an agent which inhibits or down-regulates transporter protein-mediated resistance to a chemotherapeutic agent. The agent is proposed to be used in combination therapy with a chemotherapeutic agent to reduce the risk of development of resistance to the chemotherapeutic agent and/or to overcome inherent or acquired resistance to the agent. The present invention also provides pharmaceutical compositions comprising the transporter protein inhibitor alone or in combination with a chemotherapeutic agent.

DESCRIPTION OF THE PRIOR ART

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in any country.

The development of resistance to chemotherapeutic agents during the treatment of cancer or infection by pathogens remains a major impediment to the continued, long term use of these agents. In addition, the presence of inherent or acquired resistance frequently requires larger doses of chemotherapeutic agents to be administered resulting in sometimes severe toxic side effects.

Accordingly, notwithstanding that a variety of chemotherapies are available to oncologists to reduce the rate of tumor progression, intrinsic or acquired tumor-mediated drug resistance is a major clinical obstacle that can result in the lack of tumor responsiveness in patients undergoing treatment. Multidrug resistance (MDR) is in part due to active efflux transporters belonging to the ATP-binding cassette (ABC) superfamily, such as P-glycoprotein (Pgp), multidrug resistance protein-1 (MRP-I) and breast cancer resistance protein (BCRP). For example, Pgp is over-expressed in about 30-40% of primary and more than 50% of metastatic breast cancer patient samples. More recent data have suggested that Pgp is also involved in the passage of molecules across the blood brain barrier, the intestinal wall, and in inducing apoptosis in peripheral blood mononuclear cells. The development of substrates or inhibitors of this protein, therefore, represents an active area of the pharmaceutical industry.

Clinical trials have been conducted to evaluate the efficacy of MDR-reversing agents such as Verapamil, Quinidine and Cyclosporin A. Unfortunately, however, the results have been mixed and somewhat inconclusive.

Paclitaxel, isolated from the bark of the pacific yew tree in the 1970's, is an antitumor drug that binds to beta-tubulin and inhibits its depolymerization. Significant antitumor efficacy is seen in the ovarian, breast, lung, head and neck, bladder and esophageal cancers. Docetaxel is a more potent analog of paclitaxel, and is effective in breast, ovarian, lung, gastric and prostate cancers. Both Paclitaxel and Docetaxel are substrates for Pgp- and MRP-I -mediated efflux, and their efficacy is thus compromised in cells which overexpress Pgp or MRP-I.

MDR inhibitors have not been largely successful in overcoming resistance to chemotherapeutic agents. This may have been due in part to the focus on identifying inhibitors to single MDR proteins even though many cancers are associated with the overexpression of multiple MDR proteins. Cyclosporin A has been the most promising to date and inhibits a number of MDR proteins.

There is a need to identify other inhibitors of mediators of MDR.

Intracellular bacteria and mycobacteria are particularly difficult to treat and as a result conditions such as tuberculosis *{Mycobacterium tuberculosis),* listeriosis *{Listeria monocytogenes),* bacillary dysentery *{Shigella dysenteriae)* and legionnaires disease *{Legionella pneumophila)* can pose serious clinical challenges. *Listeria* and *Shigella* are generally self-limiting and are, therefore, usually treatable with supportive therapy. Legionnaires' Disease and tuberculosis represent a greater problem. Legionnaires' Disease accounts for 1 to 8% of community-acquired pneumonias that result in hospitalization and about 4% of lethal nosocomial pneumonias. Even with appropriate treatment, mortality occurs in at least 15% of community-acquired cases and is higher among immunosuppressed or hospitalized patients.

About 10 million Americans are infected with *Mycobacterium* although only about 10% of these individuals will develop tuberculosis in their lifetime. Globally, tuberculosis is an increasing problem, especially in Africa where AIDS facilitates its spread. It is estimated that nearly 1 billion people will become infected, 200 million will become sick, and 70 million will die worldwide between now and 2020. In 1999, approximately 8.4 million cases and 2 million deaths were attributed to tuberculosis; 100,000 of those 2 million deaths occurred among children. One particular problem associated with tuberculosis is drug resistance and, since mycobacteria are intracellular parasites, the risk is that treatments with poor access to intracellular reservoirs may only partially clear the pathogen allowing the development of drug-resistant strains. Developing strategies which allow the concentration of antibiotics within myobacterial reservoirs would be of considerable use.

In accordance with the present invention, an inhibitor of Pgp-mediated resistance to chemotherapeutic agents is identified. Is proposed to be used *inter alia* in the chemotherapy of a range of conditions including cancer and infection by parasitic microorganisms and viruses.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Abbreviations used herein are defined in Table 1.

The present invention provides a flavonoid compound as well as analogs, homologs, derivatives, mimetics and functional equivalents thereof including methylated forms thereof in substantially pure form, in a plant extract, in a chemical extract or fraction and/or in combination with excipients, diluents, stabilizing molecules, penetrants and/or one or more chemotherapeutic compounds. The flavonoid of the present invention is useful in reducing the risk that a cell or group of cells will acquire resistance to a chemotherapeutic agent. The flavonoid compound is also useful in reducing inherent resistance to the chemotherapeutic compound. The flavonoid compound may be administered simultaneously or concurrently with (i.e. together with or subsequent to) the chemotherapeutic agent or it may be administered prior to, during or after administration of the chemotherapeutic agent.

The preferred flavonoid is heptamethyl-apigenin-8-O-glycoside, also known as heptamethylvitexin, methylvitexin or R-substituted vitexin wherein the R group represents a single or multiple substitution of the hydroxy! group on vitexin. Reference herein to any of the above compounds includes reference to analogs, homologs, derivatives, mimetics and/or functional equivalents thereof. It is proposed herein that the R-substituted vitexin is an inhibitor of resistance mediated by a cell transporter protein and in particular P-glycoprotein (Pgp) and/or the breast cancer resistance protein (BCRP). Accordingly, it is proposed that the flavonoid prevents or reduces export of the chemotherapeutic agent out of a cell.

Chemotherapeutic agents contemplated herein include agents used in the treatment or prophylaxis of cancer (including tumors), infection by pathogens (including potential or opportunistic pathogens) and/or the treatment or prophylaxis of a physiological or clinical condition. The chemotherapeutic agents may also target non-desired immune cells or cytokin-producing cells.

The present invention further provides compositions including pharmaceutical compositions comprising a flavonoid capable of inhibiting or reducing a transport function of at least Pgp and optionally BCRP. The compositions may also comprise one or more chemotherapeutic agents. Alternatively, the compositions may be a multi-facet composition comprising compounds which are pre-mixed prior to use or separately administered at the same or different times. In a multi-facet pharmaceutical pack, a first compartment would contain the flavonoid compound and subsequent compartments comprise one or more chemotherapeutic compounds.

The present invention further contemplates a use of the flavonoid compound in the manufacture of a medicament to reduce the risk of development of resistance to a chemical agent or to overcome inherent or acquired resistance.

Table 1 - Abbreviations

Abbreviation	Definition
ABC	ATP-binding cassette
BCRP	Breast cancer resistance protein
COL	Colchicine
DNR	Daunorubicin
EtBr	Ethidium bromide
FL-VLB	BOBIPY-FL Vinblastine
IC_{50}	50% Inhibitory concentration
MDR	Multidrug resistance
MRP-1	Multidrug resistance protein-1
Pgp	P-glycoprotein
Rh123	Rhodamine 123
VLB	Vinblastine

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graphical representation showing that heptamethylvitexin increases drug accumulation in multidrug resistant CEMTVLB $_{100}$ cells. CEMTVLB$_{10}$o cells express Pgp and pump out the chemotherapy drug Daunorubicin (DNR). The flavonoid compound HMV inhibited Pgp-ATPase activity at concentrations from 1 μM to 100 μM, leading to increased DNR accumulation in the cells.

Figure 2 is a graphical representation showing that heptamethylvitexin increases drug accumulation in multidrug resistant CEM7VLB$_{10}$o cells. CEMTVLB$_{10}$o cells also pump out Vinblastine (VBL). The flavonoid compound HMV inhibited Pgp and gave a 2-fold increase in fluorescent VLB accumulation in the cells at a concentration of 10 μM.

Figure 3 is a graphical representation showing the flavonoid heptamethylvitexin is able to reverse resistance to DNR, increasing the ability of DNR to kill cancer cells. The reversal is dose-dependent .with up to 23-fold reversal at 20 μM. Reversal of DNR resistance was as follows 50 nM: 1.5-fold, 100 nM: 1.2-fold, 1 μM: 2.5-fold, 5 μM: 6.9-fold, 10 μM: 13.1-fold, 20 μM: 23.4-fold.

Figure 4 is a graphical representation showing that flavonoid heptamethylvitexin is able to reverse resistance to VLB, and the reversal is dose-dependent, with up to 70-fold reversal at 20 μM. Reversal of VLB resistance is as follows 50 nM: 1.8-fold , 100 nM: 1.5-fold, 1 μM: 2.0-fold, 5 μM: 4.9-fold, 10 μM: 19.7-fold, 20 μM: 69.4-fold.

Figure 5 is a graphical representation showing the effect of methylated vitexins on verapamil-induced Pgp ATPase activity. Stimulation of ATPase was induced by verapamil (100 μM) in the presence and absence of increasing concentrations of methylated vitexins. Sample I is heptamethyl vitexin (HMV) and sample II contains heptamethyl vitexin. Data are presented as the average of a duplicate experiment.

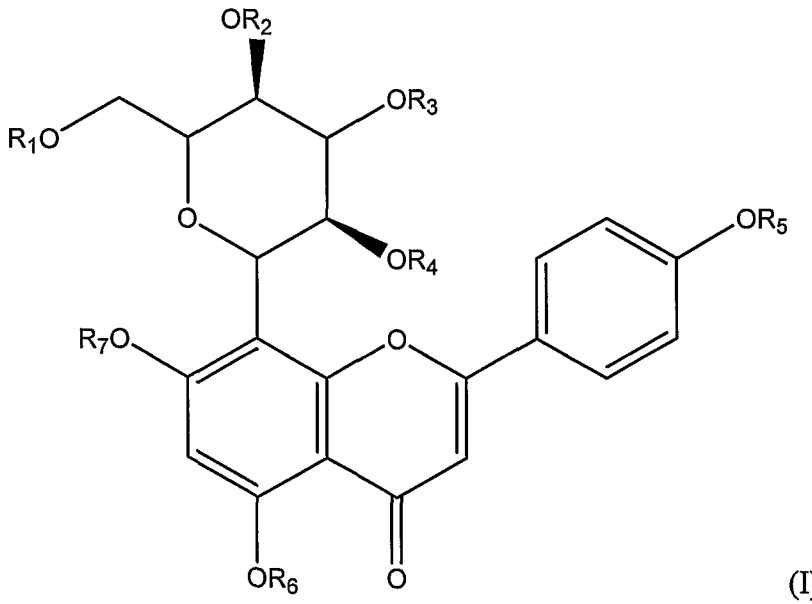
Figure 6 is a graphical representation showing the cytotoxicity of samples (I & II) containing heptamethyl vitexin (HMV). Vinblastine (VLB) was included as positive control. Data are presented as the average of a duplicate experiment.

Figure 7 is a graphical representation showing cytotoxicity of Vinblastine (VLB) toward CEM/VBL_{100} in the presence and absence of heptamethyl vitexin (HMV). Data are presented as the average of a duplicate experiment.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is predicated in part on the identification of a flavonoid compound including a methylated form thereof which is capable of inhibiting Pgp function and/or activity and optionally also BCRP function or activity. These proteins are associated with exporting from a cell, chemotherapeutic agents and hence are involved in resistance of a cell to a chemotherapeutic agent. Pgp and BCRP are referred to herein as transporter proteins. This is particularly important *inter alia* in the treatment of cancer including tumors, in the treatment or prophylaxis of infection by pathogens or potential pathogens and in the induction of immunosuppression such as during transplant operations.

Accordingly, the present invention contemplates a method for overcoming acquired or inherent resistance to a chemotherapeutic agent in a subject said method comprising administering to said subject an amount an R-substituted vitexin of general Formula I:



(I)

wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 may be the same or different and each is selected from hydrogen, -Ci-Cioalkyl, -C_2-C_{10}alkenyl, -C_2-C_{10}alkynyl, -$(CH_2)_nCOR_8$, -$(CH_2)_nR_9$, -PO_3H, -$(CH_2)_n$heterocyclyl or -$(CH_2)_n$aryl where R_8 is -OH, -NH_2, -NHd-C_3alkyl, -OC_1-C_3alkyl or -Ci-C_3alkyl and R_9 is -OH, -SH_5 -SCi-C_3alkyl, -OCi-C_3alkyl, -C_3-C_{12}cycloalkyl,

-C_3-C_{12}cycloalkenyl, -NH_2, -NHC!-C_3alkyl or -NHC(C=NH)NH_2, n is 0 or an integer from 1 to 10 and where each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHd-Csalkyl, -Od-C_3alkyl, -SH, -SCi-C_3alkyl, -CO_2H, -COzQ-Csalkyl, -$CONH_2$ or -CONHCi-C_3alkyl;

prior to, together with or subsequent to the chemotherapeutic agent.

The term "alkyl" as used herein refers to straight chain or branched hydrocarbon groups. Suitable alkyl groups include, but are not limited to, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, hexyl, heptyl, octyl, nonyl and decyl. For example, C_1-Caalkyl refers to methyl, ethyl, propyl and isopropyl. The preferred alkyl group is a methyl group.

The term "alkenyl" as used herein refers to straight chain or branched unsaturated hydrocarbon groups containing one or more double bonds. Suitable alkenyl groups include, but are not limited to, ethenyl, propenyl, isopropenyl, butenyl, pentenyl, hexenyl, heptenyl, octenyl, nonenyl and decenyl.

The term "alkynyl" as used herein refers to straight chain or branched hydrocarbon groups containing one or more triple bonds. Suitable alkynyl groups include, but are not limited to, ethynyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, octynyl, nonynyl and decynyl.

The term "cycloalkyl" as used herein, refers to cyclic hydrocarbon groups. Suitable cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, cyclodecyl, cycloundecyl and cyclododecyl.

The term "cycloalkenyl" as used herein, refers to cyclic unsaturated hydrocarbon groups having at least one double bond in the ring. Suitable cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclohexenyl, cycloheptenyl, cyclooctenyl, cyclononenyl, cyclodecenyl, cycloundecenyl and cyclododecenyl.

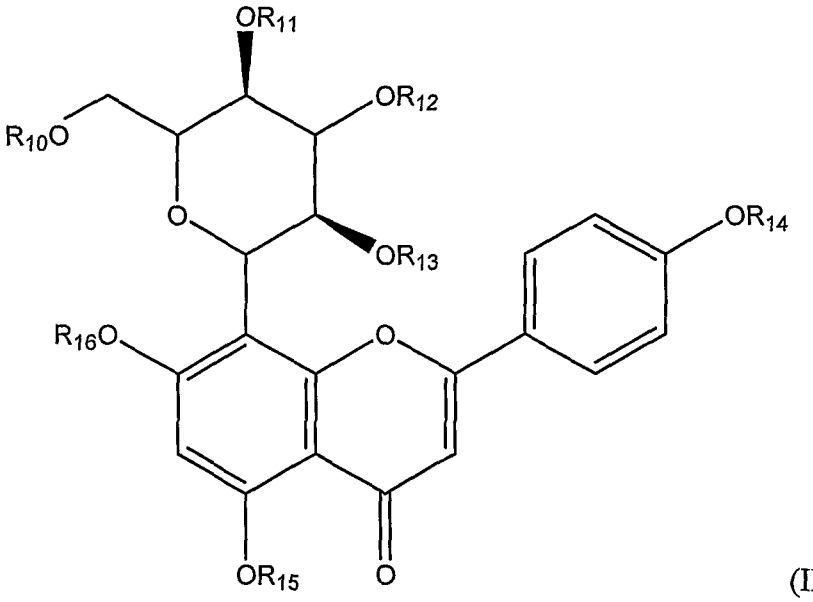
The term "heterocyclyl" as used herein refers to 5 or 6 membered cyclic hydrocarbon groups in which at least one carbon atom has been replaced by N, O or S. Optionally, the heterocyclyl group may be fused to a phenyl ring. Suitable heterocyclyl groups include, but are not limited to pyrrolidinyl, piperidinyl, pyrrolyl, thiophenyl, furanyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, pyridinyl, quinolinyl, isoquinolinyl, indolyl, benzofuranyl, benzothiophenyl, oxadiazolyl, tetrazolyl, triazolyl and pyrimidinyl.

The term "aryl" as used herein, refers to C_6-C_{10} aromatic hydrocarbon groups, for example phenyl and naphthyl.

It will also be recognized that the compounds of Formula (I) possess asymmetric centres and are therefore capable of existing in more than one stereoisomer^ form. The present invention thus also relates to compounds in one particular isomeric form at one or more asymmetric centres eg., greater than about 90% ee, such as about 95% or 97% ee or greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be naturally occurring or may be prepared by asymmetric synthesis, for example using chiral intermediates, or by chiral resolution.

In a preferred embodiment, one or more of R_1 through R_7 is each an alkyl group, consequentially forming an alkoxy group. In a most preferred embodiment, the alkyl group is a methyl group.

Accordingly, another aspect of the present invention contemplates a method for overcoming acquired or inherent resistance to a chemotherapeutic agent in a subject, said method comprising administering to said subject an amount of an R-substituted vitexin of general Formula II:



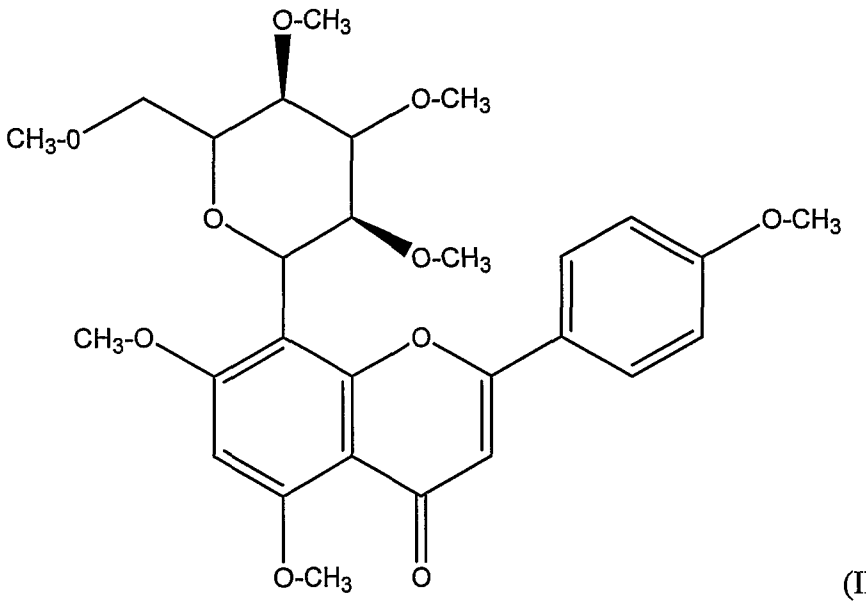
(II)

wherein R_{10} through R_{16} may be the same or different and each is a Ci-C_{10} alkyl;

prior to, together with or subsequent to the chemotherapeutic agent.

Preferably, the alkylvitexin is a methylvitexin where the alkyl group is a methyl group. Most preferably, however, each of R_{10} through R_{16} is a methyl group forming a heptamethylvitexin .

Accordingly, another aspect of the present invention provides a method for overcoming acquired or inherent resistance to a chemotherapeutic agent in a subject said method comprising administering to said subject an amount of a methylvitexin of general Formula III:



(III)

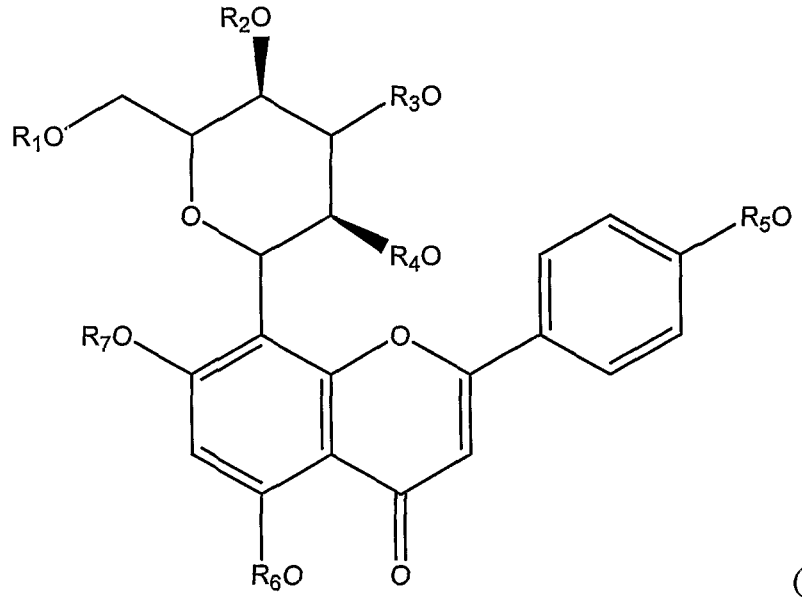
prior to, together with or subsequent to the chemotherapeutic agent.

The preferred compound of Formula (III) may be described as methylvitexin, heptamethylvitexin, heptamethyl-apigenin-8-glycoside, amongst other terms.

The present invention includes, within these terms, derivatives, analogs, homologs, stereoisomers, mimetics and functional equivalents.

Accordingly, the present invention further contemplates a method for overcoming acquired or inherent resistance to a chemotherapeutic agent in a subject, said method comprising administering to said subject an amount of heptamethylvitexin or an extract or chemical fraction comprising same or a derivative, homolog, analog, stereoisomer, mimetic or functional or structural equivalent thereof prior to, together with or subsequent to the chemotherapeutic agent.

The present invention further contemplates a method of treating a subject with cancer said method comprising administering to said subject an anti-cancer chemotherapeutic agent and an R-substituted vitexin of general Formula 1:



(I)

wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 may be the same or different and each is selected from hydrogen, $-C_1$-C_{10}alIlCyI, $-C_2$-C_{10}alkenyl, $-C_2$-C_{10}alkynyl, $-(CH_2)_nCOR_8$, $-(CH_2)_\pi R_9$, $-PO_3H$, $-(CH_2)_n$heterocyclyl or $-(CH_2)_n$aryl where R_8 is -OH, $-NH_2$, -NHQ-Csalkyl, $-OC_1$-C_3alkyl or -CrQjalkyl and R_9 is -OH, -SH, -SQ-Qjalkyl, -OQ-Csalkyl, $-C_3$-C_{12}cycloalkyl, -Cs-Cncycloalkenyl, $-NH_2$, -NHQ-Qalkyl or $-NHC(C=NH)NH_2$, n is 0 or an integer from 1 to 10 and where each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, $-NH_2$, -NHd-Csalkyl, $-OC_1$-C_3alkyl, -SH, -SC^Csalkyl, $-CO_2H$, -CO^-Csalkyl, $-CONH_2$ or -CONHCrCsalkyl.

The administration of the transporter protein inhibitor compound of Formulae I through III may be concurrent or simultaneous with the chemotherapeutic agent or prior to, during or subsequent to the administration of the chemotherapeutic agent. Consequently, the transporter protein inhibitors may be administered simultaneously or sequentially to the chemotherapeutic agent. Where sequential administration occurs, the transporter protein inhibitor and the chemotherapeutic agent may be given in either order and within seconds, minutes, hours, days or weeks apart. The administration of the R-substituted vitexin may

also be in conjunction with the administration of other transporter protein inhibitors such as other Pgp inhibitors or MDR-I-inhibitors. An example of an MDR-I inhibitor is cyclosporin A.

The transporter protein inhibitor and the chemotherapeutic agent may also be formulated together or separately. This is discussed further below. Reference to a "transporter protein inhibitor" means, overall, that the inhibitor reduces the potential for resistance to develop due to a transporter protein. Such an inhibitor may, at some doses, up-regulate transporter protein levels or activity. However, it is proposed that overall the effect is to reduce the incidence of acquired or inherent resistance development.

The transporter protein inhibitor may be chemically synthesized, chemically modified or may be purified from a plant extract. Alternatively, a parent or precursor molecule of the inhibitor may be obtained from a plant or other source and the chemically modified (e.g. methylated) into an active inhibitor form. A formulation comprising a partially purified extract of a plant containing the transporter protein inhibitor is also contemplated by the present invention.

Consequently, the present invention extends to purified, isolated, partially purified, substantially purified and plant or chemical extracts or fractions of the transport protein inhibitor.

Examples of plants from which the heptamethylvitexin or a precursor form thereof (e.g. un- or less-methylated form) may be isolated include but are not limited to *Achillea nobilis L, Achillea setacea W. et K., Adhatoda vctsica Nees* (leaves and flowers), *Ailanthus excelsa* (leaves), *Alternanthera maritima (Mart.) St. HH.* (aerial part of the plant), *Anthurium versicolor* (leaves), *Anthurium versicolor* (leaves), *Artemisia vulgaris L* (whole plant), *Arum palaestinum, Aspalathus linearis* (leaves and the stems), *Aspalathus linearis* (rooibos tea), *Beta vulgaris L* (Leaves of the sugar beet), *Bryonia alba* (roots), *Bryonia dioica* (roots), *Calendula flower, Milk-thistle fruit* and *Passionflower, Cannabis sativa* (plant), *Cannabis sativa subsp. sativa* (cannabinoid-free cannabis) (leaves and flowers), *Cayaponia*

tayuya (roots), *Cayaponia tayuya* (roots), *Chamaecytisus eriocarpus, Chamaecytisus pygmaeus* (aerial parts), *Cissus rheifolia* (leaves), *Citrus aurantium var. amara L* (sour orange leaves), *Citrus sinensis L* (sweet orange leaves), *Clinacanthus nutans, Crataegus monogyna Jacq* (leaves and flowers), *Crataegus orientalis* (leaves), *Crataegus oxyacantha L* (flowers, leaves, and bark), *Crataegus pinnatiflda* (leaves and fruits), *Crataegus pinnatifida var. psilosa* (leaves), *Crataegus tanacetifolia* (Rosaceae) (leaves, flowers, and fruits), *Crossopteryx febrifuga* (leaves), *Crotalaria anagyroides, Crotalaria paniculata wild* (flowers), *Crotalaria sessiliflora* (aerial part of the plant), *Crotalaria striata* (leaves and stem bark), *Crotalaria thebaica (Del.) Dc* (aerial parts), *Croton hovarum* (leaves), *Cyathea fauriei* (leaves), *Cyathea faurier* (leaves), *Cyathea hancockii* (leaves), *Cyathea leichhardtiana* (leaves), *Cyathea mertensiana* (leaves), *Cyathea onusta* (leaves), *Cyathea podophylla* (leaves), *Cyathea spinulosa* (leaves), *Cyathea tueckheimeii* (leaves), *Desmodium triflorum,Desmodium antartica, Dissotis rotundifolia T* (whole plant), *Dracunculus vulgaris Schott* (leaves), *Drosophyllum lusitanicum* (shoot and callus cultures), *Dysolobium apioides* (leaves), *Ecbolium linneanum* (leaves, flowers, and roots), *Erythrina crista-galli Linn,* (leaves), *Euodia daniellii* (fruits and leaves), *Fagopyrum esculentum Moeench* (buckwheat grain), *Fagopyrum esculentum Moeench, Buckwheat Hulls, Feronia elephantum Correa* (leaves), *Gaillardia aristata* (Compositae), *Gaillardia pulchella, Gentiana lutea, Glochidion zeylanicum* (leaves), *Gnidia involucrata* (aerial parts) (Thymelaeaceae), *Gonocaryum calleryanum* (leaves), *Gonocytisus angulatus* (aerial part), *Gramineae species* (stem and leaves), *Gutierrezia grandis, Helenium brevifolium* (above ground part), *Heylandia latebrosa* (leaves), *Hyparrhenia hirta* (aerial parts), *Itea japonica* (leaves), *Jatropha gossypifolia* (leaves), *Jatropha heterophylla* (aerial parts), *Kleinhovia hospita* (flowers), *Larix gmelinii* (needles), *Lavandula dentata* (aerial parts), *Lespedeza capitata* (arial portion of the plant), *Ludwigia* (Onagraceae), *Lupinus arboreus* (whole plant including roots), *Lythrum salicaria L* (purple loosestrife), *Majorana hortensis* (aerial parts), *Majorana syriaca, Marrubium vulgare* (leaves), *Melilotus indica, Mollugo cerviana* (whole plant), *Ochna japotapita* (leaves), *Ocimum gratissimum var. gratissimum, Ocimum sanctum* (aerial parts) *L, Onopordum laconicum* (aerial parts), *Orchidaceae plant, Oxera macrocalyx, Oxera neriifolia, Parkinsonia aculeata* (leaves), *Passiflora caerulea L, Passiflora family, Passiflora incarnata, Passiflora serratifolia* (leaves), *Passiflora sexflora*

(leaves), *Phenax angustifolius* (leaves), *Phoradendron tomentosum* (leaves), *Plagiomnium affim* (mosses), *Populus heterophylla L* (leaves), *Prosopis argentina* (leaves), *Prosopis chilensis* (leaves and pods), *Prosopis fiebrigii* (leaves), *Prosopis fiebrigii* (leaves), *Prosopis hasslerin* (leaves), *Prosopis reptans* (leaves), *Psophocarpus tetragonolobus (L.) DC.* (winged bean), *Pterostemon mexicanum, Pterostemon rotundifolia, Rhynchosia bracteata* (leaves), *Rhynchosia cana* (leaves), *Rhynchosia densiflora* (leaves), *Rhynchosia jacobii* (leaves), *Rhynchosia suaveolens* (leaves), *Rhynchosia sublobata* (leaves), *Rumex vesicarius, Swertia* genus such as *Swertia abyssinica, Swertia adolβ-friderici, Swertia angustifolia, Swertia bimaculata, Swertia binchuanensis, Swertia calycina, Swertia chirayta, Swertia ciliata, Swertia cincta, Swertia cordata, Swertia crassiuscula, Swertia cuneata, Swertia decora, Swertia delavajyi, Swertia dichotoma, Swertia diluta, Swertia engleri, Swertia erythrosticta, Swertia franchetiana, Swertia hispidicalyx, Swertia japonica, Swertia kilimandscharica, Swertia lactea, Swertia macrosperma, Swertia marginata, Swertia mileensis, Swertia multicaulis, Swertia mussoti, Swertia perennis, Swertia petiolata, Swertia przewalskii, Swertia off. pseudohookeri, Swertia pυbescens, Swertia punicea, Swertia racemosa, Swertia rosulata, Swertia schugananica, Swertia tashiroi, Swertia tetraptera, Swertia volkensii, Swertia wolfongiana* and *Swertia yunnanensis, Scutellaria albida* (aerial parts), *Synandrospadix vermitoxicus* (leaves), *Terminalia catappa L* (leaves), *Theobroma cacao, Trema micrantha* (leaves and branches), *Trigonella corniculata* (seeds), *Trigonella foenum-graecum linn,* (seeds), *Trigonella grandiflora, Triticum aestivum* (wheat bran, Sakha *69)* (Gramineae), *Trollius chinensis* (flowers), *Trollius chinensis Bunge* (flower), *Verbena bipinnatiβda Nutt. herb* (Verbenaceae), *Vicia species* (pods), *Vigna plants in Leguminosae* (seed coat), *Vigna radiataL* (mung bean), *Vitexpeduncularis* (leaves), *Vitexpolygama* (leaves).

Accordingly, the present invention provides a method of overcoming acquired or inherent resistance in a subject to a chemotherapeutic agent said method comprising administering to said subject a Pgp- and optionally a BCRP-inhibiting effective amount of heptamethylvitexin in purified form or contained within a plant extract, said plant being selected from *Achillea nobilis L, Achillea setacea W. et K., Adhatoda vasica Nees* (leaves and flowers), *Ailanthus excelsa* (leaves), *Alternanthera maritima (Mart.) St. HH.* (aerial

part of the plant), *Anthurium versicolor* (leaves), *Anthurium versicolor* (leaves), *Artemisia vulgaris L* (whole plant), *Arum palaestinum, Aspalathus linearis* (leaves and the stems), *Aspalathus linearis* (rooibos tea), *Beta vulgaris L* (Leaves of the sugar beet), *Bryonia alba* (roots), *Bryonia dioica* (roots), *Calendula flower, Milk-thistle fruit* and *Passionflower, Cannabis sativa* (plant), *Cannabis sativa subsp. sativa* (cannabinoid-free cannabis) (leaves and flowers), *Cayaponia tayuya* (roots), *Cayaponia tayuya* (roots), *Chamaecytisus eriocarpus, Chamaecytisus pygmaeus* (aerial parts), *Cissus rheifolia* (leaves), *Citrus aurantium var. amara L* (sour orange leaves), *Citrus sinensis L* (sweet orange leaves), *Clinacanthus nutans, Crataegus monogyna Jacq* (leaves and flowers), *Crataegus orientalis* (leaves), *Crataegus oxyacantha L* (flowers, leaves, and bark), *Crataegus pinnatifida* (leaves and fruits), *Crataegus pinnatifida var. psilosa* (leaves), *Crataegus tanacetifolia* (Rosaceae) (leaves, flowers, and fruits), *Crossopteryx febrifuga* (leaves), *Crotalaria anagyroides, Crotalaria paniculata wild* (flowers), *Crotalaria sessiliflora* (aerial part of the plant), *Crotalaria striata* (leaves and stem bark), *Crotalaria thebaica (Del.) Dc* (aerial parts), *Croton hovarum* (leaves), *Cyathea fauriei* (leaves), *Cyathea faurier* (leaves), *Cyathea hancockii* (leaves), *Cyathea leichhardtiana* (leaves), *Cyathea mertensiana* (leaves), *Cyathea onusta* (leaves), *Cyathea podophylla* (leaves), *Cyathea spinulosa* (leaves), *Cyathea tueckheimeii* (leaves), *Desmodium triflorum,Desmodium antartica, Dissotis rotundifolia T* (whole plant), *Dracunculus vulgaris Schott* (leaves), *Drosophyllum lusitanicum* (shoot and callus cultures), *Dysolobium apioides* (leaves), *Ecbolium linneanum* (leaves, flowers, and roots), *Erythrina crista-galli Linn,* (leaves), *Euodia daniellii* (fruits and leaves), *Fagopyrum esculentum Moeench* (buckwheat grain), *Fagopyrum esculentum Moeench, Buckwheat Hulls, Feronia elephantum Correa* (leaves), *Gaillardia aristata* (Compositae), *Gaillardia pulchella, Gentiana lutea, Glochidion zeylanicum* (leaves), *Gnidia involucrata* (aerial parts) (Thymelaeaceae), *Gonocaryum calleryanum* (leaves), *Gonocytisus angulatus* (aerial part), *Gramineae species* (stem and leaves), *Gutierrezia grandis, Helenium brevifolium* (above ground part), *Heylandia latebrosa* (leaves), *Hyparrhenia hirta* (aerial parts), *Itea japonica* (leaves), *Jatropha gossypifolia* (leaves), *Jatropha heterophylla* (aerial parts), *Kleinhovia hospita* (flowers), *Larix gmelinii* (needles), *Lavandula dentata* (aerial parts), *Lespedeza capitata* (arial portion of the plant), *Ludwigia* (Onagraceae), *Lupinus arboreus* (whole plant including

roots), *Lythrum salicaria L* (purple loosestrife), *Majorana hortensis* (aerial parts), *Majorana syriaca, Marrubium vulgare* (leaves), *Melilotus indica, Mollugo cerviana* (whole plant), *Ochnajapotapita* (leaves), *Ocimum gratissimum var. gratissimum, Ocimum sanctum* (aerial parts) *L, Onopordum laconicum* (aerial parts), *Orchidaceae plant, Oxera macrocalyx, Oxera neriifolia, Parkinsonia aculeata* (leaves), *Passiflora caerulea L, Passiflora family, Passiflora incarnata, Passiflora serratifolia* (leaves), *Passiflora sexflora* (leaves), *Phenax angustifolius* (leaves), *Phoradendron tomentosum* (leaves), *Plagiomnium affine* (mosses), *Populus heterophytta L* (leaves), *Prosopis argentina* (leaves), *Prosopis chilensis* (leaves and pods), *Prosopis flebrigii* (leaves), *Prosopis flebrigii* (leaves), *Prosopis hasslerin* (leaves), *Prosopis reptans* (leaves), *Psophocarpus tetragonolobus (L.) DC.* (winged bean), *Pterostemon mexicanum, Pterostemon rotundifolia, Rhynchosia bracteata* (leaves), *Rhynchosia cana* (leaves), *Rhynchosia densiflora* (leaves), *Rhynchosia jacobii* (leaves), *Rhynchosia suaveolens* (leaves), *Rhynchosia sublobata* (leaves), *Rumex vesicarius,Swertia* genus such as *Swertia abyssinica, Swertia adolfi-friderici, Swertia angustifolia, Swertia bimaculata, Swertia binchuanensis, Swertia calycina, Swertia chirayta, Swertia ciliata, Swertia cincta, Swertia cordata, Swertia crassiuscula, Swertia cuneata, Swertia decora, Swertia delavajyi, Swertia dichotoma, Swertia diluta, Swertia engleri, Swertia erythrosticta, Swertia franchetiana, Swertia hispidicalyx, Swertia japonica, Swertia kilimandscharica, Swertia lactea, Swertia macrosperma, Swertia marginata, Swertia mileensis, Swertia multicaulis, Swertia mussoti, Swertia perennis, Swertia petiolata, Swertia przewalskii, Swertia off. pseudohookeri, Swertia pubescens, Swertia punicea, Swertia racemosa, Swertia rosulata, Swertia schugananica, Swertia tashiroi, Swertia tetraptera, Swertia volkensii, Swertia wolfongiana* and *Swertia yunnanensis, Scutellaria albida* (aerial parts), *Synandrospadix vermitoxicus* (leaves), *Terminalia catappa L* (leaves), *Theobroma cacao, Trema micrantha* (leaves and branches), *Trigonella corniculata* (seeds), *Trigonella foenum-graecum linn.* (seeds), *Trigonella grandiflora, Triticum aestivum* (wheat bran, Sakha 69) (Gramineae), *Trollius chinensis* (flowers), *Trollius chinensis Bunge* (flower), *Verbena bipinnatiflda Nutt. herb* (Verbenaceae), *Vicia species* (pods), *Vigna plants in Leguminosae* (seed coat), *Vigna radiata L* (mung bean), *Vitex peduncularis* (leaves), *Vitex polygama* (leaves) or a derivative, analog, homolog, stereoisomer, mimetic or functional equivalent of said

heptamethylvitexin, wherein said heptamethylvitexin is administered simultaneously or sequentially or otherwise in conjunction with treatment with the chemotherapeutic agent.

As indicated above, a precursor molecule, such as vitexin, may also be extracted from a plant or obtained from another source and then subjected to chemical modification such as methylation.

The chemotherapeutic agents contemplated for use with the present invention are those useful in the treatment of cancer including tumors, infection by pathogenic or opportunistic pathogenic eukaryotic or prokaryotic organisms or viruses, inflammation, or any other condition requiring the administration of a chemotherapeutic agent.

Accordingly, the term "chemotherapeutic" agent is not to be construed as being limited to the treatment of cancers although the present invention is particularly useful when used in conjunction with anti-cancer agents. Examples include chemotherapeutic agents, anti¬ inflammatory drugs, anti-pathogenic agents, anti-microbial agents, anti-metabolites, anti¬ tumor antibiotics, mitotic inhibitors, steroids, sex hormones, hormone agonists and microtubule inhibitors.

Examples of anti-cancer chemotherapeutic agents include daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, Vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES).

Anti-inflammatory drags, include nonsteroidal anti-inflammatory drugs and corticosteroids.

Anti-pathogenic agent drugs include antiviral drags such as ribivirin, vidarabine, acyclovir, ganciclovir, 3TC, Abacavir, Acyclovir, Alpha interferon, AZT, Bleomycin, Capreomycin, Cidofovir, Ciprofloxacin, Cyclophosphamide, ddC, ddi, Delavirdine, Didanosine, Dronabinol, Efavirenz, Erythropoetin, Famciclovir, Filgrastim, Foscarnet, Ganciclovir, Ganciclovir Implants, Indinavir, Lamivudine, Lopinavir, Megace, Methotrexate, Nelfinavir, Nevirapine, Nonoxynol-9, Ribavirin, Ritonavir, Saquinavir, Stavudine, Sulfamethoxazole, Tenofovir, Trimethoprim, Zalcitabine and Zidovudine.

Anti-microbial agents include Amikacin, Atovaquone, Azithromycin, Clarithromycin, Clindamycin, Clofazimine, Cycloserine, Dapsone, Ethambutol, Ethionamide, Isoniazid, IVIG, Kanamycin, Metronidazole, Ofloxacin, Para Aminosalicyclic Acid, Pentamidine, Primaquine, Pyrazinamide, Pyrimethamine, Rifabutin, Rifampin, Streptomycin, Sulfadiazine, Sulfadoxine, Sulfamethazine, Trimetrexate and Triple Sulfa.

Anti-metabolites contemplated herein are substances which interfere with the body's chemical processes, such as creating proteins, DNA, and other chemicals needed for cell growth and reproduction; in cancer treatment, anti-metabolite drags disrupt DNA production, which in turn prevents cell division. Examples include Azaserine, D-Cycloserine, Mycophenolic acid, Trimethoprim, 5-fluorouracil, capecitabine, methotrexate, gemcitabine, cytarabine (ara-C) and fludarabine.

Other agents contemplated herein are anti-tumor antibiotics which interfere with DNA by stopping enzymes and mitosis or altering the membranes that surround cells. These agents work in all phases of the cell cycle. Thus, they are widely used for a variety of cancers. Examples of anti-tumor antibiotics include dactinomycin, daunorubicin, doxorubicin (Adriamycin), idarabicin, and mitoxantrone.

Mitotic inhibitors are plant alkaloids and other compounds derived from natural products. They can inhibit, or stop, mitosis or inhibit enzymes for making proteins needed for reproduction of the cell. These work during the M phase of the cell cycle. Examples of mitotic inhibitors include paclitaxel, docetaxel, etoposide (VP-16), Vinblastine, vincristine, and vinorelbine.

Steroids are natural hormones and hormone-like drugs that are useful in treating some types of cancer (lymphoma, leukemias, and multiple myeloma) as well as other illnesses. When these drugs are used to kill cancer cells or slow their growth, they are considered chemotherapy drugs. They are often combined with other types of chemotherapy drugs to increase their effectiveness. Examples include prednisone and dexamethasone.

Sex hormones, or hormone-like drugs, alter the action or production of female or male hormones. They are used to slow the growth of breast, prostate, and endometrial (lining of the uterus) cancers, which normally grow in response to hormone levels in the body. These hormones do not work in the same ways as standard chemotherapy drugs. Examples include anti-estrogens (tamoxifen, fulvestrant), aromatase inhibitors (anastrozole, letrozole), progestins (megestrol acetate), anti-androgens (bicalutamide, flutamide), and LHRH agonists (leuprolide, goserelin).

Alkylating agents work directly on DNA to prevent the cancer cell from reproducing. As a class of drugs, these agents are not phase-specific (in other words, they work in all phases of the cell cycle). These drugs are active against chronic leukemias, non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, and certain cancers of the lung, breast, and ovary. Examples of alkylating agents include busulfan, cisplatin, carboplatin, chlorambucil, cyclophosphamide, ifosfamide, dacarbazine (DTIC), mechlorethamine (nitrogen mustard), and melphalan.

Nitrogen mustard in the form of its crystalline hydrochloride it is used as a drug in Hie treatment of Hodgkin's disease, non-Hodgkin's lymphomas, and brain tumors. Nitrogen mustards cause mutations in the genetic material of cells, thereby disrupting mitosis, or cell

division. Cells vary in their susceptibility to nitrogen mustards, with rapidly proliferating tumor and cancer cells most sensitive; bone marrow, which produces red blood cells, is also sensitive, and depression of red blood cell production is a frequent side effect of nitrogen mustard therapy. The nitrogen mustards also suppress the immune response (see immunity). Other types include the aromatic mustards melphalan and chlorambucil, cyclophosphamide, HNl, Z>w-(2-chloroethyl) ethylamine HN2, Z>/,y-(2-chloroethyl) methylamine and HN3, /rø-(2-chloroethyl) amine.

Nitrosoureas act in a similar way to alkylating agents. They interfere with enzymes that help repair DNA . These agents are able to travel to the brain so they are used to treat brain tumors as well as non-Hodgkin's lymphomas, multiple myeloma, and malignant melanoma. Examples of nitrosoureas include carmustine (BCNU) and lomustine (CCNU).

Hormone agonists include Leuprolide (Lupron, Viadur, Eligard) for prostate cancer, Goserelin (Zoladex) for breast and prostate cancers and Triptorelin (Trelstar) for ovarian and prostate cancers and nafarelin acetate (Synarel).

Microtubule inhibitors include "Vinca" alkaloids, taxoids and benzimidazoles.

Accordingly, the transport protein inhibitors of the present invention are useful in overcoming acquired or inherent resistance to anti-cancer agents, alkylating agents and nitrogen mustards, antibiotics, anti-metabolites, hormonal agonists and antagonists and steroids, immunomodulators, synthetic compounds and natural products of microtubile inhibitors.

Unless otherwise indicated, the subject invention is not limited to specific formulations of components, manufacturing methods, dosage or diagnostic regimes, or the like. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

The singular forms "a", "an" and "the" include plural aspects unless the context clearly

dictates otherwise. Thus, for example, reference to "an agent" includes a single agent, as well as two or more agents; reference to "a compound" includes a single compound as well as two or more compounds; reference to "a mimetic" includes a single mimetic as well as two or more mimetics; and so forth.

In describing and claiming the present invention, the following terminology is used in accordance with the definitions set forth below.

The terms "therapeutic extract", "compound", "active agent", "chemical agent", "pharmacologically active agent", "medicament", "active" and "drug" are used interchangeably herein to refer to an R-substituted vitexin or its derivatives, analogs, homologs, stereoisomers, mimetics or functional equivalents. In particular, these terms refer to a methylvitexin that induces a desired pharmacological and/or physiological effect such as but not limited to inhibiting or reducing the activity or function of the Pgp transporter and optionally also BCRP. The terms also encompass pharmaceutically acceptable and pharmacologically active ingredients of those active agents specifically mentioned herein including but not limited to salts, esters, amides, prodrugs, active metabolites, analogs and the like. When the terms "therapeutic extract", "compound", "active agent", "chemical agent" "pharmacologically active agent", "medicament", "active" and "drug" are used, then it is to be understood that this includes the active agent *per se* as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, metabolites and analogs, as well as extracts comprising the compound.

The present invention extends to chemical extracts and fractions comprising precursor or parental forms of the inhibitors. For example, vitexin may be chemically methylated to produce methylvitexin including heptamethylvitexin.

Reference to a "therapeutic extract", "compound", "active agent", "chemical agent" "pharmacologically active agent", "medicament", "active" and "drug" may include combinations of two or more of such components, such as for example, two or more forms of the R-substituted vitexin or derivatives, analogs, homologs, stereoisomers, mimetics or

functional equivalents thereof or one or more of these components and a chemotherapeutic agent. A "combination" also includes multi-part or multi-facet combinations such as a two-part composition where the agents are provided separately and given or dispensed separately or admixed together prior to dispensation.

For example, a multi-part pharmaceutical pack may have an the vitexin compound maintained separately to an anti-cancer or anti-pathogenic or other chemotherapeutic agent.

The terms "effective amount" and "therapeutically effective amount" of an agent as used herein mean a sufficient amount of the transport protein inhibitor antagonist to provide the desired therapeutic or physiological effect or outcome including inhibiting Pgp and optionally BCRP activity. Undesirable effects, e.g. side effects, are sometimes manifested along with the desired therapeutic effect; hence, a practitioner balances the potential benefits against the potential risks in determining what is an appropriate "effective amount". The exact amount of agent required will vary from subject to subject, depending on the species, age and general condition of the subject, mode of administration and the like. Thus, it may not be possible to specify an exact "effective amount". However, an appropriate "effective amount" in any individual case may be determined by one of ordinary skill in the art using only routine experimentation. The effective amount is conveniently measured by a reduction in resistance ability to a chemotherapeutic agent or the overcoming of resistance. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

Similarly, a "pharmacologically acceptable" salt, ester, emide, prodrug or derivative of a compound as provided herein is a salt, ester, amide, prodrug or derivative that this not biologically or otherwise undesirable.

The terms "treating" and "treatment" as used herein refer to resistance to a chemotherapeutic agent or overcoming acquired or inherent resistance to a

chemotherapeutic agent. The chemotherapeutic agent includes *inter alia* anti-cancer or anti-tumor agents and anti-pathogenic agents.

The terms "cancer" and "tumor" may be used interchangeably and includes a pre-cancerous condition.

"Treating" a subject may involve prevention of resistance or overcoming resistance to a chemotherapeutic agent.

A "subject" as used herein refers to an animal, preferably a mammal and more preferably a human who can benefit from the pharmaceutical formulations and methods of the present invention. There is no limitation on the type of animal that could benefit from the presently described pharmaceutical formulations and methods. A subject regardless of whether a human or non-human animal may be referred to as an individual, patient, animal, host or recipient as well as subject. An "animal" may also be an avian species such as a poultry bird. The compounds and methods of the present invention have applications in human medicine and veterinary medicine as well as administering antibiotics or other agents in feed lots to animals or birds.

Preferred animals are humans or laboratory test animals.

Examples of laboratory test animals include mice, rats, rabbits, guinea pigs, hamsters, cats and dogs.

The term "pharmaceutically acceptable salts" refers to physiologically and pharmaceutically acceptable salts of the compounds of the invention: i.e. salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects thereto.

The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon

the area to be treated. Administration may be topical (including ophthalmic and to mucous membranes including vaginal and rectal delivery), pulmonary, e.g. by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g. intrathecal or intraventricular, administration. Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, foams and liposome-containing formulations. The pharmaceutical

compositions and formulations of the present invention may comprise one or more penetration enhancers, carriers, excipients or other active or inactive ingredients.

Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Microemulsions are included as an embodiment of the present invention. Emulsions and their uses are well known in the art and are further described in U.S. Patent 6,287,860, which is incorporated herein in its entirety.

Formulations of the present invention include liposomal formulations. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers. Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior that contains the composition to be delivered. Cationic liposomes are positively charged liposomes which are believed to interact with negatively charged DNA molecules to form a stable complex. Liposomes that are pH-sensitive or negatively-charged are believed to entrap DNA rather than complex with it. Both cationic and noncationic liposomes have been used to deliver DNA to cells.

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome comprises one or more glycolipids or is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. Liposomes and their uses are further described in U.S. Patent No. 6,287,860.

The pharmaceutical formulations and compositions of the present invention may also include surfactants. The use of surfactants in drug products, formulations and in emulsions

is well known in the art. Surfactants and their uses are further described in U.S. Patent No. 6,287,860.

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of the transporter protein inhibitor. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs. Penetration enhancers may be classified as belonging to one of five broad categories, i.e. surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants. Penetration enhancers and their uses are further described in U.S. Patent No. 6,287,860.

One of skill in the art will recognize that formulations are routinely designed according to their intended use, i.e. route of administration.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which oligonucleotides of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts and fatty acids and their uses are further described in U.S. Patent No. 6,287,860. Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether.

Compositions and formulations for parenteral, intrathecal or intraventricular administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

The formulation of therapeutic compositions and their subsequent administration (dosing) is within the skill of those in the art. Dosing is dependent on severity and responsiveness of the disease state to be treated, with the course of treatment lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved. Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates. Optimum dosages may vary depending on the relative potency of individual oligonucleotides, and can generally be estimated based on EC_{50}s found to be effective *in vitro* and *in vivo* animal models. In general, dosage is from 0.01 μg to 100 g per kg of body weight, and may be given once or more daily, weekly, monthly or yearly, or even once every 2 to 20 years. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo maintenance therapy to prevent the recurrence of the disease state, wherein the oligonucleotide is administered in maintenance doses, ranging from 0.01 μg to 100 g per kg of body weight, once or more daily, to once every 20 years.

By "pharmaceutically acceptable" carrier, excipient or diluent is meant a pharmaceutical vehicle comprised of a material that is not biologically or otherwise undesirable, i.e. the material may be administered to a subject along with the selected active agent without causing any or a substantial adverse reaction. Carriers may include excipients and other additives such as diluents, detergents, coloring agents, wetting or emulsifying agents, pH buffering agents, preservatives, and the like.

The present invention further contemplates the use of an R-substituted vitexin including a compound of general Formulae (I) through (III) in the manufacture of a medicament to reduce the risk of development of resistance to a chemotherapeutic agent or to overcome acquired or inherent resistance *to* the chemotherapeutic agent.

Preferably, the R-substitute vitexin is heptamethylvitexin or is a derivative, homolog, analog, mimetic, stereoisomer or functional equivalent thereof.

The present invention is further described by the following non-limiting Examples. In these Examples, materials and methods outlined below were employed:

Cells and Chemicals

CCRF-CEM and CEM/VLB$_{100}$ cells are available from Bill Walsh Laboratories, Sydney, Australia. K562 cells are available from the American Type Culture Collection. K562/V16 cells are derived from K562/V8 and are resistant to 16 ng/ml Vinblastine. All cell lines are grown in RPMI-1640 supplemented with 10% v/v foetal bovine serum (GIBCO BRL, Buffalo, NY, USA) at 37°C in a humidified incubator with 5% v/v CO_2. AU cells are free of mycoplasma. Daunorubicin (DNR), Vinblastine (VLB), Verapamil, rhodamine 123 (RhI 23), ethidium bromide (EtBr) and colchicine (COL) are from Sigma-Aldrich (St. Louis, MO, USA). BOBIPY-FL Vinblastine (FL-VLB) are from Molecular Probes (Eugene, OR, USA).

Drug Accumulation

5 x 10^5 cells/ml are pre-incubated for 15 min with the flavonoid compound (heptamethylvitexin) at 37^0C, followed by incubation for 90 min with 25 μM DNR, or 1 μM FL-VLB. Controls are incubated with an equivalent volume of DMSO. Cells are then washed twice and resuspended in ice-cold PBS, kept on ice and analysed immediately using a FACS Calibur Sort using CellQuest software (Becton Dickinson, Sydney, Australia). For FL-VLB, green fluorescence (FL-I) is collected using a 530/30 band pass filter. For daunorubicin red fluorescence (FL-2) was collected using a 585/42 band pass filter. In dose-dependence experiments 1, 5, 10, 20, 50 and 100 μM heptamethylvitexin was used. In experiments with FL-VLB 10 μM heptamethylvitexin was used.

CeIl ViabilityAssays

Cytotoxicity assays are performed as previously described. Briefly, 3 x 10^5 cells are plated, in triplicate, in serial 2-fold dilutions of each drug and a set concentration of heptamethylvitexin. After 72 h, cell viability was determined by incubating cells with 2 mg/ml MTS (Promega, Madison, USA), after which absorbance are measured at 490 run using a Bio-Rad Microplate Reader (Bio-Rad Laboratories, Ca, USA). The 50% inhibitory concentration (IC_{50}) was determined to be the drug concentration at which there was a 50% reduction in cell viability. Fold reversal was calculated by division of the IC_{50} obtained for cells incubated with drug alone by the IC_{50} for cells incubated with the drug in the presence of the flavonoid.

EXAMPLE 1

Effects ofheptamethylvitexin on CEM/VLB$_m$ Cells: Daunorubicin (DNR)

Heptamethylvitexin (HMV) increases drug accumulation in multidrug resistant CEMA^LB$_{10O}$ cells. The results are shown in Figure 1. CEM/VLB$_{1O}$o cells express Pgp and pump out the chemotherapy drug DNR. The flavonoid compound HMV inhibited Pgp at a concentration from 1 μM to 100 μM, leading to increased DNR accumulation in the cells.

EXAMPLE 2

Effects ofheptamethylvitexin on CEM/VLB$_{100}$ Cells: Vinblastine (VLB)

Heptamethylvitexin (HMV) increases drug accumulation in multidrug resistant CEMA'LB$_{10}$Ocells. The results are shown in Figure 2. The flavonoid compound HMV inhibited Pgp and gave a 2-fold increase in fluorescent VLB accumulation in the cells at a concentration of 10 μM.

EXAMPLE 3

Effects ofheptamethylvitexin on Cancer Cells

The flavonoid heptamethylvitexin is able to reverse resistance to DNR, increasing the ability of DNR to kill cancer cells. The results are shown in Figure 3. The reversal is dose-dependent with up to 23-fold reversal at 20. μM. Reversal of DNR resistance was as follows 50 nM: 1.5-fold, 100 nM: 1.2-fold, 1 μM: 2.5-fold, 5 μM: 6.9-fold, 10 μM: 13. 1-fold, 20 μM: 23.4-fold.

EXAMPLE 4

Effects ofheptamethylvitexin on Cancer Cells

The flavonoid heptamethylvitexin is able to reverse resistance to VLB, and the reversal is dose-dependent, with up to 70-fold reversal at 20 μM. The results are shown in Figure 4.

Reversal of VLB resistance is as follows 50 nM: 1.8-fold , 100 nM: 1.5-fold, 1 μM: 2.0-fold, 5 μM: 4.9-fold, 10 μM: 19.7-fold, 20 μM: 69.4-fold.

EXAMPLE 5

Effect of methylated vitexins on P-Glycoprotein (Pgp) ATPase activity

The effect of methylated vitexins on Pgp ATPase activity was determined using the Pgp-GIo (Trade Mark) Assay Systems from Promega Ltd. The assay detects the effects of compounds on recombinant human Pgp in a cell membrane fraction. The assay relies on the ATP dependence of the light-generating reaction of firefly luciferase, which measures the remaining unmetabolized ATP. Thus, decreases in luminescence reflects ATP consumption by Pgp and, therefore, the lower the signal, the higher the Pgp activity. Accordingly, compounds that stimulate the Pgp ATPase have significantly lower signals than untreated samples, and are typically substrates for transport by Pgp. Compounds that interact with Pgp are proposed to be potential inhibitors of its ATPase activity.

Methylated vitexins were investigated for inhibition of verapamil induced Pgp ATPase stimulation. Dose-response curves of five samples of methylated vitexins were carried out to examine the effect on Pgp ATPase activity induced by verapamil. Sample I is heptamethyl vitexin, sample II contains heptamethyl vitexin, whilst the other three samples (III, IV & V) contain penta-, terra- and trimethyl vitexins. The two samples (I & II), which contain heptamethyl vitexin, showed concentration dependent inhibition of verapamil-induced Pgp ATPase activity, with IC_{50} value of approximately 10 μM. Verapamil (100 μM), in the absence of the vitexins, exhibited 17-fold increase in Pgp ATPase activity in comparison with the basal activity. The other three samples (III, IV & V) of methylated vitexins showed no effect on Pgp ATPase activity induced by verapamil (Figure 5).

EXAMPLE 6

Cytotoxic effect of methylated vitexins

Human lymphoblastic leukemia multidrug resistant subline, VBL_{100} (CEM/VBL_{100}), selected for Vinblastine resistance, was maintained in RPMI 1640 medium supplemented with 10% v/v heat-inactivated fetal bovine serum without antibiotics at 37°C in a humidified incubator with 5% v/v of carbon dioxide. Cells were passaged regularly to maintain the population below 8 x 10^5 cells/mL. CEMA^BL_{100} cells were treated with 100 ng/mL VLB once every 4 weeks to maintain the resistance phenotype.

Cells were seeded onto 96-well plates at a density of 5 x 10^4 per well per 100 μL, followed by treatment of heptamethyl vitexin at 5 and 25 μM, respectively. A concentration response relationship of VLB was then established. Control cells were treated with vehicle (DMSO) at final concentration of 1% w/v, at which minimal effect of vehicle on cell proliferation was observed. Cells were incubated at 37^0C in a humidified incubator for 72 hrs, 20μL of CellTiter 96 (Registered Trade Mark) AQueous One Solution Reagent was added into each well, and then the plate incubated for 2-3 hours at 37°C in a humidified, 5% v/v CO_2 atmosphere. The absorbance was recorded at 490 nm using the microplate reader (POLARstar, BMG Labtech). Cytotoxic effect of the compounds was expressed as percentage of cell viability against the agent concentrations.

Results as shown in Figure 6 illustrated that heptamethyl vitexin (I) exhibited minor toxic (< 20 %) effect on cells at concentrations up to 20 μM, but significantly reduced cell viability at high concentration (ie. 85 μM). This result supports the hypothesis that heptamethyl vitexin restored VLB sensitivity against MDR,as a result of modulation of Pgp activity, but not due to toxicity to cells.

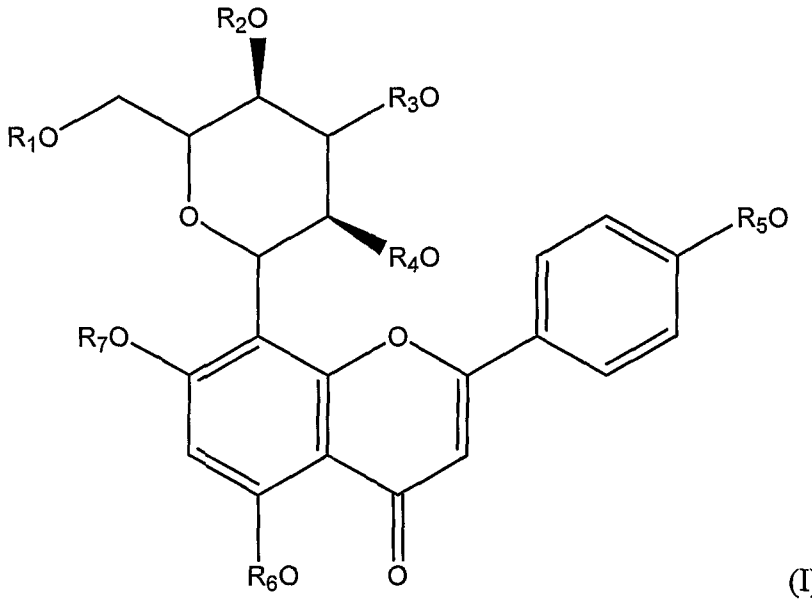
Results shown in Figure 7 indicated that heptamethyl vitexin concentration-dependently enhanced the cytotoxicity of Vinblastine. This represents an approximately 100 fold decrease in IC_{50} value of Vinblastine in the presence of 25 μM of heptamethyl vitexin. This reversal activity of heptamethyl vitexin appeared to correlate with the inhibitory

activity against verapamil induced Pgp ATPase stimulation, as shown in Figure 5, possibly by competing with verapamil for binding.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

CLAIMS

1. A method for overcoming acquired or inherent resistance to a chemotherapeutic agent in a subject said method comprising administering to said subject an amount an R-substituted vitexin of general Formula I:



(I)

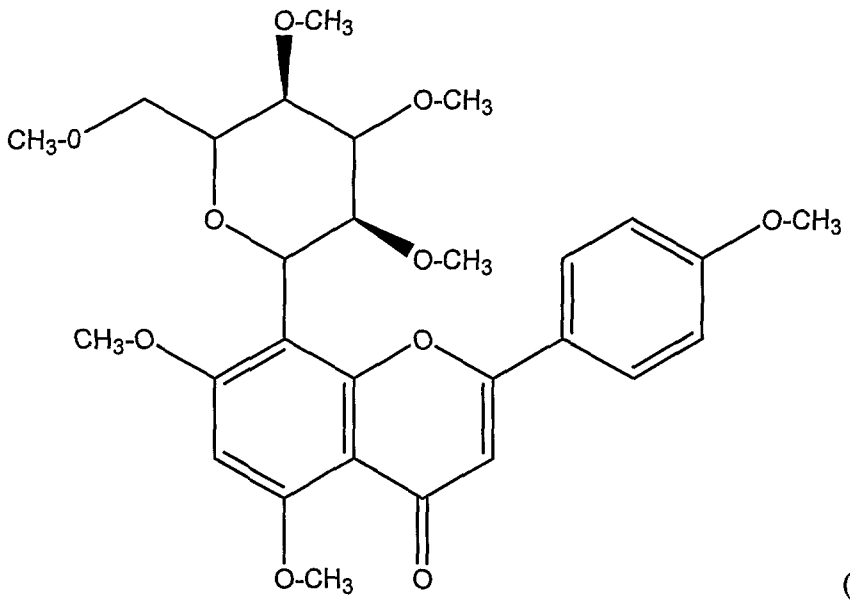
wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 may be the same or different and each is selected from hydrogen, -CrC^alkyl, -C_2-C_{10}alkenyl, -C_2-C_{10}alkynyl, -$(CH_2)_n COR_8$, -$(CH_2)_n R_9$, -PO_3H, -$(CH_2)_n$heterocyclyl or -$(CH_2)_n$aryl where R_8 is -OH, -NH_2, -NHCrCsalkyl, -OC_1-C_3alkyl or -Q-Qalkyl and R_9 is -OH, -SH, -Sd-C_3alkyl, -OCi-C_3alkyl, -Cs-C^cycloalkyl, -C_3-C_{12}cycloalkenyl, -NH_2, -NHd-dalkyl or -NHC(C=NH)NH_2 5 n is Oor an integer from 1 to 10 and where each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHQ-Csalkyl, -OQ-Csalkyl, -SH, -Sd-C_3alkyl, -CO_2H, -C0 2d-C_3alkyl, -$CONH_2$ or -CONHd-Csalkyl;

prior to, together with or subsequent to the chemotherapeutic agent.

2. The method of Claim 1 wherein each of Ri through R_7 is a Ci-C_{10} alkyl.

3. The method of Claim 2 wherein the C_1-C_{10} alkyl is methyl.

4. The method of Claim 3 wherein the R-substituted vitexin is of heptamethylvitexin of Formula (III):



(III).

5. The method of Claim 4 wherein the heptamethylvitexin is chemically synthesized.

6. The method of Claim 4 wherein the heptamethylvitexin is isolated from or contained within a plant extract or is generated by methylation of vitexin *in vitro.*

7. The method of Claim 6 wherein the plant is selected from the list consisting of *Achillea nobilis L, Achillea setacea W. et K., Adhatoda vasica Nees* (leaves and flowers), *Ailanthus excelsa* (leaves), *Alternanthera maritima (Mart.) St. HiI.* (aerial part of the plant), *Anthurium versicolor* (leaves), *Anihurium versicolor* (leaves), *Artemisia vulgaris L* (whole plant), *Arum palaestinum, Aspalathus linearis* (leaves and the stems), *Aspalathus linearis* (rooibos tea), *Beta vulgaris L* (Leaves of the sugar beet), *Bryonia alba* (roots),

Bryonia dioica (roots), *Calendula flower, Milk-thistle fruit* and *Passionflower, Cannabis sativa* (plant), *Cannabis sativa subsp. sativa* (cannabinoid-free cannabis) (leaves and flowers), *Cayaponia tayuya* (roots), *Cayaponia tayuya* (roots), *Chamaecytisus eriocarpus, Chamaecytisus pygmaeus* (aerial parts), *Cissus rheifolia* (leaves), *Citrus aurantium var. amara L* (sour orange leaves), *Citrus sinensis L* (sweet orange leaves), *Clinacanthus nutans, Crataegus monogyna Jacq* (leaves and flowers), *Crataegus orientalis* (leaves), *Crataegus oxyacantha L* (flowers, leaves, and bark), *Crataegus pinnatiflda* (leaves and fruits), *Crataegus pinnatiflda var. psilosa* (leaves), *Crataegus tanacetifolia* (Rosaceae) (leaves, flowers, and fruits), *Crossopteryx febrifuga* (leaves), *Crotalaria anagyroides, Crotalaria paniculata wild* (flowers), *Crotalaria sessiliflora* (aerial part of the plant), *Crotalaria striata* (leaves and stem bark), *Crotalaria thebaica (Del.) Dc* (aerial parts), *Croton hovarum* (leaves), *Cyathea fauriei* (leaves), *Cyathea faurier* (leaves), *Cyathea hancockii* (leaves), *Cyathea leichhardtiana* (leaves), *Cyathea mertensiana* (leaves), *Cyathea onusta* (leaves), *Cyathea podophylla* (leaves), *Cyathea spinulosa* (leaves), *Cyathea tueckheimeii* (leaves), *Desmodium triflorum,Desmodium antartica, Dissotis rotundifolia T* (whole plant), *Dracunculus vulgaris Schott* (leaves), *Drosophyllum lusitanicum* (shoot and callus cultures), *Dysolobium apioides* (leaves), *Ecbolium linneanum* (leaves, flowers, and roots), *Erythrina crista-galli Linn,* (leaves), *Euodia daniellii* (fruits and leaves), *Fagopyrum esculentum Moeench* (buckwheat grain), *Fagopyrum esculentum Moeench, Buckwheat Hulls, Feronia elephantum Correa* (leaves), *Gaillardia aristata* (Compositae), *Gaillardia pulchella, Gentiana lutea, Glochidion zeylanicum* (leaves), *Gnidia involucrata* (aerial parts) (Thymelaeaceae), *Gonocaryum calleryanum* (leaves), *Gonocytisus angulatus* (aerial part), *Gramineae species* (stem and leaves), *Gutierrezia grandis, Helenium brevifolium* (above ground part), *Heylandia latebrosa* (leaves), *Hyparrhenia hirta* (aerial parts), *Itea japonica* (leaves), *Jatropha gossypifolia* (leaves), *Jatropha heterophylla* (aerial parts), *Kleinhovia hospita* (flowers), *Larix gmelinii* (needles), *Lavandula dentata* (aerial parts), *Lespedeza capitata* (arial portion of the plant), *Ludwigia* (Onagraceae), *Lupinus arboreus* (whole plant including roots), *Lythrum salicaria L* (purple loosestrife), *Majorana hortensis* (aerial parts), *Majorana syriaca, Marrubium vulgare* (leaves), *Melilotus indica, Mollugo cerviana* (whole plant), *Ochnajapotapita* (leaves), *Ocimum gratissimum var. gratissimum, Ocimum*

sanctum (aerial parts) *L, Onopordum laconicum* (aerial parts), *Orchidaceae plant, Oxera macrocalyx, Oxera neriifolia, Parkinsonia aculeata* (leaves), *Passiflora caerulea L, Passiflora family, Passiflora incarnata, Passiflora serratifolia* (leaves), *Passiflora sexflora* (leaves), *Phenax angustifolius* (leaves), *Phoradendron tomentosum* (leaves), *Plagiomnium affine* (mosses), *Populus heterophylla L* (leaves), *Prosopis argentina* (leaves), *Prosopis chilensis* (leaves and pods), *Prosopis fiebrigii* (leaves), *Prosopis flebrigii* (leaves), *Prosopis hasslerin* (leaves), *Prosopis reptans* (leaves), *Psophocarpus tetragonolobus (L.) DC.* (winged bean), *Pterostemon mexicanum, Pterostemon rotundifolia, Rhynchosia bracteata* (leaves), *Rhynchosia cana* (leaves), *Rhynchosia densiflora* (leaves), *Rhynchosia jacobii* (leaves), *Rhynchosia suaveolens* (leaves), *Rhynchosia sublobata* (leaves), *Rumex vesicarius,Swertia* genus such as *Swertia abyssinica, Swertia adolfl-friderici, Swertia angustifolia, Swertia bimaculata, Swertia binchuanensis, Swertia calycina, Swertia chirayta, Swertia ciliata, Swertia cincta, Swertia cordata, Swertia crassiuscula, Swertia cuneata, Swertia decora, Swertia delavajyi, Swertia dichotoma, Swertia diluta, Swertia engleri, Swertia erythrosticta, Swertia franchetiana, Swertia hispidicalyx, Swertia japonica, Swertia kilimandscharica, Swertia lactea, Swertia macrosperma, Swertia marginata, Swertia mileensis, Swertia multicaulis, Swertia mussoti, Swertia perennis, Swertia petiolata, Swertia przewalskii, Swertia off. pseudohookeri, Swertia pubescens, Swertia punicea, Swertia racemosa, Swertia rosulata, Swertia schugananica, Swertia tashiroi, Swertia tetraptera, Swertia volkensii, Swertia wolfongiana* and *Swertia yunnanensis, Scutellaria albida* (aerial parts), *Synandrospadix vermitoxicus* (leaves), *Terminalia catappa L* (leaves), *Theobroma cacao, Trema micrantha* (leaves and branches), *Trigonella corniculata* (seeds), *Trigonella foenum-graecum linn,* (seeds), *Trigonella grandiflora, Triticum aestivum* (wheat bran, Sakha 69) (Gramineae), *Trollius chinensis* (flowers), *Trollius chinensis Bunge* (flower), *Verbena bipinnatifida Nutt. herb* (Verbenaceae), *Vicia species* (pods), *Vigna plants in Leguminosae* (seed coat), *Vigna radiata L* (mung bean), *Vitexpeduncularis* (leaves), *Vitexpolygama* (leaves).

8. The method of Claim 1 wherein the subject is a mammal.

9. The method of Claim 8 wherein the mammal is a human.

10. The method of Claim 1 wherein the chemotherapeutic agent is selected from daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX)$_5$ colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES).

11. The method of Claim 1 wherein the chemotherapeutic agent is a nonsteroidal anti¬inflammatory drug or corticosteroid.

12. The method of Claim 1 wherein the chemotherapeutic agent is selected from ribivirin, vidarabine, acyclovir, ganciclovir, 3TC, Abacavir, Acyclovir, Alpha interferon, AZT, Bleomycin, Capreomycin, Cidofovir, Ciprofloxacin, Cyclophosphamide, ddC, ddi, Delavirdine, Didanosine, Dronabinol, Efavirenz, Erythropoetin, Famciclovir, Filgrastim, Foscarnet, Ganciclovir, Ganciclovir Implants, Indinavir, Lamivudine, Lopinavir, Megace, Methotrexate, Nelfinavir, Nevirapine, Nonoxynol-9, Ribavirin, Ritonavir, Saquinavir, Stavudine, Sulfamethoxazole, Tenofovir, Trimethoprim, Zalcitabine and Zidovudine.

13. The method of Claim 1 wherein the chemotherapeutic agent is selected from Amikacin, Atovaquone, Azithromycin, Clarithromycin, Clindamycin, Clofazimine, Cycloserine, Dapsone, Ethambutol, Ethionamide, Isoniazid, IVIG, Kanamycin, Metronidazole, Ofloxacin, Para Aminosalicyclic Acid, Pentamidine, Primaquine, Pyrazinamide, Pyrimethamine, Rifabutin, Rifampin, Streptomycin, Sulfadiazine, Sulfadoxine, Sulfamethazine, Trimetrexate and Triple Sulfa.

14. The method of Claim 1 wherein the chemotherapeutic agent is selected from Azaserine, D-Cycloserine, Mycophenolic acid, Trimethoprim, 5-fluorouracil, capecitabine, methotrexate, gemcitabine, cytarabine (ara-C) and fludarabine.

15. The method of Claim 1 wherein the chemotherapeutic agent is selected from dactinomycin, daunorubicin, doxorubicin (Adriamycin), idarubicin, and mitoxantrone.

16. The method of Claim 1 wherein the chemotherapeutic agent is selected from paclitaxel, docetaxel, etoposide (VP-16), Vinblastine, vincristine, and vinorelbine.

17. The method of Claim 1 wherein the chemotherapeutic agent is selected from prednisone and dexamethasone.

18. The method of Claim 1 wherein the chemotherapeutic agent is selected from anti-estrogens (tamoxifen, fulvestrant), aromatase inhibitors (anastrozole, letrozole), progestins (megestrol acetate), anti-androgens (bicalutamide, flutamide), and LHRH agonists (leuprolide, goserelin).

19. The method of Claim 1 wherein the chemotherapeutic agent is selected from busulfan, cisplatin, carboplatin, chlorambucil, cyclophosphamide, ifosfamide, dacarbazine (DTIC), mechlorethamine (nitrogen mustard), and melphalan.

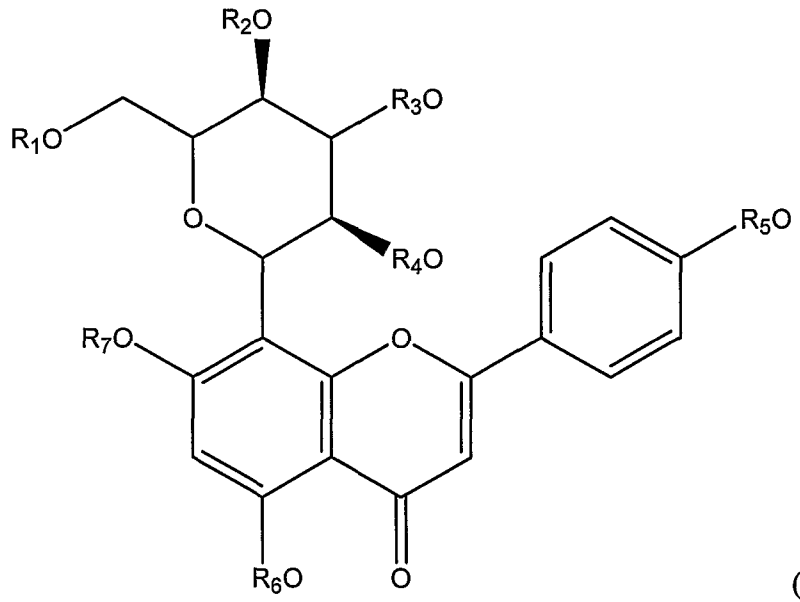
20. The method of Claim 1 wherein the chemotherapeutic agent is selected from melphalan and chlorambucil, cyclophosphamide, HNl, *b*w-(2~chloroethyl) ethylamine HN2, *b:s*-(2-chloroethyl) methylamine and HN3, /rø-(2~chloroethyl) amine.

21. The method of Claim 1 wherein the chemotherapeutic agent is selected from nitrosoureas include carmustine (BCNU) and lomustine (CCNU).

22. The method of Claim 1 wherein the chemotherapeutic agent is selected from microtubule inhibitors include "Vinca" alkaloids, taxoids and benzimidazoles.

23. Use of an R-substituted vitexin including a compound of general Formulae (I) through (III) in the manufacture of a medicament to reduce the risk of development of resistance to a chemotherapeutic agent or to overcome acquired or inherent resistance to the chemotherapeutic agent.

24. A method of treating a subject with cancer said method comprising administering to said subject an anti-cancer chemotherapeutic agent and an R-substituted vitexin of general Formula 1:



(I)

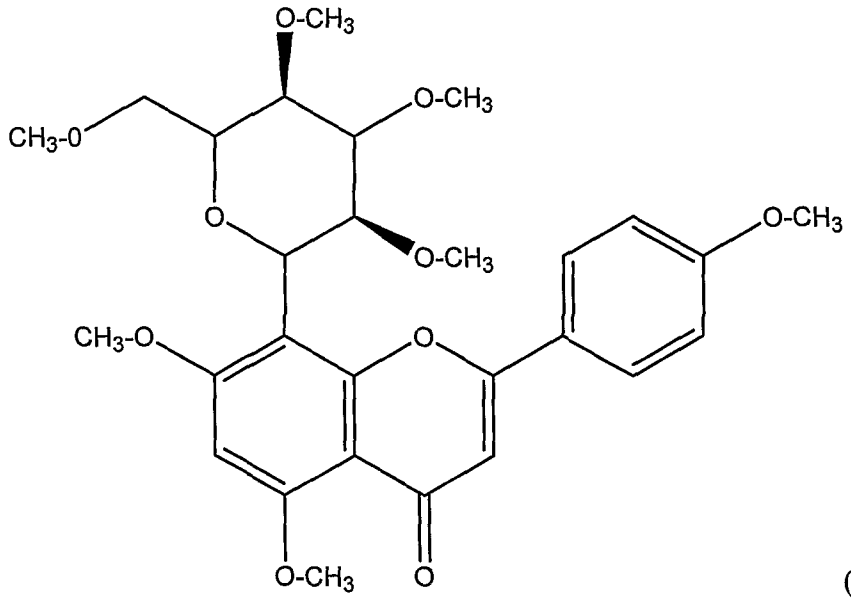
wherein R_1, R_2, R_3, R_4, R_5, R_6 and R_7 may be the same or different and each is selected from hydrogen, -d-Qoalkyl, $-C_2$-C_{10}alkenyl, $-C_2$-C_{10}alkynyl, $-(CH_2)_nCOR_8$, $-(CH_2)_nR_9$, $-PO_3H$, $-(CH_2)_n$heterocyclyl or $-(CH_2)_n$aryl where R_8 is -OH, $-NH_2$, -NHd-Csalkyl, $-OC_1$-C_3alkyl or -d-C_3alkyl and R_9 is -OH, -SH, -Sd-C_3alkyl, $-OC_1$-C_3alkyl, -Ca-Cncycloalkyl, -CrCncycloalkenyl, $-NH_2$, -NHCrC $_3$alkyl or $-NHC(C=NH)NH_2$, n is Oor an integer from

1 to 10 and where each alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl or heterocyclyl group may be substituted with one or more groups selected from -OH, -NH_2, -NHQ-Csalkyl, -Od-Csalkyl, -SH, -SC_1-C_3alkyl, -CO_2H, -COaQ-Qalkyl, -$CONH_2$ or -CONHd-Csalkyl.

25. The method of Claim 24 wherein each of Ri through R_7 is a C_1-C_{10} alkyl.

26. The method of Claim 25 wherein the C_1-Ci_0 alkyl is methyl.

27. The method of Claim 26 wherein the R-substituted vitexin is of heptamethylvitexin of Formula (III):



(III).

28. The method of Claim 27 wherein the heptamethylvitexin is chemically synthesized.

29. The method of Claim 27 wherein the heptamethylvitexin is isolated from or contained within a plant extract or is generated by methylation of vitexin *in vitro*.

30. The method of Claim 24 wherein the subject is a mammal.

31. The method of Claim 30 wherein the mammal is a human.

32. The method of Claim 24 wherein the chemotherapeutic agent is selected from daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphoramide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, Vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES).

33. The method of Claim 24 wherein the chemotherapeutic agent is a nonsteroidal anti¬inflammatory drug or corticosteroid.

34. The method of Claim 24 wherein the chemotherapeutic agent is selected from ribivirin, vidarabine, acyclovir, ganciclovir, 3TC, Abacavir, Acyclovir, Alpha interferon, AZT, Bleomycin, Capreomycin, Cidofovir, Ciprofloxacin, Cyclophosphamide, ddC, ddi, Delavirdine, Didanosine, Dronabinol, Efavirenz, Erythropoetin, Famciclovir, Filgrastim, Foscarnet, Ganciclovir, Ganciclovir Implants, Indinavir, Lamivudine, Lopinavir, Megace, Methotrexate, Nelfinavir, Nevirapine, Nonoxynol-9, Ribavirin, Ritonavir, Saquinavir, Stavudine, Sulfamethoxazole, Tenofovir, Trimethoprim, Zalcitabine and Zidovudine.

35. The method of Claim 24 wherein the chemotherapeutic agent is selected from Amikacin, Atovaquone, Azithromycin, Clarithromycin, Clindamycin, Clofazimine, Cycloserine, Dapsone, Ethambutol, Ethionamide, Isoniazid, IVIG, Kanamycin, Metronidazole, Ofloxacin, Para Aminosalicyclic Acid, Pentamidine, Primaquine,

Pyrazinamide, Pyrimethamine, Rifabutin, Rifampin, Streptomycin, Sulfadiazine, Sulfadoxine, Sulfamethazine, Trimetrexate and Triple Sulfa.

36. The method of Claim 24 wherein the chemotherapeutic agent is selected from Azaserine, D-Cycloserine, Mycophenolic acid, Trimethoprim, 5-fluorouracil, capecitabine, methotrexate, gemcitabine, cytarabine (ara-C) and fludarabine.

37. The method of Claim 24 wherein the chemotherapeutic agent is selected from dactinomycin, daunorubicin, doxorubicin (Adriamycin), idarubicin, and mitoxantrone.

38. The method of Claim 24 wherein the chemotherapeutic agent is selected from paclitaxel, docetaxel, etoposide (VP-16), Vinblastine, vincristine, and vinorelbine.

39. The method of Claim 24 wherein the chemotherapeutic agent is selected from prednisone and dexamethasone.

40. The method of Claim 24 wherein the chemotherapeutic agent is *selected from* anti-estrogens (tamoxifen, fulvestrant), aromatase inhibitors (anastrozole, letrozole), progestins (megestrol acetate), anti-androgens (bicalutamide, flutamide), and LHRH agonists (leuprolide, goserelin).

41. The method of Claim 24 wherein the chemotherapeutic agent is selected from busulfan, cisplatin, carboplatin, chlorambucil, cyclophosphamide, ifosfamide, dacarbazine (DTIC), mechlorethamine (nitrogen mustard), and melphalan.

42. The method of Claim 24 wherein the chemotherapeutic agent is selected from melphalan and chlorambucil, cyclophosphamide, HNl, tø-(2-chloroethyl) ethylamine HN2, *b*w-(2-chloroethyl) methylamine and HN3, *tr*w-(2-chloroethyl) amine.

43. The method of Claim 24 wherein the chemotherapeutic agent is selected from nitrosoureas include carmustine (BCNU) and lomustine (CCNU).

44. The method of Claim 24 wherein the chemotherapeutic agent is selected from microtubule inhibitors include "Vinca" alkaloids, taxoids and benzimidazoles.

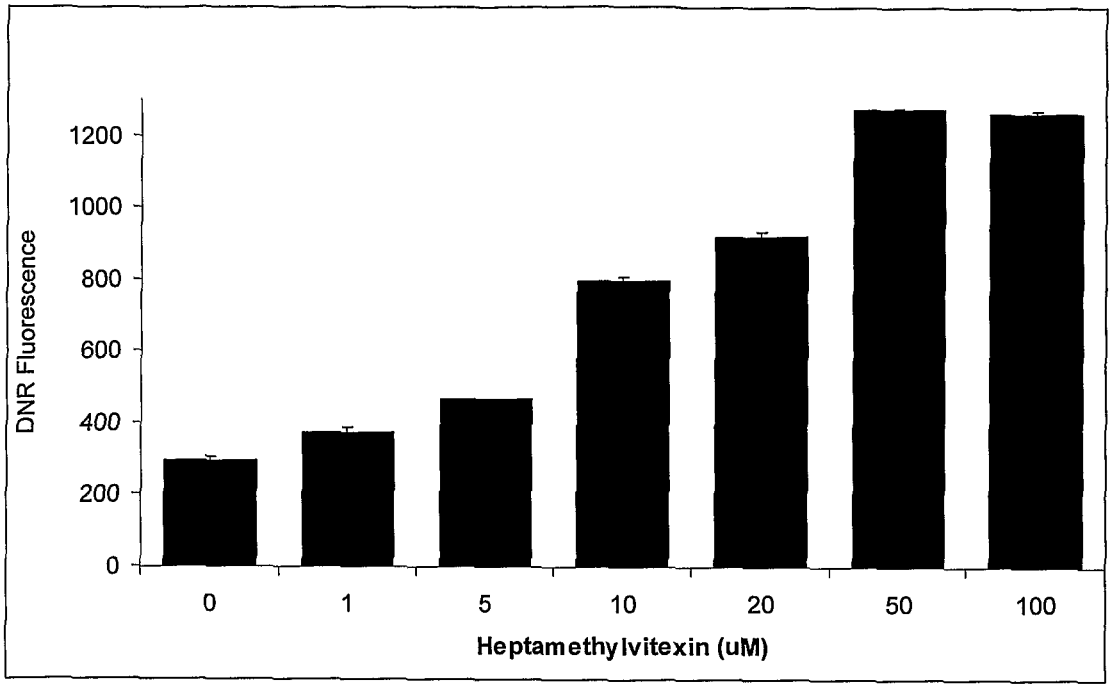

DNR Fluorescence
1200
1000
800
600
400
200
0
0
1
5
10
20
50
100
Heptamethylvitexin (uM)

Figure 1

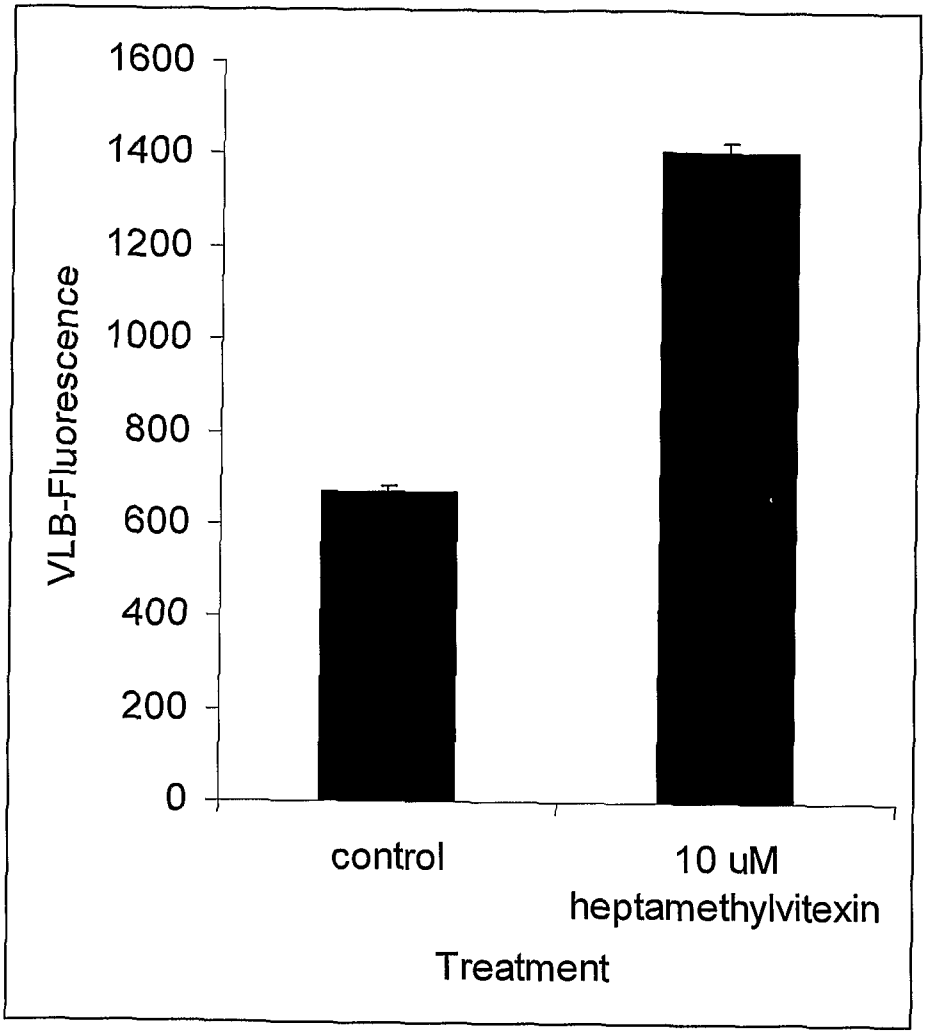

1600
1400
1200
1000
800
600
400
200
0
VLB-Fluorescence
control
10 uM heptamethylvitexin
Treatment

Figure 2

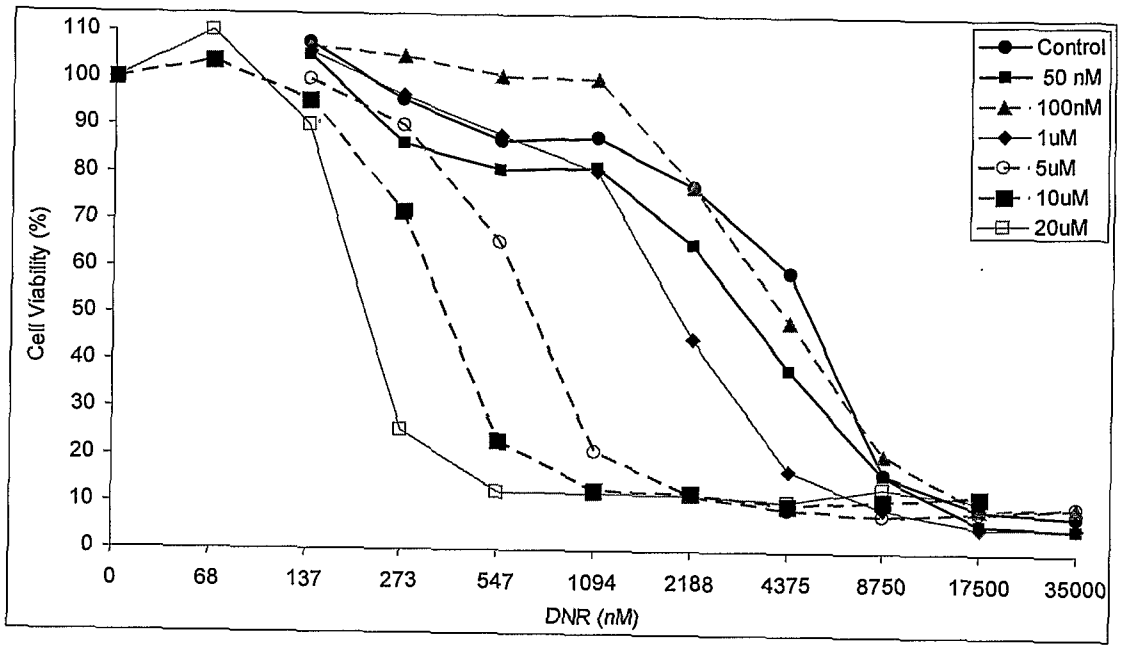

Cell Viability (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
68
137
273
547
1094
2188
4375
8750
17500
35000
DNR (nM)
Control
50 nM
100nM
1uM
5uM
10uM
20uM

Figure 3

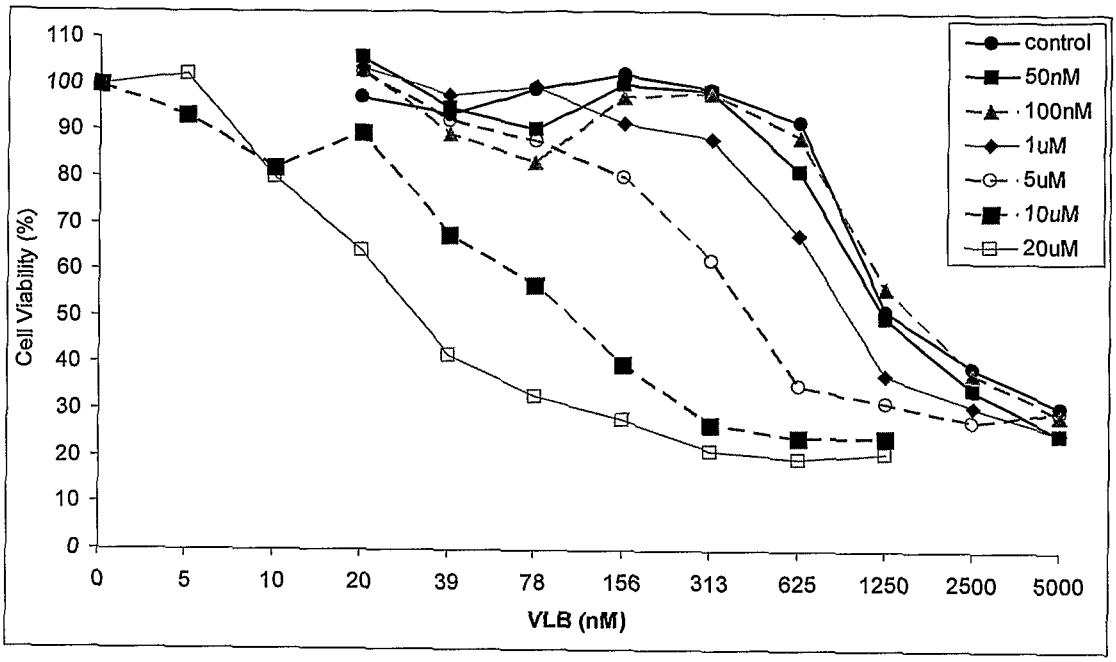

Cell Viability (%)
110
100
90
80
70
60
50
40
30
20
10
0
0
5
10
20
39
78
156
313
625
1250
2500
5000
VLB (nM)
control
50nM
100nM
1uM
5uM
10uM
20uM

Figure 4

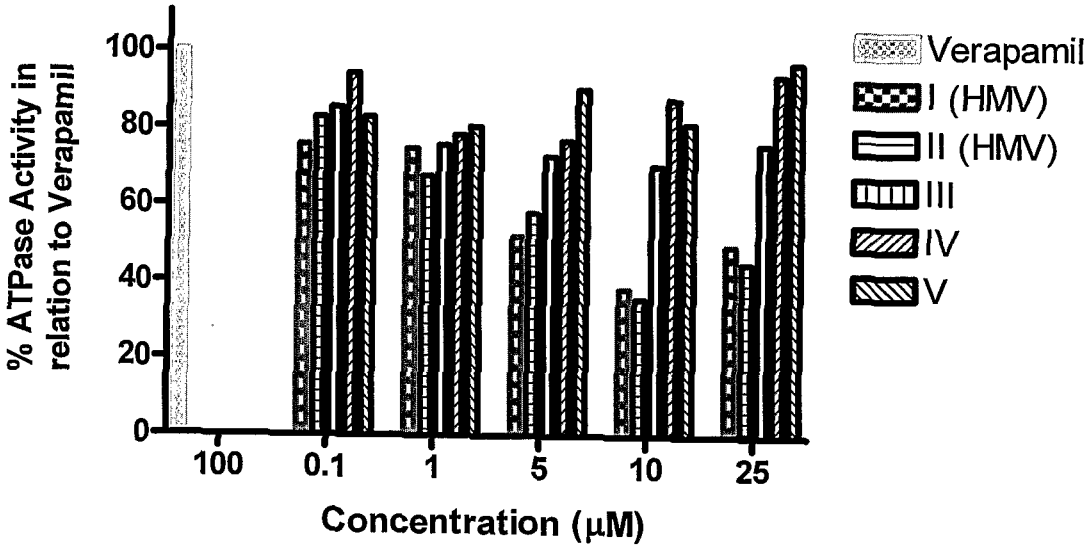

% ATPase Activity in relation to Verapamil
100
80
60
40
20
0
100
0.1
1
5
10
25
Concentration (μM)
Verapamil
I (HMV)
II (HMV)
III
IV
V

Figure 5

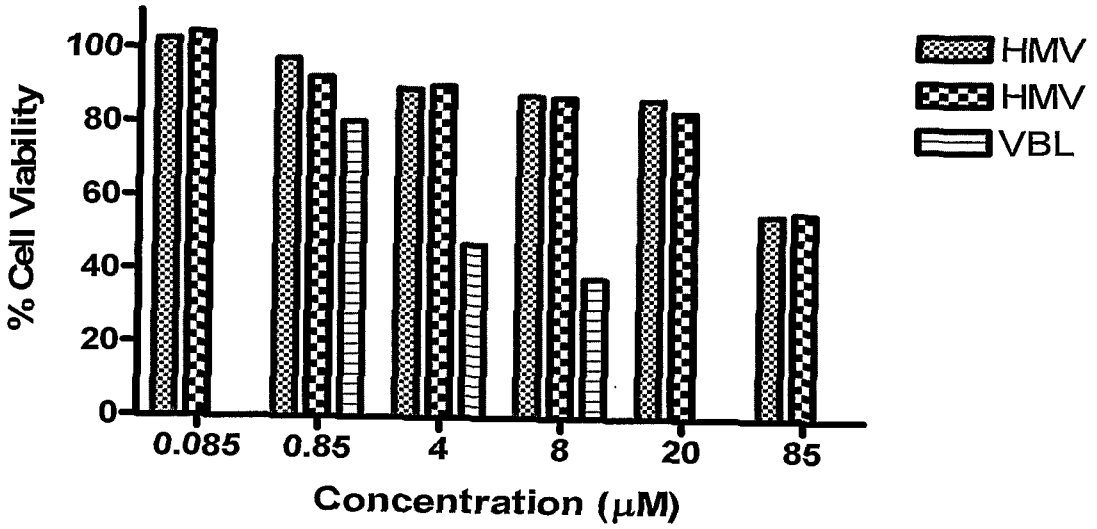

% Cell Viability
100
80
60
40
20
0
0.085
0.85
4
8
20
85
Concentration (μM)
HMV
HMV
VBL

Figure 6

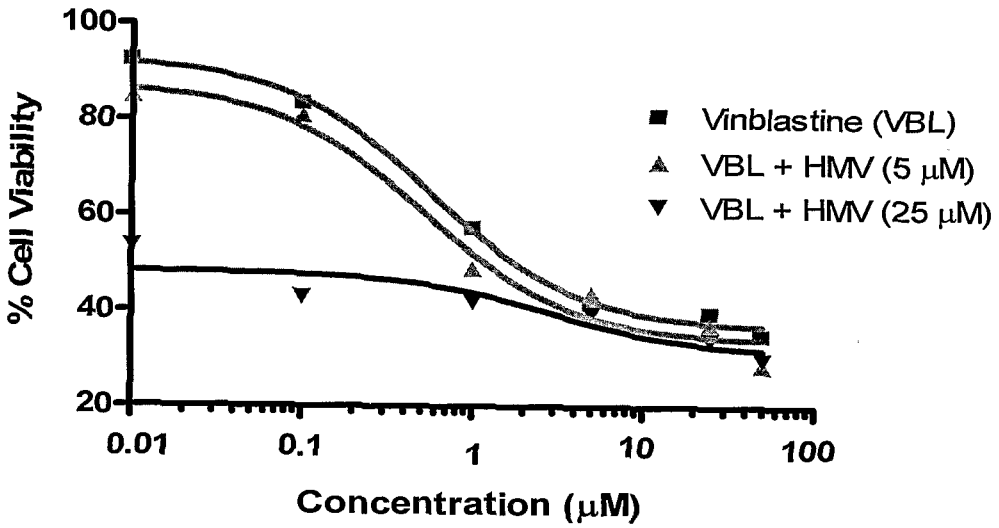

100
80
60
40
20
% Cell Viability
0.01
0.1
1
10
100
Concentration (μM)
Vinblastine (VBL)
VBL + HMV (5 μM)
VBL + HMV (25 μM)

Figure 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2006/001981

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

Λ***61K 31/353*** (2006.01) ***A61P 31/04*** (2006.01) *A61P 35/00* (2006.01)
Λ***61K31/475*** (2006.01) ***A61P 31/12*** (2006.01)
A61K 31/704 (2006.01) ***A61P 33/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MEDLINE, WPIDS, CAPLUS; vitexin, flavoniod, methylvitexin, chemotherapy, drug therapy, resistant, cancer, infection, parasite, virus.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Ren, W. et al. "Flavonoids: Promising anticancer agents" Medicinal Research Reviews, July 2003; 23(4):5 19-534.	
A	Di Pietro, A. et al. "Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters." CMLS, Cell. MoI. Life Sci., Feb 2002; 59(2):307-322.	
A	WO 1994/023715 (Sloan-Kettering institute for cancer research, et al.) 27 October 1994	

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 February 2007	0 8 MAR 2007
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**NIZNIK, TAMARA** Telephone No : (02) 6283 2422

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2006/001981

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 9423715	AU 67030/94
	US 5336685

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX