Title: COMPOSITIONS AND METHODS FOR INHIBITION OF ANGIOGENESIS AND LYMPHANGIOGENESIS

Abstract: A composition including a rifamycin derivative or a pharmaceutically acceptable salt, hydrate, or prodrug thereof is disclosed. The composition includes an amount and formulation of the rifamycin derivative or a pharmaceutically acceptable salt, hydrate, or prodrug thereof sufficient to inhibit one or more of angiogenesis and lymphangiogenesis in an organism and/or sufficient to induce drug-sensitization in or inhibition of a cancer cell in the organism.

Diagram: Figure 1

Multiple Triggers with CHOP => CHOP Sensitization => Gene Expression Changes => ROS => Start to Necroptosis

CHOP Treatment

Acquisition of CHK1/2 Resistance

Aft Activator

P P

P P

P P

P P

ROS

STO Genes

Chemotherapies (Pharmacotherapies)

CHOP Treatment

CHOP Resistance
COMPOSITIONS AND METHODS FOR INHIBITION OF ANGIOGENESIS AND LYMPHANGIOGENESIS

TECHNICAL FIELD
[001] The present disclosure relates to compositions for inhibition of angiogenesis, lymphangiogenesis, and related pathologies. In particular, it relates to compositions including rifamycin and rifamycin derivatives, such as rifabutin or rifabutin derivatives, or rifampicin and rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, or prodrugs thereof. The present disclosure also relates to methods of inhibition of angiogenesis or lymphangiogenesis, by rifamycin, rifamycin derivatives, such as rifabutin or rifabutin derivatives, or rifampicin and rifampicin derivatives, and/or pharmaceutically acceptable salts, hydrates, or prodrugs thereof, or combinations thereof to an organism.

BACKGROUND
Angiogenesis and Lymphangiogenesis

[002] Angiogenesis is the process of formation of new blood vessels from pre-existing blood vessels. Lymphangiogenesis is the process of new lymphatic vessels from pre-existing lymphatic vessels. Angiogenesis is a normal and necessary process in development and wound healing, but is also associated with a number of pathologies, including cancer, age-related macular degeneration, tumorigenesis, proliferative diabetic retinopathy, atherosclerosis, peripheral arterial disease, and rheumatoid arthritis. Significantly, angiogenesis is a fundamental step in the transformation of tumors from benign to malignant, and inhibition of angiogenesis is an important field of study. Similarly, lymphangiogenesis is associated with progression of lymphatic malignancies.

[003] A number of growth factors are known to promote and mediate angiogenesis and lymphangiogenesis. Vascular Endothelial Growth Factor ("VEGF") is a family of signaling proteins that stimulate angiogenesis, and VEGF has been shown in vitro to stimulate endothelial cell migration and cell migration. Therapeutic inhibition of endogenous VEGF is an established means of arresting tumorigenesis and progression of macular degeneration and retinopathy. Matrix Metalloproteinases, including Matrix Metalloproteinase 2 ("MMP2"), mediate the
breakdown of extracellular matrix proteins in disease processes including metastatic. Fibroblast Growth Factors, including Basic Fibroblast Growth Factor ("bFGF") are highly potent stimulators of angiogenesis. In vitro and in vivo assays assessing the expression and angiogenic effect of these proteins provide sensitive models for measurement of the anti-angiogenic effect of target compounds. As lymphangiogenesis is known to be mediated by many of the same molecular mechanisms as angiogenesis, such models are also useful predictors of anti-lymphangiogenic effect of target compounds.

[004] Inhibition of angiogenesis and lymphangiogenesis is emerging as an important method of slowing or stopping the progression of angiogenesis- and lymphangiogenesis-related pathologies. However, safe and effective methods and compounds for such inhibition are lacking.

Cancer Therapeutics

[005] Effective cancer treatment is frequently inhibited by the inability of the patient to withstand an effective dose of a therapeutic drug, by the development of resistance to therapeutic drugs by cancer cells, or both. These problems are exhibited across a wide range of cancers and therapeutic drugs. Physicians and researchers have attempted to address these problems through various approaches, such as administering multiple therapeutic drugs at once or in series, but these solutions are not optimal because they frequently pose additional risks to the patient, such as increased rates of relapse, increased chances of opportunistic infections due to increased length of treatment, and increased chances of adverse drug reactions due to exposure to more drugs.

[006] Many of these problems could be avoided or lessened by rendering the cancer cells more sensitive to one or more therapeutic drugs. However, safe and effective methods for sensitizing cancer cells in such a manner are lacking.

Rifamycin and Rifabutin

[007] Rifabutin is a member of the rifamycin class of antibiotics. Rifabutin was approved for use as an antibiotic in the United States in 1992. Although rifabutin has been tested for other antibiotic and anti-inflammatory uses, its most common use remains the treatment of
tuberculosis and other *Mycobacterium* infections. Rifampicin, another member of the rifamycin class of antibiotics, was introduced in 1967 and is also used to treat tuberculosis and similar infections.

**SUMMARY**

[008] The present disclosure, in one aspect, relates to compositions including rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicin or a rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof!, or a combination thereof. The compositions are operable to inhibit angiogenesis and/or lymphangiogenesis in a tissue. The compositions are further operable to induce drug-sensitization in a cancer cell and/or to inhibit a cancer cell in the tissue.

[009] In certain embodiments, the present disclosure is directed to methods of inhibiting angiogenesis in a tissue. Such methods include administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicin or a rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue.

[010] In certain embodiments, the present disclosure is directed to methods of inhibiting lymphangiogenesis in a tissue. The methods include administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicin or a rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit lymphangiogenesis within the tissue.

[011] In certain embodiments of the present disclosure provides methods of inhibiting angiogenesis and lymphangiogenesis in a tissue. The methods include administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicin or a rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit angiogenesis and lymphangiogenesis within the tissue.

[012] In certain embodiments of the present disclosure, methods of inhibiting angiogenesis within a tissue and sensitizing a cancer cell within the tissue to a drug are provided.
The methods comprise administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicm or a rifampinein derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit the cancer cell in the tissue to the drug certain of the cancer cell to the drug is increased. In certain embodiments, the composition is administered to the cancer cell in an amount and for a time sufficient to increase the amount of a chemotherapeutic in the drug. In certain embodiments, the composition is administered to the cancer cell in an amount and for a time sufficient to decrease activity of or inhibit a p-glycoprotein (P-gp) efflux pump in the cell. In certain embodiments, the composition is administered to the cancer cell in an amount and for a time sufficient to increase reactive oxygen species (ROS) in the cancer cell. In certain embodiments, the drug is administered to the cancer cell in an amount and for a time sufficient to inhibit the cancer cell.

[013] In certain embodiments, the present disclosure provides methods of inhibiting lymphangiogenesis within a tissue and sensitizing a cancer cell within the tissue to a drug. In further related aspects, the present disclosure methods of inhibiting angiogenesis and lymphangiogenesis within a tissue and sensitizing a cancer cell within the tissue to a drug.

[014] In certain embodiments of the present disclosure, methods of inhibiting angiogenesis within a tissue and inhibiting a cancer cell within the tissue with a drug are provided. The methods include administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicm or a rifampinein derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof!, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue, and administering a composition comprising rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, or rifampicm or a rifampinein derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, to the tissue in an amount and for a time sufficient to inhibit the cancer cell in the tissue to the drug. In certain
embodiments, the composition is administered to the cancer cell in an amount and for a time sufficient to decrease activity of or inhibit a p-glycoprotein (P-gp) efflux pump in the cell. In certain embodiments, the composition is administered to the cancer cell in an amount and for a time sufficient to increase reactive oxygen species (ROS) in the cancer cell. In certain embodiments, the present disclosure provides methods of inhibiting lymphangiogenesis within a tissue and inhibiting a cancer cell within the tissue with a drug. In certain embodiments, the present disclosure methods of inhibiting angiogenesis and lymphangiogenesis within a tissue and inhibiting a cancer cell within the tissue with a drug.

[015] The following abbreviations are used throughout the specification:

CHOP - cyclophosphamide, doxorubicin, vincristine, prednisone  
NHL - non-Hodgkin's lymphoma  
ROS - reactive oxygen species  
RTI-x - designates a rifamycin derivative in which "x" is replaced by an identification number used in the present specification to designate a particular composition.  
DOX - doxorubicin.  
VEGF - Vascular Endothelial Growth Factor A  
MMP2 - Matrix Metalloproteinase 2  
bFGF - Basic Fibroblast Growth Factor

BRIEF DESCRIPTION OF THE DRAWINGS

[016] A more complete understanding of certain embodiments of the present disclosure and advantages thereof can be acquired by referring to the following description taken in conjunction with the accompanying drawings, which depict certain embodiments of the present disclosure, and in which like numbers refer to similar components, and in which:

[017] FIGURE 1 illustrates a cellular network via which rifamycin and rifamycin derivatives, such as rifabutin or rifabutin derivatives, or rifampicin and rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, or prodrugs thereof, can cause drug-sensitization and an example drug-sensitization effect in CHOP-resistant DLBCL cells;

[018] FIGURE 2A illustrates the effects of rifabutin on growth of CHOP-sensitive (CRL2631) NHL cells and CHOP-resistant (G3) NHL cells in the presence or absence of CHOP
as demonstrated by resazurin fluorescence;

[019] FIGURE 2B illustrates the effects of rifabutin on growth of CHOP-resistant (G3) NHL cells in the absence of CHOP (top panel) as compared to a control drug as demonstrated by resazurin fluorescence and in the presence of varying dilutions of CHOP for 24 hrs (bottom panel);

[020] FIGURE 2C illustrates the effects of rifabutin on growth of another CHOP-resistant NHL cell line (SUDHL10-R) and the parental CHOP-sensitive NHL cell line (SUDHL10-S) after 24 hrs (top panel) and 48 hrs (bottom panel) of treatment as demonstrated by resazurin fluorescence;

[021] FIGURE 3A illustrates the effects of rifabutin or rifabutin derivatives RTI-79 and RTI-176 on cell growth of primary human dermal fibroblasts both with and without 2 uM Dox;

[022] FIGURE 3B illustrates the effects of doxorubicin and rifabutin on cell growth of primary human dermal fibroblasts;

[023] FIGURE 4 illustrates the effects of rifabutin on growth of CHOP-resistant lymphoma cells obtained by aspiration from a dog as demonstrated by resazurin fluorescence;

[024] FIGURE 5 illustrates the effects of rifabutin in combination with CHOP or CHOP alone on tumor burden in mm³ over time in SCID mice injected with CHOP-resistant (G3) NHL cells;

[025] FIGURE 6 illustrates the effects of CHOP or control solution with no CHOP on tumor burden in mm³ over time in SCID mice injected with CHOP-sensitive (CRL2631) NHL cells;

[026] FIGURE 7 illustrates the effects of reduced dosages of CHOP+rifabutin or control solution with no CHOP or rifabutin on tumor burden in mm³ over time in SCID mice injected with CHOP-resistant (G3) NHL cells;

[027] FIGURE 8 illustrates a Kaplan-Meier curve showing average life span for SCID mice injected with CHOP-resistant (G3) cells when treated with either doxorubicin alone (DOX) or doxonibicin i-rifabutin derivative RTI-81 (DQX+NZ);

[028] FIGURE 9 illustrates the average tumor volume of chemo-resistant SK-OV-3 xenografts in mice after control treatment with saline, treatment with 33 mg/kg DOX, and treatment with 3.3 mg/kg DOX + 25 mg/kg rifabutin over time.
FIGURE 10 illustrates the average tumor volume of multi-drug resistant cancer cell line (NCI/ADR-RES) xenografts in mice after control treatment with saline, treatment with 7 mg/kg DOXIL® and treatment with 7mg/kg DQXIL®+ 25 mg/kg RTI-79 over time.

FIGURE 11 illustrates the average tumor volume of multi-drug resistant cancer cell line (NCI/ADR-RES) xenografts in mice with multiple, large tumors after control treatment with saline, treatment with 7 mg/kg DOXIL®, and treatment with 7 mg/kg DOXIL® + 25 mg/kg RTI-79 over time.

FIGURE 12 illustrates the effects of rifabutin or RTI-79 on growth of CHOP-resistant (G3) NHL cells;

FIGURE 13 illustrates the effects of rifabutin or RTI-176 on growth of CHOP-resistant (G3) NHL cells;

FIGURE 14 illustrates the effects of rifabutin or RTI-81 on growth of CHOP-resistant (G3) NHL cells;

FIGURE 15 illustrates the interaction of rifabutin and doxorubicin on CHOP-sensitive (CRL2631) NHL cells;

FIGURE 16 illustrates the interaction of RTI-79 and doxorubicin on CHOP-sensitive (CRL2631) NHL cells.

FIGURE 17 illustrates the effects of rifabutin or RTI-82 on multidrug-resistant breast cancer (MDA-MB-231) cells;

FIGURE 18 illustrates the interaction of rifabutin with actinomycin D on multidrug resistant sarcoma (MES-SA-Dx5) cells;

FIGURE 19 illustrates the interaction of rifabutin with menadione on dexamethasone resistant multiple myeloma (MM.1R) cells;

FIGURE 20 illustrates the interaction of rifabutin and RTI-79 with and without doxorubicin at an 8:1 rifabutin or RTI-79:doxorubicin molar ratio on multi-drug resistant cancer cell line (NCI/ADR-RES) cells;

FIGURE 21 illustrates the interaction of RTI-79 and doxorubicin on multi-drug resistant T lymphoblastoid leukemia (MOLT-4) cells;

FIGURE 22 illustrates the effects of rifabutin and RTI-79 with and without doxorubicin at an 8:1 rifabutin or RTI-79:doxorubicin molar ratio on ovarian carcinoma...
FIGURE 23 illustrates the effects of rifabutin and actinomycin D on multi-drug resistant sarcoma (MES-SA-Dx5) cells.

FIGURE 24 illustrates the effects of rifabutin and menadione on dexamethasone resistant multiple myeloma (MM.1R) cells;

FIGURE 25 illustrates the interaction of rifabutin and mitoxantrone on osteosarcoma (U-2 OS) cells;

FIGURE 26 illustrates the interaction of rifabutin with gemcitabine on multi-drug resistant breast cancer (MDA-MB-231) cells;

FIGURE 27 illustrates the interaction of rifabutin with paclitaxel on myeloid leukemia cells (HL-60) cells;

FIGURE 28 illustrates the interaction of rifabutin and camptothecin on ovarian cancer (OVCAR-8) cells;

FIGURE 29 illustrates the number of viable cells present after re-exposure to CHOP of CHOP-sensitive (CRL2631) cells to a full or half dose of CHOP in the presence or absence of rifabutin;

FIGURE 30A illustrates a Western blot for phosphorylated Akt (pAkt) Akt, 14-3-3ζ, and an actin control in CHOP-sensitive (CRL2631) and CHOP-resistant (G3) cells. FIGURE 30B illustrates the effect of varying amounts of Akt Inhibitor VIII on growth of G3 cells as demonstrated by resazurin fluorescence. FIGURE 30C illustrates a Western blot for phosphorylated Akt (pAkt) Akt, 14-3-3ζ, and a Vimentin control in G3 cells exposed or not exposed to Akt Inhibitor VIII;

FIGURE 31 illustrates the amount of ROS in CHOP-sensitive (CRL2631) or CHOP-resistant (G3) cells before and after 101 ng/ml CHOP treatment (cyclophosphamide = 240 ng/ml [0.83 μM]; Doxorubicin = 33 ng/ml [0.057 μM]; Vmcristine = 0.93 ng/ml [0.0045 μM]; Prednisone = 67 ng/ml [0.828 μM].

FIGURE 32 illustrates the ROS levels in distinct populations of cells in CHOP-sensitive (CRL2631) cells purified by flow cytometry;

FIGURE 33 illustrates the number of viable cells present after treatment of low-ROS CREF2631 cells and high-ROS CRL2631 cells with 101 ng/ml CHOP treatment
(cyclophosphamide = 240 ng/ml [0.83 uM]; Doxorubicin = 33 ng/ml [0.057 uM]; Vincristine = 0.93 ng/ml [0.0045 uM]; Prednisone = 67 ng/ml [0.828 uM].)

[053] FIGURE 34 illustrates the effect on cell growth of varying amounts of CHOP in the presence or absence of 10 uM rifabutin on low-ROS CRL2631 cells as demonstrated by resazurin fluorescence;

[054] FIGURE 35 illustrates the effect of 10 uM rifabutin on ROS in CHOP-resistant (G3) cells over time;

[055] FIGURE 36A provides a western-blott showing different ABCB1 protein levels in si-ABCBI and si-NCl (control si-RNA) treated ADR-RES cells, as well as the untreated ADR-RES cells and its parental drag-sensitive strain OVCAR8;

[056] FIGURE 36B shows the effects of rifabutin (RBT) on calcein-AM efflux in OVCAR8 than in ADR-RES cells.

[057] FIGURE 36C shows the effects of 5 µM rifabutin (RBT), RTI-79, and rifampin (RMP) on calcein-AM efflux and the further effects of ABCB1 RNA-silencing;

[058] FIGURE 37A shows dose-response curves of various RTIs on calcein-AM efflux.;

FIGURE 37B shows the effects of various RTIs on 1uM doxorubicin's toxicity in G3 cells;

[059] FIGURE 37C shows the correlation between efflux inhibition effect and drag sensitizing ability for various RTI-x rifamycin derivatives;

[060] FIGURE 37D shows the comparison of doxorubicin fluorescence intensity in the NCI/ADR-RES cells with rifabutin treatment or dimethyl sulfoxide (DMSO) control.

[061] FIGURE 38A shows the effects of MDR/P-gp inhibitors and two control drugs (carboxin, nifazoxinide) on ROS in doxorabilein-sensitive OVCAR8 cells;

[062] FIGURE 38B shows the effects of MDR/P-gp inhibitors and two control drugs (carboxin, nifazoxinide) on ROS in doxorubicin-resistant ADR-RES cells;

[063] FIGURE 38C shows the effects of MDR/P-gp inhibitors and two control drugs (carboxin, nifazoxinide) on ROS in doxorubicin-resistant G3 cells;

[064] FIGURE 39 shows staining of ADR-RES cells treated with RTI-79; ADR-RES cells were infected 24 hrs with a baculovirus expressing a recombinant GFP protein fused with a
mitochondrial localization signal (green); cells were stained with CellROX to detect ROS (red) or DAPI to detect nuclei (blue);

[065] FIGURE 40 shows the effects of cell-permeable calcium modulators (BAPTA, Verapamil) and a Complex I inhibitor (Rotenone) on ROS in G3 cells;

[066] FIGURE 41A shows the effects of P-gp inhibitors (Ileserpine, Elacridar) on ROS levels in ADR and OVCAR8 cells

[067] FIGURE 41B shows the effects of RTI-79 on ROS and calcium mobilization in doxorubicin-sensitive lymphoma (CRL2631, 10S, WSU) and ovarian carcinoma (OVCAR8) cells and doxorubicin-resistant lymphoma (G3R, 10R, WSUR) cells;

[068] FIGURE 41C shows the levels of ROS and calcium mobilization in more CHOP-sensitive lymphoma (CRL2631, 10S, WSU) compared to the more resistant derivative cell lines (G3, 10R, WSU-R), and in the more doxorubicin-sensitive OVCAR8 versus the more resistant derivative cell line ADR.

[069] FIGURE 42 shows a time course of RTI-79 induction of ROS and calcium mobilization in G3 cells; and

[070] FIGURE 43 shows the effects of siRNA knockdown of P-gp on induction of ROS and mobilization of calcium.

[071] FIGURE 44 shows the effects of rifabutin on G3 and CRL2631 cells in a collagen invasion 3D assay.

[072] FIGURE 45 shows the effects of rifabutin on G3 and CRL2631 cells in a modified Boyden chamber assay.

[073] FIGURE 46A shows the effect of RTI-79, rifabutin, and rifampicin on the secretion of MMP2 in human umbilical vein endothelial cells ("HUVECs"); and

[074] FIGURE 46B shows the effect of RTI-79, rifabutin, and rifampicin on the secretion of MMP2 in human umbilical vein endothelial cells ("HUVECs").

[075] FIGURE 47 shows the effect of RTI-79 and rifabutin on HUVEC invasion in a cell invasion assay compared to negative and positive controls.

[076] FIGURE 48 shows the qualitative effects of RTI-79 and rifabutin on tube formation in a tube formation assay at 4 hours, 8 hours, and 24 hours compared to negative and positive controls.
[077] FIGURE 49A shows the qualitative effect of RTI-79 and rifabutin on HUVEC invasiveness in a three-dimensional collagen invasion assay.

[078] FIGURE 49B shows the quantitative effect of RTI-79 on HUVEC invasiveness in a three-dimensional collagen invasion assay.

[079] FIGURE 50 shows the effect of RTI-79 on angiogenesis in a chick chorioallantoic membrane ("CAM") model compared to cell media alone and to cell media containing pro-angiogenic factors.

[080] FIGURE 51A shows dextran-FITC immunohistochemical staining of endothelium formed during an in vivo matrigel plug assay wherein RTI-79 treated or untreated mice were injected with matrigel plugs containing matrigel alone, matrigel and positive control (bFGF), matrigel and negative control (sorafinib), or matrigel and RTI-79; and

[081] FIGURE 51B shows relative FITC fluorescence observed in combined blood, plasma, and dissolved matrigel plugs after removal following the in vivo matrigel plug assay.

[082] FIGURE 52A shows immunohistochemical staining of CD-31 in tumors removed from mice 14 days or 54 days after xenografting of ADR-RES ovarian cancer cells and 5 days or 45 days after twice-weekly treatment with saline or RTI-79; and

[083] FIGURE 52B shows pixel areas of CD-31 stained capillaries in the tumors.

DETAILED DESCRIPTION

[084] The present disclosure relates to compositions and methods of inhibition of angiogenesis and lymphangiogenesis. In further aspects of the present disclosure, such compositions and methods can be employed simultaneously and/or sequentially for drug-sensitization of a cancer cell and/or inhibiting a cancer cell. In still further aspects, the present disclosure relates to compositions and methods for inhibition of angiogenesis and drug-sensitization of a cancer cell, as well as compositions and methods for inhibition of angiogenesis and inhibiting a cancer cell. In still further aspects, the present disclosure related to compositions and methods for inhibition of lymphangiogenesis and drug-sensitization of a cancer cell, as well as compositions and methods for inhibition of lymphangiogenesis and inhibiting a cancer cell. In additional aspects of the present disclosure, the present disclosure relates to compositions and methods inhibition of angiogenesis and lymphangiogenesis and drug-
sensitization of a cancer cell and/or inhibiting a cancer cell. It will be understood by those of
ordinary skill in the art that these and other aspects of the present disclosure are non-limiting and
can also be related or overlapping. Where support for a specific combination of various aspects
of the present disclosure is not expressly recited, it will nonetheless be understood that these
aspects can be combined by one of ordinary skill in the art with reference to this disclosure.
These compositions and methods are described in further detail below.

[085] Unless otherwise indicated by the specific context of this specification, angiogenesis refers to the formation of new blood vessels in an organism. Angiogenesis includes the formation of new blood vessels associated with or due to one or more pathologies or disease states within the organism. Inhibition of angiogenesis refers to partial or total inhibition of angiogenesis, and/or to a relative reduction in total angiogenesis or a rate or progression of angiogenesis, as demonstrated by, for example and not limitation, decreased density of blood vessel formation in a region within a patient, such as a tissue, an organ, or a tumor, or within the substantial!)' the entire body of a patient, as well as in vivo and/or in vitro assays evidencing same. The patient can be any animal. In particular, the patient can be a mammal, such as a human, a pet mammal such as a dog or cat, an agricultural mammal, such as a horse, cow, pig, sheep, or goat, or a zoo mammal.

[086] Similarly, unless otherwise indicated by the specific context of this specification, lymphangiogenesis refers to the formation of new lymphatic vessels in an organism. Lymphangiogenesis includes the formation of new lymphatic vessels associated with or due to one or more pathologies or disease states within the organism. Inhibition of lymphangiogenesis refers to partial or total inhibition of lymphangiogenesis, and/or to a relative reduction in total lymphangiogenesis or a rate or progression of lymphangiogenesis, as demonstrated by, for example and not limitation, decreased density of lymphatic vessel formation in a region within a patient, such as a tissue, an organ, or a tumor, or within the substantially the entire body of a patient, as well as in vivo and/or in vitro assays evidencing same. The patient can be any animal. In particular, the patient can be a mammal, such as a human, a pet mammal such as a dog or cat, an agricultural mammal, such as a horse, cow, pig, sheep, or goat, or a zoo mammal.

[087] Unless otherwise indicated by the specific context of this specification, a cancer cell includes a cell of any type of cancer. Furthermore, it includes a cancer cell in a patient,
either in a cancerous growth, such as a tumor, or in isolation from other cancer cells, such as during metastasis. The patient can be any animal. In particular, the patient can be a mammal, such as a human, a pet mammal such as a dog or cat, an agricultural mammal, such as a horse, cow, pig, sheep, or goat, or a zoo mammal. Although certain embodiments herein are expressed in terms of a cancer cell, the same or similar effects can be seen in groups of cancer cells in a patient.

[088] Drug-sensitization, unless otherwise indicated by the specific context of this specification, includes increased sensitivity to a drug, decreased resistance to a drug, or potentiation of a drug's activity or efficacy. Any effect can be measured using any methods accepted in the art. in certain embodiments, drug-sensitization can be determined by an increased ability of the dmg to inhibit a cell. Cellular inhibition can include killing the cell, such as via apoptosis or necrosis, reducing the growth of the cell, thus reducing the growth of the cancer containing the cell, rendering the cell more susceptible to the immune system, preventing or reducing metastasis, reducing the size of a tumor containing the cell, or otherwise negatively affecting a cancer cell. An increased ability of the dmg to inhibit a cancer cell can be demonstrated by an ability to inhibit the cell with a reduced amount of drug or in a shorter period of time than in the absence of dmg-sensitization. in the case of drug-resistant cancer cells, which include cells with inherent or acquired resistance, dmg-sensitization can result in a renewed or newly acquired ability of the dmg to inhibit a cancer cell or type of cancer cell.

Compositions

[089] The present disclosure includes compositions for inhibition of angiogenesis, inhibition of lymphangiogenesis, and inhibition of angiogenesis and lymphangiogenesis, as well as for cancer cell drag sensitization, cancer cell inhibition, and cancer cell dmg sensitization and cancer cell inhibition. The compositions include rifamycin, rifamycin derivatives, such as rifabutin or rifabutin derivatives, rifampicin and rifampicin derivatives, pharmaceutically acceptable salts, hydrates, and prodrugs thereof, and combinations thereof. Additional rifamycin derivates include rifapentine and rifalazil.

[090] In certain embodiments, the present disclosure provides derivatives of rifabutin according to one of the following general structures:
(III), or

(IV), or
in which R can be an aikyi, aryl, or hetero aryl group.

[09] In certain embodiments, the present disclosure provides enantiomers of the general structures. In certain embodiments, it provides enantiomers with the following general chiral structures:
in which R can be a n aikyi, aryi, or hetero aryl group.

[092] In certain embodiments having general structures I or II or general chiral structures Ia or lia, R can be one of the following structures:
In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:

[094] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:

[095] in certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:
where X and R can include the following combinations:

\[ X=O, \ R = \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \quad \text{or} \quad \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \]

\[ X=\text{NH}, \ R = \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \quad \text{or} \quad \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \]

\[ X\cdot R = \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \quad \text{or} \quad \begin{array}{c}
\text{---} \\
\text{---} \\
\text{---} \\
\text{---}
\end{array} \]

[096] The structure with the general formula above can also be the following enantiomer:
In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:

![Chemical structure diagram]

RTI-181: \( R = -\text{CH}_3 

RTI-176: \( R = -\text{CO} \)

RTI-183: \( R = -\text{CONH} \)

In another embodiment, the present disclosure provides derivatives of rifabutin according to the following formula:

\[
X = \text{NH}, \quad R = -\text{CH}_2\text{R} \quad \text{or} \quad -\text{CH}_2\text{Ar} \\
X - R = -\text{N} 
\]

wherein \( X \) is a C, O, or N and \( R \) is an alkyl, aryl, or heteroaryl group.

In another embodiment, the present disclosure provides derivatives of rifabutin according to the following formula:
wherein X is a C, O, or N and R is an alkyl, aryl, or hetero-aryl group or where X and R are as follows:

$$X = \text{NH}, \quad R = \text{alkyl}, \text{aryl}, \text{or hetero-aryl}$$

[0100] In certain embodiments, a composition of the general formula above can be the following enantiomer:

[0101] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:
wherein X is a C, O, or N and R can include the structures listed below:

\[
X = \text{a} \quad \text{C, O, or N, and R can include the structures listed below:}
\]

\[
X = \text{O, R} =
\]

\[
X = \text{N, R} =
\]

[0102] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula, wherein X is a C, O, or N:

[0103] In certain embodiments, a composition with the general formula above can be the
In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:

\[
\text{X} = \text{C, O, N}
\]

[0104] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:

[0105] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:
wherein X is a C, O, or N and R is an alkyl, aryl, or hetero-aryl group or where X and R are as follows:

\[
X=O, \quad R = \begin{array}{c}
\text{or} \\
\end{array}
\]

\[
X=NH, \quad R = \begin{array}{c}
\text{or} \\
\end{array}
\]

\[
X-R = \begin{array}{c}
\text{or} \\
\end{array}
\]

[0106] In certain embodiments, the present disclosure provides derivatives of rifabutin according to the following formula:
[0107] In certain embodiments, the present disclosure provides a drug-sensitization composition comprising a series of 3,4-cyclo-rifamycm derivatives. Examples of such compositions are as follows:

![Chemical Structure 1]

or the following enantiomer:

![Chemical Structure 2]

[0108] In certain embodiments X can be CH, S, SO, SO_2 or N. Y can be H or an acetyl group. R1 can be hydrogen. R2 can be a hydroxyl or an amino (-NH_2) group. R1 and R2 together can be an oxo or imine group. R3 can be one of the following groups: hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl and heterocycloalkyl groups that can be additionally
substituted with from zero to four substituents chosen independently from halogen, hydroxy, alkoxy-alkyl, -CN, nitro, -8-alkyl, amino, alkylaraino, dialkylamino, dialkylaminoalkyl, carboxy, carboalkoxy, acyl, carboxamido, alkylsulfoxide, acylamino, phenyl, benzyl, phenoxy, and benzyloxy. In certain embodiments, R3 can be -C(=0)-R4, -C(=0)-0-R4 and -C(=0)-NH-R4 where R4 is independently selected from alkyl, alkenyl, alkynyl, cycloalkyi, aryl, heteroaryi and heterocycloalkyi groups that can be additionally substituted with from zero to four substituents chosen independently from halogen, hydroxy, alkoxy-alkyl, -CN, nitro, -S-alkyl, amino, alkylamino, dialkylamino, dialkylaminoalkyl, carboxy, carboalkoxy, acyl, carboxamido, alkylsulfoxide, acylamino, phenyl, benzyl, phenoxy and benzyloxy.

[0109] In certain embodiments, the present invention provides compositions of the following structure:

![Chemical Structure](image)

or the following enantiomer:
wherein $Y$ is H or an acetyl group and $R_4$ can be selected from alkyl, aikenyi, alkynyl, cycloalkyl, aryi, heteroaryl and heterocycloalkyl groups that can be additionally substituted with from zero to four substituents chosen independently from halogen, hydroxy, alkoxy-alkyl, -CN, nitro, -S-aikyi, amino, alkyiamino, dialkylamino, dialkylarmnoalkyl, carboxy, carboalkoxy, acyl, carboxamido, alkylsulfoxide, acyiamino, phenyl, benzyl, phenoxy and benzyloxy.

[0110] In certain embodiments, the present invention provides compositions with the following structure:
or the following enantiomer:

![Chemical Structure]

wherein Y is H, or acetyl group; Z is carbon, oxygen or nitrogen atom; and R4 is independently selected from alkyl, alkenyl, alkynyl, cycloalkyl, aryi, heteroaryl and heterocycloalkyl groups that can be additionally substituted with from zero to four substituents chosen independently from halogen, hydroxy, alkoxy-alkyl, -CN, nitro, -S-alkyl, amino, alkylamino, dialkyiamino, dialkylaminoalkyl, carboxy, carboalkoxy, acyl, carboxamido, alkylsulfoxide, acylamino, phenyl, benzyl, phenoxy and benzyloxy.

[0111] Examples of compositions for inhibition of angiogenesis, inhibition of lymphangiogenesis, drug sensitization, inhibition of a cancer cell, and for combinations of these various applications in accordance with certain aspects of the present disclosure can include those listed in Table 1. Compositions of Table 1 are designated by like names throughout this specification.

*Table 1: Rifamycin Derivatives*

<table>
<thead>
<tr>
<th>RTI- x</th>
<th>General structure</th>
<th>R</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Image</td>
<td>Chemical Structure</td>
<td>Chemical Formula</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>33</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(t-butyloxycarbonyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(benzyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>49</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(2-methoxyethyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>51</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(l-cyclobutylmethyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(isopropyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(t-ethoxyxycarbonyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>61</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>11-deoxy-l 1-imino- 4-deoxy-3,4[2-spiro-[l-(acetyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>I/II</td>
<td>Structure</td>
<td>Chemical Formula</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>64</td>
<td>I</td>
<td><img src="image" alt="Structure 1" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(n-propyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>65</td>
<td>I</td>
<td><img src="image" alt="Structure 2" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(cyclopropyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>66</td>
<td>I</td>
<td><img src="image" alt="Structure 3" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(ethyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>67</td>
<td>I</td>
<td><img src="image" alt="Structure 4" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(beRT1oyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>68</td>
<td>I</td>
<td><img src="image" alt="Structure 5" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(benzylxycarbonyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>69</td>
<td>I</td>
<td><img src="image" alt="Structure 6" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(methyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>70</td>
<td>I</td>
<td><img src="image" alt="Structure 7" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(2-methylpropyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>74</td>
<td>I</td>
<td><img src="image" alt="Structure 8" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(phenylaminocarbonyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>75</td>
<td>II</td>
<td><img src="image" alt="Structure 9" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(t-butyloxycarbonyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>76</td>
<td>II</td>
<td><img src="image" alt="Structure 10" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(ethyloxycarbonyl)piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>No.</td>
<td>Form</td>
<td>Structure</td>
<td>Chemical Formula</td>
</tr>
<tr>
<td>-----</td>
<td>------</td>
<td>-----------</td>
<td>-----------------</td>
</tr>
<tr>
<td>77</td>
<td>I</td>
<td><img src="structure1.png" alt="Image" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(ethylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>78</td>
<td>II</td>
<td><img src="structure2.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(n-propylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>79</td>
<td>II</td>
<td><img src="structure3.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(isobutylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>80</td>
<td>II</td>
<td><img src="structure4.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(benzylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>81</td>
<td>I</td>
<td><img src="structure5.png" alt="Image" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(isobutylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>82</td>
<td>I</td>
<td><img src="structure6.png" alt="Image" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(ethylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>83</td>
<td>II</td>
<td><img src="structure7.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(ethylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>84</td>
<td>I</td>
<td><img src="structure8.png" alt="Image" /></td>
<td>11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(isopropylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>85</td>
<td>II</td>
<td><img src="structure9.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(isopropylxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>86</td>
<td>II</td>
<td><img src="structure10.png" alt="Image" /></td>
<td>4-deoxy-3,4[2-spiro-[1-(phenylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
</tr>
<tr>
<td>Number</td>
<td>Structure</td>
<td>Chemical Formula</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td><img src="image1" alt="Structure 1" /></td>
<td>4-deoxy-3,4-[2-spiro-[1-(acetyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>88</td>
<td><img src="image2" alt="Structure 2" /></td>
<td>4-deoxyO,4-[2-spiro-[1-(beRTIoyl)-pipeaidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>89</td>
<td><img src="image3" alt="Structure 3" /></td>
<td>4-deoxy-3,4-[2-spiro-[1-(3,3-dimethylbutanoyl)-piperidm-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifaraycin S</td>
<td></td>
</tr>
<tr>
<td>91</td>
<td><img src="image4" alt="Structure 4" /></td>
<td>11-deoxy-1 1-in o-4-deoxy-3,4-[2-spiro-[1-(n-pentanoyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>94</td>
<td><img src="image5" alt="Structure 5" /></td>
<td>11-deoxy-1 1-imino-4-deoxy-3,4-[2-spiro-[1-(2-methylpropanoyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td><img src="image6" alt="Structure 6" /></td>
<td>11-deoxy-1 1-imino-4-deoxy-3,4-[2-spiro-[1-(3-methylbutanoyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td><img src="image7" alt="Structure 7" /></td>
<td>11-deoxy-1 1-imino-4-deoxy-3,4-[2-spiro-[1-(2-methylpropanoyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>101</td>
<td><img src="image8" alt="Structure 8" /></td>
<td>4-deoxy-3,4-[2-spiro-[1-(dimethylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>102</td>
<td><img src="image9" alt="Structure 9" /></td>
<td>4-deoxy-3,4-[2-spiro-[1-(isobuiylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
<tr>
<td>103</td>
<td><img src="image10" alt="Structure 10" /></td>
<td>4-deoxy-3,4-[2-spiro-[1-(isopropylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S</td>
<td></td>
</tr>
</tbody>
</table>
Modification of the rifamycin structure in locations corresponding to the 21-OH,
23-OH or 25-0-Ac sites of the rifabutin structures I, II, iII, IV and V do not generally affect drug-sensitization activity and thus variations with modifications at these sites or even elimination of these sites are encompassed herein. Such variations can be used to improve synthesis yields, control costs, increase water solubility, or improve pharmaceutical properties of the composition. Sites 21, 23 and 25 are located as follows:
The present disclosure also includes pharmaceutically acceptable salts, hydrates, prodrugs, and mixtures of any of the above compositions. The term "pharmaceutically acceptable salt" refers to salts whose counter ion derives from pharmaceutically acceptable non-toxic acids and bases.

The 3,4-cyclo-rifamycin derivatives which contain a basic moiety, such as, but not limited to an amine or a pyridine or imidazole ring, can form salts with a variety of organic and inorganic acids. Suitable pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) base addition salts for the compounds of the present invention include inorganic acids and organic acids. Examples include acetate, adipate, alginates, ascorbates, aspartates, benzenesulfonate (besylate), benzoate, bicarbonate, bisulfate, borates, butyrates, carbonate, camphorsulfonate, citrate, digluconates, dodecylsulfates, ethanesulfonate, fumarate, gluconate, glutamate, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrobromides, hydrochloride, hydroiodides, 2-hydiOxyethanesulfonates, isethionate, lactate, maleate, malate,
mandelate, methanesulfonate, 2-naphthalenesulfonates, nicotinates, raucate, nitrate, oxalates, pectinates, persulfates, 3-phenylpropionates, picrates, pivaiates, propionates, pamoate, pantothenate, phosphate, salicylates, succinate, sulfate, sulfonates, tartrate, p-toluenesulfonate, and the like.

[0115] The 3,4-cyclo-rifamycin derivatives which contain an acidic moiety, such as, but not limited to a carboxylic acid, can form salts with variety of organic and inorganic bases. Suitable pharmaceutically acceptable base addition salts for the compounds of the present invention include, but are not limited to, ammonium salts, metallic salts made from calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from lysine, N,N-dialkyl amino acid derivatives (e.g. N,N-dimethylglycine, piperidine-1-acetic acid and morpholine-4-acetic acid), N,N'-dibeiizylethyenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine (N-methylglucamine), t-butilamine, dicyclohexylamine, hydrabamine, and procaine.

[0116] The 3,4-cyclo-rifamycin derivatives, and salts thereof, can exist in their tautomeric form (for example, as an amide or imino ether). All such tautomeric forms are contemplated herein as part of the present invention.

[0117] The compounds described herein can contain asymmetric centers and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms. Each chiral center can be defined, in terms of absolute stereochemistry, as (R)- or (S)-. The present invention is meant to include all such possible isomers, as well as, their racemic and optically pure forms. Optically active (R)- and (S)-, or (D)- and (L)- isomers can be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefmic double bonds or other centers of geometric asymmetry, and unless specified otherwise, it is intended that the compounds include both E and Z geometric isomers.

[0118] The configuration of any carbon-carbon double bond appearing herein is selected for convenience only and unless explicitly stated, is not intended to designate a particular configuration. Thus the carbon-carbon double bond depicted arbitrarily above as $E$ can be $Z$, $E$, or a mixture of the two in any proportion.

[0119] Abbreviations as used herein have the meanings known by one skilled in the art. Specifically, Ac represent acetyl group, Boc represents t-butoxycarbonyl group, Bn represents
benzyl group, DCM represents dichloromethane, DMF represents N,N-dimethylformamide, DMSO represents dimethyl sulfoxide, Et represents ethyl group, EtOAc represents ethylacetate, Me represents methyl group, Ph represents phenyl group, TEA represents trietbylamme, TFA represents trifluoroacetic acid, THE represents tetraliydrofuran, and TMS is trimethyisiaoie group.

[0120] Compositions of the present disclosure can also include a pharmaceutically acceptable carrier, in particular a carrier suitable for the intended mode of administration, or salts, buffers, or preservatives. Rifamycin and many of its derivatives, such as rifabutin and rifampicin derivatives are poorly soluble in water. Accordingly, aqueous compositions of the present disclosure can include solubility enhancers. Compositions for oral use can include components to enhance intestinal absorption. The overall formulation of the compositions can be based on the intended mode of administration. For instance, the composition can be formulated as a pill or capsule for oral ingestion. In other examples, the composition can be encapsulated, such as in a liposome or nanoparticle. In particular, it can be encapsulated with the drug to sensitize the cancer cell, such as encapsulated in a liposome with doxorubicin. It can also be administered with a liposomal or nanoparticle drug, such as DOXIL® (doxorubicin HCl liposome injection)(Centocor Ortho Biotech Products, LP, Raritan, NX), whether encapsulated with the drug or not. It can also be separately encapsulated. The compositions can also be formulated for injection or infusion or for implantation in a biocompatible carrier.

[0121] Compositions of the present disclosure can contain a sufficient amount of rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to inhibit angiogenesis when the composition is delivered to a region susceptible to angiogenesis in the body of a patient, such as a tissue, a blood vessel, a complex of blood vessels, an organ, or a tumor. The amount can vary depending on other components of the composition and their effects on drug availability in a patient, the intended mode of administration, the intended schedule for administration, any drug toxicity concerns, drug-drug interactions, such as interactions with other medications used by the patient, or the individual response of a patient. Many compositions can contain an amount well below levels at which toxicity to normal cells or to the patient overall becomes a concern.
[0122] Compositions of the present disclosure can contain a sufficient amount of rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to inhibit lymphangiogenesis when the composition is delivered to a region susceptible to angiogenesis in the body of a patient, such as a tissue, a lymphatic vessel, a lymph node, an organ, or a tumor. The amount can vary depending on other components of the composition and their effects on drug availability in a patient, the intended mode of administration, the intended schedule for administration, any drug toxicity concerns, drug-drug interactions, such as interactions with other medications used by the patient, or the individual response of a patient. Many compositions can contain an amount well below levels at which toxicity to normal cells or to the patient overall becomes a concern.

[0123] Compositions of the present disclosure can further contain a sufficient amount of rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to cause drug-sensitization or inhibition of a cancer cell to occur when the composition is administered to a cancer cell. The amount can vary depending on other components of the composition and their effects on drug availability in a patient, the type of drug or drugs to which the cancer cell is sensitized, the amount of drug otherwise required to inhibit the cancer cell, the intended mode of administration, the intended schedule for administration, any drug toxicity concerns, drug-drug interactions, such as interactions with other medications used by the patient, or the individual response of a patient. Many compositions can contain an amount well below levels at which toxicity to normal cells or to the patient overall becomes a concern.

[0124] Compositions of the present disclosure can further include other therapeutic agents. For example, they can include one or more of the anti-angiogenic agents listed herein, particularly those described below in connection with Angiogenesis Inhibition Methods. They can additionally or alternatively contain any one or more of the chemotherapeutic agents listed herein, and, for example, can include combinations of chemotherapeutic agents and anti-angiogenic agents. The amounts of those anti-angiogenic agents and/or chemotherapeutic agents in compositions of the present disclosure can be reduced as compared to normal doses of such.
agents administered in a similar fashion.

[0125] Compositions of the present disclosure can also contain one or more drugs for which the rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, causes drug-sensitization. Example drugs are described in the current specification. In certain embodiments, compositions of the present disclosure can contain one or more other drugs commonly used in combination with the drug for which sensitization occurs. For example, certain compositions can include rifabutin or a rifabutin derivative with any CHOP drug, regardless of whether rifabutin causes drug-sensitization for that drug. In certain embodiments, the composition can contain another dmg that also causes drug sensitization, such as a drug that affects the amount or ROS, particularly superoxide, in a ceil. For example it can contain superoxide dismutase inhibitors. In certain embodiments, the composition can contain another drug that affects drug resistance or a property causing drug resistance in cancer cells. For example, it can contain drugs affecting the apoptotic pathway, such as the apoptotic pathway inhibitors for Bel-XL or mimetics for BH3 proteins.

[0126] The amount of rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, present in a composition can be measured in any of a number of ways. The amount may, for example, express concentration or total amount. Concentration can be for example, weight/weight, weight/volume, moles/weight, or moles/volume. Total amount can be total weight, total volume, or total moles. Typically, the amount can be expressed in a manner standard for the type of formulation or dosing regimen used.

[0127] The present disclosure further includes methods of identifying whether a rifamycin derivative, such as a rifabutin derivative, or a rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, is able to inhibit angiogenesis. Such methods include preparing or obtaining such a derivative, applying it to a tissue, an organ, a blood vessel, or a model thereof, or a cell that expresses or is responsive to pro-angiogenic factors, and identifying that the derivative inhibits angiogenesis, inhibits proxies or markers of angiogenesis, and/or inhibits expression of or response to pro-angiogenic
factors.

[0128] The present disclosure further includes methods of identifying whether rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, is able to sensitize a cancer cell or inhibit a cancer cell. Such methods include preparing or obtaining such a derivative, applying it to a cancer cell, and identifying that the derivative renders the cancer cell more susceptible to a chemotherapeutic in any manner described herein.

**Angiogenesis and Lymphangiogenesis Inhibition Methods**

[0129] In certain embodiments, the disclosure provides methods of inhibiting angiogenesis in a patient by administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient. In certain embodiments, the disclosure provides methods of inhibiting lymphangiogenesis in a patient by administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient. In certain embodiments, the present disclosure provides methods of inhibiting both angiogenesis and lymphangiogenesis in a patient by administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient.

[0130] In certain embodiments, the disclosure provides methods of reducing the amount of an anti-angiogenic agent administered to a patient by also administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient. Such methods may, in particular, be employed with anti-angiogenic agents that can have other harmful effects. For example, use of certain biologies, such as anti-VEGF antibodies, can be associated with the risk of severe adverse
effects. For purpose of example and not limitation, methods of the present disclosure can be employed to reducing the amount of another anti-angiogenesis agent administered, including, for example, anti-VEGF antibodies such as bevacizumab, aflibercept, soluble VEGF receptors, growth factors such as angiopoeitin 2, protease inhibitors such as Tissue Inhibitor of Metalloproteinase ("TIMP") 1-4, cytokines such as interferon-a, -β, and -γ, interleukin-4, -12, and -18, and platelet factor 4, clotting proteins such as prothrombin and antithrombin III. In certain embodiments, reducing the amount of another anti-angiogenesis agent administered by administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient will reduce unwanted effects of the anti-angiogenesis agent or reduce the risk of occurrence of unwanted effects.

[0131] The compositions disclosed herein can be delivered to a region of a patient susceptible to angiogenesis and/or lymphangiogenesis, such as a tissue, an organ, a tumor, one or more lymph nodes, and one or more blood vessels, by delivering the composition to the patient. The mode of delivery can be selected based on a number of factors, including metabolism of the rifamycin or rifamycin derivative, such as the rifabutin or rifabutin derivative, the angiogenesis-related pathology to be treated, the health of the patient, ability or inability to use particular dosing forms or schedules with the patient, preferred dosing schedule, including any adjustment to dosing schedules due to side effects of additional medications or treatments, and ease of administration. In certain embodiments, the mode of administration can be enteral, such as orally or by introduction into a feeding tube. In certain embodiments, the mode of administration can be parenteral, such as intravenously.

[0132] The dosage amounts and schedule of administration of the composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the patient can vary depending on other components of the composition and their effects on bioavailability in a patient, the intended mode of administration, the intended schedule for administration, whether other drugs, such as other anti-angiogenic agents and chemotherapeutic drugs, are administered, any drug toxicity concerns, and the patient's response to the composition. In a specific embodiment, the dosage amount and
frequency of administration of the composition can be such that levels of the rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, to the patient in the patient remain well below levels at which toxicity to the patient becomes a concern. However the amount and frequency can also be such that levels of the rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof in the region susceptible to angiogenesis remain continuously at a level sufficient to inhibit angiogenesis in the susceptible region.

[0133] Without limiting the compositions and methods of administration described herein, and without limitation by theory, in certain embodiments, the rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivatives, rifampicin or rifampicin derivative, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, can exhibit their anti-angiogenic effects on a patient by directly or indirectly inhibiting expression and release of pro-angiogenic growth factors, including bFGF, VEGF, and angiogenesis-associated enzymes, including MMP2. In certain embodiments, the rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivatives, rifampicin or rifampicin derivative, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof can additionally or alternatively inhibit angiogenesis by the induction of intracellular ROS species, such as superoxide species, that modulate downstream signalling pathways involved with angiogenesis.

[0134] In certain embodiments of the present disclosure, methods of inhibiting cancer-related angiogenesis are provided. For example, the angiogenesis related to cancer can be vascularization of a tumor. The vascularization of the tumor can be prevented, reduced, delayed, or reversed. As a result, the tumor can decrease in size, the rate of growth of the tumor can decrease, the growth of the tumor can be arrested, or the tumor can die, and malignant transformation of the tumor can be arrested, delayed, reversed, or reduced. The compositions including rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivatives, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, can be administered to a patient systemically, such as by oral
administration or intravenous infusion, or can be administered locally, such as by injection into a tumor, into the proximity of the tumor, or to the blood vessels supplying the region (such as a tissue or organ) of the patient bearing the tumor.

[0135] According to certain embodiments of the present disclosure, mechanisms of inhibiting angiogenesis related to diseases of the eye are provided. For example, the angiogenesis related to diseases of the eye can be angiogenesis associated with age-related macular degeneration or diabetic retinopathy, or both. Vascularization of the eye can be prevented, reduced, delayed, or reversed. As a result, the retinopathy or macular degeneration can be delayed, arrested, or reversed. The compositions including rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivatives, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, can be administered to a patient systemically, such as by oral administration or intravenous infusion, or can be administered locally, such as by intra-vitreal injection. In certain embodiments in which the compounds are administered by mtravitreal injection, the injection can occur weekly, or semi-monthly, or once every three weeks, or monthly, or once every six weeks, or every two months, or every three months, or as needed. In certain embodiments in which the compositions are administered by mtravitreal injection, the amount by weight of the rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivatives, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, administered is between about 0.01 mg and about 10 mg. In certain embodiments, the amount by weight administered is between about 0.1 mg and about 2.5 mg. In certain embodiments, the amount by weight is about 0.1 mg, or about 0.2 mg, or about 0.3 mg, or about 0.4 mg, or about 0.5 mg, or about 0.6 mg, or about 0.7 mg, or about 0.8 mg, or about 0.9 mg, or about 1.0 mg, or about 1.1 mg, or about 1.2 mg, or about 1.3 mg, or about 1.4 mg, or about 1.5 mg, or about 1.6 mg, or about 1.7 mg, or about 1.8 mg, or about 1.9 mg, or about 2.0 mg, or about 2.1 mg, or about 2.2 mg, or about 2.3 mg, or about 2.4 mg, or about 2.5 mg.

[0136] The methods of certain embodiments of the present disclosure relating to inhibition of angiogenesis and lymphangiogenesis can also be employed in conjunction with certain methods of drug sensitization, cancer inhibition, or drug sensitization and cancer inhibition disclosed herein, such as those disclosed below. Where the methods of these various
aspects of the present disclosure are employed in conjunction, they can be employed sequentially, concurrently, or sequentially and concurrently.

**Drug-Sensitization and Cancer Inhibition Methods**

[0137] The present disclosure further includes drug-sensitization and cancer cell inhibition methods in which a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof is administered to a cancer cell in order to sensitize the cancer cell to another drug. Such methods can augment or complement the methods of inhibition of angiogenesis and lymphangiogenesis disclosed herein. For example, unwanted angiogenesis and/or lymphangiogenesis is commonly observed in cancer and cancer-related pathologies. The methods described herein can be employed to both inhibit angiogenesis and lymphangiogenesis and drug-sensitize and/or inhibit a cancer cell.

[0138] For example, and without limiting the compositions and methods of administration described herein, the compositions and methods can prevent or reduce metastasis. Metastasis from solid tumors is a complex, multistep process whereby cancer cells must breach the basement membrane and migrate away from the primary tumor environment to invade the surrounding stroma and enter the vasculature directly or via the lymphatics. The cancer cells must then also invade another area of the body. The compositions disclosed herein can prevent or reduce metastasis by preventing or reducing any of these movements or activities of the cancer cells. Rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, can also decrease the levels of metastasis-associated cellular factors in or around cancer cells. Such factors include matrix metalloproteinase (MMP) 2 or other MMP family members and vascular endothelial growth factor (VEGF). MMP family members are involved in the breakdown of extracellular matrix in disease processes such as metastasis. VEGF is an important signaling protein involved in both vasculogenesis (the formation of the circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).

[0139] The composition can be any composition described above. In a specific embodiment, the composition can be administered with any other drug which can alternatively be present in a pharmaceutical composition as described herein. For example the other drug can
include DOXIL®.

[0140] The drug can be any drug for rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof! or a combination thereof, increases drug-sensitization. In a specific embodiment, the drug can be a chemotherapeutic. Example types of chemotherapeutics include alkylating agents, antimetabolites, anti-tumor antibiotics, hormonal agents, targeted therapies, differentiating agents and other drugs.

[0141] Example alkylating agents include nitrogen mustards such as mechlorethamine (nitrogen mustard), chlorambucil, cyclophosphamide, ifosfamide, and melphalen. Example alkylating agents further include nitrosoureas, such as streptozocin, carmustine (BCNU), and iomustine. Example alkylating agents further include alkyl sulfonates such as busulfan, triazmes, such as procarbazine and dacarbazine (DTiC) and temozolomide, and ethylén[amines, such as thiotepa and altretamine (hexamethylmelamine). Example alkylating agents further include platinum drugs, such as cisplatin, carboplatin, and oxalaplatin.

[0142] Example antimetabolites include purine antagonists such as mercaptopurine (6-MP), thioguanine (6-TG), fludarabine phosphate, clofarabine, cladribine, and pentostatin. Example antimetabolites also include pyrimidine antagonists such as fluorouracil (5-FIT), floxuridine, capecitabine, cytarabine, gemcitabine and azacitidine. Example antimetabolites further include plant alkaloids. Some plant alkaloids include topoisomerase inhibitors such as topoisomerase I inhibitors such as camptothecin, topotecan and irinotecan, or topoisomerase II inhibitors such as amsacrine, etoposide, and tenyposide. Other plant alkaloids include mitotic inhibitors such as taxanes, including paclitaxel and docetaxel, epothilones, including ixabepilone, vinca alkaloids, including vinblastine, vincristine, and vmore!bine, as well as estramustine. Example antimetabolites further include folate antimetabolites such as methotrexate and pemetrexed. Other antimetabolites include hydroxyurea.

[0143] Example anti-tumor antibiotics include anthracyclines or anthracycline analogs such as daunorubicin, doxorubicin, epirubicin, mitoxantrone, and idarubicin. Other anti-tumor antibiotics include daetinomycin, plicamycin, mitomycin, bleomycin, apicidin, and actinomycin.

[0144] Example hormonal agents include gonadotropin-releasing hormone agonists such as leuprolide and goserelin. Other example hormonal agents include aromatase inhibitors such
as aminoglutethimide, exemestane, letrozole and anastrozole. Other hormonal agents include tamoxifen and flutamide. Still other example hormonal agents include anti-estrogens such as fulvestrant, tamoxifen, and toremifene or anti-androgens such as bicalutamidine, flutamide, and nilutamide. Example hormonal agents further include progestins such as megestrol acetate, and estrogens.

[0145] Example targeted therapies include antibodies or other therapeutics that act on a molecular level such as imatinib, gefitinib, sunitinib, and bortezomib.

[0146] Example differentiating agents include retinoids such as tretinoin, bexarotene, and arsenic trioxide.

[0147] Other chemotherapeutics include L-asparaginase, phenoxodiol, rapamycin, and menadione.

[0148] In methods of the current disclosure, the cancer cell can be sensitized to a drug already known to inhibit the cancer cell, or it can be sensitized to a drug not previously used with that type of cancer cell. If the cancer cell is a drug-resistant cancer cell that has acquired resistance, it can be sensitized to a drug that previously exhibited a decreased ability to inhibit the cancer cell or cancer cells of the same type.

[0149] In certain embodiments, the composition can directly inhibit the cancer cell instead of or in addition to causing drug-sensitization.

[0150] The cancer cell that undergoes drug-sensitization or inhibition can be any type of cancer cell. It may, for instance, be a carcinoma, a sarcoma, a leukemia, a lymphoma, or a glioma. It can also be a soft cancer or a hard cancer. It can also be a cancer affecting a particular organ or tissue, such as: an immunologically-related cancer such as leukemia, lymphoma, including Non-Hodgkin's lymphoma, or Hodgkin's disease, myeloma, including multiple myeloma, sarcoma, lung cancer, breast cancer, ovarian cancer, uterine cancer, including endometrial cancer, testicular cancer, intestinal cancer, including colon cancer, rectal cancer, and small intestinal cancers, stomach cancer, esophageal cancer, oral cancer, pancreatic cancer, liver cancer, prostate cancer, glandular cancers such as adrenal gland cancer and pituitary tumor, bone cancer, bladder cancer, brain and other nervous tissue cancers, including glioma, eye cancer, including retinoblasoma, skin cancer, including basal cell carcinoma and melanoma, and kidney cancer.
[0151] The composition can be delivered to the cancer cell in a patient by delivering the composition to the patient. The mode of delivery can be selected based on a number of factors, including metabolism of the rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, or another drug in the composition, mode of administration of other drugs to the patient, such as the drug to which the cancer cell is sensitized, the location and type of cancer cell to be drug-sensitized, health of the patient, ability or inability to use particular dosing forms or schedules with the patient, preferred dosing schedule, including any adjustment to dosing schedules due to side effects of chemotherapeutics, and ease of administration, in certain embodiments, the mode of administration can be enteral, such as orally or by introduction into a feeding tube. In certain embodiments, the mode of administration can be parenteral, such as intravenously.

[0152] The dosage amounts and administration schedule of the composition can vary depending on other components of the composition and their effects on drug availability in a patient, the type of drug or drugs to which the cancer cell is sensitized, the intended mode of administration, the intended schedule for administration, when other drugs are administered, any drug toxicity concerns, and the patient's response to the drug. In a specific embodiment, the amount and frequency of rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof or combination thereof that is delivered can be such that levels in the patient remain well below levels at which toxicity to normal cells or to the patient becomes a concern. However the amount and frequency can also be such that the levels in the cancer cell remain continuously at a level sufficient to induce drug-sensitization or are at a level sufficient to induce-drug sensitization when or shortly after the drug to which the cancer cell is sensitized is delivered to it. Accordingly, the composition can be taken or administered on a regular basis during treatment with the drug to which the cancer cell is sensitized or it can be taken only a set time before, at the same time, or a set time after the drug to which the cancer cell is sensitized.

[0153] In certain embodiments, the disclosure provides methods of inhibiting a cancer cell using a drug to which the cancer cell is resistant by administering a composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or rifampicin derivative, or rifampicin derivative, or combination thereof, or another drug in the composition, mode of administration of other drugs to the patient, such as the drug to which the cancer cell is sensitized, the location and type of cancer cell to be drug-sensitized, health of the patient, ability or inability to use particular dosing forms or schedules with the patient, preferred dosing schedule, including any adjustment to dosing schedules due to side effects of chemotherapeutics, and ease of administration, in certain embodiments, the mode of administration can be enteral, such as orally or by introduction into a feeding tube. In certain embodiments, the mode of administration can be parenteral, such as intravenously.
rifampicin derivative, or a pharmaceutically acceptable salt, hydrate, and prodrug thereof, or a combination thereof, to the cancer cell. In certain embodiments, the disclosure provides methods of reducing the amount of a drug administered to a patient by also administering the composition. Such methods may, in particular, be employed with drugs that have other harmful effects. For example, use of certain alkylating agents, such as topoisomerase inhibitors, increases the later chances of leukemia in the patient. The chance of this adverse effect can be lessened if lower doses of the alkylating agent can be administered with the same therapeutic effect. Similarly, methods of the present disclosure can be used to reduce the amount of mitotic inhibitors administered, reducing the chance or amount of resulting peripheral nerve damage, or the methods can be used to reduce the amount of anti-tumor antibiotics administered, reducing the chance or amount of resulting hearing damage. In the case of anti-tumor antibiotics for which there is a total lifetime dosage limit, methods of the present disclosure can allow a patient to be treated with the drug for a longer time, increasing life expectancy or improving quality of life.

[0154] Methods of the present disclosure can also allow amounts of some chemotherapeutics administered to remain sufficiently low as to allow the patient to have children after cancer treatment. Methods of the present disclosure can further allow amounts of the chemotherapeutics administered to be lowered into a range where a drug approved for use in adults might also be used in children.

[0155] In certain embodiments in which the composition comprising rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof directly inhibits a cancer cell alone or in addition to causing drug-sensitization, the dosage and administration can be adequate to allow this inhibition. In an example embodiment, it can consist of regular administration of an amount of rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, to maintain a certain level in the patient, the blood, a tissue, or a tumor. However, dosage amounts and the administration schedule can be adjusted based on other components of the composition and their effects on drug availability in a patient, the intended mode of administration, the intended schedule for
administration, when other drugs are administered, any drug toxicity concerns, and the patient's response to the drug.

[0156] Without limiting the compositions and methods of administration described herein, in certain embodiments, rifamycin, a rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, and prodrug thereof, or combination thereof, can exhibit drug-sensitization effects on a cancer cell by directly or indirectly inhibiting an efflux pump, such as the ATP-binding cassette sub-family B member 1 (ABCB1) pump. This glycoprotein is found in the cell membrane and actively transports certain chemotherapeutics, such as doxorubicin, out of cancer cells, reducing efficacy of the drug. By inhibiting this pump, the amount of chemotherapeutic present in a cancer cell can be increased and thus the killing effect on the cancer cell can be increased.

[0157] According to certain embodiments of the present disclosure, rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, suppress ABCB1 activity, increasing the effective amount of a chemotherapeutic within a cancer cell.

[0158] Also, without limiting the compositions and methods of administration described herein, in certain embodiments, rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, can exhibit drug-sensitization effects on a cancer cell by acting on the Akt (protein kinase B)/14-3-3g/2mitochondrial electron transport chain (ETC)/reactive oxygen species (ROS) signaling network within a cell. An example of how the rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof, or combination thereof can effect this pathway in a drug-resistant cancer cell is shown in FIGURE 1. In this example, a CHOP-resistant cancer cell, such as a CHOP-resistant diffuse large B-cell lymphoma (DLBCL) cell, undergoes cellular changes such that Akt is constitutively activated. This constitutively activated Akt phosphorylates mitochondrial GSK-3β. This phosphorylated GSK-3B then binds the 14-3-3ζ protein, rendering the GSK-3B unavailable to bind to mitochondrial ETC Complex 1. GSK-3B binding to ETC Complex 1 inhibits the complex
activity, so the overall result of constitutive Akt activation is that ETC Complex 1 is not inhibited when it otherwise should be. Downregulation of Complex I activity by GSK-3β can lead to increased electron leakage from the ETC, resulting in increases in ROS.

[0159] ETC Complex 1 acts to reduce the amount of electron spillage from the ETC during mitochondrial activity. Electrons spilled in such a manner react with oxygen to produce reactive oxygen species (ROS). Thus, increased ETC Complex 1 activity and the resultant reduction in electron leakage decrease the amount of ROS in the cell. Low levels of ROS can lead to an intracellular environment that inhibits the ability of chemotherapeutics such as CHOP to induce cancer cell death by apoptosis. Thus, one effect of constitutive Akt activation is a decrease in ROS, making the cancer cell harder to kill.

[0160] According to certain embodiments of the present disclosure, rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof or combinations thereof, suppress ETC Complex 1 activity, restoring it to a more normal level. As a result, more ROS are present in the cell and the cellular environment is restored to one in which CHOP can once again induce cell death via apoptosis.

[0161] A similar effect can be seen with other chemotherapeutics or other drugs whose efficacy relies on a cellular environment with minimum amount of ROS or other factors (such as other intracellular chemicals, proteins, or conditions) resulting from a minimum amount of ROS in the cell.

[0162] As a result of this effect on the Akt/14-3-3ETC/ROS network, the present disclosure also includes methods of inducing drug-sensitization in a cancer cell by administering an amount of rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, sufficient to decrease activity of ETC Complex 1 or increase cellular levels of ROS. In particular, the disclosure includes methods of administering an amount sufficient to increase cellular levels of ROS to an amount sufficient to allow a drug to which a cancer cell is sensitized to kill, reduce the growth of, or negatively affect the cancer cell.

[0163] Although the above example relates to cancer cells that have become resistant to a drug due to abnormal Akt activity, the same methods are applicable to cancer cells that exhibit
low ROS levels for other reasons. Furthermore, the same methods can be used for drug-sensitization in cancer cells that have no ROS abnormality by increasing ROS to an abnormal level if the cancer cells then become sensitive to the drug at the abnormal ROS level.

[0164] Effects mediated by ROS described above may, in particular, be mediated by superoxide species and superoxide species can be the particular form of ROS affected.

[0165] Although some drug-sensitization or cancer cell inhibition effects can be mediated by the ROS pathway, compositions and methods of the present disclosure can act via other cellular pathways alternatively to or in addition to the Akt/14-3-3ζ/ETC/ROS network. This can be particularly true with respect to drug-sensitization to chemotherapeutics that operate in a different manner than CHOP. For example, ROS can affect the mitochondrial-directed Bcl-2 apoptosis pathway as well. Furthermore, the effect of rifabutin on ROS induction has been shown to be very rapid, whereas the effect on Akt has been shown to take at least 18 hours. Accordingly, it appears likely that an initial ROS induction event can occur, followed by a secondary downstream effect downregulating Akt. In CHOP-resistant cells, Akt is constitutively active thereby increasing Complex I activity resulting in decreases in ROS. induction of ROS by compositions and methods of the present disclosure will further promote drug sensitivity in the resistant cancer cell by downregulating the Akt pathway.

[0166] Again without limiting the compositions and methods of administration described herein, in certain embodiments, rifamycin, rifamycin derivatives, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivatives, or pharmaceutically acceptable salts, hydrates, and prodrugs thereof, or combinations thereof, can exhibit drug-sensitization effects on a cancer cell by mobilizing calcium within the cell. Increased calcium mobilization correlates with increased ROS amounts. Drug-sensitive cells often exhibit both increased levels of calcium and increased ROS levels as compared to drug-resistant cells. Typically, ROS levels rise first in such cells, followed by calcium mobilization. Accordingly, rifamycin or a rifamycin derivative, such as rifabutin or a rifabutin derivative, can directly inhibit efflux pump activity, which then causes a burst of ROS followed by calcium mobilization.

[0167] According to certain embodiments of the present disclosure, the compositions disclosed herein can inhibit a cancer cell through more than one activity. For instance, it can both decrease efflux pump activity and increase ROS. In certain embodiments, these multiple
activities can have synergistic effects.

EXAMPLES

[0168] The following examples are provided to further illustrate certain embodiments of the disclosure. They are not intended to disclose or describe each and every aspect of the disclosure in complete detail and should not be so interpreted. Unless otherwise specified, designations of cells lines and compositions are used consistently throughout these examples.

Example 1- Drug-Sensitization of CHOP-Resistant NHL Cell Lines

[0169] Several human cell lines were utilized as in vitro models of NHL, including the CRL2631 line obtained from the American Type Culture Collection (ATCC). CRL2631 was established from peripheral blood leukocytes (PBL) of a patient with DLBCL. CHOP-resistant NHL cell lines (designated G3) were generated by repeated cycles of on-off treatments with CHOP, a treatment protocol that is similar to clinical regimens.

[0170] The effects of rifabutin on cell growth of both CHOP-sensitive (CRL2631) and CHOP-resistant (G3) cells in the presence or absence of CHOP are shown in FIGURE 2A. A reduction in cell growth is demonstrated by a reduction in fluorescence emitted by the cell growth indicator dye, resazurin.

[0171] Rifabutin was confirmed to have drug-sensitization activity in clinically derived CHOP resistant cell lines. As shown in FIGURE 2A, CHOP inhibited growth of CHOP-sensitive (CRL2631) cells but had little effect on G3 cells. Rifabutin did not affect the growth of cells in the absence of CHOP, indicating low toxicities (FIGURE 2A). Rifabutin enhanced the sensitivity of CHOP-sensitive cells to CHOP as shown in FIGURE 2B, relative to a control drug. FIGURE 2C shows similar effects in another CHOP-resistant NHL line.

Example 2 - Toxicity

[0172] Doxorubicin and rifabutin or its derivatives RTI-79 and RT1-176 were applied to primary human fibroblasts to determine comparative cytotoxicity. Results are presented in FIGURE 3A and FIGURE 3B and demonstrate that rifabutin and the analogs are not toxic to normal cells.
[0173] To further test safety of rifabutin and its derivatives, rifabutin and rifabutin
derivates RTI-79 and RTI-81 were administered as an adjunct therapy to doxorubicin (DOX).

[0174] Swiss mice were dosed with levels equal to and exceeding that of intended doses. Swiss mice were given repeated weekly oral doses of rifabutin at 180 mg/kg, RTI-79 at 250 mg/kg or RTI-81 at 30 mg/kg in conjunction with intravenous 3.3 mg/kg DOX. No overt toxicity or weight loss was seen over several weeks time. Further, no significant differences between mice treated with RTI-79 with or without DOX were observed after both histological analysis of heart tissue by hematoxylin and eosin (H&E) and analysis of blood and serum for complete blood count and manual differential. Intravenous rifabutin or RTI-81 were also given repeatedly both at 75 mg/kg in conjunction with intravenously administered 3.3 mg/kg DOX and no overt toxicity or weight loss was seen over several weeks time. Further data in Example 4 below shows treatment efficacy using less than one-fifth the above oral dose of 33 mg/kg rifabutin with intravenous 3.3 mg/kg doxorubicin.

Example 3 - Drug-Sensitization of CHOP-Resistant Lymphoma Cells From Dog Model

[0175] A single lymphoma aspirate from a dog with CHOP-resistant lymphoma was tested for responsiveness to CHOP in the presence or absence of rifabutin. CHOP-responsiveness was measured by a decrease in fluorescence signal generated by resazurin. FIGURE 4 shows that growth of aspirated lymphoma cells was resistant to CHOP at doses up to 640 ng/ml, but significant growth inhibition was observed at a dose of 1280 ng/ml CHOP. The inclusion of 5 μM rifabutin significantly enhanced the sensitivity of the aspirated lymphoma cells to CHOP such that significant growth inhibition was observed at 320 and 640 ng/ml CHOP. Rifabutin had no effect on cell viability in the absence of CHOP.

Example 4 - Drug-Sensitization In Vivo

[0176] In a first efficacy study, 6-8 week old female SCID mice (7 mice per treatment
ami) were injected subcutaneously on both flanks with 1 X 10^7 G3 CHOP-resistant NHL cells. Once palpable tumors (about 50-100 cc size) appeared, therapies (CHOP or CHOP+rifabutin) were started. CHOP was administered at the maximum tolerated dose (cyclophosphamide, 40 mg/kg i.v.; doxorubicin, 3.3 mg/kg i.v.; vincristine, 0.5 mg/kg i.v.; and prednisone, 0.2 mg/kg
orally daily for 5 d) weekly for 3 weeks. Rifabutin in an amount of 100 mg/kg was administered on the day of each CHOP treatment and 24-hours later by gavage. Mouse body weight and tumor size were monitored every two days and tumor size measured by caliper. The tumor volume formula \((L*W^2)/2\) was used to calculate tumor mass.

[0177] The overall tumor burden per mouse was much lower in mice that received CHOP+rifabutin than for those receiving CHOP only treatments. CHOP treatment alone of the SCID mice harboring subcutaneous G3 lymphomas resulted in relatively fast tumor growth, as compared to tumors in CHOP+rifabutin treated mice (FIGURE 5). The dosage of rifabutin administered had little or no toxicity in the mice. Control mice injected with CRL2631 cells, in contrast, exhibited a marked decrease in tumor growth in response to CHOP alone (FIGURE 6).

[0178] A second efficacy study was conducted where mice were treated before the appearance of palpable tumors. In that experiment, one week after transplantation of CHOP-resistant G3 cells, one group (7 mice) was treated with CHOP-only and a second group (8 mice) was treated with CHOP + rifabutin. One week later, mice received a second treatment and tumors began to appear in the CHOP-only group. The two treatment groups differed not only in the tumor size but also in the number of tumors developed. More tumors appeared and grew at a significantly higher rate in CHOP-only mice compared to CHOP+rifabutin mice. The CHOP only treatment group developed tumors at 12 of 14 (85.7%) injection sites. The CHOP+rifabutin treatment group developed fewer tumors at only 6 of 16 (37.5%) injection sites. In a separate experiment, SCID mice developed G3 tumors at 35 of 42 (83.3%) injection sites when receiving no treatment; this is similar to the CHOP only treatment group. Significance was analyzed by the T test yielding a highly significant difference between the means of the tumor burdens of the two groups \((p<.01)\) at Day 7. Thus, rifabutin actually reduces the tumor take rate which could translate into more complete responses when humans are treated early with this combination.

[0179] A third study was conducted in which mice injected with CHOP-resistant G3 cells received reduced dosages of CHOP in combination with 33 mg/kg rifabutin. CHOP+rifabutin was administered weekly beginning one week post-inoculation. Control mice were given no CHOP or rifabutin. Tumor load was significantly less in mice that received even reduced CHOP dosages as compared to imtreated mice, demonstrating that rifabutin can allow the use of lower dosages of CHOP without a significant decrease in therapeutic effect (FIGURE 7).
A fourth efficacy study was conducted using DOX in combination with the rifabutin derivative RTI-81. SCID mice were injected with CHOP-resistant G3 cells in the same manner as the first efficacy study above. Treatments began 2 weeks post-inoculation and were administered twice weekly. DOX was given at 3.3 mg/kg i.v. and RTI-81 was given at 10 mg/kg by gavage. A statistically significant difference in average life-span is seen when mice were treated with doxorubicin and RTI-81 as compared to DOX alone. Mice receiving doxorubicin+RTI-81 lived 27% longer than those receiving doxorubicin only (X² = 8.6 p=0.00336 (dof=1)) (FIGURE 8). Respective mean and median lifespans for each group were: 42.6, 42 and 34.6, 33. Mice treated with doxorubicin only were 10.37 times as likely to die before those treated with doxorubicin+RTI-81. Cox proportional hazard ratio was 0.0964 with a likelihood ratio of 7.24 (p=0.00714 (dof=1, n=15).

In a fifth efficacy study, we generated xenografts of the human ovarian cancer cell line SK-OV-3, a cell line considered doxorabiem-resistant, by bilateral subcutaneous (s.c.) injection of 1X 10⁷ tumor cells to establish localized tumors in 6-8 week old female SCID mice. Using rifabutin co-administered with DOX, in vivo efficacy was assessed. Once tumor volumes were at least 75mm³ and showed consistent growth rates, therapies (DOX only 3.3 mg/kg i.v. or DOX 3.3 mg/kg i.v. + rifabutin 25 mg/kg oral) were started. Cycles of Dox or Dox + rifabutin were given once a week for 4 cycles. This cyclical dosing scheme of mouse models has precedent in the literature and is intended to mimic the cycles of DOXIL® (Centocor Ortho Biotech Products, LP, Raritan, NJ) given in the clinic. Rifabutin was administered on the day of each DOX treatment and by gavage. Mouse body weight and tumor size were monitored. As shown in FIGURE 9, after 19 days treatment, average tumor volumes were 587 mm³ for the DOX-only treatment group, and 348 mm³ for the DOX + rifabutin group. This is a 40% reduction in tumor size for the DOX + rifabutin group.

In a sixth efficacy study, we generated xenografts of multi-drug resistant ovarian cancer cell line (NCI/ADR-RES) by implantation of NCI/ADR-RES cell xenografts in the left and right flanks of nude mice, resulting in two tumors per mice. In vivo efficacy of RTI-79 was assessed by co-admmstration with DOXIL®. Once tumor volumes were at least 90 mm³ and showed consistent growth rate, therapies (DOXIL® only 7 mg/kg i.v. or DOXIL® 7 mg/kg i.v. + RTI-79 25 mg/kg oral) were started. Cycles of DOXIL® or DOXIL® + RTI-79 were given
every week for six cycles. RTI-79 was administered by oral gavage 24 and 48 hours after each DOXIL® administration. Tumor size was monitored. As shown in FIGURE 10, after 41 days the tumor volume in the RTI-79-treated mice was 66% lower than in mice receiving only DOXIL®.

[0183] In a seventh efficacy study, we generated xenografts of multi-drug resistant ovarian cancer cell line (NCI/ADR-RES) by implantation of NCI/ADR-RES cells xenografts in the left and right flanks of nude mice, resulting in two tumors per mouse. In vivo efficacy of RTI-79 was assessed by co-administration with DOXIL®. Therapies were DOXIL® only 7 mg/kg i.v. or DOXIL® 7 mg/kg i.v. + RTI-79 25 mg/kg oral. Cycles of DOXIL® or DOXIL® + RTI-79 were given every week for six cycles. RTI-79 was administered by oral gavage 24 and 48 hours after each DOXIL® administration. Tumor size was monitored. As shown in FIGURE 11, after 46 days the tumor volume in the RTI-79-treated mice was 55% lower than in mice receiving only DOXIL®. Furthermore, tumor volume in RTI-79-treated mice was reduced by 50% during the course of the study.

Example 5 - Sensitization to CHOP Using Other Rifabutin Derivatives

[0184] Several compositions of the present disclosure were tested and their effects on cell growth were measured. A reduction in cell growth is demonstrated by a reduction in fluorescence emitted by the cell growth indicator dye, resazurin. Compositions were tested on CHOP-resistant G3 NHL cells that had been cultivated in RPMI medium for five days. Prior to assay, the cells were counted by haemocytometer and cell concentration standardized to 625,000 cells/ml. Test drugs were solubilized in 100% DMSO and then diluted to final assay concentration with 0.1M phosphate buffered saline (PBS) and a final DMSO concentration of 0.5%. Cells were added to assay plates containing the test drugs (rifabutin + 148 ng/ml, 74 ng/ml, 37 ng/ml, or 0 ng/ml doxorubicin) and allowed to incubate for 96 hours at 37°C and 5% CO₂. The metabolic dye reazurin was added to the wells of the assay plate at a final concentration of 20 μg/ml and the plates were incubated for an additional 24 hours. The plates were then read in a BMG Polarstar plate reader at wavelength (573-605) and the data plotted as OD versus increasing dilutions (i.e. decreasing total amounts) of rifabutin derivative concentration.
Results of tests were performed on G3 cells to compare the effects of rifabutin and certain rifabutin derivatives on cell growth in the presence or absence of 1 µM doxorubicin (DOX) are presented in Table 2, which indicates the IC50S for selected rifamycin analogs on lymphoma cell line G3.

**Table 2: IC50S for selected rifamycin analogs on lymphoma cell line G3.**

<table>
<thead>
<tr>
<th>Analog</th>
<th>IC50 (µM)</th>
<th>IC50 (µM) with doxorubicin (1 µM)</th>
<th>Fold increase in potency over DOX alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxorubicin</td>
<td>2.36</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Rifabutin</td>
<td>&gt;64</td>
<td>.25</td>
<td>9.4</td>
</tr>
<tr>
<td>RTI-51</td>
<td>&gt;64</td>
<td>3.3</td>
<td>0.7</td>
</tr>
<tr>
<td>RTI-53</td>
<td>&gt;64</td>
<td>11.8</td>
<td>0.2</td>
</tr>
<tr>
<td>RTI-78</td>
<td>58</td>
<td>0.08</td>
<td>29.5</td>
</tr>
<tr>
<td>RTI-79</td>
<td>43</td>
<td>1.14</td>
<td>2.1</td>
</tr>
<tr>
<td>RTI-81</td>
<td>&gt;64</td>
<td>0.3</td>
<td>7.9</td>
</tr>
<tr>
<td>RTI-82</td>
<td>&gt;64</td>
<td>3.5</td>
<td>0.7</td>
</tr>
<tr>
<td>RTI-102</td>
<td>&gt;64</td>
<td>0.95</td>
<td>2.5</td>
</tr>
<tr>
<td>RTI-174</td>
<td>51</td>
<td>0.64</td>
<td>3.7</td>
</tr>
<tr>
<td>RTI-175</td>
<td>&gt;64</td>
<td>1.06</td>
<td>2.2</td>
</tr>
<tr>
<td>RTI-176</td>
<td>&gt;64</td>
<td>0.45</td>
<td>5.2</td>
</tr>
<tr>
<td>RTI-181</td>
<td>52</td>
<td>0.43</td>
<td>5.5</td>
</tr>
<tr>
<td>RTI-182</td>
<td>62</td>
<td>1.2</td>
<td>2.0</td>
</tr>
<tr>
<td>RTI-183</td>
<td>&gt;64</td>
<td>5.19</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Example data for RTI-79 and rifabutin is shown in FIGURE 12. Example data for RTI-176 and rifabutin is shown in FIGURE 13. Example data for RTI-81 and rifabutin is shown in FIGURE 14. Example data for interaction of rifabutin and doxorubicin on CRL263I cells is shown in FIGURE 15. Example data for interaction of RTI-79 and doxorubicin on CRL2631 cells is shown in FIGURE 16. These results establish that a variety of rifabutin derivatives are similarly effective at restoring doxorubicin sensitivity to CHOP-resistant cells.

**Example 6 - Drug-Sensitization of Multiple Cell Lines**

The ability of rifabutin and rifabutin derivatives to cause drug-sensitization to doxorubicin in multiple types of cancer cells was investigated by performing experiments similar to those described above. In these experiments, the following cell lines were used: CHOP-resistant NHL cell line G3, CHOP-sensitive NHL cell line CRL2631, the multi-drug resistant
sarcoma cell line MES-SA-Dx5; multi-drug-resistant breast cancer cell line MDA-MB-231, multi-drug resistant ovarian carcinoma cell line SK-OV3, multi-drug resistant ovarian cancer cell line NCI/ADR-RES, drug-sensitive ovarian cancer cell line (WCAR-5, and multi-drug resistant ovarian cancer cell line OVCAR-3. Results are presented in Table 3.

Table 3: Magnitude of potentiation observed with rifamycin analogs in combination with doxorubicin

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Tissue</th>
<th>Cell Line</th>
<th>RBT</th>
<th>RTI-51</th>
<th>RTI-53</th>
<th>RTI-79</th>
<th>RTI-81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma</td>
<td>B cells</td>
<td>G3</td>
<td>+++</td>
<td></td>
<td>++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Lymphoma</td>
<td>B cells</td>
<td>CRL CRL2631</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Uterus</td>
<td>MES-SA-Dx5</td>
<td>++</td>
<td></td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Breast</td>
<td>MDA-MB-231</td>
<td>+++</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>SK-OV3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>NCI/ADR-RES</td>
<td></td>
<td></td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
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<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Tissue</th>
<th>Cell Line</th>
<th>RBT</th>
<th>RTI-82</th>
<th>RTI-102</th>
<th>RTI-174</th>
<th>RTI-175</th>
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<tbody>
<tr>
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<td>B cells</td>
<td>G3</td>
<td>+++</td>
<td></td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>B cells</td>
<td>CRL CRL2631</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Uterus</td>
<td>MES-SA-Dx5</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Breast</td>
<td>MDA-MB-231</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>SK-OV3</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-3</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-5</td>
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<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
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<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Tissue</th>
<th>Cell Line</th>
<th>RBT</th>
<th>RTI-176</th>
<th>RTI-181</th>
<th>RTI-182</th>
<th>RTI-183</th>
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<tr>
<td>Lymphoma</td>
<td>B cells</td>
<td>G3</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>B cells</td>
<td>CRL CRL2631</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Uterus</td>
<td>MES-SA-Dx5</td>
<td>++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Breast</td>
<td>MDA-MB-231</td>
<td>+++</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>SK-OV3</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-3</td>
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<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>OVCAR-5</td>
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</tr>
<tr>
<td>Carcinoma</td>
<td>Ovarian</td>
<td>NCI/ADR-RES</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
RBT = rifabutin; + potentiation between 1.2 to 2.0 fold increase; ++ potentiation between 2.1 to 5 fold increase; +++ potentiation greater than 5 fold increase

[0187] Example data for rifabutin or RTI-82 on MDA-MB-231 cells is presented in FIGURE 17. Example data for rifabutin or RTI-79 with or without doxorubicin on SK-OV3 cells is presented in FIGURE 18. Example data for rifabutin or RTI-81 on MES-SA-Dx5 cells is presented in FIGURE 19. Example data for interaction of rifabutin or RTI-79 and doxorubicin on ADR-RES cells is shown in FIGURE 20. Example data for interaction of RTI-79 and doxorubicin on MOLT-4 cells is shown in FIGURE 21. Example data for the interaction of rifabutin or RTI-79 and doxorubicin on ovarian carcinoma OVCAR-8 cells is shown in FIGURE 22. These results establish that rifabutin and rifabutin analogs are able to induce drug-sensitization for a variety of types of cancer.

Example 7 - Sensitization to Various Chemotherapeutics Using Rifabutin and Rifabutin Derivatives

[0188] Similar tests were performed to compare the effects of rifabutin and certain rifabutin derivatives on cell growth in the presence or absence of various chemotherapeutics on various cell lines. Chemotherapeutics include: the targeted therapy bortezomib (Veleade®), the pyrimidine antagonist gemcitabine, the platinum drug cis-piatin, the anti-tumor antibiotic actinomycin D, the anti-tumor antibiotic apicidm, the topoisomerase I inhibitor camtopthecm, the anti-tumor antibiotic doxorubicin, the mitotic inhibitor vinblastine, the nitrogen mustard alkylating agent melphalen, the hormonal agent tamoxifen, the folate antimetabolite methotrexate, the toposimerase II inhibitor etoposide, phenoxodiol, the antibiotic rapamycin, and menadione. Additional cell lines used include: ovarian cancer OVCAR-8, T lymphoblastoid leukemia MOLT-4, dexamethasone-resistant multiple myeloma MM.IR, myeloid leukemia cells HL-60, osteosarcoma cells U-2 OS, and myeloma RPMI 8226. Results are shown in Table 4.

Table 4: IC50 for selected cancer cell lines and clinically relevant cancer therapeutics in interaction with Rifabutin

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Cell line</th>
<th>Therapeutic drug</th>
<th>IC50 (µM)</th>
<th>IC50 (µM) with</th>
<th>Fold increase in potency</th>
</tr>
</thead>
</table>
Rifabutin

<table>
<thead>
<tr>
<th>Diffuse large B cell lymphoma</th>
<th>G3</th>
<th>Doxorubicin</th>
<th>2.36</th>
<th>0.25</th>
<th>9.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td>G3</td>
<td>Vinblastine</td>
<td>8.00</td>
<td>1.00</td>
<td>8.0</td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td>G3</td>
<td>Mitoxantrone</td>
<td>0.46</td>
<td>0.04</td>
<td>11.5</td>
</tr>
<tr>
<td>Diffuse large B cell lymphoma</td>
<td>CRL2631</td>
<td>Doxorubicin</td>
<td>0.35</td>
<td>0.12</td>
<td>2.9</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>OVCAR-3</td>
<td>Menadione</td>
<td>&gt;32</td>
<td>10.88</td>
<td>&gt;2.9</td>
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<tr>
<td>Ovarian carcinoma</td>
<td>OVCAR-5</td>
<td>Velcade</td>
<td>0.17</td>
<td>0.08</td>
<td>2.1</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>OVCAR-8</td>
<td>Mitoxantrone</td>
<td>14.0</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>SK-OV3</td>
<td>Mitoxantrone</td>
<td>&gt;32</td>
<td>12.28</td>
<td>&gt;2.6</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>ADR-RES</td>
<td>Doxorubicin</td>
<td>&gt;32</td>
<td>6.59</td>
<td>&gt;4.9</td>
</tr>
<tr>
<td>Leukemia</td>
<td>MOLT-4</td>
<td>Doxorubicin</td>
<td>0.03</td>
<td>0.01</td>
<td>3</td>
</tr>
<tr>
<td>Leukemia</td>
<td>MOLT-4</td>
<td>Actinomycin D</td>
<td>0.04</td>
<td>&lt;0.008</td>
<td>&gt;5</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>MDA-MB-231</td>
<td>Gemcitabine</td>
<td>&gt;32</td>
<td>4.83</td>
<td>&gt;6.6</td>
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<tr>
<td>Multiple myeloma</td>
<td>MM.1R</td>
<td>Camptothecin</td>
<td>1.13</td>
<td>0.3</td>
<td>3.8</td>
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<td>MM.1R</td>
<td>Menadione</td>
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<td>2</td>
<td>2</td>
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<tr>
<td>Myeloid leukemia</td>
<td>HL-60</td>
<td>Paclitaxel</td>
<td>0.4</td>
<td>0.2</td>
<td>2</td>
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<tr>
<td>Uterine Sarcoma</td>
<td>MES-SA-Dx5</td>
<td>Actinomycin D</td>
<td>0.03</td>
<td>0.01</td>
<td>3</td>
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<tr>
<td>Osteosarcoma</td>
<td>U-2OS</td>
<td>Mitoxantrone</td>
<td>0.14</td>
<td>0.06</td>
<td>2.3</td>
</tr>
<tr>
<td>Myeloma</td>
<td>RPMI 8226</td>
<td>Paclitaxel</td>
<td>1.0</td>
<td>0.89</td>
<td>1.1</td>
</tr>
</tbody>
</table>

[0189] Example data for interaction of rifabutin with actinomycin D on MES-SA-Dx5 cells is shown in FIGURE 23. Example data for interaction of rifabutin with menadione on MM.1R cells is shown in FIGURE 24. Example data for interaction of rifabutin and mitoxantrone on U-2 OS cells is shown in FIGURE 25. Example data for interaction of rifabutin and gemcitabine on MDA-MB-231 cells is shown in FIGURE 26. Example data for interaction of rifabutin with paclitaxel on HL-60 cells is shown in FIGURE 27. Example data for interaction of rifabutin and camptothecin on OVCAR-8 cells is shown in FIGURE 28. These results demonstrate the ability of rifabutin and rifabutin derivatives to induce drug-sensitivity for a wide variety of chemotherapeutics in a wide variety of cancers.

**Example 8 - Prevention of the Emergence of CHOP resistance**

[0190] The ability of rifabutin to prevent the emergence of CHGP-resistance was determined by treating CFIOP-sensitive CRL2631 cells with eitherCHOP alone or
CHOP+rifabutin for one week. Following treatment, the cells were grown in the absence of CHOP, then their sensitivity to CHOP was assayed by retreatment with CHOP, followed by counting of viable cells. Results are shown in FIGURE 29. Rifabutin was able to significantly repress the emergence of CHOP-resistant cells at both half (0.5X) and full (IX) doses of CHOP. A 1X CHOP dose in this experiment corresponds to final concentrations of the following components: 0.83 µM 4-hydroxycyclophosphamide [4HC, a pre-activated form of cyclophosphamide], 0.057 µM doxorubicin, 0.01 µM vincristine, and 0.186 µM prednisone.

Example 9 - Effects of Rifabutin and Rifabutin derivatives on ROS

A Western blot of CHOP-sensitive (CRL2631) or CHOP-resistant (G3) lymphoma cells revealed that Akt, phosphorylated Akt, and 14-3-3ζ levels were consistent with the model proposed in FIGURE 1 (FIGURE 30A) in that Akt was markedly more active in CHOP-resistant G3 cells than in CRL2631. The model was further confirmed by treatment of CHOP-resistant (G3) cells with Akt Inhibitor VIII, which caused a dose-dependent reversal of CHOP resistance (FIGURE 30B). The inhibitory effect of Akt inhibitor VIII on the expression of phosphorylated Akt and total 14-3-3ζ protein was confirmed by Western blot (FIGURE 30C).

Additional studies further confirmed the model of FIGURE 1 by demonstrating that CHOP-sensitive (CRL2631) cells make more ROS than do CHOP-resistant (G3 cells) (FIGURE 31). Furthermore, CHOP increased ROS in CHOP-sensitive (CRL2631) cells, but not in CHOP-resistant (G3) cells (FIGURE 31).

Examination of CHOP-sensitive CRL2631 cells revealed that these cells include two distinct populations, a low-ROS population and high-ROS population (FIGURE 32). When these populations were separated, the low-ROS population proved more resistant to CHOP than high-ROS population (FIGURE 33). However, this low-ROS cell population was sensitized to CHOP by rifabutin (FIGURE 34). Rifabutin also rapidly induces ROS in CHOP-resistant (G3) cells (FIGURE 35).

Overall, these results demonstrate that, at least in the CRL2631 lymphoma cell line and cell lines derived therefrom, CHOP-resistance is mediated by ROS levels and that rifabutin and rifabutin derivatives decrease CHOP-resistance by increasing ROS.
Example 10 - Rifabutin and RTI-79 Decrease Drug Efflux and Mobilize Calcium

[0195] Rifabutin and its derivatives, such as RTI-79, showed clear inhibition of efflux pumps in NCI/ADR-RES and G3 cells when tested in calcein-AM assays. This inhibitory effect was unambiguously due to inhibition of ABCB1 pumps. The difference in pump activity between ADR-RES cells and its drug-sensitive parental strain, OVCAR-8, can be seen in FIGURES 34A and 34B.

[0196] It is known that mitigation of ABCB1 activity will lead to more effective accumulation of doxorubicin in cells (Shen, F., Chu, S., Bence, A.K., Bailey, B., Xue, X., Erickson, P.A., Montrose, M.H., Beck, W.T., and Erickson, L.C. (2008). Quantitation of doxorubicin uptake, efflux, and modulation of multidrug resistance (MDR) in MDR human cancer cells. J Pharmacol Exp Ther 324, 95-102.). Thus the inhibition on ABCB1 by RTI-79 decreases its contribution to its potentiating doxorubicin toxicity on these drug-resistant cells. This was confirmed by testing with additional rifabutin derivatives. As shown in FIGURE 36, the stronger inhibitors of ABCB1, also better re-sensitized drug-resistant cells. RTI-79 was the strongest inhibitor as well as best re-sensitizer.

[0197] Doxorubicin-sensitive (OVCAR8 ovarian) and Doxorubicin-resistant (G3 lymphoma; ADR-RES ovarian) cells were treated for 2 hrs with 10 uM RTI-79, p-glycoprotein (P-gp) inhibitors (reserpine, elacridar), or control drugs (DMSO, carboxin, nifazoxidine). Cells were then stained with the fluorescent ROS indicator, CellROX, and subjected to flow cytometry to quantitate total intracellular ROS. As FIGURE 38 shows, RTI-79 induced ROS in ovarian carcinoma and lymphoma cell lines, as did the MDR/P-gp inhibitors, reserpine, elacridar. This suggests that RTFs ability to induce ROS was the result of inhibition of efflux pumps. The degree of ROS induced by RTI-79 and P-gp inhibitors was much greater in the doxorubicin-resistant ADR-RES and G3 cell lines than in the doxorubicin-sensitive OVCAR8 cell line. Control drugs established that this effect is specific to MDR/P-gp inhibitors and RTI-79.

[0198] The intracellular origin of RT1-induced ROS was determined by staining ADR-RES cells with the red fluorescent ROS indicator, CellROX (Invitrogen), and visualizing where the ROS was concentrated by confocal microscopy. Results are presented in FIGURE 39. Mitochondria were localized by infecting cells for 24 hrs with the BacMAM mitotracker baculovirus, which expresses a GFP fused to a mitochondria localization signal. Nuclei were
stained with the blue DAPI stain. There was a good co-localization of red CellROX staining with the green GFP mitotracker, indicating that the ROS were originating from the mitochondria.

[0199] The electron transport chain (ETC) is known to be a primary generator of ROS in the cell. Most of the ROS is generated by Complexes I and III of the ETC. Inhibition of Complex I results in electrons piling up and leaking to react with oxygen to produce ROS. The effects of a Complex I inhibitor (rotenone) and a Complex III inhibitor (antimycin A) on ROS levels in the cell were tested and results are shown in FIGURE 40. Specifically, Dox-resistant G3 lymphoma cells were treated 10 mM RTI-79, BAPTA-AM (cell permeable calcium chelator), verapamil (a calcium channel blocker and P-gp inhibitor), a Complex I inhibitor (Rotenone), a Complex III inhibitor (antimycin A), or control drugs (oxaloacetate, carboxin, nifazoxinide). Rotenone, but not antimycin A, induced ROS, suggesting that RTI-79-induced and efflux pump inhibitor-induced ROS originate at Complex I of the ETC.

[0200] MDR/P-gp activity is closely associated with calcium status in the cell, so calcium modulators were tested for effects on ROS. As shown in FIGURE 40, both a cell-permeable calcium chelator (BAPTA-AM) and a calcium channel blocker (and efflux pump inhibitor) induced ROS in G3 cells. As shown in FIGURE 41A and 39B, P-gp inhibitors (Reserpine, Elacridar) induced ROS relative to control drugs (Carboxin, Nifazoxinide). As shown in FIGURE 41B, a P-gp inhibitor (Elacridar) induced calcium in a similar manner as RTI-79, indicating connections between calcium, ROS, and efflux pump activity in the mechanism of action of RTIs. Because calcium modulators induced ROS, testing was performed to investigate whether RTI-induced ROS was associated with calcium mobilization in doxorubicin-sensitive and doxorubicin-resistant cells and in resistant cells treated with RTI-79. Relatively Dox-sensitive lymphoma (CRL2631, 10S, WSU) and ovarian carcinoma (OVCA8) and more Dox-resistant lymphoma (G3R, 10R, WSUR) and ovarian carcinoma (ADR) were treated with 10 mM DMSO for 2 hrs. Dox-resistant cells were also treated with 10 mM RTI-79 for 2 hrs (G3R+RTI79; 10R+RTI-79, WSUR+RTI-79). Cells were co-stained with the cell-permeable red fluorescent ROS indicator, CellROX, and cell-permeable green fluorescent calcium indicator, Fluo-4AM, and then subjected to flow cytometry to quantitate changes in ROS and calcium levels. As shown in FIGURE 41C, levels of both ROS and calcium in doxorubicin-sensitive cells were much higher than in the resistant lines, and RTI-79 induced both ROS and calcium
mobilization in resistant cells. Thus, the ability of RTI-79 to sensitize doxorubicin-resistant cells was closely correlated with the inhibition of efflux pumps, induction of ROS, and mobilization of calcium.

[0201] To determine whether increases in ROS led to calcium mobilization or calcium mobilization resulted in ROS induction, a time course of RTI-79 treatment of G3 cells monitoring ROS and calcium was conducted. Cells were co-stained with the red fluorescent ROS indicator, CeliROX, and the green fluorescent calcium indicator, Fluo-4AM for 30 minutes and treated with 10 uM RTI-79 for 0 to 30 minutes. All samples were analyzed at the same time in flow cytometry. As shown in FIGURE 42, increases in ROS were seen as soon as 1 minute after exposure of cells to RTI-79 and gradually increase to 4 minute, level off to 5 minute, and then decrease after 6 minute, followed by increases up to 15 minute. In contrast, calcium mobilization did not occur until after 15 minutes of RTI-79 treatment, thus indicating that ROS levels increased first followed by calcium mobilization.

[0202] RTI-79 might inhibit MDR/P-gp by inducing ROS, which then increase calcium mobilization that then inhibits efflux pump activity. Alternatively, RTI-79 can first directly inhibit efflux pump activity, which then causes a burst of ROS followed by calcium mobilization. To determine which mechanism most likely involved, ADR-RES (Dox-resistant) and OVCAR8 (Dox-sensitive) ovarian carcinoma cells were transfected with siRNA to knockdown efflux pumps to determine the effect on ROS and calcium. Cells were then co-stained with CeliROX and Fluo-4AM for 1 hour. Some cells were treated with RTI-79 for 1 hour and controls (no RTI-79) were treated with DMSO. As shown in FIGURE 43, knockdown of P-gp in ADR-RES cells led to increases in both ROS and calcium mobilization, and greatly enhanced the ability of RTI-79 to increase ROS and calcium mobilization. As expected, the effect of downregulating efflux pump activity on ROS and calcium in OVCAR8 was much less than in ADR-RES, due to the lower efflux pump activity in OVCAR8 cells. However, the degree of induction of ROS and calcium mobilization by RTI-79 in P-gp knockdown cells (greater than 90% repression of P-gp expression) is much greater than what would be expected if the P-gp was the sole mechanism involved in RTI-79-induced upregulation of ROS. Thus, is it likely that RTI-79 acts not only to induce ROS and calcium mobilization through inhibition of ROS, but also acts at a second target, namely Complex I, to induce ROS.
Example 11 - Preventing or Reducing Metastasis

[0203] The effects of rifabutin on cell invasion was assessed in a collagen invasion 3D assay. Increased interest in the use of 3D culture systems has been motivated by accumulating evidence that 3D models better reflect the microenvironment of tumors and metastases and more accurately predict therapeutic response in vivo compared with conventional 2D assays. A collagen invasion 3D assay allows the rapid and quantitative assessment of invasiveness and a means to screen for drugs which alter the invasive phenotype of tumor cells. Malignant cell lines with high metastatic potential in vivo show a higher rate of invasion than non-metastatic tumor cells and normal ceils showed little or no ability to penetrate the barrier.

[0204] The CHOP-resistant G3 cell line is much more invasive in a collagen invasion 3D assay than its CHOP-sensitive parent cell line (CRL2631). Collagen matrices (1 mg/ml) were prepared as previously described in Su, S.C., et al.. Molecular profile of endothelial invasion of three-dimensional collagen matrices: insights into angiogenic sprout induction in wound healing. Am. J. Physiol. Cell Physiol, 295(5): C1215-29 (2008), incorporated in material part by reference herein, with the inclusion of either DMSO control or 10 μM Rifabutin (Rif). Cells were allowed to invade for 24 hours. Culture medium was removed and collagen gels containing invading cells were fixed in 3% glutaraldehyde in PBS for 30 minutes. Gels were stained with 0.1% tosuidine blue in 30% methanol for 10 minutes prior to destaining with water. Cell invasion density was quantified by counting fixed cultures under transmitted light using an Olympus CK2 inverted microscope equipped with eyepieces displaying a 10 x 10 ocular grid. For each condition, four random fields were selected and the number of invading cells per high power field (HPF) was counted manually at 10× magnification, corresponding to 1 mm² area.

[0205] Data are reported as mean number of invading cells per HPF (± S.D.) in FIGURE 44. G3 cells were more invasive than CRL2631 cells. The inclusion of rifabutin in the collagen matrix reduced the amount of G3 invasion by up to 30%. Less of this effect was observed for CRL2631 cells.

[0206] A modified Boyden chamber assay was used as an independent method to evaluate rifabutin's ability to suppress invasion/metastasis. G3 and CRL2631 cells were grown in the presence of 10 μM rifabutin or dose volume equivalent DMSO for 24 hours at 37 °C. Cell
invasion was assessed with a Chemieon QCM Collagen Invasion Assay (Millipore). The assay is a 96-well plate assay wherein each well is equipped with a suspended insert. Inserts contain an 8-micron membrane coated with a thin layer of polymerized collagen. Invading cells migrate through the collagen layer and attach to the bottom of the membrane. Cells were detached from the membrane and lysed prior to detection via CyQuant dye. Fluorescence intensity is proportional to number of invading cells. As shown in FIGURE 45, the presence of rifabutin resulted in decreased relative fluorescence from 170,374 to 114,395 RLU in G3 cells. In CRL2631 cells RLU decreases from 39,356 to 27,432 RLU in the presence of rifabutin (p<0.05).

[0207] The effect of RTI-79 treatment on the secretion of MMP2 and VEGF was also analyzed. Treatment with RTI-79 resulted in statistically significant decreases in both MMP2 and VEGF in U2-OS osteosarcoma cells in commercially available ELISA based assays. In U2-OS cells, MMP2 was reduced from 22.4 ng/ million cells to 10.5 ng/ million cells (p<0.01) with the addition of 5 uM RTI-79. When evaluating the effects of RTI-79 on VEGF, a decrease from 998 to 436 pg/million cells (p<0.01) was observed.

Example 12 - Rifamycin Derivative Synthesis

[0208] The 3,4-cyclo-rifamycm (rifabutin) derivatives of the current disclosure made be prepared as shown in the schemes listed below.

[0209] Scheme 1 illustrates the general preparation of 1-deoxo-l l-imino-3,4-spiro-piperidyl-rifamycms (lc) and 1-deoxo-l l-amino-3,4-spiro-piperidyl-rifamycms (ld). The compounds of (lc) are synthesized by condensation of 3-amino-4-deoxy-4-imino-rifamycin S (la) with a substituted piperidone or hexanon-type of ketone (lb) at a temperature range from 10°C to 70°C in organic solvent, such as THF or ethanol, in the presence of an excess of ammonium salt, such as ammonium acetate, in a sealed reaction tube. Reduction of 11-imino-rifamycin (lc) with reducing reagent, such as NaBH₄, in organic solvent, such as THF and EtOH at a temperature range from 0°C to room temperature produces 11-amino-rifamycin (ld). When the compound is RTI-35, the tbioether could be oxidized to sulfoxide (-SO-) or sulfone (-SO₂-) depending upon the ratio of compound 1c and oxidizing agents. When the compound is RTI-44, product is obtained by de-protection of Boc-propected-piperidine or Fmoc-protected-piperidine.

Scheme 1.
Scheme 2 illustrates the general preparation of 3,4-spiro-piperidyl-rifamycins (2c) and 1]-deoxo-11-hydroxy-3,4-spiro-piperidyl-rifamycins (2d). The compounds of (2c) are synthesized by condensation of 3-amino-4-deoxy-4-immo-rifamycin S (1a) with a substituted piperidone or hexanon-type of ketone (1b) at a temperature range from 10°C to 70°C in organic solvent, such as THF or ethanol, in the presence or absence of a catalyst, such as Zinc. Reduction of 11-oxo of ritamycin (2c) with reducing reagent, such as NaBH₄, in organic solvent, such as THF and EtOH at a temperature range from 0°C to room temperature produce 11-hydroxy-rifamycin (2d).

Scheme 2.

[0210] The intermediate of (1a) is commercially available or can be obtained from the rifamycin S. The hexanon-type of ketone or 4-substituted piperidone (1b or 2b: Z = C, or () is either commercially available or can be prepared by known procedures. The 4-oxo-piperidine-l-carboxamide (2b: X= NH) is prepared by reacting 4-oxo-piperidine-l-carbonyl chloride.

[0212] Scheme 3 illustrates the general preparation of 11-deoxo-11-hydroxyimino-3,4-spiro-piperidyi-rifarnycins (3c). The compounds of (3c) are synthesized from the reaction of 11-oxo-rifamycin compound (2c) with hydroxyammé (or its HCl salt) at a temperature range from 10°C to 70°C in organic solvent, such as THF or methanol, in the presence or absence of base, such as pyridine.
The above syntheses schemes are preferred schemes for the preparation of the indicated types of compounds. It is apparent to one skilled in art that other sequences of the reactions, and alternative reagents can be used for the synthesis of the rifamycin derivatives of the present disclosure. These alternatives for the synthesis of the derivatives are within the scope of this invention.

The following examples provide synthesis schemes for specific rifabutin derivative compositions. All starting material used in these examples are either purchased from commercial sources or prepared according to published procedures. Reagents were purchased from commercial sources and used without further purification. Reactions with moisture-sensitive reagents were performed under a nitrogen atmosphere. Concentration of solutions was performed by reduced pressure (in vacuum) rotary evaporation. Column flash chromatography was performed using silica gel 60 as stationary phase. The preparative thin-layer chromatography (TLC) was performed using glass plates (20x20 cm) of silica gel (60 F254, thickness 1 mm or 2 mm).

Proton nuclear magnetic resonance (1H-NMR) spectra were recorded on a Varian Inova 300, or 500 MHz magnetic resonance spectrometer. 1H-NMR refers to proton nuclear magnetic resonance spectroscopy with chemical shifts reported in ppm (parts per million) downfield from tetramethylsilane or referred to a residue signal of solvent (CHCl$_3$ = 7.27).
NMR spectra were recorded on Varian Inova 500 MHz spectrometer operating at 25 MHz and Chemical shifts were reported in ppm and referenced to residual solvent signals (CDCl₃ δ 77.23 for carbon)

[0216] The high resolution mass spectra (HRMS) were carried out in a Bruker-micrOTOF-Ql spectrometer, using electro spray ionization positive (ESI+) method and reported as M+H or M+Na, referring to protonated molecular ion or its sodium complex.

[0217] The following examples are for illustration purposes and are not intended to limit the scope of the invention. It will be apparent to one skilled in the art that the compounds of current invention can be prepared by a variety of synthetic routes, including but not limited to substitution of appropriate reagents, solvents or catalyst, change of reaction sequence, and variation of protecting groups.

**General procedure (A) for synthesis of compounds (Ic in scheme 1):**

[0218] In a sealed reaction tube, a reaction mixture of 3-amino-4-imino-rifamycin S (la) (0.1 mmol), piperidone or hexanone-type of ketone (lb) (0.2-0.3 mmol), and ammonium acetate (1 mmol) in THF (3 ml) was stirred at 60°C overnight under nitrogen. The reaction mixture was allowed to cool to room temperature and diluted with DCM (20 ml) and water (20 ml). The aqueous phase was extracted with DCM (2x 20 ml). The combined organic phase was washed with water (20 ml) and brine. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum. The residue was purified either by silica gel column chromatography or by silica gel preparative thin-layer chromatography with methanol in DCM as eluent to give the product as purple solid.

**General procedure (B) for synthesis of compounds (2c in scheme 1):**

[0219] In a round bottom flask with condenser, a reaction mixture of 3-amino-4-imino-rifamycin S (la) (0.1 mmol), piperidone or hexanone-type of ketone (lb) (0.2-0.3 mmol), and ammonium acetate (0.2-0.3 mmol) in THF (8 ml) was stirred at 75°C overnight under nitrogen. The reaction mixture was allowed to cool to room temperature and diluted with DCM (20 ml) and water (20 ml). The aqueous phase was extracted with DCM (2x 20 ml). The combined organic phase was washed with water (20 ml) and brine. The organic phase was dried over
anhydrous sodium sulphate, filtered and concentrated under vacuum. The residue was purified either by silica gel column chromatography or by silica gel preparative thin-layer chromatography with methanol in DCM as eluent to give the product as purple solid.

**General procedure (C) for synthesis of compounds (Id in scheme 1 and 2d in scheme 2):**

[0220] To a solution of rifamycin 11-imine or 11-oxo- compound (lc or 2c) (0.1 mmol) in THF (4 ml) was added a suspension of NaBH4 (0.2 mmol) in ethanol (4 ml) at room temperature. The reaction mixture stirred at room temperature for 1.5 hours and diluted with ethyl acetate (20 ml) and water (20 ml). The aqueous phase was extracted with ethyl acetate (2 × 20 ml). The combined organic phase was washed with water and brine. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum. The residue was purified either by silica gel column chromatography or by silica gel preparative thin-layer chromatography with methanol in DCM as eluent to give the product as purple solid.

**Preparation of RTI-33 11-deoxy-l-l-imino-4-deoxy-3, 4[2spiro-[ l-(t-butyloxycarbonyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S**

[0221] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI⁺): 890.4570 (M+H)⁺; calculated for (M+H)⁺: 890.4553; ¹H-NMR (300 MHz, CDCl₃) δ -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.50 (s, 9H), 1.6-1.85 (m, 4H), 1.88 (s, 3H), 1.9-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.33 (m, 1H), 3.49 (s, 1H), 3.60 (d, J=5 Hz, 1H), 3.68 (d, J=10 Hz, 1H), 3.6-3.8 (br, 2H), 3.95-4.1 (br, 2H), 4.72 (d, J=10 Hz, 1H), 5.07 (dd, J=12 and 7 Hz, 1H), 6.03 (dd, J=16 and 7 Hz, 1H), 6.16 (d, J=12 Hz, 1H), 6.28 id, J=10 Hz, 1H), 6.40 (dd, J=16 and 10 Hz, 1H), 8.26 (s, 1H), 8.71 (bs, 1H), 12.93 (s, 1H), 14.21 (s, 1H).

**Preparation ofRTI-35 11-deoxy-l-l-imino-4-deoxy-3, 4[2spiro-tetrahydrothiopyran-4-yl]-(lH)-imidazo-(2,5-dihydro)rifamycin S**

[0222] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI⁺): 807.3665 (M+H)⁺; calculated for (M+H)⁺: 807.3640; RTI-035A, 1H-
NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, M\(^l\)), 0.85 (d, J=7 Hz, 3H), 1.05 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.75-1.85 (m, 2H), 1.89 (s, 3H), 2.02 (s, 3H), 2.07 (s, M\(^l\)), 1.9-2.15 (m, 4H), 2.35 (s, M\(^l\)), 2.40 (m, IH), 2.75-2.9 (m, 2H), 3.00 (m, 1H), 3.09 (s, 3H), 3.15-3.3 (m, 2H), 3.34 (dd, J=7 and 2Hz, IH), 3.47 (s, IH), 3.60 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, 1H), 4.72 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 8 Hz, IH), 6.03 (dd, J=15 and 6 Hz, IH), 6.18 (d, J=12 Hz, IH), 6.30 (d, J=10 Hz, IH), 6.40 (dd, J=15 and 10 Hz, IH), 8.23 (s, IH), 8.78 (s, IH), 12.93 (s, IH), 14.21 (s, IH).

Preparation of RTI-44 U-deoxy-114mino-4-deoxy-3A[2-spiro-[piperidin-4-yl]]-(IH)4m idazo-(2,5-dihydro)rifamycin S

[0223] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 790.4078 (M+H\(^+\)); calculated for (M+H\(^+\)) : 790.4029; RTI-044C, 1H-NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.05 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.75-1.85 (m, 2H), 1.89 (s, 3H), 2.02 (s, 3H), 2.07 (s, 3H), 1.85-2.15 (m, 4H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, 3H), 3.15-3.3 (m, 2H), 3.3-3.45 (m, 4H), 3.50 (s, IH), 3.45-3.65 (br, 1H), 3.69 (d, J=10 Hz, IH), 4.75 (d, J=10 Hz, IH), 5.08 (dd, J=12 and 7 Hz, IH), 6.04 (dd, J=15 and 6 Hz, IH), 6.18 (d, J=12 Hz, IH), 6.30 (d, J=10 Hz, IH), 6.42 (dd, J=15 and 10 Hz, IH), 8.24 (v,1H), 8.82 (s, IH), 13.00 (s, IH), 14.28 (s, IH).

Preparation of RTI-46 11-deoxy-11-lmino-4-deoxy-3,4[2-spiro-cyclohexyl]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0224] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 789.4122 (M+H\(^+\)); calculated for (M+H\(^+\)) : 789.4076; RTI-046C, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, M\(^l\)), 1.44 (m, IH), 1.7-1.9 (m, 1OH), 1.89 (s, 3H), 2.01 (s, M\(^l\)), 2.06 (s, 3H), 1.95-2.1 (m, 2H), 2.33 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.08 (s, M\(^l\)), 3.34 (dd, J=7 and 3 Hz, IH), 3.45 (s, IH), 3.62 id, J=6 Hz, 1H), 3.68 id, J=10 Hz, IH), 4.75 (d, J=10 Hz, IH), 5.08 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=15 and 6 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.40 (dd, J=15 and 10 Hz, IH), 8.21 (s, IH), 8.87 (s, IH), 13.00 (s, IH), 14.33
Preparation of RTI-49 \(1\)-deoxy-\(\text{-l}\)-imino-4-deoxy-3,4[2-spiro-[l-(ben \(\Sigma\)l)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0225] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^{+}\)): 880.4535 (M+H\(^{+}\))\(^{+}\); calculated for (M+H\(^{+}\))\(^{+}\): 880.4498; RTI-049A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.60 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, m-\(\Delta\)), 1.44 (m, 1H), 1.65-1.85 (m, 2H), 1.91 (s, 3H), 2.01 (s, 3H), 2.07 (s, 3H), 2.35 (s, m-\(\Delta\)), 2.40 (m, 1H), 2.47 (t, J=6 Hz, 2H), 2.76 (d, J=6 Hz, 2H), 2.8-2.95 (m, 4H), 3.00 (m, 1H), 3.09 (s, 3H), 3.09 (s, 3H), 3.33 (dd, J=7 and 2Hz, 1H), 3.46 (s, 1H), 3.60-3.72 (m, 4H), 4.74 (d, J=10 Hz, 1H), 5.08 (dd, J=12 and 7 Hz, 1H), 6.04 (dd, J=16 and 7 Hz, 1H), 6.18 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.40 (dd, J=16 and 10 Hz, 1H), 7.3-7.45 (m, 5H), 8.22 (s,1H), 8.80 (s, 1H), 12.99 (s, 1H), 14.31 (s, 1H).

Preparation of RTI-51 \(1\)-deoxy-\(\text{-l}\)-imino-4-deoxy-3,4[2-spiro-[l-(2-methoxyethyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0226] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^{+}\)): 848.4487 (M+H\(^{+}\))\(^{+}\); calculated for (M+H\(^{+}\))\(^{+}\): 848.4447; RTI-051A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, m-\(\Delta\)), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.65-1.85 (m, 4H), 1.90 (s, 3H), 2.02 (s, 3H), 2.07 (s, 3H), 1.85-2.15 (br, 2H), 2.35 (s, 3H), 2.40 (m, 1H), 2.79 (t, J=5 Hz, 2H), 2.85-2.95 (m, 4H), 3.00 (m, 1H), 3.09 (s, 3H), 3.33 (dd, J=7 and 2Hz, 1H), 3.41 (s, 3H), 3.49 (s, 1H), 3.59 (t, J=5 Hz, 2H), 3.64 id, J=6 Hz, 1H), 3.68 (d, J= 10 Hz, 1H), 4.75 (d, J=10 Hz, 1H), 5.08 (dd, J=12 and 7 Hz, 1H), 6.04 (dd, J=15 and 7 Hz, 1H), 6.16 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.41 (dd, J=15 and 10 Hz, 1H), 8.25 (s,1H), 8.77 (s, 1H), 12.94 (s, 1H), 14.31 (s, 1H).

Preparation of RTI-53 \(1\)-deoxy-\(\text{-l}\)-imino-4-deoxy-3,4[2-spiro-[l-(2-morphoUnoethyl)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0227] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^{+}\)): 903.4904 (M+H\(^{+}\))\(^{+}\); calculated for (M+H\(^{+}\))\(^{+}\): 903.4869; RTI-Q53A, 1H-
NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, M), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.65-1.85 (m, 4H), 1.90 (s, 3H), 2.02 (s, 3H), 2.07 (s, 3H), 1.85-2.15 (br, 2H), 2.34 (s, 3H), 2.40 (m, IH), 2.5-2.65 (m, 6H), 2.74 (m, 2H), 2.85-2.95 (m, 4H), 3.00 (m, 1H), 3.09 (dd, J=7 and 2 Hz, IH), 3.49 (s, IH), 3.64 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, IH), 3.74 (d, J=5 Hz, 4H), 4.75 (d, J=10 Hz, IH), 5.08 (dd, J=12 and 7 Hz, IH), 6.04 (dd, J=15 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.40 (dd, J=15 and 10 Hz, IH), 8.25 K IH), 8.77 (s, IH), 12.94 (s, IH), 14.29 (s, II).

Preparation of RT-57 11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(cyclobutylmethyl)-piperidin-4-yl]]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0228] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 858.4690 (M+H)+; calculated for (M+H)+: 858.4655; RTI-057A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.7-1.85 (m, 8H), 1.90 (s, 3H), 1.9-2.15 (m, 4H), 2.02 (s, 3H), 2.07 (s, 3H), 2.35 (m, 3H), 2.40 (m, IH), 2.60 (m, 3H), 2.7-2.9 (br, 4H), 3.00 (m, IH), 3.09 (s, 3H), 3.34 (dd, J=7 and 2 Hz, 1H), 3.46 (s, IH), 3.63 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, IH), 4.75 (d, J=10 Hz, IH), 5.08 (dd, J=12 and 7 Hz, 1H), 6.03 (dd, J=16 and 7 Hz, IH), 6.17 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.22 (s, IH), 8.80 (s, IH), 12.95 (s, IH), 14.31 (s, IH).

Preparation of RTI-059A 11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(cyclopropylmethyl)-piperidin-4-yl]]-(IH)-imidazo-(2J-dihydro)rifamycin S

[0229] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 844.4536 (M+H)+; calculated for (M+H)+: 844.4498; RTI-059A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.18 (m, 2H), 0.57 (m, 2H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.93 (m, IH), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.7-1.85 (m, 4H), 1.90 (s, 3H), 1.95-2.15 (br, 2H), 2.02 (s, 3H), 2.07 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 2.46 (d, J=7 Hz, 2H), 2.8-3.05 (m, 5H), 3.09 (s, 3H), 3.35 (dd, J=7 and 2 Hz, 1H), 3.49 (s, IH), 3.63 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, IH), 4.74 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, 1H), 6.03 (dd, J=16 and 7 Hz, 1H), 6.17 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.40 (dd,
J=16 and 10 Hz, 1H), 8.25 (s, IH), 8.78 (s, IH), 12.93 (s, 1H), 14.31 (s, IH).

Preparation of RTI-60 11-deoxy-ll-imino-4-deoxy-3_A[2-spiro-[(isopropy)-piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0230] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 832.4542 (M+H)+; calculated for (M+H): 832.4181 (M+H)+. RTI-60A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.60 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.16 (d, J=6 Hz, 6H), 1.44 (m, IH), 1.7-1.8 (m, 4H), 1.88 (s, 3H), 1.95-2.15 (br, 2H), 2.01 (s, 3H), 2.05 (s, 3H), 2.33 (s, 3H), 2.40 (m, IH), 2.75-3.05 (m, 6H), 3.08 (s, 3H), 3.34 (dd, J=7 and 2Hz, IH), 3.47 (s, IH), 3.64 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, IH), 4.75 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.22 (s, IH), 8.76 (s, IH), 12.91 (s, IH), 14.31 (s, IH).

Preparation of RTI-61 11-deoxy-11-imino-4-deoxy-3,4[(2-spiro-[/(t-ethyloxycarbonyl)-piperidin-4-yl)]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0231] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 862.4270 (M+H)+; calculated for (M+H): 862.4240. RTI-61A, 1H-NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.30 (t, J=7 Hz, 3H), 1.44 (m, IH), 1.6-1.85 (m, 4H), 1.89 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s, 3H), 2.06 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, IH), 3.50 (s, IH), 3.61 (d, J=5 Hz, IH), 3.68 (d, J=10 Hz, IH), 3.6-3.8 (br, 2H), 4.0-4.2 (br, 2H), 4.21 (q, J=7 Hz, 2H), 4.72 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.17 (d, J=12 Hz, IH), 6.29 (d, J=10 Hz, 1H), 6.41 (dd, J=16 and 10 Hz, IH), 8.26 (s, 1H), 8.72 (bs, IH), 12.93 (s, IH), 14.21 (s, IH).

Preparation of RTI-63 11-deoxy-114mim-4-deoxy-3,4[(2-spiro-[/(acetyl)^iperidin-4-yl)]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0232] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 832.4181 (M+H)+; calculated for (M+H): 832.4134. RTI-63A, 1H-NMR
(300MHz, CDC13): -0.06 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.45 (m, IH), 1.6-1.85 (m, 4H), 1.89 (s, 3H), 2.03 (s, 3H), 2.06 (s, 3H), 2.20 (s, 3H), 2.25 (s, 3H), 2.35 (s, 3H), 4.15 (IH), 6.03 (dd, i=16 and 6 Hz, IH), 6.18 (d, i=12 Hz, IH), 6.29 (d, i=10 Hz, IH), 6.38 (m, IH), 8.25 (s, IH), 8.66 (s, 0.6H), 8.71 (s, 0.4H), 12.92 (s, IH), 14.16 (s, 0.4H), 14.19 (s, 0.6H).

Preparation of RTI-64 11-deoxy-l-imino-4-deoxy-3,4[2-spiro-[l-(n-propyl)piperidin-4-yl]]-(lH)-imidazol-(2,5-dihydro)rifamycin S

[0233] Following the general procedure (A), the title compound was obtained as a pure solid. HUMS (ESI+): 832.4552 (M+H)+; calculated for (M+H)+ : 832.4498; RTI-064A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 0.96 (t, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.55-1.65 (m, 2H), 1.7-1.85 (m, 4H), 1.90 (s, Mil 1.95-2.15 (br, 2H), 2.02 (s, 3H), 2.07 (s, 3H), 2.35 (s, M+H 2.40 (m, IH), 2.54 (m, 2H), 2.8-2.9 (m, 4H), 3.00 (m, IH), 3.09 (s, 3H), 3.35 (dd, J=7 and 2H, IH), 3.46 (s, IH), 3.62 (d, J=6 Hz, IH), 3.67 (d, J=10 Hz, IH), 4.75 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, i=16 and 7 Hz, IH), 6.17 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.21 (s, IH), 8.78 (s, IH), 12.95 (s, IH), 14.30 (s, IH).

Preparation of RTI-65 l1-deoxy-l4mino-4-deoxy-3A[2-spiro-[l-(cyclopropyl)piperidin-4-yl]]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0234] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 830.4386 (M+H)+; calculated for (M+H)+ : 830.4342; RTI-065A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.45-0.55 (m, 5H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.7-1.85 (m, 4H), 1.90 (s, M+H), 1.95-2.15 (br, 2H), 2.02 (s, 3H), 2.07 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 2.9-3.1 (m, 5H), 3.09 (s, 3H), 3.35 (dd, i=7 and 2 Hz, IH), 3.46 (s, 1H), 3.63 (d, J=6 Hz, 1H), 3.67 (d, J=10 Hz, 1H), 4.75 (d, J=10 Hz, IH), 5.08 (dd, J=12 and 7 Hz, IH), 6.04 (dd, J=16 and 7 Hz, IH), 6.17 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 8.21 (s, IH), 8.79 (s, IH), 8.79 (s, IH), 8.82 (s, IH).
Preparation of RTI-66 11-deoxy-ll-immo-4-deoxy-3A[2-spiro-[l-(eth:yl) -piperidin-4-yl]]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0235] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 818.4388 (M+H)\(^+\); calculated for (M+H)\(^+\) : 818.4342; RTI-066A, 1H-NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.18 (t, J=7 Hz, 3H), 1.44 (m, 1H), 1.7-1.85 (m, 4H), 1.88 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s, 3H), 2.8-2.95 (m, 4H), 3.00 (m, 1H), 3.09 (s, 3H), 3.35 (d, J=7 Hz, 1H), 3.46 (s, 1H), 3.63 (d, J=6 Hz, 1H), 3.67 (d, J=10 Hz, 1H), 4.75 (d, J=10 Hz, 1H), 5.08 (dd, J=12 and 7 Hz, 1H), 6.04 (dd, J=16 and 7 Hz, 1H), 6.16 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.40 (dd, J=16 and 10 Hz, 1H), 8.22 (s, 1H), 8.77 (s, 1H), 12.95 (s, 1H), 14.29 (s, 1H).

Preparation of RTI-67 11-deoxy-l 14mino-4-deoxy-3,4[2-spiro-[l-(heRTIoyl)-piperidin-4-yl]]-(lH)4imidazo-(2,5-dihydro)rifamycin S

[0236] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 916.4169 (M+Na)\(^+\); calculated for (M+Na)\(^+\) : 916.4109. RTI-67A, 1H-NMR (300MHz, CDC13): -0.07 (br, m\(\text{-}\)), 0.60 (br, m\(\text{-}\)), 0.84 (br, m\(\text{-}\)), 1.02 (d, J=7 Hz, 3H), 1.45 (m, 1H), 1.6-1.85 (m, 4H), 1.88 (s, 3H), 2.00 (s, 3H), 2.04 (s, 3H), 1.9-2.2 (m, 2H), 2.34 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.08 (s, 3H), 3.2-3.9 (br, 7H), 4.2 (br, 1H), 4.6 (br, 1H), 5.05 (br, 1H), 6.0 (br, 1H), 6.18 (br, 1H), 6.29 (br, 1H), 6.40 (br, 1H), 7.40 (m, 2H), 7.45 (m, m\(\text{-}\)), 8.25 (s, 1H), 8.6 (brs, 1H), 12.93 (s, 1H), 14.16 (s, 1H).

Preparation of RTI-68 11-deoxy-l l-imino-4-deoxy-3,4[2-spiro-[l-(benzylxycarbonyl)-piperidin-4-yl]]-(lH)4imidazo-(2,5-dihydro)rifamycin S

[0237] Following the general procedure (A), the title compound was obtained as a pure solid. FIRMS (ESI): 924.4435 (M+H)\(^+\); calculated for (M+H)\(^+\) : 924.4396; RTI-68A, 1H-NMR (300MHz, CDC13): -0.09 (d, J=7 Hz, 3H), 0.60 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.6-1.85 (m, 4H), 1.88 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s,
3.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, J=7 Hz), 3.33 (br, IH), 3.49 (s, 1H), 3.61 (d, J=5 Hz, 1H), 3.68 (d, J=10 Hz, 1H), 3.6-3.8 (br, 2H), 4.0-4.2 (m, 2H), 4.72 (d, J=10 Hz, 1H), 5.07 (dd, J=12 and 7 Hz, 1H), 5.20 (s, 2H), 6.03 (dd, J=16 and 7 Hz, 1H), 6.17 (d, J=12 Hz, 1H), 6.29 (d, J=10 Hz, 1H), 6.41 (dd, J=16 and 10 Hz, 1H), 7.38 (m, 5H), 8.26 (s, 1H), 8.70 (bs, 1H), 12.92 (s, 1H), 14.20 (s, 1H).

Preparation of RTI-69 11-deoxy-Il -imino-4-deoxy-3,4[2-spiro-[1-(methyl)-piperidin-4-yl]]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0238] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 804.4213 (M+H)+; calculated for (M+H)+: 804.4185; RTI-069A, 1H-NMR (300MHz, CDCl3): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.45 (m, IH), 1.7-1.85 (m, 4H), 1.90 (s, 3H), 1.95-2.15 (br, 2H), 2.02 (s, 3H), 2.07 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 2.49 (s, 3H), 2.7-2.95 (m, 4H), 3.00 (m, IH), 3.09 (s, 3H), 3.34 (d, J=7 Hz, 1H), 3.48 (s, IH), 3.63 id, J=6 Hz, 1H), 3.68 (d, J=10 Hz, 1H), 4.75 (d, J=10 Hz, 1H), 5.08 (dd, J=12 and 7 Hz, 1H), 6.04 (dd, J=16 and 7 Hz, 1H), 6.17 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.40 (dd, J=16 and 10 Hz, 1H), 8.23 (s, IH), 8.77 (s, 1H), 12.95 (s, IH), 14.29 (s, IH).

Preparation of RTI-70 11-deoxy-l J-imino-4-deoxy-3,4[2-spiro-[!-(2-methylpropyl)-piperidin-4-yl]]-(IH)4imidazo-(2,5-dihydro)rifamycin S

[0239] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 846.4682 (VI+H)+; calculated for (M+H)+: 846.4655; RTI-070A, 1H-NMR (500MHz, CDCl3): -0.09 (d, J=7 Hz, 3H), 0.60 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.94 (d, J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.74-1.85 (m, 3H), 1.89 (s, 3H), 1.9-2.15 (m, 4H), 2.01 (s, 3H), 2.05 (s, 3H), 2.29 (d, J=7 Hz, 2H), 2.33 (s, 3H), 2.40 (m, IH), 2.75-2.85 (m, 4H), 3.00 (m, IH), 3.08 (s, 3H), 3.33 (dd, J=7 and 2Hz, IH), 3.46 (s, IH), 3.63 (d, J=6 Hz, 1H), 3.68 (d, J=10 Hz, 1H), 4.75 (dd, J=10 and 2 Hz, IH), 5.07 (dd, J=12 and 7 Hz, 1H), 6.03 (dd, J=16 and 7 Hz, 1H), 6.16 (d, J=12 Hz, IH), 6.27 id, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.23 (s, IH), 8.78 (s, IH), 12.96 (s, IH), 14.30 (s, IH), 13C-NMR (125 MHz, CDCl3).
Preparation of RTI-74 l1-deoxy-l1-immo-4-deoxy-3,4[2-spiro-l-(phenylaminocarbonyl)-
piperidin-4-y|]-l(1H)-imidazo-(2,5 dihydro)rifamycin An S

[0240] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 909.4433 (M+H)+; calculated for (M+H)+: 909.4400; RTI-074A, 1H-NMR (300MHz, CDCl3): -0.07 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, IH), 1.6-1.85 (m, 3H), 1.89 (s,3H), 1.9-2.25 (m, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, 1H), 3.09 (s, 3H), 3.33 (m, 1H), 3.51 (s, 1H), 3.61 (d, J=6 Hz, IH), 3.68 (d, J= 10 Hz, 1H), 3.6-3.8 (br, 2H), 4.0-4.2 (br, 2H), 4.72 (d, J=10 Hz, 1H), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.40 (m, 2H), 7.15 (m, IH), 7.34 (m, 4H), 8.27 (s,1H), 8.69 (s, 1H), 12.92 (s, 1H), 14.19 (s, IH).

Preparation of RTI-77 l1-deoxy-l1-immo-4-deoxy-3,4[2-spiro-lf ethyloxycarbonyl]-
piperidin-4-y|]-l(1H)-imidazo-(2,5 dihydro)rifamycin S

[0241] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 876.4417 (M+Hf; calculated for (VI+H)+ : 876.4396; RTI-77A, 1H-NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, M43), 0.99 (t, J=7 Hz, 3H), 1.44 (m, IH), 1.6-1.85 (m, 6 H), 1.88 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, 1H), 3.49 (s, IH), 3.60 (d, J=5 Hz, 1H), 3.68 (d, J=10 Hz, IH), 3.6-3.8 (br, 2H), 4.0-4.2 (m, 4H), 4.72 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.17 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.41 (dd, J=16 and 10 Hz, IH), 8.25 (s,1H), 8.7 (bs, IH), 12.93 (s, IH), 14.20 (s, 1H).

Preparation of RTI-81 l1-deoxy-l1-immo-4-deoxy-3,4[2-spiro-l-(isobutyloxycarbonyl)-
piperidin-4-y|]-l(1H)-imidazo(2,3-dihydro)rifamycin S

[0242] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 890.4552 (M+H)+; calculated for (M+H)+: 890.4553; RTI-081, 1H-NMR (300MHz, CDC13): -0.09 id, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.98 (d,
J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.44 (m, 1H), 1.6-1.85 (m, 4H), 1.88 (s,3H), 1.9-2.15 (m, 3H), 2.01 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, 1H), 3.49 (s, 1H), 3.60 (d, J=7 Hz, 1H), 3.68 (d, J= 10 Hz, IH), 3.6-3.8 (br, 2H), 3.95 (m, 2H), 4.0-4.2 (br, 2H), 4.72 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.16 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.25 (s,1H), 8.7 (bs, IH), 12.93 (s, IH), 14,20 (s, IH).

Preparation of RTI-82 11-deoxy-l 1-imino-4-deoxy-3,4[2-spiro-1-(ethylaminocarbonyl)-piperidin-4-yl]imidazo-(2,5-dihydro)rifamycin S

[0243] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI⁺): 883.4175 (M+Na)+; calculated for (M+Na)+ : 883.4218; RTI-G82A, IH-NMR (300MHz, CDCl3): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 id. :=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.20 (t, J=7 Hz, 3H), 1.44 (m, IH), 1.6-1.85 (m, 3H), 1.88 (s,3H), 1.9-2.25 (m, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.3-3.4 (m, 3H), 3.50 (s, IH), 3.61 (d, J=6 Hz, IH), 3.68 (d, J= 10 Hz, IH), 3.6-3.7 (br, 2H), 3.8-4.0 (br, 2H), 4.52 (m, IH), 4.72 (d, J=10 Hz, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, 1H), 6.40 (m, 1H), 8.25 (s,1H), 8.69 (s, IH), 12.92 (s, IH), 14.20 (s, IH).

Preparation of RTI-83 4-deoxy-3,4[2-spiro-1-(ethylaminocarbonyl)-piperidin-4-yl]-imidazo-(2,5-dihydro)rifamycin S

[0244] Following the general procedure (B), the title compound was obtained as a pure solid. HUMS (ESF): 884.4048 (M+Na)⁴; calculated for (M+Na)⁴ : 884.4058; RTJ-083A, IH-NMR (300MHz, CDCl3): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.20 (t, J=7 Hz, 3H), 1.4-1.6 (m, 2H), 1.65-1.85 (m, 3H), 1.74 IS, 3H), 1.95-2.2 (m, 2H), 2.02 (s, 3H), 2.04 (s, 3H), 2.35 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.3-3.4 (m, 3H), 3.43 (s, IH), 3.56 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, IH), 3.7-4.0 (m, 4H), 4.50 (m, 1H), 4.72 id. :=10 Hz, 1H), 5.13 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.18 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.38 (m, 1H), 8.18 (s,1H), 8.90 (s, 1H), 14.57 (s, IH).
Preparation of RTI-84 11-deoxy-ll-imino-4-deoxy-3,4[2-spiro-[l-(isopropyloxycarbonyl)-piperidin-4-yl)]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0245] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 898.4203 (M+ Na\(^+\)); calculated for (M+ Na\(^+\))\(^+\): 898.4215; RTI-084A, 1H-NMR (300MHz, CDC\(_3\)): -0.09 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.30 (d, J=6 Hz, 6H), 1.44 (m, IH), 1.6-1.85 (m, 4H), 1.88 (s, 3H), 1.9-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, IH), 3.50 (s, 1H), 3.61 (d, J=6 Hz, IH), 3.68 (d, J=10 Hz, 1H), 3.6-3.8 (br, 2H), 4.0-4.2 (br, 2H), 4.72 (d, J=10 Hz, IH), 4.99 (m, IH), 5.07 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.28 (d, J=10 Hz, IH), 6.40 (dd, J=16 and 10 Hz, IH), 8.27 (sJH), 8.7 (bs, IH), 12.93 (s, 1H), 14.21 (s, IH).

Preparation of RTI-86 4-deoxy-3,4[2-spiro-[l-(phenylaminocarbonyl)-piperidin-4-yl)]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0246] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 932.4038 (M+ Na\(^+\)); calculated for (M+ Na\(^+\))\(^+\): 932.4058; RTI-086A, 1H-NMR (300MHz, CDC\(_3\)): -0.02 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.4-1.6 (m, 2H), 1.65-1.85 (m, 3H), 1.75 (s, 3H), 1.95-2.2 (m, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 3.00 (m, IH), 3.09 (s, 3H), 3.3 (m, IH), 3.45 (s, IH), 3.58 (d, J=6 Hz, IH), 3.67 (d, J=10 Hz, 1H), 3.8-4.2 (m, 4H), 4.72 (d, J=10 Hz, IH), 5.13 (dd, J=12 and 7 Hz, IH), 6.03 (dd, J=16 and 7 Hz, IH), 6.18 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.38 (r\(_m\), IH), 6.44 (s, 1H), 7.10 (m, 1H), 7.37 (m, 4H), 8.21 (sJH), 8.88 (s, IH), 14.56 (s, IH).

Preparation of RTI-91 11-deoxy-ll-imino-4-deoxy-3,4[2-spiro-[l-(3,3-dimethylbutanoyl)-piperidin-4-yl)]-(IH)4imidazo-(2J-dihydro)rifamycin S

[0247] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^+\)): 910.4589 (M+ Na\(^+\)); calculated for (M+ Na\(^+\))\(^+\): 910.4579; RTI-91A, 1H-NMR (300MHz, CDC\(_3\)): -0.07 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.85 id, J=7 Hz, 3H),
1.05 (m, 3H), 1.10 (s, 9H), 1.45 (m, 1H), 1.6-1.85 (m, 4H), 1.88 (s, 3H), 2.02 (s, 3H), 2.05 (s, Ml), 2.0-2.2 (m, 2H), 2.35 (s, 3H), 2.3-2.45 (m, 3H), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, 1H), 3.47 (s, 0.4H), 3.52 (s, 0.6H), 3.55-3.70 (m, 3H), 3.8-4.0 (m, 2H), 4.5 (m, 1H), 4.75 (m, IH), 5.06 (m, IH), 6.0 (m, IH), 6.17 (m, IH), 6.29 (d, J=10 Hz, IH), 6.4 (m, IH), 8.27 (s, IH), 8.63 (s, 0.6!%), 8.71 (s, 0.4!-i), 12.92 (s, IH), 14.16 (s, 0.4H), 14.20 is, 0.6!%).

**Preparation of RTI-94 11-deoxy-11-imino-4-deoxy-3,4[2-spiro-[1-(n-pentanoyl)-piperidin-4-y]]-(IH)-imidazo-(2,5-dihydro)rifamycin S**

[0248] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI⁺): 874.4644 (M+H)⁺; calculated for (M+H)⁺ : 874.4604; RTI-94A, 1H-NMR (300MHz, CDC13): -0.07 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.97 (t, J= 7Hz, 3H), 1.04 (m, 3H), 1.42 (m, 3H), 1.6-1.85 (m, 6H), 1.88 (s, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 1.9-2.2 (m, 2H), 2.35 (s, 3H), 2.3-2.45 (m, 3H), 3.00 (m, IH), 3.09 (s, 3H), 3.33 (m, IH), 3.49 (s, 0.4H), 3.53 (s, 0.6H), 3.55-3.70 (m, 3H), 3.8-4.0 (m, 2H), 4.5 (m, IH), 4.72 (m, IH), 5.06 (m, IH), 6.0 (m, IH), 6.17 (m, IH), 6.29 (d, J=10 Hz, IH), 6.4 (m, IH), 8.29 (s, IH), 8.63 (s, 0.6!-i), 8.70 (s, 0.4!-i), 12.92 (s, 1H), 14.17 (s, 0.4!-i), 14.20 (s, 0.6!-i).

**Preparation of RTI-97 11-deoxy-114imo-4-deoxy-3,4[2-spiro-[1-(2-methylpropanoyl)~piperidin-4-y]]-(IH)-imidazo-(2,5-dihydro)rifamycin S**

[0249] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI⁺): 860.4482 (M+H)⁺; calculated for (M+H)⁺ : 860.4447; RTI-97A, 1H-NMR (300MHz, CDC13): -0.07 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (m, 3H), 1.20 (d, J= 7Hz, 6H), 1.43 (m, 1H), 1.6-1.85 (m, 4H), 1.88 (s, Ml), 2.02 (s, 3H), 2.05 (s, 3H), 2.0-2.2 (m, 2H), 2.35 (s, 3H), 2.40 (m, IH), 2.89 (m, IH), 3.01 (m, IH), 3.09 (s, 3H), 3.33 (m, IH), 3.47 (s, 0.4H), 3.50 (s, 0.6H), 3.55-3.70 (m, 3H), 3.8-4.1 (m, 2H), 4.5 (m, IH), 4.72 (m, IH), 5.06 (m, IH), 6.01 (dd, J=15 and 6 Hz, IH), 6.18 (d, J=12 Hz, 1H), 6.29 (d, J=10 Hz, IH), 6.39 (m, IH), 8.25 (s, lH), 8.67 (s, 0.6H), 8.70 (s, 0.4H), 12.93 (s, IH), 14.16 (s, 0.4!-i), 14.19 (s, 0.6H).
Preparation of RTI-98 11-deoxy-11-imino-4-deoxy-3′,4′[2-spiro-1-(3-methylbutanoyl)-piperidin-4-yl]-(IH)-imidazo-(2, 5-dihydro)rifamycin S

[0250] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI+): 874.4632 \([\text{M}+\text{H}]^+\); calculated for \((\text{M}+\text{H})^+\): 874.4604. RTI-98A, 1H-NMR (300MHz, CDCl3): \(-0.07\) (d, \(J=7\) Hz, 3H), \(0.61\) (d, \(J=7\) Hz, 3H), \(0.84\) (d, \(J=7\) Hz, 3H), \(1.04\) (m, 3H), \(1.02\) (d, \(J=7\) Hz, 6H), \(1.43\) (m, 1H), \(1.6-1.85\) (m, 4H), \(1.88\) (s, 3H), \(2.02\) (s, 3H), \(2.05\) (s, 3H), \(2.0-2.2\) (m, 3H), \(2.30\) (m, 2H), \(2.35\) (s, 3H), \(2.40\) (m, 1H), \(3.00\) (m, 1H), \(3.09\) (s, 3H), \(3.33\) (m, 1H), \(3.47\) (s, G4H), \(3.50\) (s, 0.6H), \(3.55-3.70\) (m, 3H), \(3.8-4.0\) (m, 2H), \(4.5\) (m, 1H), \(4.72\) (m, 1H), \(5.06\) (m, 1H), \(6.01\) (m, 1H), \(6.17\) (d, \(J=12\) Hz, 0.6H), \(6.18\) (d, \(J=12\) Hz, 0.4H), \(6.29\) (d, \(J=10\) Hz, 1H), \(6.40\) (m, 1H), \(8.24\) (s,lH), \(8.65\) (s, 0.6H), \(8.72\) (s, 0.4H), \(12.92\) (s, 1H), \(14.16\) (s, 0.4H). 14.19 (s, 0.6H).

Preparation of RTI-101 4-deoxy-3′[2-spiro-1-(dimethylaminocarbonyl)-piperidin-4-yl]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0251] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI+): 884.4036 \((\text{M}+\text{Na})^+\); calculated for \((\text{M}+\text{Na})^+\): 884.4058. RTI-101, 1H-NMR (300MHz, CDCl3): \(-0.04\) (d, \(J=7\) Hz, \(M_{il}\)), \(0.61\) (d, \(J=7\) Hz, 3H), \(0.84\) (d, \(J=7\) Hz, 3H), \(1.04\) (d, \(J=7\) Hz, 3H), \(1.44\) (m, 1H), \(1.6\) (m, 1H), \(1.65-1.90\) (m, 3H), \(1.75\) (s, 3H), \(1.95-2.2\) (m, 2H), \(2.01\) (s, 3H), \(2.04\) (s, 3H), \(2.35\) (s, \(M_{il}\)), \(2.40\) (m, 1H), \(2.90\) (s, 6H), \(3.00\) (m, 1H), \(3.09\) (s, 3H), \(3.33\) (m, 1H), \(3.42\) (s, 1H), \(3.57\) (d, \(J=6\) Hz, 1H), \(3.6-3.8\) (m, 5H), \(4.72\) (d, \(J=10\) Hz, 1H), \(5.14\) (dd, \(J=12\) and 7 Hz, 1H), \(6.00\) (dd, \(J=16\) and 7 Hz, 1H), \(6.18\) (d, \(J=12\) FHz, 1H), \(6.27\) (d, \(J=10\) Hz, 1H), \(6.37\) (m, 1H), \(8.19\) (s,lH), \(8.96\) (s, 1H), \(14.62\) (s, 1H).

Preparation of RTI-102 4-deoxy-3′[2-spiro-1-(isobutylaminocarbonyl)-piperidin-4-yl]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0252] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI+): 912.4326 \((\text{M}+\text{Na})^+\); calculated for \((\text{M}+\text{Na})^+\): 912.4371. RTI-102, 1H-NMR (300MHz, CDCl3): \(-0.04\) (d, \(J=7\) Hz, 3H), \(0.61\) (d, \(J=7\) Hz, 3H), \(0.84\) (d, \(J=7\) Hz, \(M_{il}\)), \(0.95\) (d, \(J=7\) Hz, 6H), \(1.04\) (d, \(J=7\) Hz, 3H), \(1.44\) (m, 1H), \(1.6\) (m, 1H), \(1.65-1.90\) (m, 4H), \(1.75\) (s, 3H), \(1.95-2.2\) (m, 2H), \(2.02\) (s, 3H), \(2.05\) (s, 3H), \(2.35\) (s, 3H), \(2.40\) (m, 1H), \(3.00\) (m,
Preparation of RTI-103 4-deoxy-3,4[2-spiro-[l-(isopropylaminocarbonyl)-piperidin-4-yl]]-(IH)4midazo-(2,5-dihydro)rifamycin S

[0253] Following the general procedure (B), the title compound was obtained as a pure solid. FIRMS (ESI⁺): 912.4337 (M+ Na)+; calculated for (M+ Na)+: 912.4371; RTI-103, 1H-NMR (300MHz, CDCl₃): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.21 (d, J=7 Hz, 6H), 1.44 (m, 3H), 1.55 (m, 3H), 1.65-1.90 (m, 3H), 1.75 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 3H), 3.00 (m, 3H), 3.09 (s, 3H), 3.33 (m, 3H), 3.45 (s, 3H), 3.58 (d, J=6 Hz, 3H), 3.66 (d, J=10 Hz, 3H), 3.7-4.0 (m, 4H), 4.03 (m, 1H), 4.33 (d, J=7 Hz, 1H), 4.73 (d, J=10 Hz, 1H), 5.13 (dd, J=12 and 7 Hz, 1H), 6.00 (dd, J=16 and 7 Hz, 1H), 6.18 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.38 (m, 3H), 8.20 (s,1H), 8.89 (s, 1H), 14.59 (s, 1H).

Preparation of RTI-104 4-deoxy-3A[2-spiro-[l-methylpropylaminocarbonyl]piperidin-4-yl]]-(IH)4midazo-(2,5-dihyd)rifamycin S

[0254] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI⁺): 912.4373 (M+ Na)+; calculated for (M+ Na)+: 912.4371; RTI-104, 1H-NMR (300MHz, CDCl₃): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.95 (t, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3F), 1.18 (d, J=7 Hz, 3H), 1.4-1.6 (m, 4H), 1.65-1.85 (m, 3H), 1.75 (s, 3H), 2.0-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 3H), 3.00 (m, 3H), 3.09 (s, 3H), 3.33 (m, 3H), 3.45 (s, 3H), 3.58 (d, J=6 Hz, 3H), 3.66 (d, J=10 Hz, 3H), 3.7-4.0 (m, 5H), 4.30 (d, J=8 Hz, 1H), 4.73 (d, J=10 Hz, 1H), 5.13 (dd, J=12 and 7 Hz, 1H), 6.00 (dd, J=16 and 7 Hz, 1H), 6.18 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.38 (m, 3H), 8.20 (s,1H), 8.89 (s, 1H), 14.59 (s, 1H).
Preparation of RTI-105 4-deoxy-3A[2-spiro-[l-(t-hutylaminocarbonyl)]-piperidin-4-yl]-[lH]-imidazo-(2,5-dihydro)rifamycin S

[0255] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (EST): 912.4333 (M+ Na)+; calculated for (M+ Na)+: 912.4371; RTI-105, 1H-NMR (300MHz, CDC13): -0.05 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, M-), 1.04 (d, J=7 Hz, 3H), 1.40 (s, 9H), 1.44.6 (m, 2H), 1.7-1.85 (m, 3H), 1.75 (s, 3H), 2.0-2.15 (m, 2H), 2.01 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 1H), 3.00 (m, IH), 3.09 (s, M-), 3.33 (m, IH), 3.46 (s, IH), 3.59 (d, J=6 Hz, IH), 3.66 (d, J=10 Hz, IH), 3.7-4.0 (m, 4H), 4.43 (s, 1H), 4.73 (d, J=10 Hz, 1H), 5.13 (dd, J=16 and 7 Hz, IH), 6.00 (dd, J=16 and 7 Hz, IH), 6.18 (d, J=12 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.38 (m, IH), 8.22 (s, IH), 8.87 (s, IH), 14.60 (s, IH).

Preparation of RTI-175 ll-deoxy-ll-hydroxy-4-deoxy-3,4[2-spiro-[l-(isobutyloxycarbonyl)]-piperidin-4-yl]-[lH]imidazo-(2,5-dihydro)rifamycin S

[0256] Following the general procedure (C), the title compound was obtained as a pure solid. HRMS (ESI): 915.4334 (M+ Na)+; calculated for (M+ Na)+: 915.4368; RTI-175, 1H-NMR (300MHz, CDC13): 0.05 (d, J=7 Hz, 3H), 0.63 (d, J=7 Hz, M+), 0.85 (d, J=7 Hz, 3H), 0.96 (d, J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.40-1.60 (m, 2H), 1.7-2.1 (m, 6H), 1.93 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.24 (s, 3H), 2.40 (m, IH), 3.00 (m, IH), 3.07 (s, 3H), 3.48 (m, IH), 3.68 (s, IH), 3.5-3.8 (m, 2H), 3.86 (d, J=6 Hz, 2H), 3.85-4.1 (m, 4H), 4.95 (dd, J=12 and 4 Hz, IH), 5.05 (d, J=10 Hz, IH), 5.54 (s, 1H), 5.99 (d, J=12 Hz, IH), 6.16 (dd, J=16 and 6 Hz, IH), 6.27 (d, J=10 Hz, IH), 6.44 (dd, J=16 and 10 Hz, IH), 6.72 (s, IH), 8.07 (s, IH), 8.22 (bs, IH), 13.61 (s, IH).

Preparation of RTI-176 ll-deoxy-l-l-amino-4-deoxy-3,4[2-spiro-[l-(isobutyloxycarbonyl)]-piperidin-4-yl]-[lH]imidazo-(2,5-dihydro)rifamycin S

[0257] Following the general procedure (C), the title compound was obtained as a pure solid. HRMS (ESI): 892.4689 (M+H)+; calculated for (M+H)+: 892.4710; RTI-176 (RTI2-63B, 1H-NMR (300MHz) (CDC13): -0.05 (d, J=7 Hz, 3H), 0.64 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, M-), 0.96 (d, J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.40-1.70 (m, 2H), 1.7-1.9 (m, 4H), 1.9-
2.1 (m, 2H), 1.94 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.24 (s, 3H), 2.40 (m, IH), 2.6-2.8 (br, 2H), 3.03 (m, IH), 3.07 (s, 3H), 3.52 (m, IH), 3.67 (s, IH), 3.6-3.7 (m, 2H), 3.80 (d, J=10 Hz, IH), 3.91 (d, J=6 Hz, 2H), 3.85-4.1 (m, 2H), 4.11 (d, J=4 Hz, IH), 4.77 (s, 1H), 4.87 (dd, J=12 and 4 Hz, IH), 5.09 (d, J=10 Hz, IH), 5.98 (d, J=12 Hz, IH), 6.18 (dd, J=16 and 6 Hz, IH), 6.25 (d, J=10 Hz, IH), 6.44 (dd, J=16 and 11 Hz, IH), 8.19 (s, IH), 8.24 (bs, 1H), 13.93 (s, IH).

Preparation of RTI-181 il-deoxy-il-amino-4-deoxy-3A[2-spiro-[l-(2-methylpropyl)piperidin-4-yl]]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0258] Following the general procedure (C), the title compound was obtained as a pure solid. HRMS (ESI\(^\dagger\)) \(848.4777 \pm (M+H)⁺\); calculated for (M+H)⁺ : 848.481 1; RTI-181, 1H-NMR (300MHz, CDC13): -0.05 id. J=7 Hz, 3H), 0.63 (d, J=7 Hz, 3H), 0.92 id. J=6 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.40-1.50 (m, IH), 1.7-2.1 (m, 9H), 1.94 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.23 (s, 3H), 2.24 (m, 2H), 2.40 (m, 1H), 2.6-2.8 (m, 4H), 3.03 (m, IH), 3.07 (s, 3H), 3.50 (m, IH), 3.68 (s, IH), 3.80 (d, J=10 Hz, IH), 4.11 (d, J=4 Hz, IH), 4.76 (s, IH), 4.87 (dd, J=12 and 4 Hz, 1H), 5.09 (d, J=10 Hz, IH), 5.98 (d, J=12 Hz, IH), 6.18 (dd, J=16 and 6 Hz, 1H), 6.25 (d, J=10 Hz, IH), 6.44 (dd, J=16 and 11 Hz, IH), 8.27 (s, IH), 8.32 (s, 1H), 14.03 (s, IH).

Preparation of RTI-182 il-deoxy-il-amino-4-deoxy-3A[2-spiro-[l-(4-aminocarbonyl)piperidin-4-yl]]-(IH)-imidazo-(2,5-dihydro)rifamycin S

[0259] Following the general procedure (A), the title compound was obtained as a pure solid. HRMS (ESI\(^\dagger\)) : 889.4678 (M+H)⁺; calculated for (M+H)⁺ : 889.4713; RTI-182, 1H-NMR (300MHz, CDC13): -0.08 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.96 (d, J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.4 (m, IH), 1.60 (m, IH), 1.7-1.85 (m, 4H), 1.88 (s, 3H), 1.95-2.15 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.34 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.12 (m, 2H), 3.33 (m, IH), 3.50 (s, IH), 3.62 (d, J=5 Hz, IH), 3.67 (d, J=9 Hz, IH), 3.6-3.7 (m, 2H), 3.8-4.0 (m, 2H), 4.62 (t, J=5 Hz, 1H), 4.72 (d, J=10 Hz, 1H), 5.06 (dd, J=12 and 7 Hz, IH), 6.02 (dd, J=15 and 7 Hz, IH), 6.16 (d, J=12 Hz, IH), 6.29 (d, J=10 Hz, IH), 6.38 (m, IH), 8.27 (s, 1H), 8.67 (s, IH), 12.92 (s, 1H), 14.58 (s, 1H).
Preparation of **RTI-183** 11-deoxy-11-amino-4-deoxy-3,4-[2-spiro-[l-(isobutylaminocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S

[0260] Following the general procedure (C), the title compound was obtained as a pure solid. HRMS (ESI+): 891.4843 (M+H)+; calculated for (M+H)+ : 891.4870; RTI-183, 1H-NMR (300MHz, CDCl3): -0.05 (d, J=7 Hz, 3H), 0.64 (d, J=7 Hz, 3H), 0.85 (d, J=7 Hz, 3H), 0.94 (d, J=7 Hz, 6H), 1.04 (d, J=7 Hz, 3H), 1.48 (m, 1H), 1.7-1.89 (m, 8H), 1.94 (s, 3H), 2.01 (m, 1H), 2.04 (s, methyl), 2.08 (s, 3H), 2.24 (s, methyl), 2.40 (m, 1H), 3.03 (m, 1H), 3.07 (s, 3H), 3.09 (m, 2H), 3.52 (m, 1H), 3.55-3.75 (m, 3H), 3.75 (s, 1H), 3.81 (d, J=10 Hz, 1H), 3.85-4.0 (m, 1H), 4.13 (d, J=4 Hz, 1H), 4.62 (t, J=5 Hz, 1H), 4.77 (s, 1H), 4.88 (dd, J=12 and 4 Hz, 1H), 5.09 (d, J=10 Hz, 1H), 5.98 (d, J=12 Hz, 1H), 6.18 (dd, J=16 and 6 Hz, 1H), 6.26 (d, J=10 Hz, 1H), 6.44 (dd, J=16 and 1 Hz, 1H), 8.20 (s, 1H), 8.35 (s, 1H), 13.94 (s, 1H).

Preparation of **RTI-75** 4-deoxy-3A/[2-spiro-[l-(t-butyloxycarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S

[0261] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI+): 913.4267 (M+ Na)+; calculated for (M+ Na)+ : 913.4211; RTI-75A, 1H-NMR (300MHz, CDCl3): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, methyl), 1.04 (d, J=7 Hz, 3H), 1.40-1.60 (m, 2H), 1.51 (s, 9H), 1.7-1.85 (m, 3H), 1.75 (s, 3H), 1.9-2.1 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (m, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.33 (m, 1H), 3.43 (s, 1H), 3.57 (d, J=5 Hz, 1H), 3.67 (d, J=10 Hz, 1H), 3.6-3.8 (br, 2H), 3.9-4.1 (br, 2H), 4.72 (d, J=10 Hz, 1H), 5.13 (dd, J=12 and 7 Hz, 1H), 6.02 (dd, J=16 and 7 Hz, 1H), 6.18 id (J=12 Hz, 1H), 6.28 (d, J=10 Hz, 1H), 6.40 (dd, J=16 and 10 Hz, 1H), 8.19 (s, 1H), 8.93 (bs, 1H), 14.59 (s, 1H).

Preparation of **RTI-76** 4-deoxy-3A/[2-spiro-[l-(ethylxocarbonyl)-piperidin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S

[0262] Following the general procedure (B), the title compound was obtained as a pure solid. FIRMS (ESI+): 885.3945 (M+ Na)+; calculated for (M+ Na)+ : 885.3898; RTI-76A, 1H-NMR (300MHz, CDCl3): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.30 (t, J=7 Hz, 3H), 1.40-1.60 (m, 2H), 1.7-1.85 (m, 3H), 1.75 (s, 3H),
Preparation of RTI-78 4-deoxy-3A[2-spiro-[l-(n-propyloxycarbonyl)^iperidin-4-yl]-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0263] Following the general procedure (B), the title compound was obtained as a pure solid.  
FIRMS (ESI'): 899.3989 OAI+ Na)+; calculated for (M+ Na)+ 899.4054;  
RTI-78A, IH-NMR (300MHz, CDC13): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.99 (t, J=7 Hz, 3H), 1.04 (id, J=7 H, 3H), 1.40-1.60 (m, 2H), 1.69 (m, 2H), 1.7-1.85 (m, 3H), 1.75 (s, 3H), 1.95-2.1 (m, 2H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 2H), 3.00 (m, 3H), 3.09 (s, 3H), 3.33 (m, 3H), 3.42 (s, 3H), 3.56 (d, J=5 Hz, 3H), 3.66 (d, J=10 Hz, 3H, 4.72 (d, J=16 Hz, 3H), 5.13 (dd, J=12 and 7 Hz, 3H), 6.00 (dd, J=16 and 7 Hz, 3H), 6.18 (d, J=12 Hz, 3H), 6.27 (d, J=10 Hz, 3H), 6.40 (dd, J=16 and 10 Hz, 3H), 8.07 (s, 1H), 8.92 (bs, 1H), 14.57 (s, 1H).

Preparation of RTI-79 4-deoxy-3,4[2-spiro-[l-(isobutyloxycarbonyl)^iperidin-4-yl]J-(lH)-imidazo-(2,5-dihydro)rifamycin S

[0264] Following the general procedure (B), the title compound was obtained as a pure solid.

HRMS (ESI'): 913.4163 (M+ Na)+; calculated for (M+ Na)+ 913.4211;  
RTI-79A, IH-NMR (300MHz, CDC13): -0.03 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.97 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.40-1.60 (m, 2H), 1.7-1.85 (m, 3H), 1.75 (s, 3H), 1.9-2.1 (m, 3H), 2.02 (s, 3H), 2.05 (s, 3H), 2.35 (s, 3H), 2.40 (m, 2H), 3.00 (m, 3H), 3.09 (s, 3H), 3.33 (m, 3H), 3.42 (s, 3H), 3.56 (d, J=5 Hz, 3H), 3.66 (d, J=10 Hz, 3H, 4.72 (d, J=16 Hz, 3H), 5.13 (dd, J=12 and 7 Hz, 3H), 6.00 (dd, J=16 and 7 Hz, 3H), 6.19 (d, J=12 Hz, 3H), 6.27 (d, J=10 Hz, 3H), 6.39 (dd, J=16 and 10 Hz, 3H), 8.17 (s, 1H), 8.93 (bs, 1H), 14.57 (s, 1H).
Preparation of RTI-80 4-deoxy-3,4-[2-spiro-[1-(beRTIloxy carbonyl)-piperidin-4-yl]]-(1H-imidazo-(2,5-dihydro)rifamycin S

[0265] Following the general procedure (B), the title compound was obtained as a pure solid. HRMS (ESI+): 947.3987 (M+ Na)+; calculated for (M+ Na)+ 947.4054; RTI-80A, 1H-NMR (300MHz, CDCl3): -0.04 (d, J=7 Hz, 3H), 0.61 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.40-1.60 (m, 2H), 1.7-1.85 (m, 3H), 1.74 (s, 3H), 1.9-2.1 (m, 2H), 2.01 (s, 3H), 2.04 (s, 3H), 2.35 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.09 (s, 3H), 3.33 (br, 1H), 3.42 (br, 1H), 3.56 (d, J=8 Hz, 1H), 3.66 (d, J=10 Hz, 3H), 3.7-3.9 (br, 2H), 4.0-4.2 (br, 2H), 4.72 (d, J=10 Hz, 1H), 5.13 (dd, J=12 and 7 Hz, 1H), 5.20 (m, 2H), 6.00 (dd, J=16 and 7 Hz, 1H), 6.18 (d, J=12 Hz, 1H), 6.27 (d, J=10 Hz, 1H), 6.39 (dd, J=16 and 10 Hz, 1H), 7.39 (m, 5H); 8.16 (s, 1H), 8.93 (bs, 1H), 14.57 (s, 1H).

Preparation of RTI-197 11-deoxy-l-hydroxy-4-deoxy-3,4-[2-spiro-[l-(2-methylpropyl)-piperidin-4-yl]]-(1H-imidazo-(2,5-dihydro)rifamycin S

[0266] Following the general procedure (E), the title compound was obtained as a pure solid. HRMS (ESI+): 871.4433 (M+ Na)+; calculated for (M+ Na)+ 871.4470.

Preparation of RTI-197 11-deoxy-l1-hydroxyimino-4-deoxy-3, 4-[2-spiro-[l-(isohutyioxycarbonyl)-piperidinM-4-yl]]-(1H-imidazo-(2,5-dihydro)rifamycin S

[0267] Following the general procedure (D), the title compound was obtained as a solid. HRMS (ESI+): 906.4535 (M+ H)+; calculated for (M+ H)+ 906.4535; RTI-197, 1H-NMR (300MHz, CDCl3): -0.03 (d, J=7 Hz, M+H), 0.62 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.97 (d, J=7 Hz, 3H), 1.04 (d, 1=7 Hz, 3H), 1.35-1.40 (m, 1H), 1.7-1.8 (m, 1H), 1.85-2.1 (m, 6H), 2.00 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 2.33 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.10 (s, 3H), 3.34 (m, 1H), 3.42-3.50 (m, 2H), 3.67 (d, J=10 Hz, 1H), 3.8-3.9 (m, 4H), 3.93 (d, J=6 Hz, 2H), 4.60 (d, J=10 Hz, 1H), 5.23 (dd, J=12 and 8 Hz, 1H), 5.98 (dd, J=15 and 6 Hz, 1H), 6.30 (d, J=12 Hz, 2H), 6.40 (dd, 1=16 and 10 Hz, 1H), 8.35 (s, 1H), 8.92 (bs, 1H), 14.13 (s, 1H).
Preparation of RTI-217 11-deoxy-11-hydroxyimino-4-deoxy-3,4[2-spiro-[l-(isobutyraminocarbonyl)-penicillin-4-yl]]-(1H)-imidazo-(2,5-dihydro)rifamycin S

[0268] Following the general procedure (D), the title compound was obtained as a solid. \( \text{RMS (ESI}^+ \text{): 905.4695 (\text{M}+ \text{H})^+; calculated for (M+ H)}^+ \text{ 905.4662; } \)

RTI-217, 1H-NMR (300MHz, CDC13): -0.03 (d, J=7 Hz, 3H), 0.62 (d, J=7 Hz, 3H), 0.84 (d, J=7 Hz, 3H), 0.95 (d, J=7 Hz, 3H), 1.04 (d, J=7 Hz, 3H), 1.35-1.40 (m, IH), 1.7-1.8 (m, IH), 1.85-2.1 (m, 6H), 2.00 (s, 3H), 2.04 (s, 3H), 2.13 (s, 3H), 2.33 (s, 3H), 2.40 (m, 1H), 3.00 (m, 1H), 3.10 (s, 3H), 3.08-3.14 (m, 2H), 3.34 (m, 1H), 3.45 (s, 1H), 3.47 (d, J=6 Hz, 1H), 3.65-3.8 (m, 5H), 4.60 (m, 2H), 5.23 (dd, J=12 and 8 Hz, 1H), 5.98 (dd, J=16 and 7 Hz, 1H), 6.30 (d, J=12 Hz, 2H), 6.40 (dd, J=16 and 10 Hz, 1H), 8.34 (s, IH), 8.89 (s, IH), 14.14 (s, IH).

Example 13: Preparation of a Rifabutin Derivative Modified on Alternative Sites

[0269] Biotin-glycine-substituted rifabutin derivative RTI-173 contains a substitution at the 21-hydroxy site, yet has a similar activity as rifabutin on G3 cells when combined with doxorubicin, suggesting that this site can be modified without affecting drug-sensitization or cancer inhibition effects. Biotin-glycine-linked rifabutin derivative (RTI-173) has the following formula:

![RTI-173](image)

RTI-173 was prepared by the following method:
[0270] A solution of Glycine-rifabutin (240 mg, 0.27 mmole) in DMF (2 ml) was added to a solution of biotin (65 mg, 0.27 mmol), DMAP (33 mg, 0.27 mmol) and EDCI (52 mg, 0.27 mmole) in DMF (3 ml) at room temperature. The reaction mixture stirred at room temperature overnight and diluted with DCM (40 ml) and washed with water and brine. The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum. The residue was purified by silica gel column chromatography with methanol in DCM as eluent to give 108 mg of the product as purple solid. HRMS (ESI⁺): 152.5538 (M⁺Na)⁺; calculated for (M+ Na⁺) 152.5304.

**Example 14 - MMP2 and VEGF Secretion Assay**

[0271] Human umbilical vein endothelial cells ("FFUVECs") were cultured in endothelial growth medium containing M199 supplemented with 15% FBS, 400 ng/ml bovine brain extract, 100 ng/ml heparin, and antibiotics. Cells were maintained for no more than 6 passages. For assays, HUVECs were plated in a 24-well plate at 100,000 cells per well and treated with either dimethylsulfoxide ("DM SO") as a negative control, Sorafenib, a known inhibitor of MMP2 and VEGF secretion in osteosarcoma, at a concentration of 0.1µM as a positive control, rifabutin at a concentration of 10µM, rifampicin at a concentration of 10µM, or RTI-79 at concentrations of 10, 5, 1, and 0.5 µM. Treatments were added to the media. Media supematents were harvested after 48 hours and assayed for MMP2 and VEGF.

[0272] Enzyme-linked immunosorbent assays ("ELISAs") for secreted MMP2 and VEGF were performed and quantitated. As illustrated in FIGURE 46A and FIGURE 46B, respectively, secretion of MMP2 and VEGF was reduced by RTI-79, rifabutin, and rifampicin to
levels below or comparable to positive control.

Example 15 - Cell Invasion Assay

[0273] HUVEC cells were grown in the presence of RTI-79 at a concentration of 1µM, 5µM, or 10µM, rifabutin 1µM, 5µM, or 10µM, a dose equivalent volume of DM80 as a negative control, or resveratrol at a concentration of 40µM for 24 hours at 37°C in a Chemleon® QCM Collagen Invasion Assay (Millipore). The Chemleon® assay apparatus is a modified 96-well plate wherein each well is equipped with a suspended insert containing an 8µm membrane coated with a thin layer of polymerized collagen. Invading cells migrate through the collagen coating and attach to the bottom of the membrane.

[0274] After 24 hours, the assay membranes were removed and the attached cells were detached and lysed. CyQuant dye was added to the lysed cell preparations, and fluorescence intensity was measured and quantified. Fluorescence intensity is proportional to the number of invading cells.

[0275] Relative fluorescence intensity for the cell invasion assays is shown in FIGURE 47. As shown, RTI-79 and rifabutin inhibited cell invasion relative to negative control, while 10µM RTI-79 inhibited cell invasion to levels comparable to inhibition with 40µM resveratrol. Note that this dose of resveratrol has been observed to be slightly toxic to HUVEC cells after 72 hours.

Example 16 - Tube Formation Assay

[0276] To assess the effect of rifabutin and derivatives on reorganization in angiogenesis, tube formation assays were performed. Tube formation assays measure the ability of endothelial cells plated at subconfluent densities with extracellular matrix support to form capillary-like structures. After plating in the tube formation, the endothelial cells attach and generate mechanical forces on the surrounding extracellular support matrix to form tracks that facilitate cellular migration. The resulting cords of cells ultimately arrange into hollow lumens.

[0277] For each tube formation assay, 75µL of Matrigel was added to a 96-well tissue culture plate and allowed to gelate for 1 hour at 37°C. HUVEC cells were plated at a density of 25,000 cells per well in 100µE of media with 10% serum. RTI-79 or rifabutin were added to the
treatment assays at concentrations of 1µM, 5µM, or 10µM; sorafenib was added at a concentration of 5µM to positive anti-angiogenic control assays; and DMSO was added to a negative control assays. Wells were fixed and imaged at 4, 8, or 24 hours.

[0278] Representative 3X magnification micrographs of the tube formation assays are provided in FIGURE 48. These micrographs illustrate that while tubular networks form for each treatment, including positive control, the networks observed with RTI-79 treatment and rifabutin treatment are not as branched and are less organized than those observed with DMSO treatment, indicative of disrupted sprouting angiogenesis. A dose response is apparent for RTI-79 treatment. Treatment effect is pronounced at 8 hours, and while all tubular networks are deteriorating at 24 hours, this effect is more pronounced in the treated cells.

**Example 17 - Three-Dimensional Collagen Invasion Assays**

[0279] A three-dimensional collagen invasion assay was employed to assess the effects of RTI-79 and rifabutin on cell invasion as a proxy for angiogenesis. Three-dimensional culture system modeling can more closely reflect the microenvironment of tumors and metastases more closely than two-dimensional models.

[0280] Three-dimensional collagen matrices containing pro-angiogenic factor S1P were prepared in 96 well plates. HUVECs were plated at 40,000 cells per well in media containing pro-angiogenic factors VEGF and FGF with or without additional treatments. Treatments include: 20, 10, 5, 2.5, 1.25, 0.6, and 0.3 µM RTI-79 or DMSO as a negative control. HUVECs were allowed to sprout and invade matrices for 24 hours prior to fixation and imaging at 20X.

[0281] Representative micrographs of the three dimensional collagen invasion assays are provided in FIGURES 49A and 49B. As shown in FIGURE 49A, inclusion of RTI-79 in the collagen matrix decreased HUVEC invasion. Control cells treated with DMSO appear spindle shaped and some cell bodies have migrated out of the focal plane as indicated by blurred cells. Cells in treatment groups tend to remain in a single focal plane and have a rounded as opposed to spindle-shaped appearance. As shown in FIGURE 49B, inclusion of RTI-79 in the collagen matrix at 20, 10, and 5 µM resulted in decreased HUVEC cell invasion in terms of number of invading structures per 20x field (p<0.01). Inclusion of 10 µM RTI-79 also resulted in decreased distance over which the cells invaded.
Example 18 - Chick Chorioallantoic Membrane Model Assay

[0282] The chick chorioallantoic membrane ("CAM") model was employed to evaluate the anti-angiogenic effects of RTI-79.

[0283] Eggs were windowed three days after fertilization. Ten days after fertilization, cortisine acetate-treated filter paper disks were placed on the chorioallantoic membrane of the eggs. The disks were saturated with cell medium alone, cell medium containing the supernatant of U2-OS cells grown cultured for 72 hours at a plating density of 100,000 cells/air, or cell medium containing RTI-79 at a concentration of 5μM. U2-OS cell supernatant contains pro-angiogenic factors. Three days after placement of the saturated disks, the chorioallantoic membranes were fixed with 4% paraformaldehyde, excised, and photographed.

[0284] Representative photographs for each treatment arm of the CAM assay are provided in FIGURE 50. As shown, treatment with medium containing RTI-79 at a concentration of 5μM resulted in decreased blood vessel development relative to treatment with cell medium alone and treatment with cell medium containing the supernatant of U2-OS cells.

Example 19 - In Vivo Matrigel Angiogenesis Assay

[0285] Matrigel was injected into mice to form a biocompatible "plug" in which angiogenesis could be observed. 1mL of Matrigel was injected into the groin region of female three-month old C57 mice. All Matrigel treatments except the Matrigel-only negative control contained 150 ng/mL of bFGF to promote angiogenesis. The Matrigel injections also contained sorafenib at a concentration of 10μM or RTI-79 at a concentration of 10μM. Half of the mice injected with RTI-79-containing matrigel also received daily gavage of RTI-79 at a dose of 25 mg/kg/day.

[0286] Ten days after matrigel injection, the mice were injected intravenously with FITC-dextran. 30 minutes after FITC-dextran injection, the matrigel plugs and blood were harvested from each mouse. Plasma was isolated from blood and utilized to normalize FITC concentration per mouse. The plugs were imaged and weighed, then dissolved in dispase overnight. FITC fluorescence from the dissolved plugs and the harvested plasma were measured with a fluorescent plate reader, and the observed fluorescence for each treatment arm was quantitated to determine FITC staining of the plugs normalized to the weight of the plugs and the
FiTC within the plasma.

[0287] Representative micrographs of the FITC-stained matrigel plugs are shown in FIGURE 51A. Inclusion of RTI-79 in the Matrigel injection and RTI-79 gavage decreased the formation of blood vessels relative to Matrigel treated only with bFGF. FIGURE 51B shows the relative FITC fluorescence per gram matrigel plug weight relative to the FITC fluorescence observed in the plasma (RLU plug/g plug/RLU in plasma). Inclusion of RTI-79 in the Matrigel injection and RTI-79 gavage decreased relative FITC fluorescent of the plug as compared to Matrigel treated only with bFGF.

**Example 20: In Vivo Tumor Histochemistry Assay**

[0288] Anti-angiogenic effects of RTI-79 were assessed in vivo by immunohistochemical staining of tumor xenografts.

[0289] Xenografts of ADR-RES ovarian cancer cells were injected into nude mice. The mice receive twice weekly gavage of either saline or 25 mg/kg RTI-79 beginning 9 days after xenograft injection. Tumors were harvested 5 days or 45 days after treatment initiation and fixed in formalin, embedded in paraffin, and sectioned into 7μt sections. The sections were stained with anti-CD31 antibody and counterstained with hematoxylin.

[0290] To quantitate blood vessel formation in the tumors, 4 fields of view at a magnification of 10x were micrographed, and the area of anti-CD31 staining was determined and averaged by Image! analysis.

[0291] Representative micrographs of the tumors are provided in FIGURE 52A. FIGURE 52B shows the quantitative analysis of anti-CD-31 staining in the tumors of mice gavaged with saline and the mice gavaged with RTI-79. As shown, the tumors of saline gavaged mice exhibit greater density of CD-31 staining than did the tumors of mice gavaged with RTI-79.

**Example 21: Example Rifabutin and Rifabutin Derivative Compositions and Methods of Administration to a Patient Exhibiting or at Risk of Pathology-Related Angiogenesis**

[0292] Compositions comprising rifamycin, rifamycin derivative, such as rifabutin or a
rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, can be prepared as described herein. In particular, compositions can be formulated in tablets or capsules for oral use. These tablets or capsules can be extended release tablets or capsules to provide a more stable and continuous supply of the rifamycin or rifamycin derivative to a region exhibiting or susceptible to angiogenesis in the patient. Tablets or capsules can contain at least 10 mg, at least 50 mg, at least 100 mg, at least 150 mg, or at least 200 mg of rifamycin or rifamycin derivative. Combinations tablets or capsules with other drugs can be prepared, particularly if the recommended dosing schedule for those drugs is similar to that of the rifamycin or rifamycin derivative.

[0293] Compositions can also be formulated for intravenous injection as well. In general, the amount of rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof, or combination thereof, can be lower in a dose formulated for intravenous injection than in a dose formulated for oral administration because intravenous injection avoids the need for absorption through the intestines. Injectable doses of the compositions can be provided in multi-use containers or in single-use containers. These containers can be compatible for use with standard intravenous needles and syringes as well as intravenous drip systems. Single-use containers can contain the entire amount of rifamycin or rifamycin derivative administered. Alternatively, they can contain amounts appropriate for daily doses. Single-use containers can contain at least 1 mg, at least 5 mg, at least 10 mg, at least 50 mg, at least 100 mg, or at least 150 mg of rifamycin or rifamycin derivative. Multi-use containers can be designed to allow administration of these same amounts of rifamycin or rifamycin derivative. Injectable compositions can further contain other injectable agents.

[0294] Compositions comprising rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof, or combination thereof can be administered to patients exhibiting or at risk of pathology-related angiogenesis in the form of any compositions described in this example or elsewhere herein or any any other form. In certain embodiments, the rifamycin or rifamycin derivatives can be administered orally to patients exhibiting or at risk of pathology-
related angiogenesis. In particular, they can be administered in the form of tablets or capsules. The rifamycin or rifamycin derivative can be administered such that the patient receives at least 50 mg/adult human/week, at least 100 mg/adult human-\(^\text{2}\) week, at least 150 mg/adult human/week, or at least 300 mg/adult human/week. Amounts can be reduced for children. For example, a child under age 5 might receive one quarter or less of an adult human dose. A child age 5 to age 10 can receive one half to one quarter the adult human dose. A child age 10 or over over can receive three quarters to one half the adult human dose. In another embodiment, the rifamycin or rifamycin derivative can be administered such that the patient receives at least 0.5 mg/kg/week, at least 1 mg/kg/week, at least 2 mg/kg/week, at least 5 mg/kg/week, at least 10 mg/kg/week, at least 20 mg/kg/week, at least 30 mg/kg/week, at least 50 mg/kg/week or at least 100 mg/kg/week.

[0295] Compositions comprising rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof, or a combination thereof, administered orally in this fashion can be administered weekly, daily, or multiple times per day. The dosing schedule can be adjusted so as to maintain minimal blood concentrations for a period of time, particularly with extended release formulations. Alternatively, maintenance of minimal blood concentrations can not be necessary for some methods of treatment and dosing can instead be designed to achieve a total blood concentration for a shorter period of time, such as for four hours or less. Although amounts are expressed as weekly totals, it will be understood that the compositions do not have to be administered for a full week. For example, a patient can receive a single dose in connection with an anti-angiogenic treatment and can not receive a further dose until much later, with another anti-angiogenic treatment, or not at all. Furthermore, it is possible to administer the weekly total through various combinations of doses on various days. For example, it can be possible to administer doses only every other day or every few days. Doses also need not be the same each day. In certain embodiments, the patient can be provided with a pack of varying-dose tablets or capsules labeled by day (e.g. Day 1, Day 2, etc.), by portions of the day (e.g. Day 1 morning, Day 1 evening, etc.), or by week (e.g. Week 1, Week 2, etc.) and instructed to begin taking the tablets or capsules at a specified time dictated by the schedule for administration of an anti-angiogenic agent.
[0296] In certain embodiments, the composition can comprise rifabutin or RTI-79 administered orally in one to three doses of rifabutin or RTI-79 in 100 mg to 300 mg amounts over a period of up to 48 hours. A single oral dose of 300 mg rifabutin causes a mean (±SD) peak plasma concentration (Cmax) of 375 (±267) ng/mL (range 141 to 1033 ng/mL). The plasma elimination of rifabutin is biphasic with an initial half-life of approximately 4 hours, followed by a mean terminal half-life of 45 (±17) hours (range 16 to 69 hours). The rifabutin derivative RTI-79 is expected to present similar results. Maximal RTI-79 plasma concentration is reached within 3 hours of administration. Accordingly, appropriate dosages for variations of this example using intravenously injected rifabutin or RTI-79 rather than orally administered forms can be calculated. In an alternative embodiment, rifamycin or a rifamycin derivative, such as rifabutin or RTI-79, can be administered in a method that matches the pharmacokinetics of the rifamycin or rifamycin derivative to that of the anti-angiogenic agent also administered to the patient.

[0297] In another alternative embodiment, a composition comprising rifamycin, rifamycin derivative, such as rifabutin or a rifabutin derivative, rifampicin or rifampicin derivative, or pharmaceutically acceptable salt, hydrate, or prodrug thereof! or combination thereof, such as rifabutin or RTI-79, can be administered in amounts similar to those described herein to reduce or prevent metastatic transformation of tumors due to angiogenesis.

* * *

[0298] Although only exemplary embodiments of the invention are specifically described above, it will be appreciated that modifications and variations of these examples are possible without departing from the spirit and intended scope of the invention. For example, various specific formulations including components not listed herein and specific methods of administering such formulations can be developed using the ordinary skill in the art. Numeric amounts expressed herein will be understood by one of ordinary skill in the art to include amounts that are approximate!') or about those expressed. Furthermore, the term "or" as used herein is not intended to express exclusive options (either/or) unless the context specifically indicates that exclusivity is required; rather "or" is intended to be inclusive (and/or).
CLAIMS

1. A composition comprising a rifamycin derivative or a pharmaceutically acceptable salt, hydrate, or prodnig thereof in an amount and formulation sufficient to inhibit one or more of angiogenesis and lymphangiogenesis in an organism.

2. The composition of claim 1, where R the rifamycin derivative has the following formula:

\[ \text{(I),} \]

wherein \( R \) comprises one of the following structures:

\[ \text{R = -H, } \]

\[ \text{structures:} \]

- \( -\text{H} \)
- \( -\text{alkyl} \)
- \( -\text{aromatic} \)
- \( -\text{alkyl ether} \)
- \( -\text{alkyl amine} \)
3. The composition of claim 1, wherein the rifamycin derivative has the following formula:

![Chemical Structure Diagram]

(III),

wherein R comprises one of the following structures:

- \(-\text{H}\)
- \(-\text{H}\)
- \(-\text{H}\)
- \(-\text{H}\)
- \(-\text{H}\)
- \(-\text{H}\)
4. The composition of claim 1, wherein the rifamycin derivative has the following formula:

\[
\begin{align*}
\text{wherein } X & \text{ is a C, O, or N and comprises one of the following structures:} \\
X=O, & \quad R = \text{ } & & \text{ or } \\
X=NH, & \quad R = \text{ } & & \text{ or } \\
X-R & = \text{ } & & \text{ or }
\end{align*}
\]
5. The composition of claim 1, wherein the rifamycin derivative has the following formula:

\[
\begin{align*}
\text{wherein } X &\text{ is a } C, O, \text{ or } N \text{ and } R \text{ comprises one of the following structures:} \\
X=O, \quad R &= \quad \text{or} \\
X=\text{NH}, \quad R &= \quad \text{or} \\
X-\text{R} &= \quad \text{or}
\end{align*}
\]
6. The composition of claim 1, wherein the rifamycin derivative has the following formula:

![Chemical structure diagram]

wherein R comprises one of the following structures:
7. The composition of claim 1, wherein the rifamycin derivative has the following formula:

\[ \text{Rifamycin Derivative} \]

wherein R comprises

\[ \text{Acetal Group} \]
8. The composition of claim 1, wherein the rifamycin derivative has the following formula:

wherein \( X \) is a C, O, or N and \( R \) comprises one of the following structures:

\[
\begin{align*}
X=O, & \quad R = \text{structure 1} \\
X=NH, & \quad R = \text{structure 2} \\
X=\text{other}, & \quad R = \text{structure 3}
\end{align*}
\]

9. The composition of claim 1, further comprising a pharmaceutically acceptable carrier, a salt, a buffer, a preservative, or a solubility enhancer.

10. A composition comprising a rifabutin derivative or a pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to both inhibit one or more of angiogenesis and lymphangiogenesis in an organism and to induce drug-sensitization in or inhibition of a cancer cell in the organism.
11. The composition of claim 10, further comprising the drug for which the rifabutin derivative is operable to induce drug-sensitization in a cancer cell.

12. The composition of claim 10, further comprising one or more chemotherapeutic drugs.

13. The composition of claim 12 wherein the chemotherapeutic drug comprises an alkylating agent, an antimetabolite, an anti-tumor antibiotic, a hormonal agent, a targeted therapy, or a differentiating agent.

14. A method of inhibiting angiogenesis in an organism comprising administering rifamycin or a rifamycin derivative to the organism in an amount and for a time sufficient to inhibit angiogenesis in the organism.

15. A method of inhibiting lymphangiogenesis in an organism comprising administering rifamycin or a rifamycin derivative to the organism in an amount and for a time sufficient to inhibit lymphangiogenesis in the organism.

16. A method of inhibiting both angiogenesis and lymphangiogenesis in an organism comprising administering rifamycin or a rifamycin derivative to the organism in an amount and for a time sufficient to inhibit both angiogenesis and lymphangiogenesis in the organism.

17. A method of treating a pathology associated with angiogenesis or lymphangiogenesis in an organism comprising administering rifamycin or a rifamycin derivative to the organism in an amount and for a time sufficient to inhibit the angiogenesis or lymphangiogenesis.

18. The method of claim 17, wherein the pathology is selected from the group consisting of cancer, age-related macular degeneration, diabetic retinopathy, rheumatoid arthritis, and combinations thereof.

19. A method of inhibiting angiogenesis within a tissue and sensitizing a cancer cell within the tissue to a drug comprising administering rifamycin or a rifamycin derivative to the
tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue and to sensitize the cancer cell to the drug.

20. The method of claim 19, wherein administering rifamycin or rifamycin derivative to the tissue comprises administering the rifamycin or a rifamycin derivative to a patient in whom the tissue is located.

21. The method of claim 19, further comprising administering rifamycin or rifamycin derivative to the tissue before the drug to which the cancer cell is sensitized.

22. The method of claim 19, further comprising administering rifamycin or rifamycin derivative to the tissue concurrently with the dmg to which the cancer cell is sensitized.

23. The method of claim 19, further comprising administering rifamycin or rifamycin derivative to the tissue after the drug to which the cancer cell is sensitized.

24. The method of claim 19, further comprising administering the rifamycin or rifamycin derivative to the tissue a second or greater time.

25. The method of claim 19, wherein administering rifamycin or rifamycin derivative to the tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue and to sensitize the cancer cell to the drug comprises rendering the cancer cell susceptible to a therapeutic effect of the dmg at a lower dose than in the absence of rifamycin or rifamycin derivative.

26. The method of claim 19, wherein administering rifamycin or rifamycin derivative to the tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue and to sensitize the cancer cell to the dmg comprises rendering the cancer cell susceptible to a therapeutic effect of the drug that the cancer cell would not be susceptible to in the absence of rifamycin or rifamycin derivative.

27. The method of claim 19, wherein the drug comprises a chemotherapeutic and wherein administering rifamycin or rifamycin derivative to the tissue in an amount and for a time sufficient to inhibit angiogenesis within the tissue and to sensitize the cancer cell to the drag
comprises rendering the cancer cell susceptible to death or a decrease in growth due to the
chemotherapeutic.

28. The method of claim 19, wherein the cancer cell is a carcinoma, a sarcoma, as
leukemia, a lymphoma, or a glioma.

29. The method of claim 19, wherein the cancer cell is a metastatic cancer cell.

30. A method of inhibiting angiogenesis within a tissue and inhibiting a cancer cell
within the tissue with a drug comprising:

administering rifamycin or rifamycin derivative to the tissue in an amount and for a time
sufficient to inhibit angiogenesis within the tissue;

administering rifamycin or rifamycin derivative to the cancer cell in an amount and for a
time sufficient to sensitize the cancer cell to the drug; and

administering the drug to the cancer cell in an amount and for a time sufficient to inhibit
the cancer cell, wherein the amount or time are less than that required to achieve the same
inhibition in the absence of rifamycin or rifamycin derivative.

31. The method of claim 30, wherein administering rifamycin or rifamycin derivative
to the cancer cell comprises administering the rifamycin or rifamycin derivative to a patient in
whom the cancer cell is located.

32. The method of claim 30, wherein administering rifamycin or rifamycin derivative
to the tissue comprises administering the rifamycin or rifamycin derivative to a patient in whom
the tissue is located.

33. The method of claim 30, wherein administering rifamycin or rifamycin derivative
to the cancer cell comprises administering the rifamycin or rifamycin derivative to a patient in
whom the cancer cell is located, and wherein wherein administering rifamycin or rifamycin
derivative to the tissue comprises administering the rifamycin or rifamycin derivative to a patient in
whom the tissue is located.
34. The method of claim 30, further comprising administering the rifamycin or rifamycin derivative concurrently with the drug.

35. The method of claim 30, further comprising administering the rifamycin or rifamycin derivative before administering the drug.

36. The method of claim 30, further comprising administering the rifamycin or rifamycin derivative after administering the drug.

37. The method of claim 30, further comprising administering rifamycin or rifamycin derivative to the cancer cell a second or greater time.

38. The method of claim 30, wherein the drug is a chernotherapeutic drug.

39. The method of claim 30, wherein the inhibition is death of the cancer cell.

40. The method of claim 30, wherein the inhibition is a decrease in growth of the cancer cell, leading to a decrease in growth of the cancer containing the cancer cell.

41. The method of claim 30, wherein the cancer cell is a carcinoma, a sarcoma, as leukemia, a lymphoma, or a glioma.

42. The method of claim 30, wherein the cancer cell is a metastatic cancer cell.
Multiple Treatments with CHOP

CHOP Treatment → CHOP Sensitization

Gene Expression Changes

↑ ROS

↓ ROS

Block to Apoptosis

↓ CHOP Treatment → CHOP Resistance

Acquisition of CHOP Resistance

Akt Activation

Akt

β-Catenin

GSK3

ETC Complex I

Chemosensitizers (rifabutin)

↓ 14-3-3ζ

↓ P
FIGURE 3B

![Graph showing cell growth (% control) versus compound concentration (uM) for different treatments: Doxorubicin, Doxorubicin + 10 uM Rifabutin, and Rifabutin.]
FIGURE 9

SK-OV-3 xenograft

- Tumor Volume (mm$^3$)
- Days Post-Treatment

- Saline
- Dox Only
- Dox + Rifabutin
FIGURE 12

Cell Growth (% Control) vs. Compound Concentration (μM)

- Rifabutin
- Rifabutin + 1 μM DOX
- RTI-79
- RTI-79 + 1 μM DOX
FIGURE 15

- Rifabutin
- Rifabutin + 0.2 uM DOX
- Vehicle + 0.2 uM DOX
FIGURE 25

- Mitoxantrone + 10 uM Rifabutin
- Mitoxantrone
- Vehicle
- Rifabutin
FIGURE 27

Cell Growth (% Control) vs. Compound Concentration (μM)

- Paclitaxel
- Paclitaxel + 10 μM Rifabutin
**FIGURE 28**

A line graph showing cell growth (% control) against compound concentration (uM). The graph compares different treatments:
- Camptothecin
- Camptothecin + 25 uM Rifabutin
- Vehicle
- Vehicle + 25 uM Rifabutin
FIGURE 31

[Bar chart showing ROS (G.ROX fluorescence) levels for Control, CHOP 1 hr, CHOP 24 hr, 265s (CHOP Sensitive), Control, CHOP 1 hr, CHOP 24 hr, G5 (CHOP Resistant).]
FIGURE 34

- 2631 (low ROS)+DMSO
- 2631 (low ROS)+Rifabutin

Resazurin Fluorescence vs. CHOP (ng/ml)
FIGURE 35

[Bar chart showing ROS (CellROX fluorescence) over time of Rifabutin treatment (min).]
FIGURE 37A

![Graph showing inhibition (%) against concentration (µM) for various compounds: RBT, RMP, RTI-51, RTI-53, RTI-78, RTI-79, RTI-174, RTI-181, RTI-183.](image-url)
FIGURE 37D

Fluorescence Intensity

RBT  control
FIGURE 38A

OVCA198 Ovarian Carcinoma

FIGURE 38B

ADR Ovarian Carcinoma
FIGURE 38C

[Diagram showing ROS levels for different treatments, including DMSO, RTI-75, Reserpine, Eburidil, Carbazin, and Nisazolidine. The bar for G3 Lymphoma is significantly higher than the others.]
FIGURE 43

- **OVCAR8 ROS**
  - Control siRNA
  - P-gp siRNA
  - No RTI-79
  - 10 uM RTI-79

- **ADR ROS**
  - Control siRNA
  - P-gp siRNA
  - No RTI-79
  - 10 uM RTI-79

- **OVCAR8 Calcium**
  - Control siRNA
  - P-gp siRNA
  - No RTI-79
  - 10 uM RTI-79

- **ADR Calcium**
  - Control siRNA
  - P-gp siRNA
  - No RTI-79
  - 10 uM RTI-79
FIGURE 46A

MMP2

* <0.01
** <0.001
**** <0.0001

Response

Ctrl  79-10uM  79-5uM  79-1uM  79-0.4uM  10uM Rbt  10uM Rftp  5uM Sor.
**FIGURE 46B**

![VEGF Bar Chart]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>0.09</td>
</tr>
<tr>
<td>79-10uM</td>
<td>0.07</td>
</tr>
<tr>
<td>79.5uM</td>
<td>0.06</td>
</tr>
<tr>
<td>79.1uM</td>
<td>0.05</td>
</tr>
<tr>
<td>79-0.4uM</td>
<td>0.04</td>
</tr>
<tr>
<td>10uM Rbt</td>
<td>0.06</td>
</tr>
<tr>
<td>10uM RfrMP</td>
<td>0.07</td>
</tr>
<tr>
<td>5uM Sor.</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* and ** indicate significance at p < 0.05 and p < 0.01, respectively.
**FIGURE 47**

**HUVEC Cell Invasion**

- **No Drug**: High fluorescence
- **10μM T39**: Moderate fluorescence
- **5μM T39**: Lower fluorescence
- **1μM T39**: Lower fluorescence
- **10μM Rif**: Low fluorescence
- **5μM Rif**: Low fluorescence
- **1μM Rif**: Low fluorescence
- **40μM Resveratrol**: Low fluorescence

*\( p = 0.0133 \)

**\( p = 0.0014 \)
FIGURE 48

[Image of a figure with different treatments and time points]
FIGURE 49B

RTI-79 inhibits invasion of HUVECs into Collagen

* p < 0.01
FIGURE 50

RTI-79 in media

Media alone

U2-OS media
FIGURE 52A

Anti CD-31

Saline

RTI-79

FIGURE 52B

Area of CD-31 stained capillaries

Pixel Area

0 5000 10000 15000 20000 25000 30000

5 days 45 Days

Saline

79 only
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/395 (2015.01)
CPC - A61K 31/395 (2015.05)
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)
IPC(8) - A61K 31/395; A61P 31/04 (2015.05)
CPC - A61K 31/395, 31/445; C07D 471/04, 498/22 (2015.05)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/649; 514/183, 314; 540/460 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Search terms used: rifamycin derivative spiro piperidinyl angiogenesis lymphangiogenesis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>US 20110059136 A1 (BORODY) 10 March 2011 (10.03.2011) entire document</td>
<td>1, 2, 9-13</td>
</tr>
</tbody>
</table>

** Further documents are listed in the continuation of Box C. **

* 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 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 25 June 2015
Date of mailing of the international search report 23 JUL 2015

Name and mailing address of the ISA/US
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Facsimile No. 571-273-3201

Authorized officer:
Blaine R. Copenheaver
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. □ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. □ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1, 2, and 9-13 have been analyzed subject to the restriction that the claims read on the composition of claim 1 of the instant invention as described in the Lack of Unity of Invention (See Box IV). The claims are restricted to a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to inhibit one or more of angiogenesis and lymphangiogenesis in an organism, wherein the rifamycin derivative is Formula (I), wherein R comprises H.

See Extra Sheet

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1, 2, 9-13

**Remark on Protest**

- □ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- □ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- □ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: Claims 1-13 are drawn to compositions comprising rifamycin derivatives.

Group II: Claims 14-42 are drawn to methods thereof.

The first invention of Group I is restricted to a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to inhibit one or more of angiogenesis and lymphangiogenesis in an organism, wherein the rifamycin derivative is Formula (I), wherein R comprises methyl. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "y" group(s) will result in not being the only claimed invention to be searched/examined.

The inventions listed in Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof, are not present in Group II and the special technical features of Group II, methods of inhibiting and treating, are not present in Group I.

The Groups I and II formulae do not share a significant structural element, requiring the selection of alternative formulae as well as the rifamycin derivative.

The Groups I and II share the technical features of a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to inhibit one or more of angiogenesis and lymphangiogenesis in an organism; and a composition comprising a rifabutin derivative or a pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to both inhibit one or more angiogenesis and lymphangiogenesis in an organism and to induce angiogenesis-associated increase in or inhibition of a cancer cell in the organism. However, these shared technical features do not represent a contribution over the prior art.

Specifically, "Rifampicin as an Oral Angiogenesis Inhibitor Targeting Hepatic Cancers" to Shichiri et al. teach a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to inhibit or more of angiogenesis and lymphangiogenesis in an organism (Abstract, Rifampicin, a semisynthetic antibiotic derived from the rifamycins...; Pg. 4761, Col. 1, 1st para., animals were moved to a cage supplied with sterile drinking water containing either 0.2 mg/mL rifampicin/0.25% DMSO or 0.25% DMSO alone. Tumor growth during the treatment period was monitored by measuring the tumor mass on each animal using vernier calipers twice a week.; Pg. 4765, Col. 2, last para., Down-regulation of angiogenesis-associated genes by rifampicin.; Pg. 4766, Fig. 5, Rifampicin and rifamycins down-regulate growth, migration, and angiogenesis-associated genes. ; Fig. 5 shows Rifampicin); and a composition comprising a rifabutin derivative or a pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to both inhibit one or more angiogenesis and lymphangiogenesis in an organism and to induce drug-sensitization on or in or inhibition of a cancer cell in the organism (Abstract, Rifampicin, a semisynthetic antibiotic derived from the rifamycins...; Rifampicin and rifabutin are semi-synthetic derivatives of rifamycins; Pg. 4761, Col. 1, 1st para., animals were moved to a cage supplied with sterile drinking water containing either 0.2 mg/mL rifampicin/0.25% DMSO or 0.25% DMSO alone. Tumor growth during the treatment period was monitored by measuring the tumor mass on each animal using vernier calipers twice a week.; Pg. 4765, Col. 2, last para., Down-regulation of angiogenesis-associated genes by rifampicin.; Pg. 4766, Fig. 5, Rifampicin and rifamycins down-regulate growth, migration, and angiogenesis-associated genes. ; Fig. 5 shows Rifampicin).

Additionally, "Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis" to Hoenrauff teaches a composition comprising a rifamycin derivative or pharmaceutically acceptable salt, hydrate, or prodrug thereof in an amount and formulation sufficient to inhibit one or more angiogenesis and lymphangiogenesis in an organism (Pg. 677, Col. 1, Para. 3. Another unique feature of doxycycline in treating lymphatic filariasis is its ameliorating effect on disease. In short, the diameter of scrotal lymph vessels, a marker for lymphangiogenesis induced by filarial worms... Preceding this event by several months, treated patients also display a reduction in the plasma levels of VEGFs [10] which are essential for angiogenesis (VEGF-A) as well as lymphangiogenesis (both VEGF-A and VEGF-C, [60-62]) and produced by cells of the immune system in response to bacterial stimuli.; Pg. 676, Col. 2, Para. 2, Doxycycline in lymphatic filariasis. All studies in bancroftian filariasis (W. bancrofti) used a daily dose of 200mg of doxycycline.; Pg. 677, Col. 2, Para. 5. However, the exploitation of antibiotics is only at its beginning. For example, rifampicin has shown antibacterial (and antifilarial) activity and is proving to be just as successful as doxycycline in animal models...). In fact, a combination of the two drugs reduces Wolbachia loads to a higher extent and in a shorter time in a murine filariasis model...).

The inventions listed in Groups I and II therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.