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(54) **Title**: PHARMACEUTICAL COMPOSITIONS AND METHODS FOR COUNTERING CHEMOTHERAPY INDUCED CARDIOTOXICITY

(57) **Abstract**: This disclosure provides methods and pharmaceutical compositions for reducing or eliminating cardiotoxicity, particularly cardiotoxicity induced by a cancer treatment or other therapy. In some cases, the methods and compositions prevent or reduce cardiotoxicity caused by anthracycline treatment. The methods provided herein often comprise administering a protective agent such as myricetin, tricetin, robinetin, ficetin, vitexin, quercetin, dihydrorobinetin, kaempferol, 7,3',4',5'-tetrahydroxyflavone, and myricitrin in conjunction with the administration of a cancer drug or other treatment. They may comprise administering a protective agent in combination with dexrazoxane. The compositions provided herein include co-formulations of a protective agent with a different protective agent or with a cancer treatment (e.g., anthracycline drug).

PHARMACEUTICAL COMPOSITIONS AND METHODS FOR COUNTERING CHEMOTHERAPY INDUCED CARDIOTOXICITY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the priority of U.S. provisional application Serial Number 62/291,480, filed February 4, 2016, and U.S. provisional application Serial Number 62/348,102, filed June 9, 2016, each of which is hereby incorporated by reference herein in its entirety for all purposes.

BACKGROUND

Cardiotoxicity and congestive heart failure are serious side-effects of oncological therapies, most prominently those comprising anthracyclines, which are administered to greater than one million cancer patients per year and half of all childhood cancer patients. Adverse cardiac side effects are also observed in patients treated with protein kinase inhibitors and antibody-based biologics that target protein kinase. Certain reductions in heart failure rates have been achieved by capping the maximal doses of anthracyclines and by changing their administration schedules, all of which severely limits the therapeutic potentials of these anticancer agents. The cardiotoxicity of cancer drugs can also preclude those patients with pre-existing cardiac conditions from receiving treatment.

Anthracyclines are generally a class of compounds that have the structural core of anthracene. They often are highly effective chemotherapeutics and therefore are used for the treatment of many cancers, including leukemias, lymphomas, breast, uterine, ovarian, bladder cancer, and lung cancers and are often used in childhood cancer treatment regimens. Some anthracycline drugs include doxorubicin, daunorubicin, idarubicin, and epirubicin. Although the exact mechanisms may yet to be validated, anthracyclines have been reported to work by inhibiting DNA and RNA synthesis; promoting free radical formation through redox cycling, with iron promoting the conversion of superoxide into hydroxyl radicals; inhibiting topoisomerases (e.g., topoisomerases IIα and/or IIβ); and evicting histones from open chromosomal areas.

A common side effect of anthracycline use is associated with cardiotoxicity, which is dose dependent and may also result from cumulative exposures. Cardiotoxicity, in some

instances, may result from the formation of toxic reactive oxygen species through redox cycling during the metabolism of anthracyclines and from the formation of double-stranded DNA breaks caused by inhibition of topoisomerase II. The reactive oxygen species (ROS) may activate apoptotic pathways, leading to cell death in both cancer and normal cells. Cardiomyocytes may be sensitive to the oxidative stress. Cardiac mitochondria can be easily injured by anthracycline and anthracycline-iron complexes, which have a high affinity for dianionic phospholipid cardiolipin that is present at a high concentration in the inner mitochondrial membrane.

Some protein kinase inhibitors-including small molecule and biologic inhibitors may also cause cardiotoxicity. Protein kinase inhibitors are a wide class of compounds that inhibits the activity of protein kinases and can be used in cancer treatments. Tyrosine kinases regulate a variety of cellular functions including cell growth (e.g., epidermal growth factor ("EGFR") and dysregulation may lead to certain forms of cancer. Inhibition of such tyrosine protein kinases may be accomplished by using small molecules that bind to the ATP pocket of a given protein kinase, blocking it from catalyzing the phosphorylation of target proteins. Small molecules may cause cardiotoxicity by: (1) selectively inhibiting kinases that also play a role in heart cells (e.g., on-target side effects); (2) targeting multiple kinases in the same pathway (e.g., impacting off-target kinases); and (3) inhibiting non-kinase targets that play a role in heart function; small molecules may also cause cardiotoxicity through a different mechanism. Cardiotoxicity of TKI inhibitors such as imatinib mesylate (Gleevec®), Nilotinib (Tasigna®), sorafenib (Nexavar®), sunitinib (Sutent®) and dasatinib (Sprycel®) has been reported previously (Chu *et al.*, Lancet (2007) 370:2011-2019; Xu *et al.*, Hematol Rev. (2009) Mar1; 1(1): e4; Kerketla *et al.*, Nature Medicine (2006) 12:908-916).

Protein kinase activity may also be inhibited by biologic drugs such as monoclonal antibodies against receptor protein kinases. These therapeutics may exert efficacy by preventing receptor protein kinases from activating and are generally able to bind cell surface antigens with high specificity. Several monoclonal antibodies target receptor protein kinases that play an important role in heart function and thus may cause cardiotoxicity as a result. Trastuzumab and bevacizumab are examples of monoclonal antibodies that can cause cardiotoxicity (e.g., heart failure resulting from cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress). Proteasome inhibitor

chemotherapy compounds (e.g., bortezomib) are also known to be associated with cardiotoxicity and heart failure.

Currently, the bisdioxopiperazine dexrazoxane (DEX) is the only drug approved for reducing the incidence of cardiotoxicity and heart failure in cancer patients receiving anticancer agents. Despite its clinical effect, DEX is only approved for the treatment of patients with metastatic breast cancer who have already received accumulated dose of 300-500 mg/m^2 anthracyclines like doxorubicin or epirubicin. DEX is not approved for use in children and adolescents, it is particularly disheartening to find reports of high incidences of heart failure in anthracycline-treated young children in their later life of post-cancer. Further, the limited indication approval and use are also testament of DEX's shortcomings, which include interfering with antitumor efficacy of anthracyclines, inducing secondary malignancies, and causing blood and bone marrow disorders.

Given the serious impact that many cancer therapies exert on heart function, there exists a clear clinical need for developing an effective drug that prevents, alleviates, or eliminates cardiotoxicity caused by anthracyclines, protein kinase inhibitors (e.g., tyrosine kinase inhibitor), proteasome inhibitors and other cancer treatments. Of particular importance is the development of drugs that can prevent or reduce cancer drug-induced cardiotoxicity without significantly interfering with the anticancer action of the cancer drug. Also important is the development of cardioprotective drugs that do not cause serious side effects such as neutropenia, worsening of heart problems, or increased risk of secondary malignancies. These potential drugs will significantly improve existing cancer therapy, not only by protecting from potential heart injuries in cancer patients, but also by enabling chemotherapy doses optimized to achieve maximum-anti-cancer effects.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 generally depicts a method of reducing cancer treatment-induced cardiotoxicity in a patient by co-administering a cancer treatment and protective agent to the patient.

Fig. 2 generally depicts co-administration of a cancer treatment, dexrazoxane (DEX), and a protective agent.

Fig. 3 depicts the effects of mock treatment, doxorubicin (DOX), myricetin, or a co-administration of doxorubicin and myricetin on cell survival in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) 3 days following treatment.

Fig. 4A-B depicts the effects of doxorubicin (DOX) (4A), or a co-administration of doxorubicin and myricetin (4B) on mitochondrial health in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) 2 days following treatment.

Fig. 5 depicts the effects of mock treatment, doxorubicin, or a co-administration of doxorubicin and myricetin on contractility in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) 3 days following treatment.

Fig. 6A-C depict a chart providing the raw data (6A) or normalized data (6B) for the experiments depicted in Fig. 3, or the raw data for the experiments depicted in Fig. 5 (6C).

Fig. 7A-C depict the effects of myricetin (7A), myricitrin/myricetrin (7B), or dihydromyricetin (7C) on doxorubicin-induced apoptosis at increasing concentrations in human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CM) at 3 days following treatment.

Fig. 8 is a graph illustrating the protective effect of myricetin (MYR; 100μM) on doxorubicin (DOX)-induced cardiotoxicity at increasing concentrations of doxorubicin for 72 hours. Y-axis, percentage of cell survival; and X-axis, increasing concentrations of DOX.

Fig. 9 is a graph illustrating percentage rescue by increasing concentrations (X-axis) of myricetin (MYR; circle) and dexrazoxane (DEX; square) of human induced pluripotent stem cell-derived cardiomyocytes treated with 0.5μM of doxorubicin (DOX).

Fig. 10 depicts the protective effects of myricetin against doxorubicin (DOX)-induced contractility dysfunction in cardiomyocytes, represented in a scale of beat rates (per minute; left panel), duration (in second; center panel) and peak height (in arbituary unit; right panel) for mock treated, DOX (0.5μM), DOX plus DEX (100μM), or DOX plus MYR (100μM) after 48 hours of treatment.

Fig. 11 depicts the effect of myricetin (MYR) on DOX-induced DNA double strand break in human iPSC-derived cardiomyocytes treated with DMSO, DOX alone (0.5μM), DOX plus DEX (100μM), or DOX plus MYR (100μM), measured after 48 hours of the treatment, presented in percentage of γH2AX-positive cells quantified for each condition (right) and representative images of the cells (left).

Fig. 12 depicts the effect of myricetin (MYR) on doxorubicin (DOX)-induced sarcomere disruption shown in representative images for mock treated (DMSO; left), DOX alone (0.5μM; center), or DOX plus MYR (100μM; right).

Fig. 13 depicts the effect of myricetin (MYR) on inhibition of topoisomerases IIα and β (TOPOIIα and TOPOIIβ) compared with that of dexrazoxane (DEX).

Fig. 14 depicts the effects of myricetin (MYR) and dexrazoxane (DEX) on TOPOIIβ protein degradation illustrated in a graph (top) and representative images (bottom).

Fig. 15 depicts the effect of myricetin (MYR) and dihydromyricetin (DHM) on topoisomerases IIβ (TOPOIIβ) enzymatic inhibition and relative potency thereof as illustrated in a decatenation gel (top) and a graph (bottom).

Fig. 16 is a graph illustrating relative potency of MYR and DHM in rescuing cardiomyocytes from DOX-induced cell death.

Fig. 17 is a graph illustrating relative potency of MYR and DHM in rescuing cardiomyocytes from DOX-induced double strand break.

Fig. 18 is a graph illustrating the effect of MYR on RNA expression levels of TOPOIIα (right) and TOPOIIβ (left) as demonstrated in cardiomyocytes treated with DOX alone or DOX plus MYR.

Fig. 19 depicts two graphs illustrating potency of myricetin (MYR) in protecting cardiomyocytes from epirubicin (EPI; left) and idarubicin (IDA; right)-induced cytotoxicity.

Fig. 20 is a graph illustrating the effect of myricetin (MYR) on sunitinib (SUN)-induced cell death.

Fig. 21 is a graph illustrating the effect of myricetin (MYR) on sorafenib (SOR)-induced contractile dysfunction.

Fig. 22 is a graph illustrating the effect of myricetin (MYR) on bortezomib (BOR)-induced cell death.

Fig. 23 is a graph illustrating the lack of effect of myricetin (MYR) on DOX's anticancer activity.

Fig. 24 depicts the effect of MYR on DOX-induced contractile dysfunction in mice measured in percentage of fractional shortening (left) and ejection fraction (right).

Fig. 25 depicts the effects of DOX, DEX, and various protective agents on mitochondrial toxicity in cardiomyocytes derived from human induced pluripotent stem cells.

Fig. 26 depicts the effects of DOX, DEX, and various protective agents on apoptosis in cardiomyocytes derived from human induced pluripotent stem cells.

Fig. 27A-D depict the effects of mock treatment (27A), DEX (27B), a co-administration of doxorubicin and dexrazoxan (27C), or a co-administration of doxorubicin and vitexin (27D), on mitochondrial health in human induced pluripotent stem cell-derived cardiomyocytes.

Fig. 28A-B depict the effects of DOX, or co-administration of DOX with various concentrations of vitexin (VIT) on the electrophysiological activity in human induced pluripotent stem cell-derived cardiomyocytes over a three-day time period (left), or at a 30-hour time point (right)

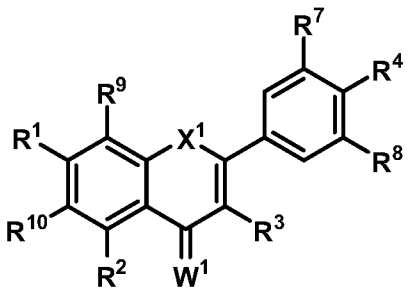
Fig. 29A-B depict the effects of co-administration of doxorubicin with kaempferol (KAE; left) and vitexin (VIT; right) on viability in MDA-MB-231 metastatic breast cancer cells.

BRIEF SUMMARY

This disclosure provides composition, kits, and methods for protecting the heart and for preventing heart failure in patients treated with anthracyclines, protein kinase inhibitors and/or biologic agents. By minimizing the risk of potentially devastating heart failure in cancer patient under chemotherapy, conventional cancer treatment can achieve improved efficacy and safety with the invention described herein.

The compositions include one or more protective agents with or without an anticancer agent. The kits often include one or more protective agents, and sometimes anticancer agents as well. The methods include methods of reducing, preventing, or eliminating cardiotoxicity induced by a drug or other therapy including cancer treatments.

In some aspects, this disclosure provides a pharmaceutical composition comprising a protective agent of according to Formula 1,



Formula 1

wherein:

X^1 is CR^5R^6, NR^5, O, S, C=O, or C=S;

each of R^1, R^2, R^3, R^5, R^6, R^9, and R^{10} is independently alkyl, alkenyl, alkynyl, alkoxy, acyl, acyloxy, carboxylic acid, ester, amine, amide, carbonate, carbamate, nitro,

thioether, thioester, cycloalkyl, heteroalkyl, heterocyclyl, monosaccharide, aryl, or heteroaryl, any of which is substituted or unsubstituted, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H;

R^4, R^7 and R^8 are alkoxy, hydroxyl or H;

W^1 is O or S; or

a salt thereof.

In some aspects, X^1 can be O or S; each of R^1, R^2, R^3, R^9, and R^{10} can be independently alkoxy, cycloalkyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and each of R^4, R^7 and R^8 can be independently alkoxy, hydroxyl or H.

In some aspects, X^1 is O; each of R^1, R^2, R^3, R^9, and R^{10} can be independently alkoxy, cycloalkyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and each of R^4, R^7 and R^8 can be independently alkoxy, hydroxyl or H.

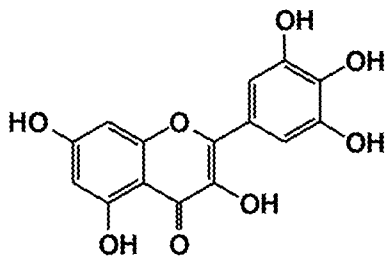
In yet other aspects, X^1 is O; each of R^1 and R^2 can be independently hydroxyl or H; each of R^3, R^9 and R^{10} can be independently cycloalkyl, heterocyclyl, hydroxyl, or H; R^4 is hydroxyl; and each of R^7 and R^8 can be independently hydroxyl or H.

In yet other aspects, X^1 is O; R^1 is hydroxyl; each of R^2 and R^3 can be independently hydroxyl or H; R^9 and R^{10} are H; R^4 is hydroxyl; and each of R^7 and R^8 can be independently hydroxyl or H.

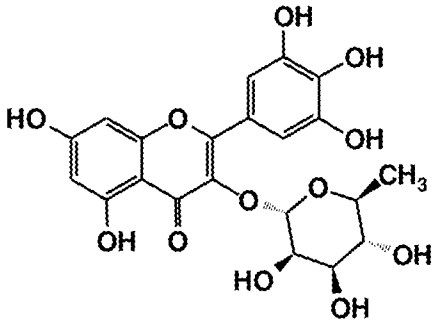
In yet other aspects, X^1 is O; R^1 is hydroxyl; each of R^2 and R^3 can be independently hydroxyl or H; R^9 can be heterocyclyl or H; of R^{10} is H; R^4 can be independently hydroxyl or H; and each of R^7 and R^8 can be independently hydroxyl or H.

In yet other aspects, X^1 is O; R^1 is hydroxyl; each of R^2 and R^9 can be independently hydroxyl or H; R^3 can be cycloalkyl, hydroxyl or H; R^{10} is H; R4 is hydroxyl; and each of R^7 and R^8 can be independently hydroxyl or H. In one embodiment, cycloalkyl of R^3 can be a monosaccharide.

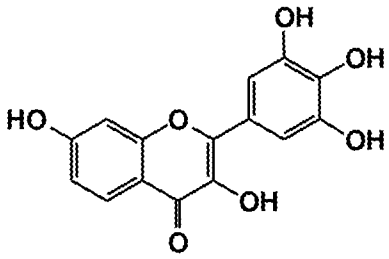
In some embodiments, the pharmaceutical composition may comprise myricetin and is a compound according to the following formula.



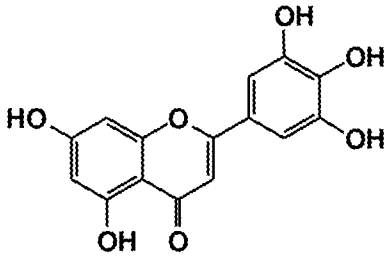
In some embodiments, the pharmaceutical composition may comprise myricetrin/myricitrin and is a compound according to the following formula.



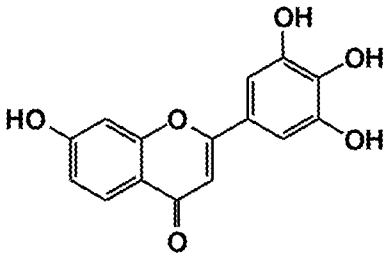
In some embodiments, the pharmaceutical composition may comprise robinetin and is a compound according to the following formula.



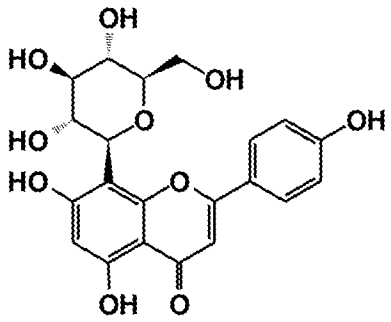
In some embodiments, the pharmaceutical composition may comprise tricetin and is a compound according to the following formula.



In some embodiments, the pharmaceutical composition may comprise 7,3',4',5'-tetrahydroxyflavone and is a compound according to the following formula.



In some embodiments, the pharmaceutical composition comprises ficetin. In some embodiments, the pharmaceutical composition comprises quercetin. In some embodiments, the pharmaceutical composition comprises kaempferol. In some embodiments, the protective agent within the pharmaceutical composition can be a compound with the following structure:



In a particular example, the protective agent within the pharmaceutical composition can be vitexin.

In some embodiments, the pharmaceutical composition may include one or more chemotherapy drug(s) (anticancer agent) or biologic agent(s). In some embodiments, the pharmaceutical composition can include a chemotherapy drug. In some embodiments, the pharmaceutical composition may include one or more chemotherapy drug(s) (anticancer agent) and one or more of the protective agent(s) selected from the group consisting of myricetin, tricetin (5,7,3',4',5'-pentahydroxyflavone), robinetin, ficetin, vitexin, 7,3',4',5'-tetrahydroxyflavone, and myricetrin.

In some embodiments, the pharmaceutical composition may comprise an anthracycline or salt thereof. In some embodiments, the anthracycline can be daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin. In some embodiments, the anthracycline is doxorubicin. In some embodiments, the anthracycline is epirubicin. In some embodiments, the anthracycline is idarubicin.

In some embodiments, the chemotherapy drug can be a protein kinase inhibitor. In some embodiments, the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

In some embodiments, the chemotherapy drug can be a proteasome inhibitor. In a particularly example, the proteasome inhibitor can be bortezomib.

In some embodiments, the protein kinase inhibitor can be a tyrosine kinase inhibitor. In some embodiments, for example, the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib, mesylate, and nilotinib. In a particular example, the tyrosine kinase inhibitor is sorafenib. In another particular example, the tyrosine kinase inhibitor is sunitinib.

In some embodiments, the chemotherapy drug can be a biologic agent. In some embodiments, the biologic agent is an antibody. In some embodiments, the antibody can be adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab. In one particular example, the antibody is trastuzumab.

In some embodiments, the pharmaceutical composition can be a liquid composition. In some embodiments, the pharmaceutical composition can be a capsule, a gel capsule, or a liposome. In some embodiments, the pharmaceutical composition can be a tablet.

In some embodiments, the pharmaceutical composition may also include dexrazoxane as an additional protective agent.

In some embodiments, the pharmaceutical composition can comprise at least 1 mg of one or more protective agents. In some embodiments, the pharmaceutical composition can

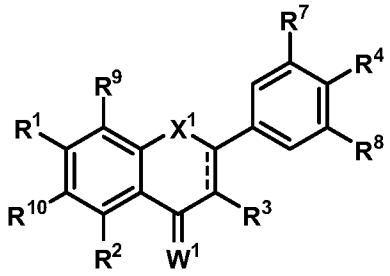
comprise between 0.1 mg and 200 mg of one or more protective agents. In some embodiments, the pharmaceutical composition can comprise between 0.1 mg and 300 mg of one or more protective agents.

In some embodiments, two protective agents are present and co-formulated together. In some embodiments, the two protective agents can be present as distinct entities within the pharmaceutical composition. In some embodiments, the pharmaceutical composition can comprise the chemotherapy drug and the chemotherapy drug is co-formulated with one of the two protective agents.

In some aspects, this disclosure provides a pharmaceutical composition comprising

(a) a protective agent selected from the group consisting of:

a compound according to Formula 2,



Formula 2

wherein:

$\equiv$ represents a single or double bond;

X^1 is CR^5R^6, NR^5, O, S, C=O, or C=S;

each of R^1, R^2, R^3, R^5, R^6, R^9, and R^{10} is independently alkyl, alkenyl, alkynyl, alkoxy, acyl, acyloxy, carboxylic acid, ester, amine, amide, carbonate, carbamate, nitro, thioether, thioester, cycloalkyl, heteroalkyl, heterocyclyl, aryl, or heteroaryl, any of which is substituted or unsubstituted, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H;

R^4, R^7 and R^8 are hydroxyl;

W^1 is O or S;

or a salt thereof; and

(b) a chemotherapy drug or a biologic agent.

In some embodiments, the pharmaceutical composition may comprise an anticancer agent or a chemotherapy drug. In some embodiments, the protective agent is selected from

the group consisting of myricetin, tricetin, robinetin, ficetin, vitexin, dihydrorobinetin, 7,3',4',5'-tetrahydroxyflavone, and myricetrin.

In some embodiments, the pharmaceutical composition may comprise one or more protective agents. In some embodiments, the pharmaceutical composition may comprise myricetin. In some embodiments, the pharmaceutical composition may comprise myricetrin. In some embodiments, the pharmaceutical composition may comprise robinetin. In some embodiments, the pharmaceutical composition may comprise dihydrorobinetin. In some embodiments, the pharmaceutical composition may comprise vitexin. In some embodiments, the pharmaceutical composition may comprise tricetin. In some embodiments, the pharmaceutical composition comprises quercetin. In some embodiments, the pharmaceutical composition comprises kaempferol.

In some embodiments, the pharmaceutical composition comprises an anthracycline or salt thereof. In some embodiments, the anthracycline is daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin. In some embodiments, the anthracycline is doxorubicin. In some embodiments, the anthracycline is epirubicin. In some embodiments, the anthracycline is idarubicin.

In some embodiments, the chemotherapy drug can be a protein kinase inhibitor. In some embodiments, the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

In some embodiments, the chemotherapy drug is a proteasome inhibitor. In a particular example, the proteasome inhibitor is bortezomib.

In some embodiments, the protein kinase inhibitor is a tyrosine kinase inhibitor. In some embodiments, the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib, mesylate, and nilotinib. In a particular example, the tyrosine kinase inhibitor is sorafenib. In another particular example, the tyrosine kinase inhibitor is sunitinib.

In some embodiments, the chemotherapy drug is a biologic agent. In some embodiments, the biologic agent is an antibody. In some embodiments, the antibody is adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin,

catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab. In a particular example, the antibody is trastuzumab. In a particular example, the antibody is bevacizumab.

In some embodiments, the pharmaceutical composition may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, or 300 mg of one or more protective agents.

In some embodiments, the pharmaceutical composition may comprise between 0.1 mg and 50 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 10 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 20 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 30 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 40 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 50 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 100 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 1 mg and 200 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 40 mg and 300 mg of the protective agent. In some embodiments, the pharmaceutical composition may comprise between 50 mg and 400 mg of the protective agent.

In some embodiments, the pharmaceutical composition may comprise the chemotherapy drug; and the chemotherapy drug and the protective agent are mixed within the pharmaceutical composition.

In some embodiments, the pharmaceutical composition comprises the chemotherapy drug wherein the dose of the chemotherapy drug is at least 0.1 mg. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug wherein the dose of the chemotherapy drug is between 0.01 mg and 50 mg. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug wherein the dose of the chemotherapy drug is between 0.01 mg and 100 mg. In some embodiments, the

pharmaceutical composition comprises the chemotherapy drug wherein the dose of the chemotherapy drug is between 0.01 mg and 200 mg.

In some embodiments, the pharmaceutical composition comprises the biologic agent at a dose of at least 50 mg. In some embodiments, the pharmaceutical composition comprises a biologic agent at a dose of between 0.1 mg and 100 mg. In some embodiments, the pharmaceutical composition comprises a biologic agent at a dose of between 0.1 mg and 200 mg.

In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 1:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 2:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 3:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 4:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 5:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 6:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 7:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 8:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 9:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 10:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and a molar ratio of the protective agent to the chemotherapy drug is at least 20:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and wherein a molar ratio of the protective agent to the chemotherapy drug is at least 100:1. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and wherein a molar ratio of the protective agent to the chemotherapy drug is at least 1:2. In some embodiments, the

pharmaceutical composition comprises the chemotherapy drug and wherein a molar ratio of the protective agent to the chemotherapy drug is at least 1:3. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and wherein a molar ratio of the protective agent to the chemotherapy drug is at least 1:4. In some embodiments, the pharmaceutical composition comprises the chemotherapy drug and wherein a molar ratio of the protective agent to the chemotherapy drug is at least 1:5.

This disclosure provides methods for administering to a subject any of the pharmaceutical compositions disclosed herein. In some aspects, this disclosure provides a method for preventing, reducing, or eliminating cardiotoxicity or heart failure in general. In some aspects, this disclosure provides a method for preventing, reducing, or eliminating cardiotoxicity induced by a chemotherapy drug or biologic agent in a subject, the method comprising: administering one or more protective agent according to Formula 1, to the subject, thereby preventing, reducing, or eliminating the cardiotoxicity induced by the chemotherapy drug or biologic agent in the subject. In some cases, the pharmaceutical composition comprises a compound selected from the group consisting of such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin.

In some aspects, this disclosure provides a method for preventing, reducing, or eliminating cardiotoxicity induced by a chemotherapy drug or biologic agent in a subject, the method comprising: administering at least one protective agent according to Formula 1 or Formula 2, to the subject, thereby preventing, reducing, or eliminating the cardiotoxicity induced by the chemotherapy drug or biologic agent in the subject.

In some embodiments, the subject is administered a chemotherapy drug or biologic agent prior to the administering of one or more protective agent(s) according Formula 1 or 2, to the subject.

In some embodiments, the subject is administered a chemotherapy drug or biologic agent following the administering of at least two protective agents of Formula 1 or 2 to the subject.

In some aspects, this disclosure provides a method for treating cancer, the method comprising: (a) administering a chemotherapy drug or biologic agent to a subject, wherein the subject has cancer and the chemotherapy drug or biologic agent is capable of causing cardiotoxicity in the subject; and (b) administering at least one protective agent according to

Formula 1 or Formula 2 to the subject, wherein the protective agent prevents, reduces, or eliminates the cardiotoxicity in the subject.

In some embodiments, the subject has a human suffering from cancer. In some embodiments, the cancer is bladder cancer, bone cancer, a brain tumor, breast cancer, esophageal cancer, gastrointestinal cancer, leukemia, liver cancer, lung cancer, lymphoma, myeloma, ovarian cancer, prostate cancer, a sarcoma, stomach cancer, or thyroid cancer.

In some embodiments, prior to the administration of the protective agent, the subject has a cardiac condition or has a history of having a cardiac condition. In some embodiments, the administration of the protective agent reduces the risk of the subject experiencing cardiotoxicity induced by the chemotherapy drug or biologic agent. In some embodiments, the administration of the protective agent reduces the risk of the subject experiencing cardiotoxicity induced by the chemotherapy drug or biologic agent by at least 30%,40%, 50%, 60%, 70%, 80%, 90%, or 95%. In some embodiments, the cardiotoxicity may comprise cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress. In some embodiments, the cardiotoxicity is cardiac tissue damage. In some embodiments, the cardiotoxicity is electrophysiological dysfunction.

In some embodiments, the chemotherapy drug used in the methods described herein may comprise an anthracycline or a salt thereof. In some embodiments, the anthracycline is daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin. In some embodiments, the anthracycline is doxorubicin. In some embodiments, the anthracycline is epirubicin. In some embodiments, the anthracycline is idarubicin.

In some embodiments, the chemotherapy drug used in the methods described herein is a protein kinase inhibitor. In some embodiments, the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

In some embodiments, the protein kinase inhibitor is a tyrosine kinase inhibitor. In some embodiments, the protein kinase inhibitor is a tyrosine kinase inhibitor. In some embodiments, the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib,

mesylate, and nilotinib. In a particular example, the tyrosine kinase inhibitor is sorafenib. In another particular example, the tyrosine kinase inhibitor is sunitinib.

In some aspect, the chemotherapy drug is a proteasome inhibitor. In one particular example, the proteasome inhibitor is bortezomib.

In some embodiments, the biologic agent used in the methods described herein can be an antibody. In some embodiments, the antibody is adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab. In one particular example, the antibody is trastuzumab.

In some embodiments, the subject according to the methods described herein has a decreased QTc interval after administering the protective agent. In some cases, the protective agent is selected from the group consisting of such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin. In one particular example, the protective agent is myricetin.

In some embodiments, the chemotherapy drug and protective agent of Formula 1 or Formula 2 are administered concurrently to the subject. In some embodiments, the chemotherapy drug and protective agent are administered sequentially to the subject. In some embodiments, the protective agent is administered to the subject prior to the administration of the chemotherapy drug. In some embodiments, the protective agent is administered to the subject after the administration of the chemotherapy drug.

In some embodiments, at least two protective agents of Formula 1 or Formula 2 can be administered. For example, the at least two protective agents can be selected from the group consisting of such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin.

In some embodiments, one or more protective agent(s) can further comprise dexrazoxane.

This disclosure provides a method for treating or preventing organ damage in a subject comprising: administering one or more protective agents selected from the group consisting of such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin to a subject with organ damage, thereby treating or preventing organ damage in the subject.

This disclosure also provides kits. In some aspects, this disclosure provides a kit comprising: (a) a protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin; and (b) a chemotherapy drug or a biologic agent.

In some aspects, this disclosure provides a kit comprising: (a) a protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin; (b) a chemotherapy drug or a biologic agent; and (c) dexrazoxane. In some embodiment, the protective agent is myricetin.

DETAILED DESCRIPTION

Certain cancer drugs (e.g., anthracycline drugs, protein kinase inhibitors) and other therapies can cause cardiotoxicity in patients. For example, anthracycline-induced cardiotoxicity occurs when the drug such as doxorubicin intercalates the DNA upon a cleavage of DNA by topoisomerase II enzymes thereby effectively preventing TOPOIIα or β from ligating the cleaved strands back together.

This disclosure provides pharmaceutical compositions and methods that may prevent, reduce or eliminate such cardiotoxicity and that may also prevent, reduce or eliminate organ damage caused by cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress. Many of the compositions and methods provided herein relate to the administration of a specific protective agent in conjunction with one or more cancer treatments, thereby reducing the risk that the cancer treatment will cause or aggravate cardiotoxic events in a patient. The protective agents described herein include such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and/or derivatives or salts thereof. In some cases, the protective agents may be flavonoids. In some cases, the protective agent may be administered in combination with a different protective agent. In some cases, the protective agent may be administered in combinations such as combinations including dexrazoxane and another protective agent.

The present disclosure may enable cancer patients - including heart healthy patients and patients with pre-existing cardiac conditions - to receive a desired dosage of a therapy (e.g., an anthracycline or salt thereof) without having the dosage regimen significantly altered

by the risk of cardiotoxicity. Another advantage of the present disclosure is that it may enable a larger patient population to receive a given therapy, such as certain patients with pre-existing cardiac conditions or with age limits. In addition, the reduction or prevention of cardiotoxicity may enable a cancer patient to avoid having to take a medication to treat a heart condition. Overall, the advantages presented herein may help to facilitate a better therapeutic outcome for patients.

The pharmaceutical compositions and methods (including methods of use) provided herein generally relate to reducing, eliminating or preventing cardiotoxicity caused by chemotherapeutic drugs, biologic agents, or radiation therapy; they can also be used to reduce or eliminate organ damage caused by electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress. **Figure 1** depicts a general schematic of some embodiments of the methods provided herein. The top panel shows a cancer treatment **[110]**, such as a chemotherapeutic drug, biologic agent, or radiation therapy, being administered to a patient **[120]**, who develops cardiotoxicity and is then gradually given reduced doses of the cancer treatment over time **[130]**. Therefore, the cardiotoxicity associated with administration of the cancer treatment **[110]** in the absence of a protective agent **[140]** may limit the patient population that is eligible to receive treatment. In the bottom panel, the cancer treatment **[110]** is co-administered with a protective agent **[140]**, such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin and myricitrin to a patient, e.g., **[151]** who experiences reduced cardiotoxicity, or no cardiotoxicity at all **[160]**, thereby enabling the patient to tolerate the dosage regimen. Although separate vehicles for the cancer treatment and protective agent are depicted, in some cases the cancer treatment and protective agent are co-formulated together. The co-administration of the cancer treatment **[110]** with the protective agent **[140]** may enable a larger patient population **[150]** to receive the cancer treatment, including healthy patients and patients with pre-existing cardiac conditions **[152, 153]**.

Figure 2 also depicts a general schematic of embodiments provided herein. The top panel shows a cancer treatment **[210]** (e.g., a chemotherapeutic drug, a biologic agent, or radiation therapy), and dexrazoxane **[220]** being co-administered to a patient **[230]** who then experiences some cardiotoxicity over time **[240]**. The co-administration of the cancer treatment **[210]** and dexrazoxane **[220]** in the absence of the protective agent **[250]** may limit the patient population that is eligible to receive treatment. In the bottom panel, the cancer treatment **[210]**, the dexrazoxane **[220]**, and a protective agent **[250]** (such as myricetin,

vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin) are administered to a patient **[261]** who experiences reduced cardiotoxicity, or no cardiotoxicity at all **[270]**. In this embodiment, the co-administration of the protective agent **[250]** with the cancer treatment **[210]** and dexrazoxane **[220]** may enhance the activity of dexrazoxane to prevent, alleviate, or eliminate cardiotoxicity in a patient **[261]**, thereby enabling a larger patient population **[260]** to receive treatment, including patients without and those with pre-existing cardiac conditions **[262, 263]**. In some cases, the protective agent, the dexrazoxane and/or the cancer treatment are administered separately; in some cases, they are administered concurrently or as co-formulations. Generally, the co-formulations and methods provided herein may reduce the cardiotoxicity induced in patients by chemotherapeutic drugs, biologic agents, or radiation therapy.

The compositions provided herein may include a co-formulation of two or more protective agents. For example, the co-formulation may comprise such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin, and dexrazoxane. In some cases, the compositions may include a co-formulation of a protective agent (e.g., such as myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin) with a certain cancer treatment (e.g., chemotherapeutic drug or biologic agent). In some cases, provided herein are kits that contain at least two protective agents (or a protective agent and a cancer treatment) as separate components, often along with instructions for use.

METHODS

Provided herein are methods for administering to a patient, particularly a cancer patient, a pharmaceutical composition that can reduce, eliminate or prevent cardiotoxicity caused by a cancer treatment (e.g., chemotherapeutic drugs, biologic agents or radiation therapy). The methods provided herein also comprise treating cancer in a patient using at least one of the compositions provided herein. In some cases, the patient may be heart-healthy; in some cases, the patient is at-risk for a cardiac condition.

The methods provided herein generally comprise administering to a patient a pharmaceutical composition comprising at least one protective agent described herein, or at least one protective agent and a cancer treatment (e.g., anthracycline drug, protein kinase inhibitor, biologic agent, or radiation therapy). The protective agent and cancer treatment may also be combined with a different cardioprotective agent (e.g., dexrazoxane). In some

cases, the protective agent and cancer treatment may be co-formulated, in that they are mixed within the same pharmaceutical composition (e.g., tablet, capsule, liposome, liquid, or vapor); in some cases, they exist as distinct entities.

Subjects

The methods and compositions disclosed herein are generally used to prevent, reduce, treat, or eliminate cancer treatment-induced cardiotoxicity in a subject. The subject may be any human patient, particularly a cancer patient, a patient at risk for cancer, or a patient with a family or personal history of cancer. In some cases, the patient is in a particular stage of cancer treatment. For example, a pharmaceutical composition described herein can be administered to a human patient with early or late stage cancer in order to reduce cardiotoxicity caused by a cancer treatment.

The cancer patients may have any type of cancer. Examples of cancer can include, but are not limited to, adrenal cancer, anal cancer, basal cell carcinoma, bile duct cancer, bladder cancer, cancer of the blood, bone cancer, a brain tumor, breast cancer, bronchus cancer, cancer of the cardiovascular system, cervical cancer, colon cancer, colorectal cancer, cancer of the digestive system, cancer of the endocrine system, endometrial cancer, esophageal cancer, eye cancer, gallbladder cancer, a gastrointestinal tumor, kidney cancer, hematopoietic malignancy, laryngeal cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, mesothelioma, cancer of the muscular system, Myelodysplastic Syndrome (MDS), myeloma, nasal cavity cancer, nasopharyngeal cancer, cancer of the nervous system, cancer of the lymphatic system, oral cancer, oropharyngeal cancer, osteosarcoma, Kaposi sarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, rectal cancer, renal pelvis cancer, cancer of the reproductive system, cancer of the respiratory system, sarcoma, salivary gland cancer, skeletal system cancer, skin cancer, small intestine cancer, stomach cancer, testicular cancer, throat cancer, thymus cancer, thyroid cancer, a tumor, cancer of the urinary system, uterine cancer, vaginal cancer, or vulvar cancer. The term ‘lymphoma’ may refer to any type of lymphoma including B-cell lymphoma (e.g., diffuse large B-cell lymphoma, follicular lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, marginal zone B-cell lymphoma, Burkitt lymphoma, lymphoplasmacytic lymphoma, hairy cell leukemia, or primary central nervous system lymphoma) or a T-cell lymphoma (e.g., precursor T-lymphoblastic lymphoma, or peripheral T-cell lymphoma). The term ‘leukemia’ may refer to any type of leukemia including acute leukemia or chronic

leukemia. Types of leukemia include acute myeloid leukemia, chronic myeloid leukemia, acute lymphocytic leukemia, acute undifferentiated leukemia, or chronic lymphocytic leukemia. In some cases, the cancer patient does not have a particular type of cancer. For example, in some instances, the patient may have a cancer that is not breast cancer.

Examples of cancer include cancers that cause solid tumors as well as cancers that do not cause solid tumors. Furthermore, any of the cancers mentioned herein may be a primary cancer (e.g., a cancer that is named after the part of the body where it first started to grow) or a secondary or metastatic cancer (e.g., a cancer that has originated from another part of the body).

A patient at risk of cancer may be at risk because of a particular condition such as a pre-cancerous condition. Pre-cancerous conditions include but are not limited to actinic keratosis, Barrett's esophagus, atrophic gastritis, ductal carcinoma in situ, dyskeratosis congenita, sideropenic dysphagia, lichen planus, oral submucous fibrosis, solar elastosis, cervical dysplasia, leukoplakia, and erythroplakia). In some cases, a patient may be at risk of cancer because of cell or tissue dysplasia (e.g., an abnormal change in cell number, abnormal change in cell shape, abnormal change in cell size, or abnormal change in cell pigmentation).

A patient at risk of cancer may be a patient that was exposed to a carcinogenic agent. Such patients may include patients with exposure to known or probable carcinogens (e.g., acetyl aldehyde, asbestos, or tobacco products), or patients exposed to ionizing radiation (e.g., gamma radiation, beta-radiation, X-radiation, or ultraviolet radiation). In some cases, a patient at risk of cancer is at risk because of a family history of cancer.

The methods and compositions disclosed herein may also be used to prevent, reduce, or eliminate cardiotoxicity in patients with a history of cancer, particularly patients who have been administered cancer treatments (e.g., anthracycline drugs, protein kinase inhibitors, proteasome inhibitors, or biological agents) with cardiotoxic effects. Examples of a patient with a history of cancer include, but are not limited to, a patient in remission, a patient in complete remission, a patient with relapsed cancer or a patient with recurring cancer.

The methods and compositions disclosed herein are generally used in a patient that has been administered, or is currently being administered, a cardiotoxicity-inducing agent (e.g., a cancer treatment). Non-limiting examples of cardiotoxicity-inducing agents are described elsewhere herein and may include cancer treatments, chemotherapeutic drugs, anthracyclines (e.g., doxorubicin, epirubicin, and idarubicin), protein kinase inhibitors (e.g., tyrosine kinase inhibitor), biologic agents (e.g., trastuzumab), or radiation therapy, as well as

any cancer treatment otherwise known to cause cardiotoxicity. In some examples, a pharmaceutical composition disclosed herein is administered to a cancer patient with previous exposure to a cancer treatment known to have cardiotoxic effects, in order to reduce the risk of cardiotoxicity associated with the patient's current cancer treatment regimen. In some cases, the pharmaceutical composition is administered to a cancer patient in order to reduce or off-set cumulative effects of prior exposures to cancer treatment or drugs, or to other agents that cause cardiotoxicity. In some examples, a pharmaceutical co-formulation comprising myricetin and anthracycline may be administered to a prostate cancer patient who also has dilated cardiomyopathy caused by a previous cancer treatment. In another example, a pharmaceutical co-formulation comprising vitexin may be administered to a lung cancer patient who is being concurrently treated with an anthracycline. In yet another example, a pharmaceutical co-formulation comprising robinetin may be administered to a breast cancer patient. In yet another example, a pharmaceutical co-formulation comprising tricetin may be administered to a Kaposi sarcoma cancer patient. In yet another example, a pharmaceutical co-formulation comprising ficetin may be administered to a breast cancer patient. In yet another example, a pharmaceutical co-formulation comprising 7,3',4',5'-tetrahydroxyflavone may be administered to a breast cancer patient. In yet another example, a pharmaceutical co-formulation comprising myricitrin may be administered to a breast cancer patient. In yet another example, a pharmaceutical co-formulation comprising myricetin and anthracycline may be administered to a liver cancer patient who also has dilated cardiomyopathy caused by a previous cancer treatment. In yet another example, a pharmaceutical co-formulation comprising myricetin and doxorubicin may be administered to a sarcoma cancer patient.

In some cases, the methods and compositions herein may be used to alleviate cardiotoxicity that is not caused by a cancer treatment. As such, the patient may have or be at risk of having, cardiotoxicity induced by a drug that is not specifically for cancer, such as a protein kinase inhibitor. Such patients may have a condition such as a neurological or cardiac disorder. In some cases, the condition may be a condition treatable by a protein kinase inhibitor.

In some cases, the patient may have organ damage or be at risk of having organ damage. For example, the patient may have organ damage (or be at risk of organ damage) as a result of cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress. For such patients, the methods and compositions provided

herein may reduce or eliminate the organ damage caused by cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress.

In some cases, patients treated by any of the methods or compositions described herein may have heart disease, or have a family history of heart disease. Examples of heart disease include, but are not limited to, arrhythmogenic cardiomyopathy, arterial disease, Brugada Syndrome, congenital heart disease, dilated cardiomyopathy, heart palpitations, heart valve disease, hypertensive heart disease, hypertrophic cardiomyopathy, long QT syndrome, rheumatic heart disease, or vascular disease. In some cases, the heart disease is caused by a cardiotoxic agent (e.g., anthracycline). For example, the heart disease may be caused by any of the cardiotoxic agents mentioned herein. In one particular example, a pharmaceutical co-formulation comprising myricetin and doxorubicin may be administered to a breast cancer patient who also has hypertrophic cardiomyopathy. In another example, a co-formulation of one or more of compound selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin may be administered to a cancer patient experiencing cardiotoxicity from a previously administered chemotherapy drug.

A patient treated by any of the methods or compositions described herein may be of any age and may be an adult, infant or child. In some cases, the patient is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 years old, or within a range therein (e.g., between 2 and 20 years old, between 20 and 40 years old, or between 40 and 90 years old). A particular class of patients that may benefit is patients over the age of 40. Another particular class of patients that may benefit is pediatric patients, who may be at life risk of chronic heart symptoms. Furthermore, a patient treated by any of the methods or compositions described herein may be male or female.

Any of the compositions disclosed herein may also be administered to a non-human subject, such as a laboratory or farm animal. Non-limiting examples of a non-human subject

include a dog, a goat, a guinea pig, a hamster, a mouse, a pig, a non-human primate (e.g., a gorilla, an ape, an orangutan, a lemur, or a baboon), a rat, a sheep, a cow, or a zebrafish.

Drug Administration

The disclosure provided herein describes methods to prevent, reduce, or eliminate cancer treatment-induced cardiotoxicity in patients by administering to a patient one or more protective agents of Formula 1, Formula 2 or derivative or salt thereof. The disclosure herein also describes methods to prevent, reduce, or eliminate cancer treatment-induced cardiotoxicity in patients by administering to a patient one or more protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin (or derivative or salt thereof). The disclosure provided herein also describes methods of administering to a subject, wherein the subject has cancer and the cancer treatment is capable of causing cardiotoxicity and organ damage in the subject, and administering one or more protective agents (or derivative or salt thereof) selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin, wherein the protective agent prevents, reduces, or eliminates the cardiotoxicity in the subject.

Methods disclosed herein can further comprise administering to the patient a combination of dexrazoxane (or derivative or salt thereof) and a protective agent according to Formula 1, Formula 2, or derivative or salt thereof; the combined agents may be administered as a co-formulation or separately. In some aspects, the methods comprise administering to the patient a combination of dexrazoxane (or derivative or salt thereof) and myricetrin (or derivative or salt thereof); the combined agents may be administered as a co-formulation or separately.

Methods disclosed herein can further comprise administering to the patient combined agents comprising a combination of dexrazoxane (or derivative or salt thereof) and a protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin. (or derivative or salt thereof); the combined agents may be administered as a co-formulation or separately.

The protective agents may be administered to the subject or patient in any combination of a compound of Formula 1 or Formula 2. In some cases, only one protective agent (e.g., myricetin or a derivative or salt thereof) is administered to a subject or patient. In some cases, only one protective agent (e.g., myricitrin or a derivative or salt thereof) is

administered to a subject or patient. In some cases, only one protective agent (e.g., vitexin or a derivative or salt thereof) is administered to a subject or patient. In some cases, only one protective agent (e.g., robinetin or a derivative or salt thereof) is administered to a subject or patient. In some cases, only one protective agent (e.g., tricetin or a derivative or salt thereof) is administered to a subject or patient. In some cases, only one protective agent (e.g., 7,3',4',5'-tetrahydroxyflavone or a derivative or salt thereof) is administered to a subject or patient. In a particular example, a subject or patient described herein may be administered a therapeutically effective dose of myricetin (or derivative or salt thereof). In another example, a subject or patient described herein may be administered a therapeutically effective dose of robinetin (or derivative or salt thereof). In yet another example, a subject or patient described herein may be administered a therapeutically effective dose of vitexin (or derivative or salt thereof).

In some cases, two protective agents (or derivative or salt thereof) selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin, and dexrazoxane are administered to a subject. In cases where two or more protective agents are administered to a patient, the protective agents may be administered as distinct entities or in a co-formulation. For example, a patient experiencing cardiotoxicity may be administered a therapeutically effective co-formulation of myricetin and robinetin; myricetin and dexrazoxan; or other co-formulation described herein. The two or more protective agents may be administered simultaneously or sequentially. In some cases, the two or more protective agents may be administered sequentially in a particular order. For example, a patient may first be administered myricetin and subsequently administered dexrazoxane, or may first be given dexraxozane and then given myricetin.

In some cases, an anticancer agent (e.g., chemotherapeutic drug, biologic agent, protein kinase inhibitor, radiation therapy) (or other treatment) and one or more protective agents of Formula 1 or Formula 2 (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, and myricitrin) may be administered to a patient. In cases where a cancer treatment (or other treatment) and at least two protective agents are administered to a patient, the cancer treatment (or other treatment) and the at least two protective agents (or derivative or salt thereof) may be administered as co-formulations in any combination. For example, a patient may be administered a co-formulation of a

protective agent and a chemotherapeutic drug or a co-formulation containing one or more chemotherapeutic drugs and at least two protective agents.

In some cases, a patient or subject may be administered one or more protective agents (or derivative or salt thereof) and one or more cancer treatments (or other treatment) simultaneously. For example, the method may comprise administering to a patient a protective agent and a chemotherapy as separate entities, but simultaneously.

In some cases, a patient or subject may be administered one or more protective agents of Formula 1 or Formula 2 (or derivative or salt thereof) and one or more cancer treatments (or other treatment) sequentially. For example, the protective agent may be administered prior to administration of the cancer treatment (or other treatment). For example, a cancer patient may be administered a therapeutically effective dose of myricetin to prevent cardiotoxicity, and subsequently administered a chemotherapeutic drug (e.g., doxorubicin). In another example, a cancer patient may be administered a therapeutically effective dose of myricitrin to prevent cardiotoxicity, and subsequently administered a chemotherapeutic drug (e.g., doxorubicin). In yet another example, a cancer patient may be administered a therapeutically effective dose of vitexin to prevent cardiotoxicity, and subsequently administered a chemotherapeutic drug (e.g., doxorubicin). In another example, a cancer patient may be administered a therapeutically effective dose of robinetin to prevent cardiotoxicity, and subsequently administered a chemotherapeutic drug (e.g., doxorubicin). In another example, a cancer patient may be administered a therapeutically effective dose of tricetin to prevent cardiotoxicity, and subsequently administered a chemotherapeutic drug (e.g., doxorubicin). In other examples, the cancer treatment (or other treatment) is administered to the patient or subject prior to administration of the protective agent(s) of Formula 1 or Formula 2. In some cases, the patient is administered the one or more protective agents prior to receiving cancer treatment (or other treatment) and then is administered one or more protective agents following the cancer treatment.

In cases of sequential administration, there may be a delay period between administration of the one or more protective agents and the one or more cancer treatments (or other treatments). For example, the protective agent may be administered minutes, hours, days, or weeks prior to administration of a cancer treatment or other treatment (e.g., at least 5 minutes, at least 10 minutes, at least 30 minutes, at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours, at least 5 hours, at least 6 hours, at least 7 hours, at least 8 hours, at least 9 hours, at least 10 hours, at least 1 day, at least 2 days, at least 3 days, at least 5 days, at

least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 2 months, at most 2 months, at most 1 month, at most 3 weeks, at most 2 weeks, at most 1 week, at most 6 days, at most 5 days, at most 4 days, at most 3 days, at most 2 days, at most 1 day, at most 12 hours, at most 6 hours, at most 4 hours, at most 3 hours, at most 2 hours or at most 1 hours prior to administration of the cancer treatment). In some cases, the protective agent has been administered to the patient at least 1 day prior to the cancer treatment. In some cases, the protective agent has been administered at most 1 day prior to the cancer treatment. In some cases, the protective agent is administered at most within 2 hours after the cancer treatment. In some cases, the protective agent is administered at most within 4 hours after the cancer treatment. In some cases, the protective agent is administered at most within 6 hours after the cancer treatment. In some cases, the protective agent is administered at most within 12 hours after the cancer treatment. In some cases, the protective agent is administered at most within 1 day after the cancer treatment. In some cases, the protective agent is administered at most within 2 days after the cancer treatment. In some cases, the protective agent is administered at most within 3 days after the cancer treatment. In some cases, the protective agent is administered at most within 4 days after the cancer treatment. In some cases, the protective agent is administered at most within 5 days after the cancer treatment.

The compounds of the current disclosure (e.g., the protective agents of Formula 1) can be administered to a patient every time the patient is dosed with an anticancer agent with a dosage regimen described herein. For example, the protective agent may be administered to a patient within 24 hours every time before the patient is scheduled to be dosed with an anticancer agent. In some cases, the protective agent can be administered to a patient within 48 hours every time before the patient is scheduled to dosed with an anticancer agent. In some cases, the protective agent can be administered concurrently to a patient every time the patient is dosed with an anticancer agent. In some cases, the protective agent can be administered to a patient every time the patient has been dosed with an anticancer agent within at least 24 hours following the cancer treatment.

The compounds of the current disclosure may be administered by any of the accepted modes of administration of agents having similar utilities, for example, by cutaneous, oral, topical, intradermal, intrathecal, intravenous, subcutaneous, intramuscular, intra-articular, intraspinal or spinal, nasal, epidural, rectal, vaginal, or transdermal/transmucosal routes. The most suitable route will depend on the nature and severity of the condition being treated. Subcutaneous, intradermal and percutaneous injections can be routes for the compounds of

this disclosure. Sublingual administration may be a route of administration for compounds of this disclosure. Intravenous administration may be a route of administration for compounds of this disclosure. In a particular example, the pharmaceutical composition provided herein may be administered to a patient orally. In another particular example, the pharmaceutical composition comprising a protective agent provided herein may be administered to a patient intravenously (via, e.g., injection or infusion). In another particular example, the pharmaceutical composition comprising a protective agent provided herein may be administered to a patient intramuscularly. In a particular example, the pharmaceutical composition comprising a protective agent provided herein may be administered to a patient nasally.

A pharmaceutical composition (*e.g.,* for oral administration or for injection, infusion, subcutaneous delivery, intramuscular delivery, intraperitoneal delivery, sublingual delivery, or other method) may be in the form of a liquid. A liquid pharmaceutical composition may include, for example, one or more of the following: a sterile diluent such as water, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils that may serve as the solvent or suspending medium, polyethylene glycols, glycerin, propylene glycol or other solvents; antibacterial agents; antioxidants; chelating agents; buffers and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. The use of physiological saline is preferred, and an injectable pharmaceutical composition is preferably sterile. In another embodiment, for treatment of an ophthalmological condition or disease, a liquid pharmaceutical composition may be applied to the eye in the form of eye drops. A liquid pharmaceutical composition may be delivered orally.

For oral formulations, at least one of the compounds or agents described herein can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, and if desired, with diluents, buffering agents, moistening agents, preservatives, coloring agents, and flavoring agents. The compounds may be formulated with a buffering agent to provide for protection of the compound from low pH of the gastric environment and/or an enteric coating. A compound included in a pharmaceutical composition may be formulated for oral delivery with a flavoring agent, *e.g.*, in a liquid, solid or semi-solid formulation and/or with an enteric coating. In some cases, the compounds of this disclosure may be solubilized and encapsulated (e.g., in a liposome or a biodegradable polymer), or

used in the form of microcrystals coated with an appropriate nontoxic lipid. In some cases, the compounds of this disclosure may be solubilized and encapsulated in a liposome, micelle or the both.

A pharmaceutical composition comprising any one of the compounds or agents described herein may be formulated for sustained or slow release (also called timed release or controlled release). Such compositions may generally be prepared using well known technology and administered by, for example, oral, rectal, intradermal, or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain the compound dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane. Excipients for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Non-limiting examples of excipients include water, alcohol, glycerol, chitosan, alginate, chondroitin, Vitamin E, mineral oil, and dimethyl sulfoxide (DMSO). The amount of compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release, and the nature of the condition, disease or disorder to be treated or prevented.

The disclosure provided herein also describes methods for preventing, reducing, or eliminating organ damage in a subject by administering to a patient one or more protective agents of Formula 1 or Formula 2. The protective agent of Formula 1 or Formula 2 for preventing, reducing, or eliminating organ damage in a subject can include without limitation myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, or myricitrin (or derivative or salt thereof). In particular, the organ damage may be caused by cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress, leading to heart failure. For example, a pharmaceutical composition comprising a compound of Formula 1 (i.e., protective agent) may be administered to a patient that is experiencing cancer treatment-induced heart failure, wherein further hear failure is prevented by the administration of the pharmaceutical composition.

The pharmaceutical methods and compositions described herein prevent, reduce, or eliminate cancer treatment-induced cardiotoxicity in a patient. Accordingly, the methods and compositions provided herein enable a patient (e.g., a heart-healthy patient, a patient with cardiac disease) to receive a higher dosage of a therapy without having the dosage regimen significantly altered by the risk of cardiotoxicity. In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of

greater than 0.1 mg/m^2, 1mg/m^2, 2 mg/m^2, 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, 6 mg/m^2, 7 mg/m^2, 8 mg/m^2, 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2, 36 mg/m^2, 37 mg/m^2, 38 mg/m^2, 39 mg/m^2, 40 mg/m^2, 41 mg/m^2, 42 mg/m^2, 43 mg/m^2, 44 mg/m^2, 45 mg/m^2, 46 mg/m^2, 47 mg/m^2, 48 mg/m^2, 49 mg/m^2, 50 mg/m^2, 100 mg/m^2, 150 mg/m^2, 200 mg/m^2, 300 mg/m^2, 350 mg/m^2, 400 mg/m^2, 450 mg/m^2, 500 mg/m^2, 750 mg/m^2, 1000 mg/m^2, 1250 mg/m^2, 1500 mg/m^2, 1750 mg/m^2, or 2000 mg/m^2 of chemotherapeutic drug (e.g., anthracycline, doxorubicin or derivative or salt thereof) to a patient.

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of about 0.1 mg/m^2, 0.2 mg/m^2, 0.3 mg/m^2, 0.4 mg/m^2, 0.5 mg/m^2, 0.6 mg/m^2, 0.7 mg/m^2, 0.8 mg/m^2, 0.9 mg/m^2, 1 mg/m^2, 1.1 mg/m^2, 1.2 mg/m^2, 1.3 mg/m^2, 1.4 mg/m^2, 1.5 mg/m^2, 1.6 mg/m^2, 1.7 mg/m^2, 1.8 mg/m^2, 1.9 mg/m^2, 2 mg/m^2, 2.1 mg/m^2, 2.2 mg/m^2, 2.3 mg/m^2, 2.4 mg/m^2, 2.5 mg/m^2, 2.6 mg/m^2, 2.7 mg/m^2, 2.8 mg/m^2, 2.9 mg/m^2, 3 mg/m^2, 3.1 mg/m^2, 3.2 mg/m^2, 3.3 mg/m^2, 3.4 mg/m^2, 3.5 mg/m^2, 3.6 mg/m^2, 3.7 mg/m^2, 3.8 mg/m^2, 3.9 mg/m^2, 4 mg/m^2, 4.1 mg/m^2, 4.2 mg/m^2, 4.3 mg/m^2, 4.4 mg/m^2, 4.5 mg/m^2, 4.6 mg/m^2, 4.7 mg/m^2, 4.8 mg/m^2, 4.9 mg/m^2, 5 mg/m^2, 5.1 mg/m^2, 5.2 mg/m^2, 5.3 mg/m^2, 5.4 mg/m^2, 5.5 mg/m^2, 5.6 mg/m^2, 5.7 mg/m^2, 5.8 mg/m^2, 5.9 mg/m^2, 6 mg/m^2, 6.1 mg/m^2, 6.2 mg/m^2, 6.3 mg/m^2, 6.4 mg/m^2, 6.5 mg/m^2, 6.6 mg/m^2, 6.7 mg/m^2, 6.8 mg/m^2, 6.9 mg/m^2, 7 mg/m^2, 7.1 mg/m^2, 7.2 mg/m^2, 7.3 mg/m^2, 7.4 mg/m^2, 7.5 mg/m^2, 7.6 mg/m^2, 7.7 mg/m^2, 7.8 mg/m^2, 7.9 mg/m^2, 8 mg/m^2, 8.1 mg/m^2, 8.2 mg/m^2, 8.3 mg/m^2, 8.4 mg/m^2, 8.5 mg/m^2, 8.6 mg/m^2, 8.7 mg/m^2, 8.8 mg/m^2, 8.9 mg/m^2, 9 mg/m^2, 9.1 mg/m^2, 9.2 mg/m^2, 9.3 mg/m^2, 9.4 mg/m^2, 9.5 mg/m^2, 9.6 mg/m^2, 9.7 mg/m^2, 9.8 mg/m^2, 9.9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2, 36 mg/m^2, 37 mg/m^2, 38 mg/m^2, 39 mg/m^2, 40 mg/m^2, 41 mg/m^2, 42 mg/m^2, 43 mg/m^2, 44 mg/m^2, 45 mg/m^2, 46 mg/m^2, 47 mg/m^2, 48 mg/m^2, 49 mg/m^2, 50 mg/m^2, 51 mg/m^2, 52 mg/m^2, 53 mg/m^2, 54 mg/m^2, 55 mg/m^2, 56 mg/m^2, 57 mg/m^2, 58 mg/m^2, 59 mg/m^2, 60 mg/m^2, 61 mg/m^2, 62 mg/m^2, 63 mg/m^2, 64 mg/m^2, 65 mg/m^2, 66 mg/m^2, 67 mg/m^2, 68 mg/m^2, 69 mg/m^2, 70 mg/m^2, 71 mg/m^2, 72 mg/m^2, 73 mg/m^2, 74 mg/m^2, 75 mg/m^2, 76 mg/m^2, 77 mg/m^2, 78 mg/m^2, 79 mg/m^2, 80 mg/m^2, 81 mg/m^2, 82 mg/m^2, 83

mg/m^2, 84 mg/m^2, 85 mg/m^2, 86 mg/m^2, 87 mg/m^2, 88 mg/m^2, 89 mg/m^2, 90 mg/m^2, 91 mg/m^2, 92 mg/m^2, 93 mg/m^2, 94 mg/m^2, 95 mg/m^2, 96 mg/m^2, 97 mg/m^2, 98 mg/m^2, 99 mg/m^2, or 100 mg/m^2 of a biologic agent to a patient.

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of about 0.1 mg/m^2, 1 mg/m^2, 2 mg/m^2, 3mg/m^2, 4 mg/m^2, 5 mg/m^2, 6 mg/m^2, 7 mg/m^2, 8 mg/m^2, 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2, 36 mg/m^2, 37 mg/m^2, 38 mg/m^2, 39 mg/m^2, 40 mg/m^2, 41 mg/m^2, 42 mg/m^2, 43 mg/m^2, 44 mg/m^2, 45 mg/m^2, 46 mg/m^2, 47 mg/m^2, 48 mg/m^2, 49 mg/m^2, 50 mg/m^2, 51 mg/m^2, 52 mg/m^2, 53 mg/m^2, 54 mg/m^2, 55 mg/m^2, 56 mg/m^2, 57 mg/m^2, 58 mg/m^2, 59 mg/m^2, 60 mg/m^2, 61 mg/m^2, 62 mg/m^2, 63 mg/m^2, 64 mg/m^2, 65 mg/m^2, 66 mg/m^2, 67 mg/m^2, 68 mg/m^2, 69 mg/m^2, 70 mg/m^2, 71 mg/m^2, 72 mg/m^2, 73 mg/m^2, 74 mg/m^2, 75 mg/m^2, 76 mg/m^2, 77 mg/m^2, 78 mg/m^2, 79 mg/m^2, 80 mg/m^2, 81 mg/m^2, 82 mg/m^2, 83 mg/m^2, 84 mg/m^2, 85 mg/m^2, 86 mg/m^2, 87 mg/m^2, 88 mg/m^2, 89 mg/m^2, 90 mg/m^2, 90 mg/m^2, 95 mg/m^2, 100 mg/m^2, 110 mg/m^2, 120 mg/m^2, 130 mg/m^2, 140 mg/m^2, 150 mg/m^2, 160 mg/m^2, 170 mg/m^2, 180 mg/m^2, 190 mg/m^2, 200 mg/m^2, 300 mg/m^2, 400 mg/m^2, 500 mg/m^2 of the protective agent drug of Formula 1 or Formula 2 (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and/or a derivative or salt thereof).

The daily fixed dose of protective agent described herein, or collective dose of a combination of protective agents can be greater than 0.1 mg, 1mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 150 mg, 200 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 750 mg or higher of the protective agent (or any derivative or salt thereof). In some cases, the protective agent or agents is selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and/or a derivative or salt thereof. In a particular example, administering a pharmaceutical composition to a patient can comprise administering a co-formulation of a chemotherapy drug (e.g., doxorubicin) with at least 10

mg of myricetin. In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of myricetin (or any derivative or salt thereof) to a patient.

In another example, administering a pharmaceutical composition to a patient can comprise administering a co-formulation of a chemotherapy drug (e.g., doxorubicin) with at least 10 mg of myricetrin. In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43

mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of myricetrin (or any derivative or salt thereof) to a patient.

In yet another example, administering a pharmaceutical composition to a patient can comprise administering a co-formulation of a chemotherapy drug (e.g., doxorubicin) with at least 10 mg of vitexin. In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of vitexin (or any derivative or salt thereof) to a patient.

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45

mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of robinetin (or any derivative or salt thereof).

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of tricetin (or any derivative or salt thereof).

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45

mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1 g of 7,3',4',5'-tetrahydroxyflavone (or any derivative or salt thereof).

In some cases, administering a pharmaceutical composition herein to a patient can comprise administering a daily dose of 0.1 mg, 0.2 mg, 0.3 mg, 0.4 mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1 mg, 1.1 mg, 1.2 mg, 1.3 mg, 1.4 mg, 1.5 mg, 1.6 mg, 1.7 mg, 1.8 mg, 1.9 mg, 2 mg, 2.1 mg, 2.2 mg, 2.3 mg, 2.4 mg, 2.5 mg, 2.6 mg, 2.7 mg, 2.8 mg, 2.9 mg, 3 mg, 3.1 mg, 3.2 mg, 3.3 mg, 3.4 mg, 3.5 mg, 3.6 mg, 3.7 mg, 3.8 mg, 3.9 mg, 4 mg, 4.1 mg, 4.2 mg, 4.3 mg, 4.4 mg, 4.5 mg, 4.6 mg, 4.7 mg, 4.8 mg, 4.9 mg, 5 mg, 5.1 mg, 5.2 mg, 5.3 mg, 5.4 mg, 5.5 mg, 5.6 mg, 5.7 mg, 5.8 mg, 5.9 mg, 6 mg, 6.1 mg, 6.2 mg, 6.3 mg, 6.4 mg, 6.5 mg, 6.6 mg, 6.7 mg, 6.8 mg, 6.9 mg, 7 mg, 7.1 mg, 7.2 mg, 7.3 mg, 7.4 mg, 7.5 mg, 7.6 mg, 7.7 mg, 7.8 mg, 7.9 mg, 8 mg, 8.1 mg, 8.2 mg, 8.3 mg, 8.4 mg, 8.5 mg, 8.6 mg, 8.7 mg, 8.8 mg, 8.9 mg, 9 mg, 9.1 mg, 9.2 mg, 9.3 mg, 9.4 mg, 9.5 mg, 9.6 mg, 9.7 mg, 9.8 mg, 9.9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 36 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg, 45 mg, 46 mg, 47 mg, 48 mg, 49 mg, 50 mg, 100 mg, 500 mg, 750 mg, or 1g of ficetin (or any derivative or salt thereof).

The pharmaceutical methods and compositions described herein prevent, reduce, or eliminate cancer treatment-induced cardiotoxicity in a patient. Accordingly, the methods and compositions provided herein enable a patient to receive a therapy more frequently without having the dosage regimen significantly altered by the risk of cardiotoxicity. The daily dose of a chemotherapeutic drug, biologic agent or protective agent within the pharmaceutical composition provided herein may be administered to a patient in one or more doses per day. In some cases, the daily dose of the chemotherapeutic drug may be administered in a single dose. In some cases, the daily dose of the chemotherapeutic drug may be divided into 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses per day. For example, the daily dose of chemotherapeutic drug (e.g., doxorubicin) can be divided into 3 doses per day. In some cases, the daily dose of the chemotherapeutic drug may be divided into at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 infusions per hour. In some cases, each infusion of a composition comprising a chemotherapeutic drug may last for at least 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, or 6 hours. In some cases, the daily dose of the biologic agent may be administered

in a single dose. In some cases, the daily dose of the biologic agent may be divided into 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 doses per day. For example, the daily dose of biologic agent (e.g., bevacizumab) can be divided into 3 doses per day. In some cases, the daily dose of the biologic agent may be divided into at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, or 60 infusions per hour. In some cases, each infusion of a composition comprising a biologic agent may last for at least 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, or 6 hours. In some cases, the daily dose of the protective agent may be administered in a single dose. In some cases, the daily dose of the protective agent may be divided into 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 doses per day. For example, the daily dose of protective agent (e.g., myricetin) can be divided into 3 doses per day. In some cases, the daily dose of the protective agent may be divided into at least 1, 2, 3, 4, 5, or 6 infusions per hour. In some cases, each infusion of a composition comprising one or more protective agent(s) may last for at least 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, or 6 hours.

The pharmaceutical compositions described herein may be administered to a patient one or more times per day. In some cases, the pharmaceutical composition may be administered to a patient one time per day. In some cases, the pharmaceutical composition may be administered to a patient at least 2 times, 3 times, 4 times 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 11 times, 12 times, 13 times, 14 times, 15 times, 16 times, 17 times, 18 times, 19 times, 20 times, 21 times, 22 times, 23 times, or 24 times per day. For example, a pharmaceutical composition may be administered to a patient 3 times per day.

The pharmaceutical compositions described herein may be administered to a patient for one or more days. In some cases, the pharmaceutical composition may be administered to a patient for one day. In some cases, the pharmaceutical composition may be administered to the patient for at least 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 20 years, 30 years, 40 years, or 50 years. For example, a cancer patient may be administered a pharmaceutical co-formulation of doxorubicin and myricetin for a period

of at least 1 year. In some cases, the pharmaceutical composition may be administered to a patient for two or more consecutive days. In some cases, the pharmaceutical composition may be administered to a patient for two or more non-consecutive days. For example, a patient may be administered a pharmaceutical composition every day, consecutively, for 4 days. In another example, a patient may be administered a pharmaceutical composition on day 1, day 3, day 7, and day 15. In some cases, when a patient is administered a pharmaceutical composition over a period of time, the dosage amount administered to the patient on one day can be different from the dosage amount administered to the patient on a subsequent day. For example, a patient may be administered 5 mg of a pharmaceutical composition on the first day, and administered 10 mg of a pharmaceutical composition on a subsequent day.

The pharmaceutical compositions described herein may be effective over time. In some cases, the pharmaceutical composition may be effective for one or more days. In some cases, the duration of efficacy of the pharmaceutical composition is over a long period of time. In some cases, the efficacy of the pharmaceutical composition may be greater than 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or 1 month.

Methods provided herein can further comprise administering to the patient dexrazoxane (or any derivative or salt thereof) as part of any of the pharmaceutical compositions described herein. Such methods allow for the administration to a patient a pharmaceutical composition containing at least one protective agent and dexrazoxane, wherein the co-formulation of at least one protective agent and dexrazoxane can provide a greater protective effect as compared to the administration of dexrazoxane alone. In some cases, the administration of any of the pharmaceutical compositions described herein can reduce the likelihood of cardiotoxicity across a patient pool by as much as 1%, 2%, 3%, 4%, 5%, 6%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%. For example, if there is an 80% likelihood that patients in a patient pool that are administered dexrazoxane will experience cardiotoxicity, administering to the patients a co-formulation of myricetin and dexrazoxane can reduce the likelihood of experiencing cardiotoxicity by 75%,

resulting in a 20% likelihood that the patients will experience cardiotoxicity. This greater protective effect may also enable a larger population of patients, including those with pre-existing cardiac conditions, to receive a cancer treatment (e.g., doxorubicin) to which they would otherwise be precluded. In some cases, the dexrazoxane may be co-formulated within the pharmaceutical composition, in that it is mixed within the pharmaceutical composition, or exist as a distinct entity. In some cases, the cancer treatment, protective agent, and dexrazoxane may be administered concurrently. In some cases, the cancer treatment, protective agent, and dexrazoxane may be administered sequentially. In one example, a cancer patient may be administered a co-formulation of chemotherapeutic drug, dexrazoxane, and myricetin in a single dose at least one time per day. In another example, a cancer patient may be administered dexrazoxane, and subsequently administered myricetin.

The dose of dexrazoxane (or any derivative or salt thereof) administered within the pharmaceutical composition can be greater than 0.1 mg/m^2, 1mg/m^2, 2 mg/m^2, 3 mg/m^2, 4 mg/m^2, 5 mg/m^2, 6 mg/m^2, 7 mg/m^2, 8 mg/m^2, 9 mg/m^2, 10 mg/m^2, 11 mg/m^2, 12 mg/m^2, 13 mg/m^2, 14 mg/m^2, 15 mg/m^2, 16 mg/m^2, 17 mg/m^2, 18 mg/m^2, 19 mg/m^2, 20 mg/m^2, 21 mg/m^2, 22 mg/m^2, 23 mg/m^2, 24 mg/m^2, 25 mg/m^2, 26 mg/m^2, 27 mg/m^2, 28 mg/m^2, 29 mg/m^2, 30 mg/m^2, 31 mg/m^2, 32 mg/m^2, 33 mg/m^2, 34 mg/m^2, 35 mg/m^2, 36 mg/m^2, 37 mg/m^2, 38 mg/m^2, 39 mg/m^2, 40 mg/m^2, 41 mg/m^2, 42 mg/m^2, 43 mg/m^2, 44 mg/m^2, 45 mg/m^2, 46 mg/m^2, 47 mg/m^2, 48 mg/m^2, 49 mg/m^2, 50 mg/m^2, 51 mg/m^2, 52 mg/m^2, 53 mg/m^2, 54 mg/m^2, 55 mg/m^2, 56 mg/m^2, 57 mg/m^2, 58 mg/m^2, 59 mg/m^2, 60 mg/m^2, 61 mg/m^2, 62 mg/m^2, 63 mg/m^2, 64 mg/m^2, 65 mg/m^2, 66 mg/m^2, 67 mg/m^2, 68 mg/m^2, 69 mg/m^2, 70 mg/m^2, 71 mg/m^2, 72 mg/m^2, 73 mg/m^2, 74 mg/m^2, 75 mg/m^2, 76 mg/m^2, 77 mg/m^2, 78 mg/m^2, 79 mg/m^2, 80 mg/m^2, 81 mg/m^2, 82 mg/m^2, 83 mg/m^2, 84 mg/m^2, 85 mg/m^2, 86 mg/m^2, 87 mg/m^2, 88 mg/m^2, 89 mg/m^2, 90 mg/m^2, 91 mg/m^2, 92 mg/m^2, 93 mg/m^2, 94 mg/m^2, 95 mg/m^2, 96 mg/m^2, 97 mg/m^2, 98 mg/m^2, 99 mg/m^2, 100 mg/m^2, 150 mg/m^2, 200 mg/m^2, 300 mg/m^2, 350 mg/m^2, 400 mg/m^2, 450 mg/m^2, 500 mg/m^2, 750 mg/m^2, 1 g/m^2, 5 g/m^2, 10 g/m^2, or higher. In a particular example, administering a pharmaceutical composition to a patient can comprise administering a co-formulation of a protective agent of Formula 1 or Formula 2 (e.g., myricetin) with 50 mg/m^2 of dexrazoxane.

In some cases, administering a pharmaceutical composition described herein to a patient can comprise administering a dose of about 0.1 mg/kg, 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg, 0.6 mg/kg, 0.7 mg/kg, 0.8 mg/kg, 0.9 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3mg/kg , 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8

mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, 21 mg/kg, 22 mg/kg, 23 mg/kg, 24 mg/kg, 25 mg/kg, 26 mg/kg, 27 mg/kg, 28 mg/kg, 29 mg/kg, 30 mg/kg, 31 mg/kg, 32 mg/kg, 33 mg/kg, 34 mg/kg, 35 mg/kg, 36 mg/kg, 37 mg/kg, 38 mg/kg, 39 mg/kg, 40 mg/kg, 41 mg/kg, 42 mg/kg, 43 mg/kg, 44 mg/kg, 45 mg/kg, 46 mg/kg, 47 mg/kg, 48 mg/kg, 49 mg/kg, 50 mg/kg, 51 mg/kg, 52 mg/kg, 53 mg/kg, 54 mg/kg, 55 mg/kg, 56 mg/kg, 57 mg/kg, 58 mg/kg, 59 mg/kg, 60 mg/kg, 61 mg/kg, 62 mg/kg, 63 mg/kg, 64 mg/kg, 65 mg/kg, 66 mg/kg, 67 mg/kg, 68 mg/kg, 69 mg/kg, 70 mg/kg, 71 mg/kg, 72 mg/kg, 73 mg/kg, 74 mg/kg, 75 mg/kg, 76 mg/kg, 77 mg/kg, 78 mg/kg, 79 mg/kg, 80 mg/kg, 81 mg/kg, 82 mg/kg, 83 mg/kg, 84 mg/kg, 85 mg/kg, 86 mg/kg, 87 mg/kg, 88 mg/kg, 89 mg/kg, 90 mg/kg, 90 mg/kg, 95 mg/kg, 100 mg/kg, 110 mg/kg, 120 mg/kg, 130 mg/kg, 140 mg/kg, 150 mg/kg, 160 mg/kg, 170 mg/kg, 180 mg/kg, 190 mg/kg, 200 mg/kg, 300 mg/kg, 400 mg/kg, 500 mg/kg of a protective agent of Formula 1 or Formula 2. In some aspects, the protective agent can be selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and a derivative or salt thereof. In one embodiment, the patient is administered intravenously with a protective agent at 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3mg/kg , 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, 20 mg/kg, 21 mg/kg, 22 mg/kg, 23 mg/kg, 24 mg/kg, 25 mg/kg, 26 mg/kg, 27 mg/kg, 28 mg/kg, 29 mg/kg, 30 mg/kg, 31 mg/kg, 32 mg/kg, 33 mg/kg, 34 mg/kg, 35 mg/kg, 36 mg/kg, 37 mg/kg, 38 mg/kg, 39 mg/kg, 40 mg/kg, 41 mg/kg, 42 mg/kg, 43 mg/kg, 44 mg/kg, 45 mg/kg, 46 mg/kg, 47 mg/kg, 48 mg/kg, 49 mg/kg, 50 mg/kg, 60 mg/kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, or 100 mg/kg, 150 mg/kg, or 200 mg/kg. In one embodiment, the patient is administered with myricetin at a dose between about 0.5 mg/kg and about 50 mg/kg at least 10 minutes before administering an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered with myricetin at a dose between about 0.5 mg/k and about 100 mg/kg at least 10 minutes before administering an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with myricetin at a dose between about 0.5 mg/kg and about 200mg/kg at least 30 minutes prior to the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with myricetin at a dose between about 0.5 mg/kg and about 200 mg/kg at least 1 hour prior to the administration of an

anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with myricetin at a dose between about 0.5 mg/kg and about 200 mg/kg of myricetin at least 2 hours before the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with a dose between about 0.5 mg/kg and about 200 mg/kg of myricetin at least 4 hours prior to the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with myricetin at a dose between about 0.5 mg/kg and about 200 mg/kg of myricetin at least 6 hours before the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, the patient is administered intravenously with myricetin at a dose between about 0.5 mg/kg and about 200 mg/kg of myricetin within 6 hours after the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin). In one embodiment, myricetin is administered orally at a dose between 0.5 mg/kg and 200 mg/kg at least 0.5, 1, 2, 3, 4, 5, or 6 hour(s) prior to the administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin).

In some aspects, the patient is administered, for example, intravenously or orally with a protective agent (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, or myricitrin) at a dose between about 0.5 mg/kg and about 200 mg/kg at least 4 hours prior to the first administration of an anthracycline (e.g., doxorubicin, epirubicin, or idarubicin) after the patient has been diagnosed with cancer.

Patient Response

The methods and compositions provided herein prevent, reduce, or eliminate cardiotoxicity in a patient caused by chemotherapeutic drugs, biologic agents, or radiation therapy. Furthermore, administering to a patient a pharmaceutical composition disclosed herein may also prevent, reduce or eliminate cancer treatment-induced organ damage (e.g., organ damage caused by cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, or oxidative stress).

The methods and compositions disclosed herein may generally reduce cardiotoxicity in a patient. Examples of cardiotoxicity can include, but are not limited to, mitochondrial toxicity, apoptosis, electrophysiological dysfunction (e.g., QT prolongation), mechanical dysfunction (e.g., reduced cardiac ejection fraction), oxidative stress, cardiac tissue damage (e.g., damage caused by oxidative stress, mitochondrial damage, or damage caused by an

increase in the flux of reactive oxygen species), and cytotoxic injury to any organ (e.g., liver, kidney, or pancreas) that is not the heart.

Mitochondrial toxicity can refer to any damage that decreases the number of the active mitochondria within a given cell, tissue, organ, or organism. In some cases, mitochondrial toxicity can be measured using an *in vitro* assay. One such method that can be used for measuring mitochondrial toxicity is by co-exposing cells to (1) a cell-permeable fluorescent dye that indicates cellular nuclei, and (2) tetramethylrhodamine methyl ester (TMRM), a cell-permeable fluorescent dye that is sequestered by active mitochondria. Mitochondrial toxicity can be calculated as the fraction of TMRM-positive cells to the total number of cell nuclei. As measured by the *in vitro* assay, cancer treatment-induced mitochondrial toxicity may be greater than 1%, 2%, 3%, 4%, 5%, 6%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% in cardiomyocytes, as compared to untreated controls. For example, exposing cardiomyocytes to 1micromolar of doxorubicin for at least 48 hours can cause 100% mitochondrial toxicity, as compared to untreated control. The pharmaceutical methods and compositions described herein generally reduce cancer treatment-induced mitochondrial toxicity. As measured by the *in vitro* assay, exposing cardiomyocytes to any of the pharmaceutical compositions described herein can reduce mitochondrial toxicity as much as 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% as compared to cardiomyocytes exposed to a cancer treatment in the absence of a protective agent. For example, exposing cardiomyocytes to a co-formulation of 1micromolar doxorubicin and 115 micromolar of myricetin for at least

48 hours can reduce mitochondrial toxicity by 30%, as compared to cardiomyocytes exposed to 1 micromolar of doxorubicin.

Apoptosis can refer to a process by which a cell undergoes programmed cell death. Detectable changes within a cell undergoing apoptosis include, but are not limited to, the translocation of cytochrome C from the mitochondria, diminished mitochondrial function, changes in membrane structure, increased proteolytic activity, and DNA fragmentation. In some cases, apoptosis can be measured using an *in vitro* assay. One such method that can be used for measuring apoptosis is by co-exposing cells to (1) a cell-permeable fluorescent dye that indicates cellular nuclei, and (2) CellEvent Caspase 3/7 Detection Reagent, a fluorogenic substrate for the activated caspase 3 that is uniquely present in apoptotic cells. Percentage apoptosis can be calculated as the fraction of CellEvent-positive cells to the total number of cell nuclei. As measured by the *in vitro* assay, cancer treatment-induced apoptosis may be greater than 1%, 2%, 3%, 4%, 5%, 6%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% in cardiomyocytes, as compared to untreated controls. For example, exposing cardiomyocytes to 1micromolar of doxorubicin for at least 48 hours can cause 100% apoptosis, as compared to untreated control.

The pharmaceutical methods and compositions described herein may generally reduce cancer treatment-induced apoptosis. As measured by the *in vitro* assay, exposing cardiomyocytes to any of the pharmaceutical compositions described herein can reduce apoptosis as much as 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% as compared to cardiomyocytes exposed to a cancer treatment in the absence of a protective agent. For example, exposing cardiomyocytes to a co-formulation of 1micromolar doxorubicin and 115 micromolar of myricetin for at least 48

hours can reduce mitochondrial toxicity by 30%, as compared to cardiomyocytes exposed to 1 micromolar doxorubicin.

Electrophysiological dysfunction can refer to any damage wherein the flow of ions through a biological tissue is disrupted. For example, administering to a cancer patient a chemotherapeutic drug (e.g., doxorubicin) may cause an acute myocardial infarction, wherein ions can no longer flow through the damaged cardiac tissue resulting in a conduction block. In some cases, electrophysiological dysfunction can comprise prolongation of the QT interval, and can be measured using an *in vivo* assay. The QT interval can be used to describe the time between the start of the Q wave and the end of the T wave in an electrocardiogram. QT prolongation may indicate delayed ventricular repolarization, and can predispose the heart to early after-depolarizations (EADs) leading to re-entrant arrhythmia (e.g., Torsades de Pointes). A QT interval may also depend on the length of the cardiac cycle (RR), the amount of time between the onset of one QRS complex and the onset of the next QRS complex. A corrected QT (QTc) interval may be used to represent a QT interval that has been corrected to account for the cycle length. Bazett's formula ($QTc=QT/\sqrt{RR}$), Fridericia's formula ($QTc=QT/\sqrt[3]{RR}$), or a regression analysis method ($QTc=QT+0.154(1-RR)$) may all be used to calculate the QTc interval from the QT interval.

Administration of a chemotherapeutic drug, biologic agent, or radiation therapy to a patient may cause QTc prolongation, above the baseline QTc interval of the patient, in the absence of a protective agent. The baseline QTc interval for a patient is the QTc interval measured in the patient prior to the administration of any drug. For example, administering to a patient a chemotherapeutic drug (e.g., doxorubicin) in the absence of a protective agent can cause a QTc prolongation of 40 milliseconds (ms) above the baseline QTc interval for the patient. In some cases, administration of a chemotherapeutic drug, biologic agent, or radiation therapy to a patient, particularly in the absence of a protective agent, may cause a QTc prolongation of at least 1 ms, 2 ms, 3 ms, 4 ms, 5 ms, 6 ms, 7 ms, 8 ms, 9 ms, 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 35 ms, 40ms, 45 ms, 50 ms, 55 ms, 60 ms, 65 ms, 70 ms, 75 ms, 80 ms, 85 ms, 90 ms, 95 ms, or 100 ms above the baseline QTc interval of the patient.

Administration of any of the pharmaceutical compositions described herein can limit the cancer treatment-induced QTc prolongation experienced by the patient, above the baseline QTc interval of the patient. For example, administering to a patient a co-formulation of a chemotherapeutic drug (e.g., doxorubicin) and a protective agent (e.g., myricetin) can cause a QTC prolongation of less than 5 ms. In some cases, the pharmaceutical compositions

described herein can cause less than a 100 ms, 95 ms, 90 ms, 85 ms, 80 ms, 75 ms, 70 ms, 65 ms, 60 ms, 55 ms, 50 ms, 45 ms, 40 ms, 35 ms, 30 ms, 25 ms, 20 ms, 15 ms, 10 ms, 9 ms, 8 ms, 7 ms, 6 ms, 5 ms, 4 ms, 3 ms, 2 ms, or 1 ms increase in QTc prolongation above the baseline QTc interval of the patient.

In some cases, electrophysiological dysfunction can also comprise diminished electrical activity, and can be measured using an *in vitro* assay. Multielectrode arrays (MEAs) are devices that contain multiple planar conductive electrodes on which cells (e.g., cardiomyocytes) may be contacted. Although the size and shape of the electrical recording measured from an MEA can depend on several factors (e.g., cell homogeneity, contact between the cell and an electrode, geometry of an MEA), temporal changes can be measured by the electrode to provide information on the electrical activity of the contacting cells (e.g., percentage of active electrodes, field potential duration, and beat rate).

Exposing cardiomyocytes to a chemotherapeutic drug, biologic agent, or radiation therapy, in the absence of a protective agent, may cause a temporal decrease in the percentage of active electrodes (e.g., an electrode that is able to measure some electrical activity from the contacting cell), as measured by the *in vitro* assay. For example, exposing cardiomyocytes to 1 micromolar of doxorubicin for at least 24 hours can cause a 50% decrease in the number of active electrodes, as compared to time zero. In some cases, exposing cardiomyocytes to a cancer treatment (e.g., doxorubicin)in the absence of a protective agent (e.g., myricetin) can cause as much as a 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% reduction in the number of active electrodes, as measured by the *in vitro* assay. In a particular example, exposing cardiomyocytes to 1 μM doxorubicin for at least 24 hours can cause as much as a 50% reduction in the number of active electrodes.

The pharmaceutical methods and compositions described herein generally reduce cancer treatment-induced electrophysiological dysfunction (e.g., decrease in the number of active electrodes). As measured by the *in vitro* assay, exposing cardiomyocytes to any of the pharmaceutical compositions described herein may induce less than a 1%, 2%, 3%, 4%, 5%,

6%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% decrease in the number of active electrodes, as compared to cardiomyocytes exposed to a cancer treatment in the absence of a protective agent. For example, exposing cardiomyocytes to a co-formulation of 1 μM doxorubicin and 100 μM myricetin for at least 24 hours can induce less than a 5% decrease in the number of active electrodes.

The pharmaceutical methods and compositions described herein generally reduce the risk that the patient will experience cardiotoxicity with the administration of a cancer treatment. In some cases, the pharmaceutical methods and compositions described herein can reduce the risk of cardiotoxicity in the patient by 100%, 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1%. In some cases, the pharmaceutical methods and compositions disclosed herein may reduce the risk of cardiotoxicity in the patient by greater than 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. For example, if a patient has a 90% risk for experiencing QT prolongation when administered a chemotherapeutic drug (e.g., doxorubicin, epirubicin, or idarubicin) in the absence of a protective agent (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, myricitrin and/or a derivative or salt thereof), the patient may experience a 50% reduction of risk for QT prolongation when the protective agent is administered separately or as a co-formulation with a chemotherapeutic drug, resulting in a

45% risk for QT prolongation in the patient. For example, in one particularly embodiment, the patient is administered intravenously with a protective agent (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, myricitrin and/or a derivative or salt thereof) at a dose between about 0.5 mg/kg and about 100 mg/kg at least 30 minute, 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, or 6 hours prior to the administration of chemotherapeutic drug (e.g., doxorubicin, epirubicin, or idarubicin), wherein the risk for QT prolongation is reduced by at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% as compared to that of control that did not receive the protective agent.

The effect of anthracycline-induced cardiotoxicity on contractility can be also assessed by measuring fractional shortening (FS) and ejection fraction (EF) which are indices of systolic function. An anthracycline such as doxorubicin can have a profound impact on contractile properties. However, a patient administered with a protective agent of Formula 1 or Formula 2 (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and/or a derivative or salt thereof) can experience significantly reduced, e.g., doxorubicin-induced cardiotoxicity as observed by marked improvements in FS and EF. For example, myricetin can rescue anthracycline-induced FS and EF dysfunction by at least 30%, 40%, 50%, 60%, 70%, 80%, 90% in the patient as compared to a control group that has been treated with anthracycline, but not dosed with the protective agent.

The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 10% of the stated number or numerical range.

The term "therapeutically effective amount" may generally refer to the amount (or dose) of a compound or other therapy that is minimally sufficient to prevent, reduce, treat or eliminate a condition, or risk thereof, when administered to a subject in need of such compound or other therapy. In some instances, the term "therapeutically effective amount" may refer to that amount of compound or other therapy that is sufficient to have a prophylactic effect when administered to a subject. The therapeutically effective amount may vary; for example, it may vary depending upon the subject's condition, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, all of which may be determined by one of ordinary skill in the art.

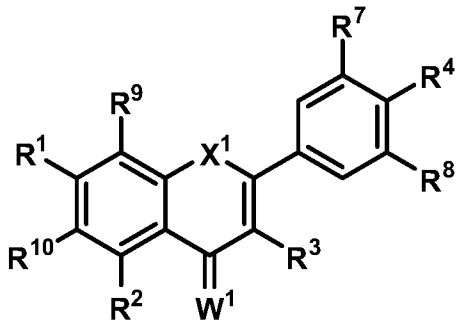
COMPOSITIONS

The pharmaceutical compositions disclosed herein may comprise a protective agent disclosed in Formula 1 or Formula 2 (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin and/or a derivative or salt thereof). The pharmaceutical composition may comprise one or more protective agents in any combination, two or more agents in any combination, three or more protective agents in any combination, or four or more protective agents in any combination. In some cases, the pharmaceutical composition can be a co-formulation of at least two protective agents (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, myricetin (myricitrin), dexrazoxane, and/or a derivative or salt thereof), or a co-formulation of at least one protective agent and a cancer treatment (e.g., chemotherapeutic drug, biologic agent, protein kinase inhibitor or radiation therapy). The protective agents within the pharmaceutical composition may reduce, eliminate or prevent cardiotoxicity induced by the cancer treatment. Additionally, the protective agents within the pharmaceutical composition may also reduce, eliminate or prevent organ damage induced by the cancer treatment. In one example, this disclosure provides a co-formulation comprising myricetin and dexrazoxane. In another example, this disclosure provides a co-formulation comprising the chemotherapeutic drug doxorubicin and myricetin.

In some cases, at least one of the protective agents in the composition may be a flavonoid, or a derivative thereof. Generally, a flavonoid may be any compound with a 15-carbon skeleton backbone consisting of two phenyl and one heterocyclic ring. Flavonoids may belong to any of the following classes of compounds including, but not limited to, anthroxanthins, flavanones, flavonols, flavanonols, flavans, anthocyanadins, bioflavonoids, isoflavonoids, isoflavones, isoflavanes, isoflavandiols, isoflavenes, or neoflavonoids. Non-limiting examples of flavonoids include ayanin, carlinoside, dihydrodaidzein, dihydroobavatin, irigenin, isoanhydroicaritin, isokurarinone, isoxanthohumol, gardenin, lupiwighteone, methoxypuerarin, mirificin, myricetin, myricetrin (myricitrin), dihydromyricetin, pyrroside, kaempferol, quercetin, swertisin, syzalterin, tricetin, ficetin, robinetin, dihydrorobinetin, 7,3',4',5'-tetrahydoxylflavone, 5,7,3',4',5'-pentahydoxyflavone or thevetiaflavone. In one example, a pharmaceutical composition disclosed herein may comprise the flavone such as 7,3',4',5'-tetrahyodxyflavone and tricetin. In another example, a pharmaceutical composition disclosed herein may comprise the flavonol such as myricetin, ficetin, robinetin, quercetin and kaempferol. In another example, a pharmaceutical

composition disclosed herein may comprise myricetrin. In an additional example, a pharmaceutical composition disclosed herein may comprise the flavanolol such as dihydromyricetin and dihydrorobinetin. In yet another example, a pharmaceutical composition disclosed herein may comprise a co-formulation of dexrazoxane and the flavonoid myricetin. In particular, the flavonoid myricetin may regulate mitochondrial toxicity in the heart by altering the activity of pyruvate dehydrogenase kinase (PDK4), a protein that may regulate enzymatic activity in cardiac tissue

In some cases, the pharmaceutical compositions described herein may comprise a compound according to Formula 1,



Formula 1

wherein:

X^1 is CR^5R^6, NR^5, O, S, C=O, or C=S;

each of R^1, R^2, R^3, R^5, R^6, R^9, and R^{10} is independently alkyl, alkenyl, alkynyl, alkoxy, acyl, acyloxy, carboxylic acid, ester, amine, amide, carbonate, carbamate, nitro, thioether, thioester, cycloalkyl, heteroalkyl, heterocyclyl, monosaccharide, aryl, or heteroaryl, any of which is substituted or unsubstituted, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H;

R^4, R^7 and R^8 are alkoxy, hydroxyl or H;

W^1 is O or S; or

a salt thereof.

In some aspects, X^1 can be O or S; each of R^1, R^2, R^3, R^9, and R^{10} can be independently alkoxy, cycloalkyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and each of R^4, R^7 and R^8 can be alkoxy, hydroxyl or H.

In some aspects, X^1 can be O; each of R^1, R^2, R^3, R^9, and R^{10} can be independently alkoxy, cycloalkyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and each of R^4, R^7 and R^8 can be alkoxy, hydroxyl or H.

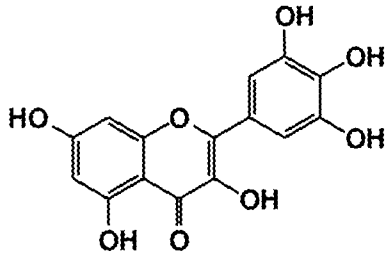
In yet other aspects, X^1 can be O; each of R^1 and R^2 can be independently hydroxyl or H; each of R^3, R^9 and R^{10} can be cycloalkyl, heterocyclyl, hydroxyl, or H; R^4 can be hydroxyl; and R^7 and R^8 can be hydroxyl or H.

In yet other aspects, X^1 can be O; R^1 can be hydroxyl; each of R^2 and R^3 can be independently hydroxyl or H; R^9 and R^{10} can be H; R^4 can be hydroxyl; and R^7 and R^8 can be hydroxyl or H.

In yet other aspects, X^1 is O; R^1 is hydroxyl; each of R^2 and R^3 can be independently hydroxyl or H; R^9 can be heterocyclyl or H; of R^{10} is H; R^4 can be independently hydroxyl or H; and each of R^7 and R^8 can be independently hydroxyl or H.

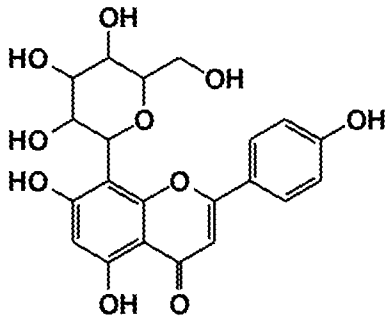
In yet other aspects, X^1 is O; R^1 is hydroxyl; each of R^2 and R^9 can be independently hydroxyl or H; R^3 can be cycloalkyl, hydroxyl or H; R^{10} is H; R4 is hydroxyl; and each of R^7 and R^8 can be independently hydroxyl or H. In one embodiment, cycloalkyl of R^3 can be a monosaccharide.

In a particular example, the compound can be of the following formula:

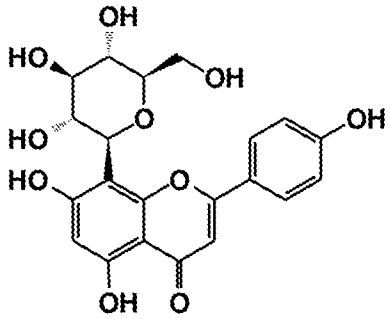


In a particular example, the compound can be myricetin. In one particular example, the compound can be robinetin. In one particular example, the compound can be tricetin. In one particular example, the compound can be 7,3',4',5'-tetrahydroxyflavone. In one particular example, the compound can be ficetin. In one particular example, the compound can be kaempferol. In one particular example, the compound can be quercetin.

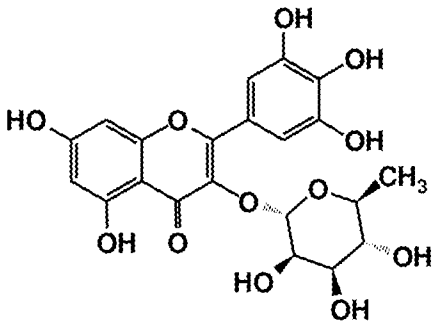
In a particular example, a protective agent within the pharmaceutical composition can be a compound with the following structure:



In a particular example, a protective agent within the pharmaceutical composition can be vitexin, wherein vitexin has the following structure:



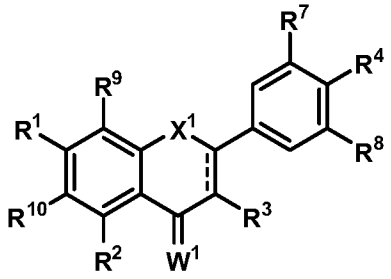
In a particular example, the compound may be a compound according to Formula 1, wherein R^1 is hydroxyl, R^2 is hydroxyl, R^3 is monosaccharide, R^4 is hydroxyl, R^7 is hydroxyl, R^8 is hydroxyl, R^9 is H, R^{10} is H, X^1 is O, and W^1 is O, or a salt thereof. In a particular example, the compound can be of the following formula:



In a particular example, the compound can be myricetrin/myricitrin.

In some cases, the monosaccharide can be a natural or unnatural sugar molecule. Non-limiting examples of a monosaccharide include glucose, dextrose, fructose, galactose mannose, ribose, deoxyribose, D-allose, L-allose, D-altrose, L-altrose, D-fucose, L-fucose, D-gulose, L-gulose, D-sorbose, D-tagatose, D-arabinose, L-arabinose, D-lyxose, L-lyxose, rhamnose, D-ribose, ribulose, sucroribulose, D-xylose, D-erythrose, L-erythrose, erythrulose, D-threose, and L-threose.

In some cases, the pharmaceutical compositions described herein may comprise a compound according to Formula 2,



Formula 2

wherein:

X^1 is CR^5R^6, NR^5, O, S, C=O, or C=S;

===== represents a single or double bond;

each of R^1, R^2, R^3, R^5, R^6, R^9, and R^{10} is independently alkyl, alkenyl, alkynyl, alkoxy, acyl, acyloxy, carboxylic acid, ester, amine, amide, carbonate, carbamate, nitro, thioether, thioester, cycloalkyl, heteroalkyl, heterocyclyl, monosaccharide, aryl, or heteroaryl, any of

which is substituted or unsubstituted, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H;

R^4, R^7 and R^8 are hydroxyl;

W^1 is O or S;

or a salt thereof.

In one particular example, the pharmaceutical compositions Formula 2 may comprise a dihydrorobinetin.

In some cases, the cancer treatment within the pharmaceutical composition described herein may be a chemotherapeutic drug (e.g., anthracyclines, protein kinase inhibitors, and proteasome inhibitors). Generally, the chemotherapeutic drug may be a drug that can induce cardiotoxicity in a patient or subject. Non-limiting examples of an anthracycline may include daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin. Non-limiting examples of a protein kinase inhibitor may include a tyrosine kinase inhibitor, afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib. Non-limiting examples of tyrosine kinase inhibitors that cause cardiotoxicity include dasatinib, imatinib, lapatinib, mesylate, nilotinib, sorafenib and sunitinib. Non-limiting example of proteasome inhibitors include bortezomib.

In some cases, a pharmaceutical composition disclosed herein may comprise a co-formulation of an anthracycline (e.g., doxorubicin) and a compound of Formula 1 or Formula 2 (e.g., myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, dihydrorobinetin, myricitrin, and/or a derivative or salt thereof). For example, the pharmaceutical composition comprises a co-formulation of doxorubicin and myricetin. In another example, the pharmaceutical composition disclosed herein may comprise a co-formulation of a protein kinase inhibitor or proteasome inhibitor (e.g., afatinib or bortezomib) and myricetin. In another example, the pharmaceutical composition disclosed herein may comprise a co-formulation of tyrosine kinase inhibitor and a protective agent. In one embodiment, the pharmaceutical composition disclosed herein may comprise a co-

formulation of sunitinib and myricetin. In another example, the pharmaceutical composition disclosed herein may comprise a co-formulation of sorafenib and myricetin.

In some cases, the cancer treatment within the pharmaceutical composition described herein may be a biologic agent (e.g., an antibody). Non-limiting examples of a biologic agent include adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab. For example, a pharmaceutical composition disclosed herein may comprise a co-formulation of bevacizumab and myricetin. For example, a pharmaceutical composition disclosed herein may comprise a co-formulation of trastuzumab and myricetin.

The compounds of the current disclosure, or their pharmaceutically acceptable salts may contain one or more asymmetric centers and may thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that are defined, in terms of absolute stereochemistry, as (*R*)- or (*S*)- or, as (D)- or (L)- for amino acids. The present invention is meant to include all such possible isomers, as well as their racemic and optically pure forms. A "stereoisomer" refers to a compound made up of the same atoms bonded by the same bonds but having different three-dimensional structures, which are not interchangeable. The present disclosure contemplates various stereoisomers and mixtures thereof and includes "enantiomers", which refers to two stereoisomers whose molecules are nonsuperimposeable mirror images of one another. Optically active (+) and (-), (*R*)- and (*S*)-, or (D)- and (L)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques, for example, chromatography and fractional crystallization. Conventional techniques for the preparation/isolation of individual enantiomers include chiral synthesis from a suitable optically pure precursor or resolution of the racemate (or the racemate of a salt or derivative) using, for example, chiral high pressure liquid chromatography (HPLC). When the compounds described herein contain olefinic 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.

When desired, the (R)- and (S)-isomers of the compounds of the present disclosure, if present, may be resolved by methods known to those skilled in the art, for example by formation of diastereoisomeric salts or complexes which may be separated, for example, by crystallization; via formation of diasteroisomeric derivatives which may be separated, for

example, by crystallization, gas-liquid or liquid chromatography; selective reaction of one enantiomer with an enantiomer-specific reagent, for example enzymatic oxidation or reduction, followed by separation of the modified and unmodified enantiomers; or gas-liquid or liquid chromatography in a chiral environment, for example on a chiral support, such as silica with a bound chiral ligand or in the presence of a chiral solvent. Alternatively, a specific enantiomer may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer to the other by asymmetric transformation.

Compounds may be dosed in their enantiomerically pure form. In some examples, the compound has an enantiomeric excess greater than about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99%. Compounds may be dosed in their diasteriomerically pure form. In some examples, the compound has a diasteriomeric excess greater than about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99%.

Stereocenters may be defined using the Cahn–Ingold–Prelog priority rules. Compounds may have stereocenters in the *R*-configuration. Compounds may have stereocenters in the *S*-configuration.

Some compounds may exhibit polymorphism. It is to be understood that the present disclosure encompasses any racemic, optically-active, polymorphic, or stereoisomeric form, or mixtures thereof, of a compound of the disclosure, which possesses the useful properties described herein, it being well known in the art how to prepare optically active forms (for example, by resolution of the racemic form by recrystallization techniques, by synthesis from optically-active starting materials, by chiral synthesis, or by chromatographic separation using a chiral stationary phase).

In certain particular embodiments, more than one compound of the current disclosure may be administered at a time to a subject. In some embodiments, two compounds of the current disclosure in combination may act synergistically or additively, and either compound may be used in a lesser amount than if administered alone.

In certain embodiments, compounds disclosed herein and/or pharmaceutical compositions thereof can be used in combination therapy with other therapeutic agents. The compounds disclosed herein and/or pharmaceutical compositions thereof and the therapeutic agent can act additively or, more preferably, synergistically. In some embodiments, compounds disclosed herein and/or pharmaceutical compositions thereof are administered concurrently with the administration of another therapeutic agent. For example, compounds

disclosed herein and/or pharmaceutical compositions thereof may be administered together with another therapeutic agent. In other embodiments, compounds disclosed herein and/or pharmaceutical compositions thereof are administered prior or subsequent to administration of other therapeutic agents.

The compounds of the present disclosure, or their pharmaceutically acceptable salts, are generally administered in a therapeutically effective amount. The amount of the compound actually administered may be determined by a physician or caregiver, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the compound administered and its relative activity, the age, weight, the response of the individual patient, the severity of the patient's symptoms, and the like.

The present disclosure further provides salts of any compound described herein. The term "salt" refers to salts derived from a variety of organic and inorganic counter ions well known in the art. Salts include, for example, acid-addition salts and base-addition salts. The acid that is added to a compound to form an acid-addition salt can be an organic acid or an inorganic acid. Inorganic acids from which salts can be derived include, for example, hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Organic acids from which salts can be derived include, for example, acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. A base that is added to a compound to form a base-addition salt can be an organic base or an inorganic base. In some cases, a salt can be a metal salt. In some cases, a salt can be an ammonium salt. Inorganic bases from which salts can be derived include, for example, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Organic bases from which salts can be derived include, for example, primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, basic ion exchange resins, and the like.

Acid addition salts can arise from the addition of an acid to a compound described herein. In some cases, the acid can be organic. In some cases, the acid can be inorganic. Non-limiting examples of suitable acids include hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, nitrous acid, sulfuric acid, sulfurous acid, a phosphoric acid, nicotinic acid, isonicotinic acid, lactic acid, salicylic acid, 4-aminosalicylic acid, tartaric acid, ascorbic acid, gentisinic acid, gluconic acid, glucaronic acid, saccaric acid, formic acid,

benzoic acid, glutamic acid, pantothenic acid, acetic acid, propionic acid, butyric acid, fumaric acid, succinic acid, citric acid, oxalic acid, maleic acid, hydroxymaleic acid, methylmaleic acid, glycolic acid, malic acid, cinnamic acid, mandelic acid, 2-phenoxybenzoic acid, 2-acetoxybenzoic acid, embonic acid, phenylacetic acid, N-cyclohexylsulfamic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, 2-hydroxyethanesulfonic acid, ethane-1,2-disulfonic acid, 4-methylbenzenesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 2-phosphoglyceric acid, 3-phosphoglyceric acid, glucose-6-phosphoric acid, and an amino acid. Non-limiting examples of suitable acid addition salts include a hydrochloride salt, a hydrobromide salt, a hydroiodide salt, a nitrate salt, a nitrite salt, a sulfate salt, a sulfite salt, a phosphate salt, a hydrogen phosphate salt, a dihydrogen phosphate salt, a carbonate salt, a bicarbonate salt, a nicotinate salt, an isonicotinate salt, a lactate salt, a salicylate salt, a 4-aminosalicylate salt, a tartrate salt, an ascorbate salt, a gentisinate salt, a gluconate salt, a glucaronate salt, a saccarate salt, a formate salt, a benzoate salt, a glutamate salt, a pantothenate salt, an acetate salt, a propionate salt, a butyrate salt, a fumarate salt, a succinate salt, a citrate salt, an oxalate salt, a maleate salt, a hydroxymaleate salt, a methylmaleate salt, a glycolate salt, a malate salt, a cinnamate salt, a mandelate salt, a 2-phenoxybenzoate salt, a 2-acetoxybenzoate salt, an embonate salt, a phenylacetate salt, an N-cyclohexylsulfamate salt, a methanesulfonate salt, an ethanesulfonate salt, a benzenesulfonate salt, a p-toluenesulfonate salt, a 2-hydroxyethanesulfonate salt, an ethane-1,2-disulfonate salt, a 4-methylbenzenesulfonate salt, a naphthalene-2-sulfonate salt, a naphthalene-1,5-disulfonate salt, a 2-phosphoglycerate salt, a 3-phosphoglycerate salt, a glucose-6-phosphate salt, and an amino acid salt.

Metal salts can arise from the addition of an inorganic base to a compound described herein. The inorganic base consists of a metal cation paired with a basic counterion, such as, for example, hydroxide, carbonate, bicarbonate, or phosphate. The metal can be an alkali metal, alkaline earth metal, transition metal, or main group metal. Non-limiting examples of suitable metals include lithium, sodium, potassium, caesium, cerium, magnesium, manganese, iron, calcium, strontium, cobalt, titanium, aluminium, copper, cadmium, and zinc. Non-limiting examples of suitable metal salts include a lithium salt, a sodium salt, a potassium salt, a caesium salt, a cerium salt, a magnesium salt, a manganese salt, an iron salt, a calcium salt, a strontium salt, a cobalt salt, a titanium salt, an aluminium salt, a copper salt, a cadmium salt, and a zinc salt. Ammonium salts can arise from the addition of ammonia or

an organic amine to a compound described herein. Non-limiting examples of suitable organic amines include triethyl amine, diisopropyl amine, ethanol amine, diethanol amine, triethanol amine, morpholine, N-methylmorpholine, piperidine, N-methylpiperidine, N-ethylpiperidine, dibenzyl amine, piperazine, pyridine, pyrrazole, pipyrrazole, imidazole, pyrazine, pipyrazine, ethylenediamine, N,N'-dibenzylethylene diamine, procaine, chloroprocaine, choline, dicyclohexyl amine, and N-methylglucamine. Non-limiting examples of suitable ammonium salts can be a triethyl amine salt, a diisopropyl amine salt, an ethanol amine salt, a diethanol amine salt, a triethanol amine salt, a morpholine salt, an N-methylmorpholine salt, a piperidine salt, an N-methylpiperidine salt, an N-ethylpiperidine salt, a dibenzyl amine salt, a piperazine salt, a pyridine salt, a pyrrazole salt, a pipyrrazole salt, an imidazole salt, a pyrazine salt, a pipyrazine salt, an ethylene diamine salt, an N,N'-dibenzylethylene diamine salt, a procaine salt, a chloroprocaine salt, a choline salt, a dicyclohexyl amine salt, and a N-methylglucamine salt.

The term "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions of the disclosure is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The term "pharmaceutically acceptable excipient" is intended to include vehicles and carriers capable of being co-administered with a compound to facilitate the performance of its intended function. The use of such media for pharmaceutically active substances is well known in the art. Examples of such vehicles and carriers include solutions, solvents, dispersion media, delay agents, emulsions and the like. Any other conventional carrier suitable for use with the multi-binding compounds also falls within the scope of the present disclosure.

In making the compositions of this disclosure, the active ingredient can be diluted by an excipient. Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, PEG, polyvinylpyrrolidone, cellulose, water, sterile saline, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents;

emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the disclosure can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

In some cases, the pharmaceutical compositions described herein may comprise an excipient that can provide long term preservation, bulk up a formulation that contains potent active ingredients, facilitate drug absorption, reduce viscosity, add flavoring, or enhance the solubility of the pharmaceutical composition. Non-limiting examples of excipients can include anti-adherents, binders (e.g., sucrose, lactose, starches, cellulose, gelatin, or polyethylene glycol), coatings (e.g., hydroxypropyl methylcellulose or gelatin), disintegrants, dyes, flavors (e.g., mint, peach, raspberry, or vanilla), glidants, lubricants, preservatives (e.g., acids, esters, phenols, mercurial compounds, or ammonium compounds), sorbents, or vehicles (e.g., petroleum or mineral oil).

Formulations

The pharmaceutical compositions disclosed herein may be any type of formulation including solid formulations comprising a compound of Formula 1 or Formula 2.

In some cases, the solid formulation comprises at least 0.01 mg, 0.1 mg, 1 mg, 2mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, or 1000 mg of one or more protective agent of Formula 1 or Formula 2 formulated singly or in combination with a chemotherapeutic drug or biologic.

In some cases, the solid formulation may comprise at least 0.1 mg, 1 mg, 2mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1 g, 5 g, 10 g, 25 g, 50 g or 100 g of one or more protective agents (e.g., myricetin, and/or a derivative or salt thereof). For example, a pharmaceutical composition described herein may be a 100 mg solid co-formulation of myricetin (75 g of the 100 mg dose) and doxorubicin (25 mg of the 100 mg dose).

In some cases, the solid formulation (or other type of formulation) can comprise at least 0.1 mg, 1 mg, 2mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350

mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, or 1000 mg of dexrazoxane. For example, a pharmaceutical composition described herein may comprise a 100 mg solid co-formulation of myricetin (75mg of the 100 mg dose) and dexrazoxane (25 mg of the 100 mg dose).

The pharmaceutical compositions disclosed herein may be a liquid formulation. In some cases, the liquid formulation can comprise at least 0.1 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, 300 mg/ml, 350 mg/ml, 400 mg/ml, 450 mg/ml, 500 mg/ml, 550 mg/ml, 600 mg/ml, 650 mg/ml, 700 mg/ml, 750 mg/ml, 800 mg/ml, 850 mg/ml, 900 mg/ml, 950 mg/ml, or 1000 mg/ml concentration of one or more protective agent(s) of Formula 1 or Formula 2 formulated singly or in combination with either a chemotherapeutic drug or biologic agent. For example, a pharmaceutical composition described herein may comprise a 100 mg/mL concentration of the protective agent myricetin and a 50 mg/mL concentration of doxorubicin.

In some cases, the liquid formulation may comprise at least 0.1 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, 300 mg/ml, 350 mg/ml, 400 mg/ml, 450 mg/ml, 500 mg/ml, 550 mg/ml, 600 mg/ml, 650 mg/ml, 700 mg/ml, 750 mg/ml, 800 mg/ml, 850 mg/ml, 900 mg/ml, 950 mg/ml, or 1000 mg/ml concentration of myricetin, or derivative or salt thereof. For example, a pharmaceutical composition described herein may comprise 100 mg/mL concentration of myricetin.

In some cases, the liquid formulation can comprise at least 0.1 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 20 mg/ml, 30 mg/ml, 40 mg/ml, 50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, 250 mg/ml, 300 mg/ml, 350 mg/ml, 400 mg/ml, 450 mg/ml, 500 mg/ml, 550 mg/ml, 600 mg/ml, 650 mg/ml, 700 mg/ml, 750 mg/ml, 800 mg/ml, 850 mg/ml, 900 mg/ml, 950 mg/ml, or 1000 mg/ml concentration of dexrazoxane co-formulated with one or more protective agent.

In some cases, a pharmaceutical composition described herein may comprise at least 2 protective agents. The molar ratio of one protective agent to at least one other protective agent can be about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about

1:8, about 1:9, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:1,000, about 1:10,000, or about 1:>10,000.

In some cases, a pharmaceutical composition described herein may comprise a cancer treatment (e.g., chemotherapeutic drug or biologic agent) and at least one protective agent. The molar ratio of the cancer treatment to at least one other protective agent can be about >10,000:1, about 10,000:1, about 1,000:1, about 100:1, about 90:1, about 80:1, about 70:1, about 60:1, about 50:1, about 40:1, about 30:1, about 20:1, about 10:1, about 9:1, about 8:1, about 7:1, about 6:1, about 5:1, about 4:1, about 3:1, about 2:1, about 1:1, about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:20, about 1:30, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:1,000, about 1:10,000, or about 1:>10,000

Kits

In some cases, the pharmaceutical compositions disclosed herein may be assembled into kits. In some cases, the kit can comprise a protective agent, wherein the protective agent may exist as distinct entities within the kit or as a co-formulation. For example, the kit may comprise one or more protective agents selected from the group consisting of myricetin, tricetin, robinetin, ficetin, vitexin, dihydrorobinetin, 7,3',4',5'-tetrahydroxyflavone, myricitrin, and dexrozoxane. In some cases, the kit can comprise at least two protective agents, wherein the two protective agents may exist as distinct entities within the kit or as a co-formulation. For example, the kit may comprise at least two protective agents selected from the group consisting of myricetin, tricetin, robinetin, ficetin, vitexin, dihydrorobinetin, 7,3',4',5'-tetrahydroxyflavone, myricitrin, and dexrozoxane. In a particular example, the kit may comprise a co-formulation of myricetin and dexrazoxane. In some cases, the kit can comprise a cancer treatment and at least one protective agent, wherein the cancer treatment and at least one protective agent may exist as distinct entities within the kit or as a co-formulation. For example, the kit may comprise a cancer treatment and myricetin and/or a derivative thereof. For example, the kit may comprise a cancer treatment and robinetin and/or a derivative thereof. For example, the kit may comprise a cancer treatment and dihydrorobinetin and/or a derivative thereof. For example, the kit may comprise a cancer treatment and tricetin and/or a derivative thereof. For example, the kit may comprise a cancer

treatment and ficetin and/or a derivative thereof. For example, the kit may comprise a cancer treatment and 7,3',4',5'-tetrahydroxyflavone and/or a derivative thereof.

In one embodiment, the kit may comprise a co-formulation of doxorubicin and myricetin.

In some cases, the kit may also comprise instructions for use. The kit may also comprise vials, tubes, needles, packaging, or other materials.

Kits with unit doses of one or more of the compounds described herein, usually in oral or injectable doses, are provided. Such kits may include a container containing the unit dose, an informational package insert describing the use and attendant benefits of the drugs in treating the disease, and optionally an appliance or device for delivery of the composition.

The kit may further comprise any device suitable for administration of the composition. For example, a kit comprising an injectable formulation of pharmaceutical compositions may comprise a needle suitable for subcutaneous administration and an alcohol wipe for sterilization of the injection site.

In some cases, kits may be provided with instructions. The instructions may be provided in the kit or they may be accessed electronically. The instructions may provide information on how to use the compositions of the present disclosure. The instructions may further provide information on how to use the devices of the present disclosure. The instructions may provide information on how to perform the methods of the disclosure. In some cases, the instructions may provide dosing information. The instructions may provide drug information such as the mechanism of action, the formulation of the drug, adverse risks, contraindications, and the like. In some cases, the kit is purchased by a physician or health care provider for administration at a clinic or hospital. In some cases, the kit is purchased by a laboratory and used for screening candidate compounds.

EXAMPLES

Example 1. Myricetin provides long-term cardioprotection (cell viability)

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Samples were either mock-treated, treated with 1.25μM doxorubicin, treated with myricetin, or co-treated with 1.25 μM of doxorubicin and myricetin for 72 hours. Following treatment, the samples were incubated Hoeschst 33342 to indicate

cell nuclei. Cells were imaged using the INCell Analyzer2200, and images were analyzed to quantify the total number of cells and plotted as a percentage of total cells normalized to control (**left**), where each data point was obtained from three biological replicates. Representative images (**Fig. 3, right**) are presented for each sample, where an increase in Hoechst 33342 signal represents an increase ion cell viability.

Cardiomyocytes were either mock-treated, treated with 1.25μM doxorubicin, treated with myricetin, or co-treated with 1.25μM of doxorubicin and myricetin for 72 hours, and subsequently stained to detect total number of cells (**Fig. 3**). Myricetin was a potent protector of cell viability. Cardiomyocytes treated with 1.25 μM doxorubicin, in the absence of myricetin, exhibited a 62.6% reduction in the number of total cells, whereas cardiomyocytes co-treated with myricetin and 1.25μM doxorubicin exhibited a 27.57% reduction the number of total cells, as compared to mock-treated control. Cardiomyocytes treated with myricetin, in the absence of doxorubicin, exhibited no significant difference in the number of total cells, as compared to mock-treated control. Error bars represent standard deviation. Representative images are presented for each sample, where an increase in Hoechst 33342 signal represents an increase in cell viability. Cardiomyocytes treated with 1.25μM doxorubicin (**Fig. 3, right: bottom left panel**), in the absence of myricetin, exhibited a reduction in Hoechst 33342 signal, whereas cardiomyocytes co-treated with 79μM myricetin and 1.25μM doxorubicin (**Fig. 3, right: bottom right panel**) exhibited less reduction in Hoechst 33342 signal, as compared to mock-treated control (**Fig. 3, right: top left panel**). Cardiomyocytes treated with myricetin (**Fig. 3, right: top right panel**), in the absence of doxorubicin, exhibited no significant difference in Hoechst 33342 signal, as compared to mock-treated control.

Example 2. Effects of myricetin on doxorubicin-induced cardiotoxicity 2 days following treatment (mitochondrial toxicity)

Human iPSC-derived cardiomyocytes were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Cardiomyocytes were treated with 1.25μM doxorubicin (**Fig. 4A**), or co-treated with 1.25μM of doxorubicin and 79μM myricetin (**Fig. 4B**) for 2 days. Following treatment, the samples were incubated with a tetramethylrhodamine methyl ester (TMRM) dye to indicate mitochondrial health, and Hoechst 33342 to identify cell nuclei. Cells were imaged using the INCell Analyzer2200. Representative images are presented for each sample, wherein a decrease in TMRM signal

indicates an increase in mitochondrial toxicity. Myricetin was a potent protector against doxorubicin-induced mitochondrial toxicity, as indicated by a greater TMRM signal in cells co-treated with 1.25μM doxorubicin and 79μM myricetin (**Fig. 4B**) as compared to cells treated with 1.25μM doxorubicin in the absence of myricetin (**Fig. 4A**).

Example 3. Effects of myricetin on doxorubicin-induced cardiotoxicity 3 days following treatment (contractility)

Human iPSC-derived cardiomyocytes were prepared as described above. Samples were either mock-treated, treated with 1.25μM doxorubicin, treated with 79μM myricetin, or co-treated with 1.25 μM of doxorubicin and 79μM myricetin for 72 hours. Following treatment, videos of beating cardiomyocytes were captured using Pulse, and analyzed to quantify beat rate (**Fig. 5; left**) from plots of cell contraction, where each data point was obtained from three biological replicates. Representative plots of cell contraction (**Fig. 5; right**) are presented for each sample. Myricetin was a potent protector of cell contractility. Mock-treated cardiomyocytes contracted at 33.33 beats per minute, whereas treatment with 1.25μM doxorubicin, in the absence of myricetin, completely inhibited contraction. Cardiomyocytes treated with myricetin, or co-treated with myricetin and 1.25μM doxorubicin, contracted at 39.33 or 37.33 beats per minute, respectively. **Fig. 6A-C** depicts a chart providing the raw data (**6A**) or normalized data (**6B**) for the experiments depicted in **Fig. 3**, or the raw data for the experiments depicted in **Fig. 5** (**6C**).

Example 4. Effects of various flavonols and flavones on doxorubicin-induced cardiotoxicity 3 days following treatment (apoptosis)

Cardiomyocytes were prepared as described above. Cells were co-treated with 1μM of doxorubicin and either myricetin (**Fig. 7A**), myricetrin (**Fig. 7B**), or dihydromyricetin (**Fig. 7C**) for 3 days. Following treatment, the samples were incubated with a CellEvent dye to indicate apoptosis-positive cells, and a second dye to identify cell nuclei. Cells were imaged using the INCell Analyzer2200, and images were analyzed to quantify the percentage of apoptotic cells. Data are presented from two independent sets of screening where each data point was obtained from triplicate.

Cardiomyocytes co-treated with doxorubicin and either myricetin (**Fig. 7A**), myricitrin (**Fig. 7B**), or dihydromyricetin (**Fig. 7C**) exhibited protective effects against

apoptosis, with half minimal inhibitory concentrations (IC50; e.g., the drug concentration that induces 50 percent apoptosis) of 20.46 μM, 38.48 μM, 40.48 μM, respectively.

Example 5. Myricetin reduces DOX's cytotoxicity in cardiomyocytes

To assess the effect of MYR against DOX-induced cytotoxicity, human iPSC-derived cardiomyocytes were mock-treated (triangle) or treated with 100μM of myricetin (MYR; circle) and increasing concentrations of doxorubicin (DOX) for 72 hours, and then incubated with dyes that indicate mitochondrial health (TMRM, Life Technologies) and cellular nuclei (Hoechst33342, Life Technologies). Cells were imaged using INCell Analyzer2200 (GE). Total number of healthy cells were counted and plotted as percentage of mock-treatment control. Lethal concentration at which 50% of cells were killed (LC50) by doxorubicin was shifted from 0.41μM in mock-treated to 1.29μM in MYR-treated conditions for iPSC cardiomyocytes **(Fig. 8)**. Data are presented from multiple independent sets of screening where each data point was obtained from triplicate. (n=3). Y-axis: percentage of cell survival; and X-axis: increasing concentrations of DOX (**Fig. 8**).

Example 6. Myricetin protects against DOX-induced cell death in cardiomyocytes

To measure the rescue rates from the DOX-induced cell death in cardiomyocytes, the protective effect of myricetin was directly compared with that of dexraxozane (DEX; standard of care). Human iPSC-derived cardiomyocytes were treated with 0.5μM of Doxorubicin and increasing concentrations of myricetin (MYR, circle) or dexraxozane (DEX, square). After 72 hours of treatment, cells were incubated with dyes that indicate mitochondrial health (TMRM, Life Technologies) and cellular nuclei (Hoechst33342, Life Technologies). Cells were imaged using INCell Analyzer2200 (GE). Total number of healthy cells were counted and plotted as percentage of doxorubicin-treatment control. Half maximal effective concentration (EC50) for MYR was 7.50μM **(Fig. 9)**. In contrast, DEX did not exhibit any significant rescues from DOX-induced cytotoxicity. (n=3).

Example 7. Myricetin protects against DOX-induced contractility dysfunction and DNA double strand break in cardiomyocytes

To assess the protective effect of myricetin on the contractility of heart cells, cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes.

Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Human iPSC-derived cardiomyocytes were then treated with DMSO, DOX (0.5μM), DOX plus DEX (100μM), or DOX plus MYR (100μM). After 48 hours of treatment, videos of beating cardiomyocytes were captured with Pulse (Cellogy). DOX treatment induced dysfunction in cardiomyocyte contraction as evidenced by reduction in beating, duration, and peak height. This contractile dysfunction was significantly corrected by MYR as compared to DEX **(Fig. 10)**. Data are presented from multiple independent sets of experiments where each data point was obtained from 6 samples (n=6). Student T-Test (unpaired, two-tailed) was used to determine the significance of the difference.

To determine whether MYR protects against DOX-induced DNA double strand break in cells, human iPSC-derived cardiomyocytes were treated with DMSO, DOX (0.5μM), DOX plus DEX (100μM), or DOX plus MYR (100μM). After 48 hours of treatment, cells were immunostained with antibody against γH2AX (EMD Millipore) to detect double strand break. Cells were then imaged using INCell Analyzer2200 (GE) and percentages of γH2AX-positive cells were quantified for each condition. While DEX exhibited little or no protection against DOX-induced double strand break in the tested heart cells, MYR conferred significant protection from DOX-related DNA damage **(Fig. 11)**. Student T-Test (unpaired, two-tailed; n=6).

Example 8. MYR Protects against sarcomere disruption by DOX

DOX-induced cell death is often manifested by severity of structural disruptions of cardiomyocyte organization (e.g., sarcomere). To assess the protective effect of MYR against DOX-induced sarcomere disruption, human iPSC-derived cardiomyocytes were treated with DMSO, DOX (0.5μM), or DOX plus MYR (100μM). After 72 hours of treatment, cells were immunostained with antibody against Cardiac Troponin T (Abcam) to show sarcomeric organization in of the heart cells. As shown in **Fig. 12**, MYR conferred significant protection against DOX-induced sarcomere disruption in cardiomyocytes, suggesting that the protective effects of MYR against DOX-induced cell death are well manifested by the structural integrity of the cardiomyocytes.

Example 9. Myricetin is a potent inhibitor of TOPOIIα and β

To gain insights into a molecular mechanism of myricetin (MYR) and that of dexraxozane (DEX) on cardioprotection, the effect of these two compounds on topoisomerases II (i.e., TOPOIIα and β), an apparent target of DOX, was assessed.

200ng of kinetoplast DNA (kDNA) was incubated with one enzymatic unit of TOPOIIα or TOPOIIβ enzyme (Inspiralis) and with various concentrations of MYR or DEX at 37°C for 30min. The reaction was then separated on 1% agarose gel for visualization of decatenated DNA (bottom band). The efficiency of catalytic inhibition was quantified by measuring the relative intensity of the band.

MYR and DEX exhibited 50% inhibition (IC50) of TOPOIIα enzyme activity at concentrations of 1.18μM and 52.70μM, respectively **(Fig. 13;** n=3). IC50 of TOPOIIβ enzyme activity for MYR and DEX were 2.07μM and 34.43μM, respectively **(Fig. 13;** n=3). The data suggest that MYR is a significantly more potent inhibitor than DEX for both topoisomerases IIα and β.

Example 10. Unlike DEX, MYR does not induce TOPOII protein degradation

To further distinguish molecular mechanisms of MYR from those of DEX and also to determine whether the inhibitory effects of MYR on TOPOII observed in the decatenation assays above is due to degradation of TOPOII proteins, human iPSC-derived cardiomyocytes were treated with DMSO, DEX (100μM), or MYR (100μM) for 24 hours, and immunostained with antibody against topoisomerase IIβ (BD Biosciences).

Cells were imaged using INCell Analyzer2200 (GE) and topoisomerase IIβ protein levels were quantified. Student T-Test (unpaired, two-tailed) was used to determine the significance of the difference.

As shown in **Fig. 14**, treatment with DEX resulted in marked disappearance of TOPOIIβ in iPSC-CMs, whereas MYR exerted no effect on topoisomerase IIβ protein levels (**Fig. 14**) (n=3). The results confirmed the hypothesis that DEX can negatively affect the stability of topoisomerases IIβ (TOPOIIβ), which may lead to the depletion of these enzymes from the heart cells, effectively resulting in prevention of DNA damage generated by poisonous effects on these enzymes by the anthracycline. These results also confirmed that the mechanism by which MYR confer protection from anthracycline-induced toxicity is

entirely independent and can be distinguished from that of DEX. Further, the effect of MYR observed in topoisomerase inhibition is not due to TOPOIIβ protein degradation or depletion of the enzyme from DOX's debilitating effects on the heart cells. It can be concluded that inhibition of topoisomerase II activity, particularly without affecting the stability of TOPOII enzymes, is an important factor for MYR's ability to confer cardioprotection.

Example 11. Neither DHM nor DHR inhibit TOPOIIα or TOPOIIβ

Since the ability of MYR to confer cardioprotection against DOX-induced toxicity is independent from DEX, it was further investigated to determine whether other flavonoid compounds have a similar effect on topoisomerase II activity like MYR.

First, MYR (flavonol) and dihydromyricetin (flavanonol) were tested for their inhibitory effect on topoisomerase II enzymatic function. Dihydromyricetin (DHM) shares a similar chemical structure except for the presence of a single bond in the major C-ring of the flavonoid scaffold.

200ng of Kinetoplast DNA (kDNA) was incubated with one enzymatic unit of TOPOIIβ and different concentrations of MYR (circle) or DHM (triangle) at 37°C for 30 min **(Fig. 15)**. The reaction was then separated on 1% agarose gel for visualization of decatenated DNA (bottom band) and the catalytic inhibition efficiency was quantified by measuring the relative intensity of the band. Surprisingly, DHM did not inhibit TOPOIIβ (n=3) **(Fig. 15)** or TOPOIIα enzymatic activity, even at extreme concentrations (> 200 μM).

Further, this result on DHM was confirmed in separate experiments with dihydrorobinetin (DHR) and robinetin (ROB) in which DHR, like DHM, showed no inhibitory activity toward these topoisomerases, while robinetin, like MYR, displayed a high level of inhibition on both TOPOIIβ and TOPOIIα. These data indicate that the structural difference in the C-ring of the flavone/flavonoid scaffold plays an important role in TOPOII inhibition.

Example 12. MYR is 2-fold more potent in protecting DOX-induced cell death than DHM

Next, the ability of MYR to confer cardioprotection was directly compared with that of DHM as these two compounds display distinctive property in their structures and TOPOII inhibition activity. Cell samples were prepared by differentiating induced pluripotent stem

cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Human iPSC-derived cardiomyocytes were treated with 0.5μM of Doxorubicin and increasing concentrations of myricetin (MYR, circle) or dihydromyricetin (DHM, triangle). After 72 hours of treatment, cells were incubated with dyes that indicate mitochondrial health and cellular nuclei. Cells were then imaged and total number of healthy cells were counted and plotted as percentage of Doxorubicin-treatment control as described above.

As illustrated in **Fig. 16**, MYR exhibited 2-fold greater potency in protecting DOX-induced cell death than DHM as half maximal effective concentrations (EC50) for MYR and DHM were 7.50μM and 13.96μM, respectively. (n=3) Based on these results, it was concluded that a double bond in C ring of the flavone/flavonoid scaffold enhances potency for the cardioprotective properties by conferring the inhibitory effects on topoisomerases II.

These observations were followed up by a DOX-induced DNA double strand break assay. Human cardiomyocytes were treated with 0.5μM of doxorubicin and with increasing concentrations of MYR (circle) or DHM (triangle). After 48 hours of treatment, cells were immunostained with antibody against γH2AX (EMD Millipore) to detect DNA double strand break. Cells were imaged using INCell Analyzer2200 (GE) and percentages of γH2AX-positive cells was quantified for each condition. Consistent with its cell death rescue rate, MYR was 2-fold more potent in protecting DOX-induced double strand break than DHM. Concentrations at which DOX-induced double strand break was reduced to 50% (IC50) for MYR and DHM were 5.28μM and 11.30μM, respectively (**Fig. 17**). (n=3)

To investigate the effect of myricetin on cardiomyocytes exposed to doxorubicin, mRNA expression levels were determined in the cells treated with DOX alone, myricetin alone, and DOX plus myricetin. Surprisingly while myricetin did not have any effect on TOPOIIβ mRNA expression by itself, DOX significantly repressed TOPOIIβ expression at 24 and 48 hours (**Fig. 18**). However, in the presence of myricetin, TOPOIIβ expression was restored to a level close to normal by myricetin, effectively preventing any transcription alteration by DOX (**Fig. 18**). This data suggested that there appeared to be a synergistic effect between DOX and myricetin on TOPOIIβ expression. With respect to expression of TOPOIIα, DOX slowly repressed expression of TOPOIIα over time. In the presence of DOX, however, myricetin further repressed TOPOIIα, suggesting a differential effect of

myricetin on these topoisomerases II at molecular and cellular levels. Combined down regulatory effect of myricetin and DOX on TOPOIIα is larger than what was observed with DOX alone.

Example 13. Cardioprotective properties of MYR analogs

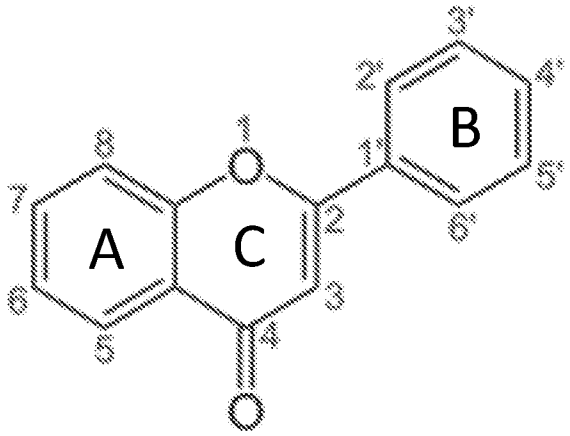
To further investigate the relationship between the structure (e.g., flavone/flavonol scaffold) and biological activity (e.g., cardioprotection, TOPOII inhibition, etc.), a group of additional flavonoid compounds related to myricetin were identified and tested for their activity.

I. Identification of flavonoids with cardio-protective properties mediated through TOPOII inhibition

Anthracycline-induced cardiotoxicity occurs when the drug such as doxorubicin intercalates the DNA upon a cleavage of DNA by topoisomerase II enzymes, thereby effectively preventing TOPOIIα or β from ligating the cleaved DNA strands back together. Therefore, a working hypothesis was proffered based on cardioprotective properties of flavonoids being mediated through topoisomerases IIα and β (TOPOIIα and TOPOIIβ) inhibition.

A systematic study on the hydroxyl substituents of the MYR scaffold was conducted for biological activity. The objective was to explore chemical space around MYR to identify which substituents (e.g., hydroxyl, alkoxyl, or heterocyclic) are required at various positions and to determine which chemical structure(s) is the essential component for being a cardioprotectant.

With respect to biological activity, a biochemical decatenation assay was used as described above to assess TOPOIIα and TOPOIIβ inhibition. Doxorubicin treated human iPSC-derived cardiomyocytes were employed to measure the protective effect of these analogs on cardiomyocytes.



Flavone/Flavonol Scaffold

Starting from the bare flavone, 48 myricetin analog compounds with hydroxyl substituents present or missing at the 3, 5, 7, 3', 4', or 5' positions were identified (myricetin is the compound with all six hydroxyl substituents present). In addition to the 48 myricetin analogs was chromone which is devoid of the B-ring of flavone, and dihydromyricetin and dihydrorobinetin (DHR) which both lack the double bond in the C-ring.

Because substituents can be incorporated into the flavone scaffold at positions 8 and/or 6 on the A-ring similar to vitexin, and also at positions 2' and/or 6' on the C-ring, hydroxyl, alkoxy, alkyl and heterocyclic, halides were contemplated for analysis. The study also included chemical moieties other than hydroxyl substituents present in the MYR scaffold (Formula 1), such as alkoxy (particularly methoxy), alkyl (methyl), heterocyclic, or halides at 3, 5, 7, 3', 4', and/or 5' positions.

This study led to the identification of the minimum structure based on the MYR scaffold required for end point activity. Among the compounds of specific combination of hydroxyl groups in 3, 5, 7, 3', 4', 5' positions selected for biological activity for cardioprotection (e.g., TOPOIIβ inhibition, and DNA double strand break), a certain group of compounds with specific combinations of substituents present or missing at the 3, 5, 7, 3', 4', 5' positions was found to be critical for biological properties as a cardioprotectant with decreased cytotoxicity.

TABLE 1

ID	Compound Name	iPSC-CM protection Max Effect (%)	EC50 (μM)	Toxicity	Rescue	TOPOIIβ Inhibition	TOPOIIα Inhibition
1	3,5,7,3',4',5'-hexahydroxyflavone (myricetin)	**78**	**14.48**	-	++++	+++	+++
2	3,7,3',4',5'-pentahydroxyflavone (robinetin)	**64**	**12.62**	-	++++	+++	+++
3	5,7,3',4',5'-pentahydroxyflavone (tricetin)	**56**	**17.19**	*	+++	+++	+++
4	3,5,7,3',4'-pentahydroxyflavone (quercetin)	**58**	**20.5**	*	++	+++	+++
5	3,7,3',4'-tetrahydroxyflavone (fisetin)	**36**	**16.32**	*	++	+++	+++
6	7,3',4',5'-tetrahydroxyflavone	**71**	**17.13**	-	+++	-	-
7	3,5,7,4'-tetrahydroxyflavone (kaempferol)	**46**	**26.01**	-	++	-	-
8	3',4',5'-trihydroxyflavone	**64**	**43.01**	-	+	-	-
9	5,7,3',4'-tetrahydroxyflavone (luteolin)	**62**	**9.67**	*	+++	-	-
10	3,7,4'-trihydroxyflavone (resokaempferol)	**27**	**3.26**	*	+	-	-
11	7,3',4'-trihydroxyflavone	**24**	**6.25**	*	+	-	-
12	3,3',4'-trihydroxyflavone	**16**	**6.43**	*	+	-	-
13	5,7,4'-trihydroxyflavone (apigenin)	†	-	-	-	N/A	N/A
14	3',4'-dihydroxyflavone	†	-	*	-	N/A	N/A
15	7,4'-dihydroxyflavone	†	-	*	-	N/A	N/A
16	3,4'-dihydroxyflavone	†	-	*	-	N/A	N/A
17	4'-hydroxyflavone	†	-	-	-	N/A	N/A

18	3,7,3'-trihydroxyflavone	†	-	*	-	N/A	N/A
19	3,5,7-trihydroxyflavone	†	-	*	-	N/A	N/A
20	3,7-dihydroxyflavone	†	-	*	-	N/A	N/A
21	7,3'-dihydroxyflavone	†	-	*	-	N/A	N/A
22	3,3'-dihydroxyflavone	†	-	*	-	N/A	N/A
23	5,7-dihydroxyflavone	†	-	*	-	N/A	N/A
24	7-hydroxyflavone	†	-	*	-	N/A	N/A
25	3-hydroxyflavone	†	-	*	-	N/A	N/A
26	3',5'-dihydroxyflavone	†	-	*	-	N/A	N/A
27	3'-hydroxyflavone	†	-	*	-	N/A	N/A
28	flavone	†	-	*	-	N/A	N/A
29	chromone	†	-	-	-	N/A	N/A
30	dihydrorobinetin	**53**	**14.02**	-	+++	-	-
31	3'-O-methylmyricetin	**76**	**58.7**	-	+	-	-
32	4'-O-methylmyricetin	**68**	**48.6**	-	+	-	-
33	3',5'-O-dimethylmyricetin	†	-	*	-	-	-
34	3',4',5'-O-trimethylmyricetin	†	-	*	-	-	-
35	3',4',5'-O-trimethylrobinetin	†	-	*	-	-	-
36	7,3',4',5'-O-tetramethylrobinetin	†	-	*	-	-	-
37	3,7,3',4',5'-O-pentamethylrobinetin	†	-	*	-	-	-
38	7-hydroxy-4-chromone	†	-	*	-	-	-

+ Compounds exhibited positive effects on respective biological properties

- Compounds exhibited negative effects on respective biological properties

† Compounds failed to exhibit > 30% protection Max Effect at 10 μM or 100 μM on initial screen.

* Compounds exhibited cytotoxicity at 100μM

N/A, Experiment not performed as compounds exhibited cytotoxicity and no cardioprotection activity

Minimum requirements of hydroxyl substituents for TOPOIIβ inhibition and cardioprotective effects

As shown in Table 1 above, the common features of the TOPOIIβ inhibitors (**1** – **5**) allowed an inference that hydroxyl substituents are required at positions 3, 7, 3', and 4' in order for flavonoid compounds to inhibit TOPOIIβ. The only exception is tricetin (**3**) which does not have the 3-hydroxyl substituent; all of the other four TOPOIIβ inhibitors have hydroxyl substituents at positions 3, 7, 3', and 4'. Furthermore, the common features of the cardioprotective compounds (**1** – **12**) in Table 1 above, allowed an additional inference that the 4' hydroxyl substituent on the B-ring may be an essential feature, along with two of the other three hydroxyl substituents at positions 3, 7, and 3', for cardioprotective activity, with the hydroxyl at position 7 preferred; the only exception being compound **8** which does not have hydroxyls at positions 3 and 7, yet has all three hydroxyl substituents at positions 3', 4', and 5' on the B-ring. Moreover, considering toxicity of the tested compounds (see **Table 1**), one can deduce a trend that cardioprotective compounds (**1** – **12**) which have all three 3', 4', and 5' hydroxyl substituents on the B-ring do not exhibit toxic effects at concentrations less than 100 μM, whereas those cardioprotective compounds which have hydroxyl substituents only at positions 3' and 4' do indeed exhibit toxic effects at concentrations less than 100 μM. Again, the one exception to this trend was tricetin (**3**), which exhibits some toxic effects at concentrations less than 100 μM despite containing all three hydroxyl substituents on the B-ring. Of the two cardioprotective compounds which only have the 4' hydroxyl substituent on the B-ring (kaempferol **7** and resokaempferol **10**), kaempferol did not show toxic effects below 100 μM, whereas resokaempferol exhibited toxic effects at concentrations below 100 μM. Based on this analysis, it was concluded that:

(1) for cardioprotection, 4' hydroxyl substituent on B-ring is required, along with one of the following, (a) two of the three hydroxyl substituents at positions 3, 7, and 3', with position 7 preferred, or (b) all three hydroxyl substituents at positions 3', 4', and 5' on the B-ring;

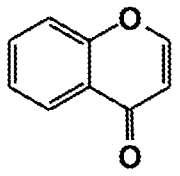
(2) for cardiotoxicity, 3', 4', and 5' hydroxyl substituents on the B-ring are preferable to 3' and 4' hydroxyl substituents on the B-ring, to alleviate toxic effects at concentrations below 100 μM; or, 4' hydroxyl *only* on the B-ring, along with all three 3, 5, and 7 hydroxyl substituents on the A/C ring system; and

(3) for TOPOIIβ inhibition, all four hydroxyl substituents at positions 3, 7, 3', and 4' are required. Tricetin (**3**) does not follow these requirements and is an outlier.

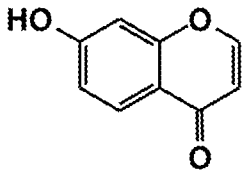
Analysis on B-ring

It is readily apparent from the compounds listed in Table 1 that the 4' position on the B-ring requires a hydroxyl substituent for cardioprotection. Of the twelve compounds (**1** – **12**) that passed the initial screen, all of them have the 4'-hydroxyl substituent. Moreover, of the sixteen compounds (**13** – **28**) that did not pass the initial screen, eleven (**18** – **28**) are absent the 4'-hydroxyl substituent. The remaining five 4'-hydroxyl compounds (**13** – **17**) that did not pass the initial screen have minimal substitution, *e.g.* only the 4'-hydroxyl as in compound **17**, or only one other hydroxyl substituent along with the 4'-hydroxyl as in compounds **14**, **15**, and **16**. Compound **13** only has one of the required hydroxyl substituents from the set of 3, 7, and 3' described above; therefore, it also does not meet the minimum requirements for cardioprotective activity. In summary, the presence of a hydroxyl substituent at position 4' on the B-ring is a necessary but not sufficient condition for flavonoid compounds to be cardioprotective. This structural requirement strongly hints at the presence of a hydrogen-bond between the 4' hydroxyl on the B-ring of the protective agent in complex with the biological target.

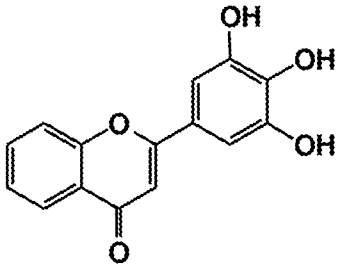
1. Chromone-related compounds



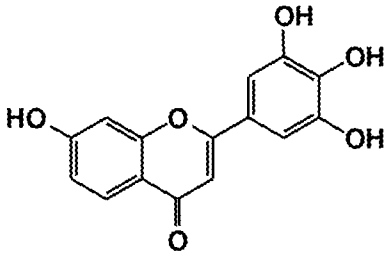
Chromone (CHR)



7-hydroxy-4-chromone



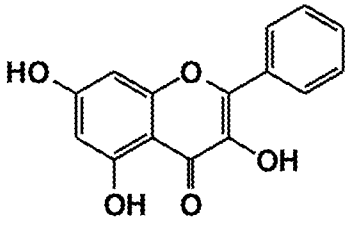
3',4',5'-trihydroxyflavone



7,3',4',5'-tetrahydroxyflavone

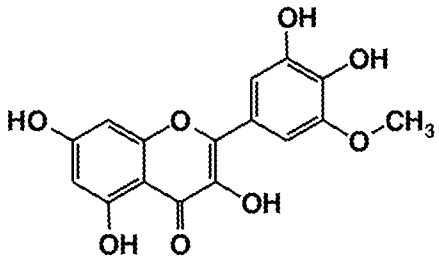
Both chromone (**29**) and 7-hydroxy-4-chromone (**38**), which each entirely lacks the B-ring of the flavone scaffold, showed no positive effect in cardiac protection. Nor did either compound confer TOPOIIβ or α inhibition (Table 1). Furthermore, 7-hydroxy-4-chromone exhibited a high level of cytotoxicity at 100 μM. Comparing these two B-ring null compounds with the corresponding tri-substituted B-ring flavone compound (**8** and **6**, respectively), it was concluded that the presence of the B-ring is required for cardiac protection.

Next, the observation obtained from 7-hydroxy-4-chromone was further explored in 3,5,7-trihydroxyflavone having the B-ring, but lacking all B-ring substituents. 3,5,7-trihydroxyflavone exhibited neither cardioprotection nor TOPOII inhibition and displayed generalized cytotoxicity, indicating that one or more moieties are required in the B-ring for the cardioprotection activity.

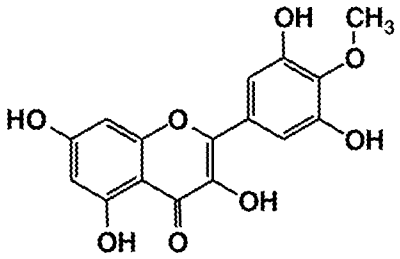


3,5,7-trihydroxyflavone

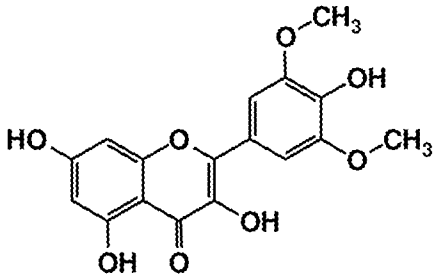
2. Methoxy substituents on B-ring



3'-O-methylmyricetin



4'-O-methylmyricetin



3',5'-O-dimethylmyricetin

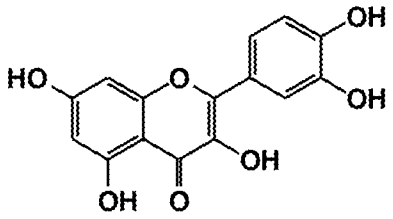
Since the B-ring appeared to be an essential component for biological activity, compounds having the B-ring with various positional combinations with either hydroxyl and/or methoxy group were tested for their activity.

3'-O-methylmyricetin having methoxy at the 3' position failed to inhibit TOPOII enzymes, but conferred cardioprotection without showing generalized cytotoxicity. However, it exhibited a significant loss of potency for cardioprotection (EC50, ~59 μM). Similarly, 4'-O-methylmyricetin having methoxy at 4' position conferred cardioprotection without TOPOII inhibition. This compound displayed a loss of potency for cardioprotection (EC50, ~ 48.7μM) as compared to that of MYR. This suggests that the presence of a single methoxy substituent at 3' or 4'of the B-ring, is an important factor for cardioprotection. Confirming this observation, 3',5'-O-dimethylmyricetin, lacking a methoxy substituent at position 4 but having a methoxy at positions 3' and 5' of the B-ring, displayed neither cardioprotection nor TOPOIIα and TOPOIIβ inhibition. This compound also exhibited significant cytotoxicity. Other compounds having multiple methoxy replacements at positions 3', 4', and 5' were also tested for cardioprotection and TOPOII inhibition. For

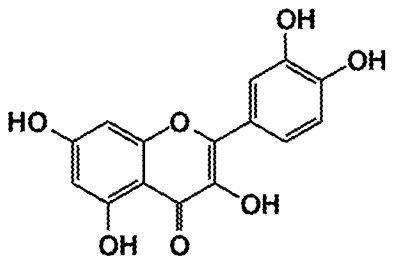
example, all of 3‘,4’,5'-O-trimethylmyricetin, 3',4',5'-O-trimethylrobinetin, 3,7,3',4',5'-O-pentamethylrobinetin, 7,3',4',5'-O-tetramethylrobinetin entirely failed to display cardioprotection or TOPOII inhibition. All exhibited increased levels of cytotoxicity at 100μM.

Accordingly, replacing 4’ or 3’ hydroxyl with methoxy significantly reduces potency and results in complete loss in TOPOII inhibition. Further, because methoxy substitution slightly enlarges and extends the compound from the B-ring, it was postulated that having a larger substituent extending from the B-ring, even at a marginal level, may pose a steric hindrance for the interaction between TOPOII enzyme and the compound. Thus, hydroxyls groups in the B-ring (3’,4’,5’) appears to be critical components that lead to cardioprotection and may play an important role in TOPOII enzyme inhibition.

3. Quercetin and Kaempferol

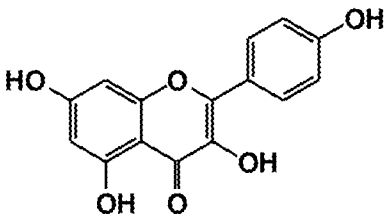


or



Quercetin

Quercetin conferred cardioprotection and exhibited TOPOII inhibition. However, a high level of general cytoxicity to cardiomyocytes was observed at a concentration of 100 μM.



Kaempferol

Kaempferol displayed a moderate level of cardioprotection without some level of cytotoxicity at 100μM, but did not exhibit any inhibitory effect on TOPOIIα or TOPOIIβ. Kaempferol, however, displayed decreased potency and failed to achieve the maximum 50% rescue rate.

It was inferred from the data that removing 3' (or 5') hydroxyl may not necessarily result in loss of TOPOII inhibition, but leads to increased cytotoxicity and reduced potency as observed in quercetin. However, these data led to the conclusion that removing 3' 4', or 5' hydroxyl group from the B-ring result in a marked reduction in potency and/or loss of TOPOII inhibition, particularly at position 4'.

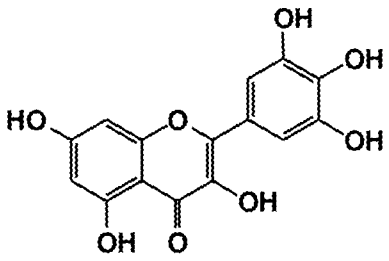
In sum, replacing one or two 3', 4', or 5' hydroxyls with an alkoxy (e.g., methoxy) group renders the compound cytotoxic. Removal of 3' and 5' hydroxyl groups from the MYR scaffold, as observed in kaempferol or removal of either 3' or 5' hydroxyl as in quercetin may reduce potency for cardioprotection and render the compound cytotoxic. However, removing *all* hydroxyls on the B-ring results in complete loss of cardioprotection and TOPOII inhibition, and causes severe cytotoxicity as observed in 3,5,7-trihydroxyflavone. Further, 4' hydroxyl of the B-ring appears to be required for the enhanced physical attributes leading to increased potency for cardioprotection with TOPOII inhibition and minimal cytotoxicity.

Accordingly, the preferred substituents for the B-ring are –OH in all 3', 4' and 5' positions in order to ensure potency and minimal toxicity as demonstrated by myricetin and robinetin.

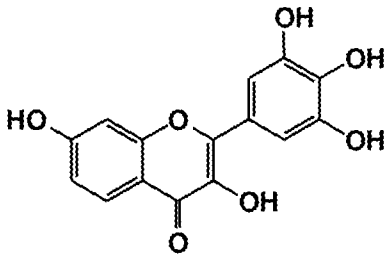
A and C-Ring Analysis

Substituents on the heterobicyclic A/C ring system of the flavone-flavonol scaffold was assessed for the cardioprotective activity. Based on the observations made on the B-ring, a subset of compounds having hydroxyls on the B-ring with various combinations with –OH at 3, 5, 7, positions of the A-C ring were tested.

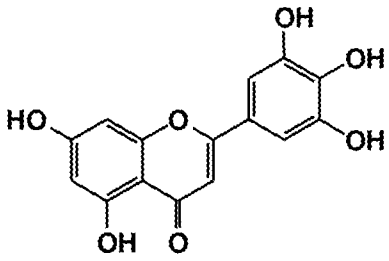
1. Myricetin, Robinetin and Tricetin



Myricetin (MYR)

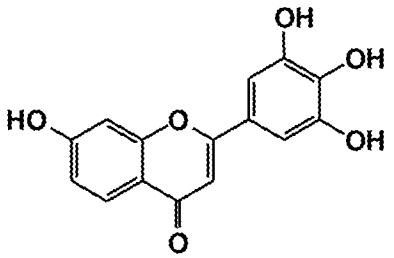


Robinetin (ROB)



Tricetin (TRI)

MYR (3,5,7,3',4',5'-hexahydroxyflavone) and ROB (3,7,3',4',5'-pentahydroxyflavone) showed equivalent levels of cardioprotection with an EC50 about 10-20 μM and TOPOIIβ and TOPOIIα inhibition at less than 10μM. Similarly, tricetin (5,7,3',4',5'-pentahydroxyflavone) lacking –OH at position 3 also displayed cardioprotection and TOPOII inhibition with a low level of cytotoxicity at 100μM. Further, 7,3',4',5'-tetrahydroxyflavone lacking –OH at both positions 3 and 5 of the A/C ring system displayed cardioprotection, but did not inhibit TOPOII enzymes.



7,3',4',5'-tetrahydroxyflavone

However, 3',4',5'-trihydroxyflavone and other compounds having no –OH at the position 7 failed to display potency less than 30μM for cardioprotection or TOPOII inhibition. These data suggested that hydroxyl (-OH) at position 7 of the A-ring may be required for cardioprotection, but not sufficient as at least one –OH group at 3 and/or 5 position can greatly enhance activity (e.g., potency and/or TOPOII inhibition) of these compounds for cardioprotection. Thus, hydroxyls in the A/C-ring (3,7) system play an important role for cardioprotection and TOPOII inhibition, provided that 3',4',5' hydroxyls are present on B-ring. Particularly –OH at position 7 in the A-ring appears to be critical for the activity.

Example 14. Protective effects of MYR on anticancer agents

1. Anthracyclines

MYR protects against epirubicin-induced cell death and idarubicin-induced cell death

Epirubicin and idarubicin are anthracyclines that are associated with heart failure in patients. In addition to doxorubicin described above, the effect of MYR was tested on epirubicin- and idarubicin-induced heart injury. As illustrated in **Fig. 19**, human iPSC-

derived cardiomyocytes were mock-treated (triangle) or treated with 100μM of MYR (circle) and increasing concentrations of epirubicin or idarubicin for 72 hours, and then incubated with dyes that indicate mitochondrial health and cellular nuclei as describe above. Cells were imaged and total number of healthy cells were counted and plotted as percentage of mock-treatment control.

Lethal concentration at which 50% of cells were killed by epirubicin (LC50) was shifted from 0.49μM in mock-treated to 1.28μM in MYR-treated conditions, showing that MYR effectively protected against epirubicin-induced cell death in cardiomyocytes (**Fig. 19, left**). (n=3)

Similarly, LC50 of Idarubicin was shifted from 0.59μM in mock-treated to 1.04μM in MYR-treated conditions, indicating that MYR also protected against idarubicin-induced cell death (**Fig. 19, right**). (n=3)

2. Protein Kinase and Proteasome Inhibitor

MYR protects against Bortezomib-, Sunitinib- and Sorafenib-induced cell death

Cardiotoxicity may result from the formation of toxic reactive oxygen species (ROS) through redox cycling caused by various anticancer agent. The reactive oxygen species (ROS) may activate apoptotic pathways, leading to cell death in both cancer and normal cells. Cardiomyocytes may be particularly sensitive to the oxidative stress and cardiac mitochondria may be easily disrupted by cancer agents like anthracycline, TKI or proteasome inhibitors. According to the data presented above, it was hypothesized that the ability of MYR and its analogs described herein to protect heart cells can be multifaceted: (1) protection by interacting TOPOII enzymes in the heart cells as in anthracyclines; and (2) the effect exerted independently from the molecular mechanism of TOPOII (e.g., ROS chelation, promoting mitochondrial integrity). To determine whether MYR confers cardioprotection on non-anthracycline drugs, the compound was tested for its ability to protect heart cells against protein kinase inhibitor-induced cytotoxicity.

Sunitinib and sorafenib are tyrosine kinase antagonists used to treat a wide range of cancers including leukemia and sarcoma. However, sunitinib and sorafenib have been reported to cause adverse events like heart failure in patients. Tyrosine kinases are enzymes responsible for the activation of many proteins involved in signal transduction pathways. These proteins are activated via phosphorylation, a step the TKIs are known to target for inhibition.

Bortezomib is a proteasome inhibitor used to treat multiple myeloma and lymphoma. In some cancer, the proteins that normally destroy cancer cells are broken down prematurely. Bortezomib interrupts this process, allowing those proteins to disrupt the dividing cancer cells.

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media day 3, before performing experiments. Human stem cell derived cardiomyocytes were then treated DMSO, sunitinib (10μM) or sunitinib plus increasing concentrations of MYR (1 to 100μM) for 72 hours, and then incubated with dyes that indicate mitochondrial health and cellular nuclei. Cells were imaged and total number of healthy cells were counted and plotted as percentage of sunitinib-treatment control. MYR displayed protection against sunitinib-induced cell death in cardiomyocytes (**Fig. 20**). (n=3). Similarly, MYR successfully corrected more than 80% of cardiac dysfunction in 5μM sorafenib treated cardiomyocytes (**Fig. 21**). Treatment with 100μM myricetin also rescued bortezomib-induced cardiotoxicity (**Fig. 22**). These data suggest that MYR protects against protein kinase inhibitor-induced cardiomyocyte cell death.

Example 15. No interference with Doxorubicin's anti-cancer activity

Bisdioxopiperazine dexrazoxane (DEX) is the only drug available for reducing the incidence of heart failure in cancer patients receiving anticancer agents. Despite its clinical effect, DEX is associated with several side effects such as interfering with antitumor efficacy of anthracyclines, inducing secondary malignancies, and causing blood and bone marrow disorders. These limitations severely limit its use for certain cancer patients.

The effect of MYR was investigated to determine whether the compound has the similar shortcomings to those observed in DEX. Breast cancer cells (MDA-MB-231) were mock-treated or treated with 100μM of MYR and with increasing concentrations of doxorubicin for 72 hours (**Fig. 23**). Cell viability assay was conducted (CellTiter-Glo, Promega). Luminescence was recorded via Synergy HT (Biotek) microplate reader and plotted as percentage of mock-treated control. Essentially no difference was observed in cell viability (LC50) between mock-treated (0.53μM) versus MYR-treated (0.48μM), indicating that MYR does not interfere with doxorubicin's anti-cancer activity (**Fig. 23**) (n=3).

Example 16. *In vivo* validation of cardioprotection against DOX-induced toxicity

An acute anthracycline-induced cardiotoxicity model was established in 9-10 week old C57BL/6 mice obtained from The Jackson Laboratory. Animals were divided into three groups: saline treated (n=8), Doxorubicin treated (n=16) or Doxorubicin+MYR treated (n=17). Doxorubicin (20 mg/kg), MYR (40 mg/kg) and saline were administered via a single intraperitoneal injection. MYR was administered 30 minutes prior to doxorubicin treatment. General health of the animals was monitored on a daily basis throughout the course of the study. Mice were anesthetized using isoflurane (~1.0%) and transthoracic echocardiography was performed using the VevoLAZR Imaging system (VisualSonics Inc., Toronto, Canada) at day -4 to obtain baseline measurements and then at day 5 following the treatments. Left ventricular (LV) M-mode images were obtained in the two-dimensional short axis view close to the papillary muscles. Tracings of endocardial tissue during systole and diastole were made off line. These data were then used to calculate fractional shortening (FS) and ejection fraction (EF) which are global indices of systolic function.

Contractile properties were unaltered in the saline group during the course of the study. In contrast, doxorubicin treatment had a profound impact on contractile properties. In this group, FS and EF decreased significantly with time ($P < 0.001$) by 15% and 19%, respectively. MYR treatment significantly reduced the doxorubicin-induced cardiotoxicity ($P < 0.05$) as observed by improvement of FS and EF by 7% and 10% respectively (**Fig. 24**). At 2-fold higher concentration than doxorubicin, MYR elicited 52% rescue of FS and 49% rescue of EF dysfunction caused by doxorubicin (**Fig. 24**).

Example 17. Effect of various protectants (including vitexin) on doxorubicin-induced cardiotoxicity regarding mitochondrial toxicity

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Samples were either mock treated, treated with 1μM of doxorubicin, or treated with 1μM of doxorubicin and the indicated drug for 48 hours. Following treatment, the samples were incubated with a tetramethylrhodamine methyl ester (TMRM) dye to indicate mitochondrial health, and a second dye to identify cell nuclei. Cells were imaged using the INCell Analyzer2200, and images were analyzed by CellProfiler to quantify the percentage of TMRM-negative cells. Representative data are presented for protective agents from two independent sets of screens where each data point was obtained

from three biological replicates. Data normalization was performed by re-calibrating data based on the mock-treated sample (0% mitochondrial toxicity) and the 1μM doxorubicin-treated sample (100% mitochondrial toxicity).

Cardiomyocytes were either mock-treated ('No treat'), treated with 1μM doxorubicin ('Dox 1μM'), or treated with 1 μM doxorubicin and the indicated drug, and subsequently stained to detect mitochondrial health (**Fig. 25**). Cardiomyocytes exposed to 17μM kaempferol ('KAE 17μM') exhibited a decrease in mitochondrial toxicity of at least 60%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM'). Cardiomyocytes exposed to either 0.76 μM ambroxol ('AMB 0.76μM'), 10μM mesalamine ('MES 10μM'), or 50μM N-acetyl cysteine ('NAC 50μM') exhibited a decrease in mitochondrial toxicity of at least 40%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM'). Cardiomyocytes exposed to either 160 μM dexrazoxane ('Dex 160μM') or 115μM vitexin ('VIT 115μM') exhibited a decrease in mitochondrial toxicity of at least 30%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM').

Example 18. Effect of various protectants (including vitexin) on doxorubicin-induced cardiotoxicity (apoptosis)

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media day 3, before performing experiments. Samples were either mock-treated, treated with 1μM of doxorubicin, or treated with 1μM of doxorubicin and the indicated drug for 48 hours. Following treatment, the samples were incubated with a TUNEL dye to indicate apoptosis-positive cells, and a second dye to identify cell nuclei. Cells were imaged using the INCell Analyzer2200, and images were analyzed by CellProfiler to quantify the percentage of apoptosis-positive cells. Representative data are presented for protective agents from two independent sets of screens where each data point was obtained from three biological replicates. Data normalization was performed by re-calibrating data based on the mock-treated sample (0% apoptosis) and the 1 micromolar doxorubicin-treated sample (100% apoptosis).

Cardiomyocytes were either mock treated ('No treat'), treated with 1μM doxorubicin ('Dox 1μM'), or co-treated with 1μM doxorubicin and the indicated drug, and subsequently stained to detect apoptosis (**Fig. 26**). Cardiomyocytes treated with 115 μM vitexin ('VIT 115

μM') exhibited a decrease in apoptosis of at least 60%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM'). Cardiomyocytes exposed to either 160μM dexrazoxane ('Dex 160μM'), 0.76 μM ambroxol ('AMB 0.76μM'), or 50μM N-acetyl cysteine ('NAC 50μM') exhibited a decrease in apoptosis of at least 50%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM'). Cardiomyocytes exposed to either 17 μM kaempferol ('KAE 17μM') or 10 μM mesalamine ('MES 10μM') exhibited a decrease in apoptosis of at least 40%, as compared to cardiomyocytes treated with doxorubicin in the absence of a protective agent ('Dox 1μM').

Example 19. Vitexin provides long-term cardioprotection (mitochondrial health)

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Samples were either mock-treated (**Fig. 27A**), treated with 1μM of doxorubicin (**Fig. 27B**), co-treated with 1μM of doxorubicin and 16μM dexrazoxane (**Fig. 27C**), or co-treated with 1μM of doxorubicin and 116 μM dexrazoxane (**Fig. 27D**) for 7 days. Following treatment, the samples were incubated with a tetramethylrhodamine methyl ester (TMRM) dye to indicate mitochondrial health. Cells were imaged using the INCell Analyzer2200, and images were analyzed by CellProfiler to quantify the percentage of TMRM-negative cells. Representative images are presented for each sample, wherein loss of TMRM signal represents mitochondrial toxicity.

Cardiomyocytes exposed to either doxorubicin (**Fig. 27B**) or co-treated with doxorubicin and dexrazoxane (**Fig. 27C**) exhibited an increase in mitochondrial toxicity as indicated by a noticeable decrease in TMRM-positive cells as compared to mock-treated cardiomyocytes (**Fig. 27A**). Treatment of cardiomyocytes with doxorubicin and vitexin (**Fig. 27D**) demonstrated improved long term mitochondrial protection, as compared to cardiomyocytes exposed to either doxorubicin (**Fig. 27B**) or doxorubicin and dexrazoxane (**Fig. 27C**).

Example 20. Vitexin provides dose-dependent cardioprotection (electrophysiological activity)

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Samples were either mock-treated with 0.1% DMSO, treated

with 1 μM doxorubicin, or co-treated with 1 μM doxorubicin and various concentrations of vitexin (e.g., 11.6 μM, 37 μM, or 116 μM). Following treatment, the percentage of active electrodes in each sample was measured for 72 hours. Percentage of active electrodes was quantified and graphed in a time course (**Fig. 28A**). The average number of active electrodes per well was quantified and graphed at 30 hours after the treatment (**Fig. 28B**, n=6, standard deviation is shown as error bars).

Cardiomyocytes exposed to 1 μM doxorubicin, in the absence of vitexin, exhibited about a 50% decrease in the number of active electrodes 24 hours after drug treatment (time zero), and about a 95% decrease, relative to time zero, in the number of active electrodes 30 hours after drug treatment (**Fig. 28A**). Cardiomyocytes co-exposed to doxorubicin and vitexin exhibited a dose-dependent increase in the percentage of active electrodes (**Fig. 28A**). At 24 hours following drug treatment, cardiomyocytes co-exposed to 1 μM doxorubicin and either 11.6 μM, 37 μM, or 116 μM vitexin exhibited about a 50%, about a 25%, or about a 0% decrease in the number of active electrodes, respectively. At 30 hours following treatment, samples that were co-exposed to 1 μM doxorubicin and 116 μM vitexin exhibited had a statistically significant higher average number of active electrodes (about 10 active electrodes) as compared to samples that were exposed to 1 μM doxorubicin in the absence of vitexin (about 2 active electrodes) (**Fig. 28B**).

Example 21. Protectants do not inhibit doxorubicin-mediated death of breast cancer cells

MDA-MB-231 cells (metastatic breast cancer) were cultured for 1 day before performing experiments. Samples treated with either increasing concentrations of Doxorubicin (e.g., 0 μM, 0.016 μM, 0.05 μM, 0.16 μM, 0.5 μM, 1.6 μM, 5 μM, 16 μM, or 50 μM), or co-treated with increasing concentrations of doxorubicin and the indicated protective agent for 72 hours. Cells were subsequently lysed with CellTiter-Glo reagent to identify metabolically active (e.g., viable) cells, wherein the luminescence measured from the lysed cell suspension is directly proportional to the number of viable cells present in the culture. Percentage cell death was quantified by measuring the decrease in luminescence. XLFit was used for curve fitting. Averages from triplicate are graphed and standard deviation is shown as error bars.

MDA-MB-231 cells co-treated with increasing concentrations of doxorubicin and either dexrazoxane, ambroxol, kaempferol (**Fig. 29A**), mesalamine, N-acetyl cysteine, or

vitexin (**Fig. 29B**) showed no significant difference in the percentage of cell death as compared to cells that were treated with doxorubicin in the absence of a protective agent. These results indicate that the pharmaceutical compositions described herein do not confer a protective benefit to MDA-MB-231 breast cancer cells, as measured by the *in vitro* assay.

Example 22. Protectants do not inhibit doxorubicin-mediated death of lung cancer cells

A549 cells (lung cancer) were cultured for 1 day before performing experiments. Samples treated with either increasing concentrations of Doxorubicin (e.g., 0 μM, 0.016 μM, 0.05 μM, 0.16 μM, 0.5 μM, 1.6 μM, 5 μM, 16 μM, or 50 μM), or co-treated with increasing concentrations of doxorubicin and the indicated drug for 72 hours. Cells were subsequently lysed with CellTiter-Glo reagent to identify metabolically active (e.g., viable) cells, wherein the luminescence measured from the lysed cell suspension is directly proportional to the number of viable cells present in the culture. Percentage cell death was quantified by measuring the decrease in luminescence. XLFit was used for curve fitting. Averages from triplicate are graphed and standard deviation is shown as error bars.

A549 cells co-treated with increasing concentrations of doxorubicin and either dexrazoxane, ambroxol, kaempferol, mesalamine, N-acetyl cysteine, or vitexin showed no significant difference in the percentage of cell death as compared to cells that that were treated with doxorubicin in the absence of a protective agent. These results indicate that the pharmaceutical compositions described herein do not confer a protective benefit to A549 lung cancer cells, as measured by the *in vitro* assay.

Example 23. Acute toxicity of various protectants (including vitexin) on electrophysiology

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at 3 day, before performing experiments. Samples were either mock-treated with 0.1% DMSO, or treated with increasing concentrations of the indicated drug for at least 20 minutes. Cardiomyocytes were treated with the hERG potassium channel blocker E4031 as a control. Following treatment, the beat period and field potential duration (FPD) were measured in each sample using the MEA.

At lower drug concentration, Cardiomyocytes exposed to either dexrazoxane, ambroxol, chenodeoxycholic acid, deferoxamine, N-acetyl cysteine, naringenin, or vitexin

exhibited no appreciable difference in beat period or field potential duration, as compared to control samples. At higher concentrations, cardiomyocytes exposed to either chenodeoxycholic acid or naringenin exhibited beating cessation from acute drug toxicity.

Example 24. Long term toxicity of various protectants (including vitexin) on electrophysiology

Cell samples were prepared by differentiating induced pluripotent stem cells into cardiomyocytes. Cells were cultured for 4 days post-differentiation, changing media at day 3, before performing experiments. Samples were either mock-treated with 0.1% DMSO, or treated with various concentrations of the indicated drug. Following treatment, the percentage of active electrodes in each sample was measured for at least 5 days. Percentage of active electrodes was quantified and graphed in a time course.

Cardiomyocytes exposed to either ambroxol, kaempferol, mesalamine, or vitexin showed no observable decrease in the number of active electrodes relative to the mock-treated sample. Cardiomyocytes exposed to the clinically-approved cardioprotectant dexrazoxane exhibited a long-term, dose-dependent cardiotoxic effect. Cardiomyocytes exposed to either 167 μM or 500 μM dexrazoxane exhibited about a 25% or 50% reduction in the number of active electrodes at about 2 days post-treatment, respectively. At about 3 days post-treatment, cardiomyocytes exposed to either 167 μM or 500 μM dexrazoxane exhibited about a 50% or 100% reduction in the number of active electrodes, respectively.

Example 25. Treatment of breast cancer in a patient with heart disease by oral administration of a pill containing doxorubicin and vitexin

A patient, with a history of heart disease, is diagnosed with breast cancer. Due to an increased risk for heart failure, the patient is unable to receive the standard treatment regimen of doxorubicin, which is known to induce cardiotoxicity. Instead, the caregiver administers a co-formulation of doxorubicin (10mg) and vitexin (100mg). An echocardiogram is performed and blood flow rate is measured to determine if the therapy has a cardiotoxic effect in the patient. The patient shows no indication of cardiac dysfunction. Exhibiting no signs of cardiotoxicity, the patient is able accept higher doses of treatment over the next several

weeks. The patient subsequently undergoes a tissue biopsy which shows no indication of breast cancer.

Example 26. Treatment of liver cancer in a patient by intravenous administration of doxorubicin, dexrazoxane and vitexin

A patient is diagnosed with liver cancer. The caregiver administers to the patient a co-formulation of doxorubicin (5mg/mL) and dexrazoxane (50mg/mL). An electrocardiogram is performed to determine if the dexrazoxane is successfully mitigating cardiotoxic effects in the patient. The patient presents with a 20ms QT prolongation. To enhance the activity of dexrazoxane, the caregiver administers to the patient a co-formulation of doxorubicin (5mg/mL) and vitexin (100mg/mL). Following treatment, an electrocardiogram is performed, and the patient exhibits no signs of QT prolongation. The patient is able to continue receiving treatment over several weeks after which a tissue biopsy is performed to confirm the liver cancer has been eradicated.

Example 27. Treatment of lung cancer in a patient with bradycardia by oral administration of a pill containing doxorubicin and myricetin

A patient is diagnosed with stage II lung cancer, and presents with bradycardia. Due to an increased risk for heart failure, the patient is unable to receive the standard treatment regimen of doxorubicin, which is known to affect cardiac contraction and induce bradycardia. Instead, the caregiver administers a co-formulation of doxorubicin (10mg) and myricetin (100mg). An electrocardiogram is used to monitor the patient's heart rate. The patient shows no indication of cardiac dysfunction. Exhibiting no signs of cardiotoxicity, the patient is able accept higher doses of treatment over the next several weeks. The lung cancer is down-staged to stage 1, and the cancer is successfully removed with surgery. Upon follow-up, a tissue biopsy is performed and shows no sign of cancer.

Example 28. Treatment of liver cancer in a patient by intravenous administration of a solution containing doxorubicin, dexrazoxane and myricetin

A patient is diagnosed with liver cancer. The caregiver administers to the patient a co-formulation of doxorubicin (5mg/mL) and dexrazoxane (50mg/mL). An electrocardiogram is performed to determine if the dexrazoxane is successfully mitigating cardiotoxic effects in the patient. The patient presents with a 20ms QT prolongation. To

enhance the activity of dexrazoxane, the caregiver administers to the patient a co-formulation of doxorubicin (5mg/mL) and myricetrin (50mg/mL). Following treatment, an electrocardiogram is performed, and the patient exhibits no signs of QT prolongation. The patient is able to continue receiving treatment over several weeks after which a tissue biopsy is performed to confirm the liver cancer has been eradicated.

Example 29. Treatment of lung cancer in a patient with bradycardia by oral administration of a pill containing myricetin

A patient is diagnosed with stage II lung cancer, and presents with bradycardia. Due to an increased risk for heart failure, the patient is unable to receive the standard treatment regimen of doxorubicin, which is known to affect cardiac contraction and induce bradycardia. Instead, the caregiver administers myricetin (100mg) 24 hours before administration of doxorubicin (10mg). An electrocardiogram is used to monitor the patient's heart rate. The patient shows no indication of cardiac dysfunction. Exhibiting no signs of cardiotoxicity, the patient is able accept higher doses of treatment over the next several weeks. The lung cancer is down-staged to stage 1, and the cancer is successfully removed with surgery. Upon follow-up, a tissue biopsy is performed and shows no sign of cancer.

Example 30. Treatment of liver cancer in a patient by intravenous administration of a solution containing doxorubicin, dexrazoxane and myricetin

A patient is diagnosed with liver cancer. The caregiver administers to the patient a co-formulation of doxorubicin (5mg/mL) and dexrazoxane (50mg/mL). An electrocardiogram is performed to determine if the dexrazoxane is successfully mitigating cardiotoxic effects in the patient. The patient presents with a 20ms QT prolongation. To enhance the activity of dexrazoxane, the caregiver administers to the patient a myricetin (100mg) 24 hours prior to administration of doxorubicin (5mg/mL) and (100mg/mL) intravenously. Following treatment, an electrocardiogram is performed, and the patient exhibits no signs of QT prolongation. The patient is able to continue receiving treatment over several weeks after which a tissue biopsy is performed to confirm the liver cancer has been eradicated.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific

embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled. Such modifications are intended to fall within the scope of the appended claims.

5

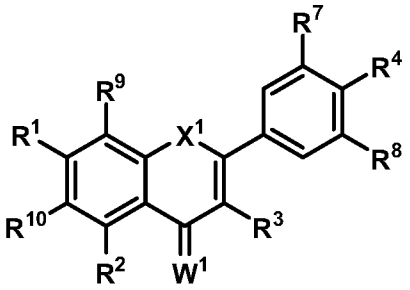
All references, patent and non-patent, cited herein are incorporated herein by reference in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

10

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition comprising an anticancer agent and a protective agent according to Formula 1,



Formula 1

wherein:

X^1 is CR^5R^6, NR^5, O, S, C=O, or C=S;

each of R^1, R^2, R^3, R^5, R^6, R^9, and R^{10} is independently alkyl, alkenyl, alkynyl, alkoxy, acyl, acyloxy, carboxylic acid, ester, amine, amide, carbonate, carbamate, nitro, thioether, thioester, cycloalkyl, heteroalkyl, heterocyclyl, aryl, or heteroaryl, any of which is substituted or unsubstituted, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H;

R^4, R^7 and R^8 are alkoxy, hydroxyl or H;

W^1 is O or S; or

a salt thereof.

2. The pharmaceutical composition of claim 1, wherein

X^1 is O or S;

each of R^1, R^2, R^3, R^9, and R^{10} is independently alkoxy, cycloalkyl, heterocyclyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and

each of R^4, R^7 and R^8 is alkoxy, hydroxyl or H.

3. The pharmaceutical composition of claim 2, wherein

X^1 is O;

each of R^1, R^2, R^3, R^9, and R^{10} is independently alkoxy, cycloakyl, heterocyclyl, halogen, hydroxyl, sulfhydryl, nitro, nitroso, cyano, azido, or H; and

each of R^4, R^7 and R^8 are alkoxy, hydroxyl or H.

4. The pharmaceutical composition of claim 3, wherein

X^1 is O;

each of R^1, R^2, and R^3 is independently hydroxyl or H;

each of R^9 and R^{10} is cycloalkyl, heterocyclyl, or H;

R^4 is hydroxyl; and

R^7 and R^8 are methoxy, hydroxyl or H.

5. The pharmaceutical composition of claim 4, wherein

X^1 is O;

R^1 is hydroxyl;

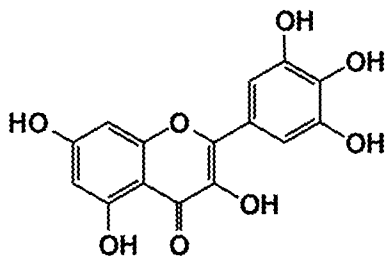
each of R^2 and R^3 is independently hydroxyl or H;

R^9 and R^{10} are H;

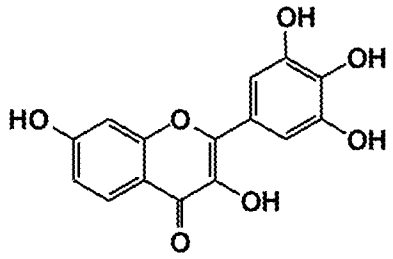
R^4 is hydroxyl; and

R^7 and R^8 are hydroxyl or H.

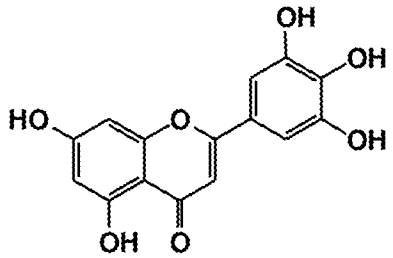
6. The pharmaceutical composition of claim 5, wherein the protective agent is myricetin and is a compound according to the formula:



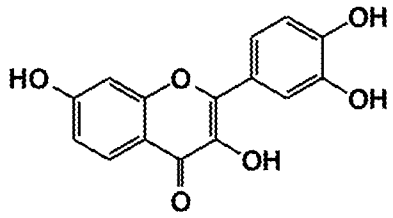
7. The pharmaceutical composition of claim 5, wherein the protective agent is robinetin and is a compound according to the formula:



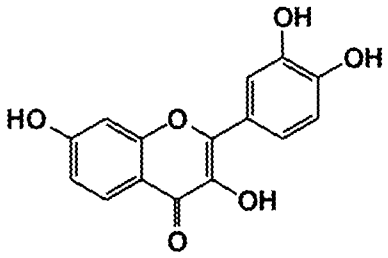
8. The pharmaceutical composition of claim 5, wherein the protective agent is tricetin and is a compound according to the formula:



9. The pharmaceutical composition of claim 5, wherein the protective agent is ficetin and is a compound according to the formula:



or



10. The pharmaceutical composition of claim 1, wherein the protective agent is selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin.

11. The pharmaceutical composition of claim 1, wherein the protective agent is kaempferol or quercetin.

12. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition is a tablet.

13. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition is a liquid composition.

14. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition is a capsule, a gel capsule, or a liposome.

15. The pharmaceutical composition of claim 1, wherein the protective agent is a topoisomerase IIα and β inhibitor.

16. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises at least 10 mg of at least one protective agent.

17. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises at least 50 mg of at least one protective agent.

18. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises at least 100 mg of the protective agent.

19. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises at least 200 mg of at least one protective agent.

20. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition comprises between 0.1 mg and 50 mg of at least two protective agents of Formula 1.

21. The pharmaceutical composition of any one of claims 1-20, wherein the anticancer agent is an anthracycline or salt thereof.

22. The pharmaceutical composition of claim 21, wherein the anthracycline is daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin.

23. The pharmaceutical composition of claim 22, wherein the anthracycline is doxorubicin.

24. The pharmaceutical composition of claim 22, wherein the anthracycline is epirubicin.

25. The pharmaceutical composition of any one of claims 1-20, wherein the anticancer agent is a protein kinase inhibitor.

26. The pharmaceutical composition of claim 25, wherein the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

27. The pharmaceutical composition of any one of claims 1-20, wherein the anticancer agent is bortezomib.

28. The pharmaceutical composition of claim 25, wherein the protein kinase inhibitor is a tyrosine kinase inhibitor.

29. The pharmaceutical composition of claim 28, wherein the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib, mesylate, and nilotinib.

30. The pharmaceutical composition of claim 29, wherein the tyrosine kinase inhibitor is sorafenib.

31. The pharmaceutical composition of claim 29, wherein the tyrosine kinase inhibitor is sunitinib.

32. The pharmaceutical composition of any one of claims 1-20, wherein the anticancer agent is a biologic agent.

33. The pharmaceutical composition of claim 32, wherein the biologic agent is an antibody.

34. The pharmaceutical composition of claim 33, wherein the antibody is adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab.

35. The pharmaceutical composition of claim 34, wherein the antibody is trastuzumab.

36. The pharmaceutical composition of claim 1, further comprising a second protective agent, wherein the second protective agent is dexrazoxane.

37. A method of treating a subject suffering from cancer, comprising administering to the subject a pharmaceutical composition of any one of claims 1-36.

38. A method for preventing, reducing, or eliminating cardiotoxicity induced by an anticancer agent or biologic agent in a subject, the method comprising: administering to the subject an effective amount of at least one protective agent according to Formula 1 and the anticancer agent or biologic agent, thereby preventing, reducing, or eliminating the cardiotoxicity induced by the chemotherapy drug or biologic agent in the subject.

39. The method of claim 38, wherein the anticancer agent or biologic agent is administered to the subject prior to the administration of the at least one protective agent according to Formula 1.

40. The method of claim 38, wherein the anticancer agent or biologic agent is administered to the subject subsequent to the administration of the at least one protective agent according to Formula 1.

41. The method of claim 38, wherein the anticancer agent or biologic agent is administered to the subject concurrently with the at least one protective agent according to Formula 1.

42. The method of claim 38, wherein the anticancer agent or biologic agent and the at least one protective agent are formulated in one liquid composition.

43. The method of claim 38, wherein the anticancer agent or biologic agent and the at least one protective agent are formulated in a tablet.

44. The method of claim 38, wherein the at least one protective agent is administered orally.

45. The method of claim 38, wherein the at least one protective agent is administered intravenously.

46. The method of claim 38, wherein the subject suffers from cancer.

47. The method of claim 46, wherein the subject is a human subject.

48. The method of claim 46, wherein the cancer is bladder cancer, bone cancer, a brain tumor, breast cancer, esophageal cancer, colorectal cancer, leukemia, liver cancer,

lung cancer, lymphoma, myeloma, ovarian cancer, prostate cancer, a sarcoma, stomach cancer, or thyroid cancer.

49. The method of claim 48, wherein the cancer is breast cancer.

50. The method of claim 48, wherein the cancer is leukemia.

51. The method of claim 48, wherein the cancer is a sarcoma.

52. The method of claim 51, wherein the sarcoma is Kaposi sarcoma.

53. The method of claim 38, wherein the anticancer agent is a protein kinase inhibitor.

54. The method of claim 53, wherein the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

55. The method of claim 53, wherein the protein kinase inhibitor is a tyrosine kinase inhibitor.

56. The method of claim 55, wherein the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib, mesylate, and nilotinib.

57. The method of claim 56, wherein the tyrosine kinase inhibitor is sorafenib.

58. The method of claim 56, wherein the tyrosine kinase inhibitor is sunitinib.

59. The method of claim 38, wherein the anticancer agent is a proteasome inhibitor (e.g., bortezomib).

60. The method of claim 38, wherein the anticancer agent is a biologic agent.

61. The method of claim 60, wherein the biologic agent is an antibody.

62. The method of claim 61, wherein the antibody is adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab.

63. The method of claim 62, wherein the antibody is trastuzumab.

64. The method of claim 38, further comprising administering a second protective agent.

65. The method of claim 64, wherein the second protective agent is dexrazoxane.

66. The method of claim 38, wherein the at least one protective agent according to Formula 1 is administered to the subject at least 24 hours before the administration of the anticancer agent or biologic agent.

67. The method of claim 38, wherein the at least one protective agent according to Formula 1 is administered to the subject at least 1, 2, 3, 4, 5, 6, 6, 7, 9, 10, 11 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 48 hours before the administration of the anticancer agent or biologic agent.

68. The method of claim 38, wherein the at least one protective agent according to Formula 1 is administered to the subject concurrently with the anticancer agent or biologic agent.

69. The method of claim 38, wherein the at least one protective agent according to Formula 1 is administered at least 1, 2, 3, 4, 5, 6, 6, 7, 9, 10, 11 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 48 hours after the administration of the anticancer agent or biologic agent to the subject.

70. The method of claim 38, wherein the at least one protective agent according to Formula 1 is administered to the subject in a molar ratio of at least 1:5 units of the anticancer agent to the protective agent

71. The method of claim 38, wherein the at least one protective agent is a topoisomerase IIα or β inhibitor.

72. The method of claim 71, wherein the at least one protective agent is a topoisomerase IIα and topoisomerase β inhibitor.

73. The method of claim 38, wherein the anticancer agent is an anthracycline or salt thereof.

74. The method of claim 73, wherein the anthracycline is daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin.

75. The method of claim 74, wherein the anthracycline is doxorubicin.

76. The method of claim 74, wherein the anthracycline is epirubicin.

77. The method of claim 74, wherein the anthracycline is idarubicin.

78. The method of claim 74, wherein the at least one protective agent is a topoisomerase IIα or β inhibitor.

79. The method of claim 38, wherein the at least one protective agent is selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin.

80. A method for treating cancer and preventing cardiotoxicity from one or more anticancer agent, the method comprising:

(a) administering a chemotherapy drug or biologic agent to a subject suffering from cancer and receiving the chemotherapy drug or biologic agent known to cause cardiotoxicity in the subject; and

(b) administering to the subject an effective amount of a protective agent according to Formula 1 or pharmaceutically acceptable salts thereof, wherein the protective agent prevents, reduces, or eliminates the cardiotoxicity in the subject.

81. The method of claim 80, wherein the chemotherapy drug is an anthracycline or salt thereof.

82. The method of claim 81, wherein the anthracycline is daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, or valrubicin.

83. The method of claim 82, wherein the anthracycline is doxorubicin.

84. The method of claim 82, wherein the anthracycline is epirubicin.

85. The method of claim 82, wherein the anthracycline is idarubicin.

86. The method of claim 80, wherein the protective agent is a topoisomerase II inhibitor.

87. The method of claim 86, wherein the protective agent is a topoisomerase IIα or β inhibitor.

88. The method of claim 80, wherein the protective agent is selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin.

89. The method of claim 80, wherein the subject is a human subject.

90. The method of claim 80, wherein the cancer is bladder cancer, bone cancer, a brain tumor, breast cancer, esophageal cancer, colorectal cancer, leukemia, liver cancer, lung cancer, lymphoma, myeloma, ovarian cancer, prostate cancer, a sarcoma, stomach cancer, or thyroid cancer.

91. The method of claim 90, wherein the cancer is breast cancer.

92. The method of claim 90, wherein the cancer is leukemia.

93. The method of claim 90, wherein the cancer is a sarcoma.

94. The method of claim 93, wherein the sarcoma is Kaposi sarcoma.

95. The method of claim 80, wherein the human subject has a cardiac condition or has a history of having a cardiac condition.

96. The method of claim 80, wherein the cardiotoxicity comprises cardiac tissue damage, electrophysiological dysfunction, mitochondrial toxicity, apoptosis, and oxidative stress.

97. The method of claim 96, wherein the cardiotoxicity is cardiac tissue damage.

98. The method of claim 80, wherein the chemotherapy drug is a protein kinase inhibitor.

99. The method of claim 98, wherein the protein kinase inhibitor is afatinib, axitinib, bosutinib, cabozantinib, carfilzomib, ceritinib, cobimetanib, crizotinib, dabrafenib, dasatinib, erlotinib, everolimus, gefitinib, ibrutinib, imatinib, lapatinib, lenvatinib, nilotinib, nintedanib, osimertinib, palbociclib, pazopanib, pegaptanib, ponatinib, regorafenib, ruxolitinib, sirolimus, sorafenib, sunitinib, tofacitinib, tofacitinib, temsirolimus, trametinib, vandetanib, vemurafenib, or vismodegib.

100. The method of claim 80, wherein the protein kinase inhibitor is a tyrosine kinase inhibitor.

101. The method of claim 100, wherein the tyrosine kinase inhibitor is selected from the group consisting of sorafenib, sunitinib, bosutinib, gefitinib, dasatinib, dabrafenib, vemurafenib, imatinib, lapatinib, mesylate, and nilotinib.

102. The method of claim 101, wherein the tyrosine kinase inhibitor is sorafenib.

103. The method of claim 101, wherein the tyrosine kinase inhibitor is sunitinib.

104. The method of claim 80, wherein the anticancer agent is a proteasome inhibitor (e.g., bortezomib).

105. The method of claim 80, wherein the biologic agent is an antibody.

106. The method of claim 105, wherein the antibody is adotrastuzumabemtansine, alemtuzumab, bevacizumab, blinatumomab, brentuximab vedotin, catumaxomab, cetuximab, gemtuzumab ozogamicin, ibritumomab tiuxetan, ipilimumab, necitumumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, ramucirumab, rituximab, tositumomab-I131, or trastuzumab.

107. The method of claim 106, wherein the antibody is trastuzumab.

108. The method of claim 80, wherein the subject has a decreased QTc interval after administering the protective agent.

109. The method of claim 80, wherein the chemotherapy drug and the protective agent of Formula 1 are administered concurrently to the subject.

110. The method of claim 80, wherein the chemotherapy drug and the protective agent of Formula 1 are administered sequentially to the subject.

111. The method of claim 110, wherein the protective agent is administered to the subject prior to the administration of the chemotherapy drug.

112. The method of claim 110, wherein the protective agent is administered to the subject after the administration of the chemotherapy drug.

113. A method for treating or preventing organ damage in a subject comprising: administering to the subject an effective amount of a protective agent of Formula 1 to a subject receiving an anticancer agent comprising at least one of anthracycline, tyrosine kinase inhibitor, or trastuzumab.

114. A method for preventing organ damage or cardiac failure in a subject comprising: administering to a subject an effective amount of a protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin.

115. A kit comprising:

a. a protective agent selected from the group consisting of myricetin, vitexin, robinetin, tricetin, ficetin, 7,3',4',5'-tetrahydroxyflavone, and myricitrin; and

b. a chemotherapy drug or a biological agent,

wherein the chemotherapy drug or the biological agent causes cardiotoxicity.

116. The kit of claim 115, further comprising dexrazoxane.

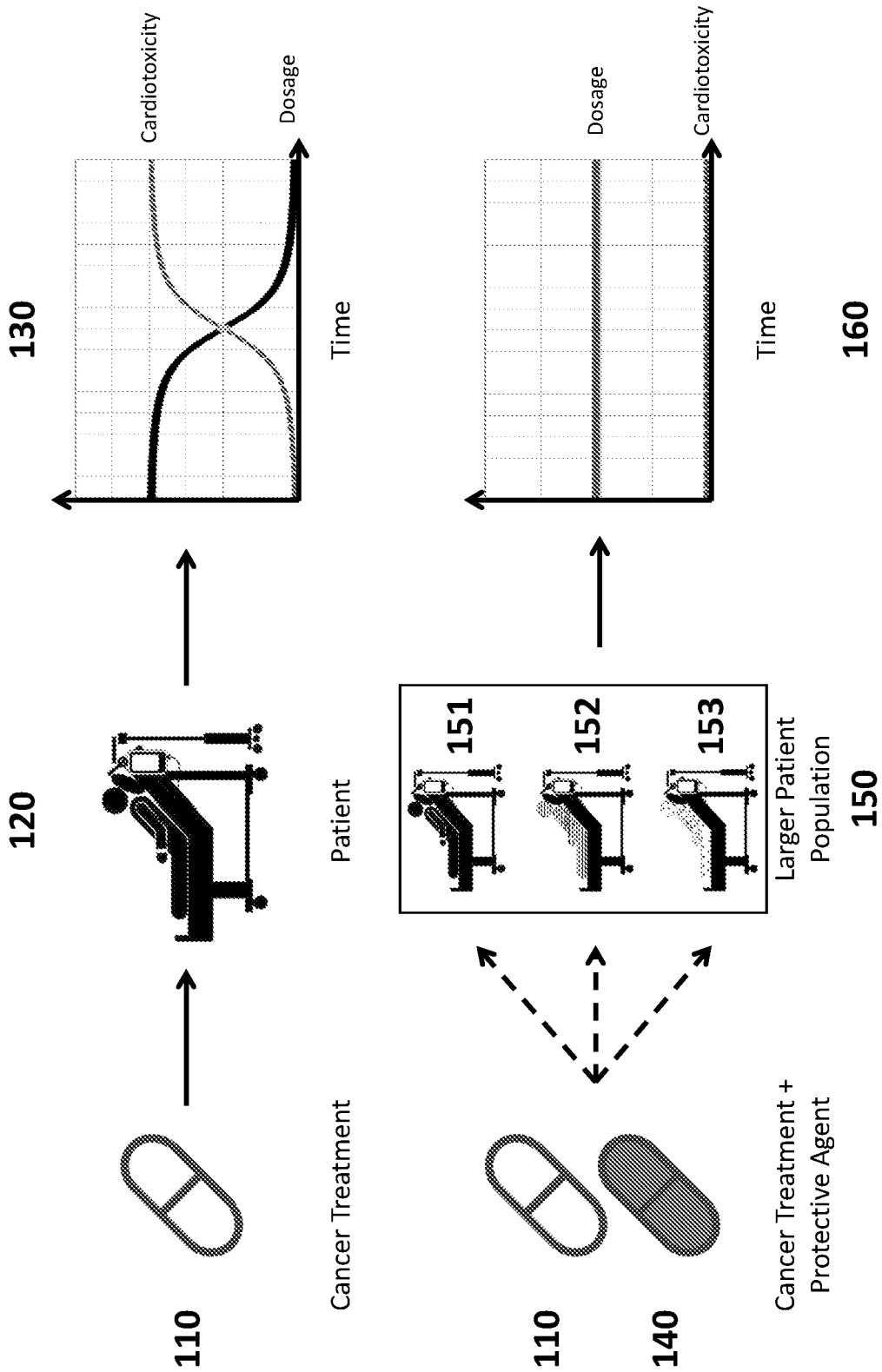

Cardiotoxicity
Dosage
Time
130
Patient
120
Cancer Treatment
110
151
152
153
Larger Patient Population
150
Cancer Treatment + Protective Agent
110
140
Dosage
Cardiotoxicity
Time
160

Fig. 1

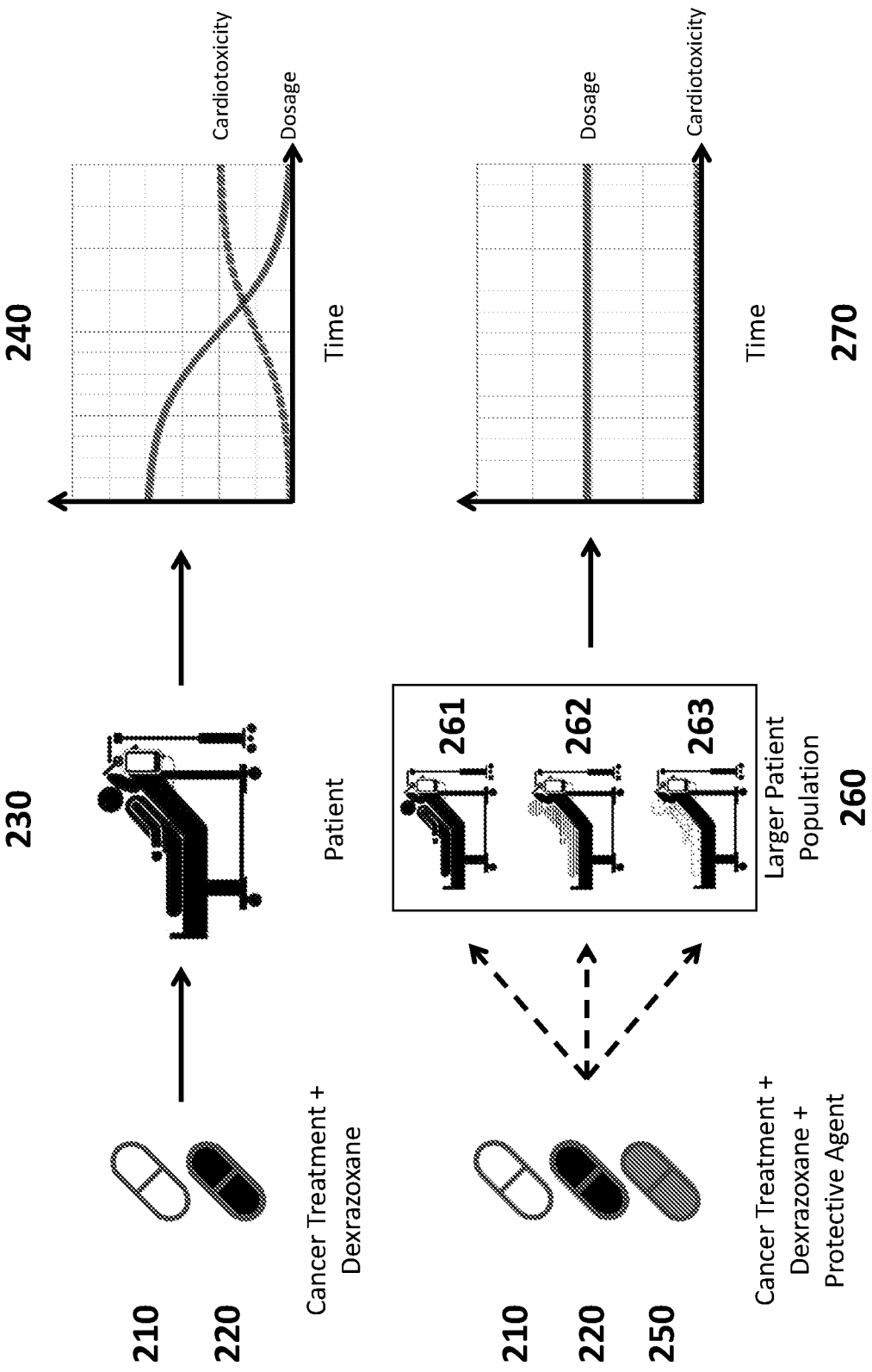

210
220
Cancer Treatment + Dexrazoxane
230
Patient
240
Cardiotoxicity
Dosage
Time
210
220
250
Cancer Treatment + Dexrazoxane + Protective Agent
261
262
263
Larger Patient Population
260
Dosage
Cardiotoxicity
Time
270

Fig. 2

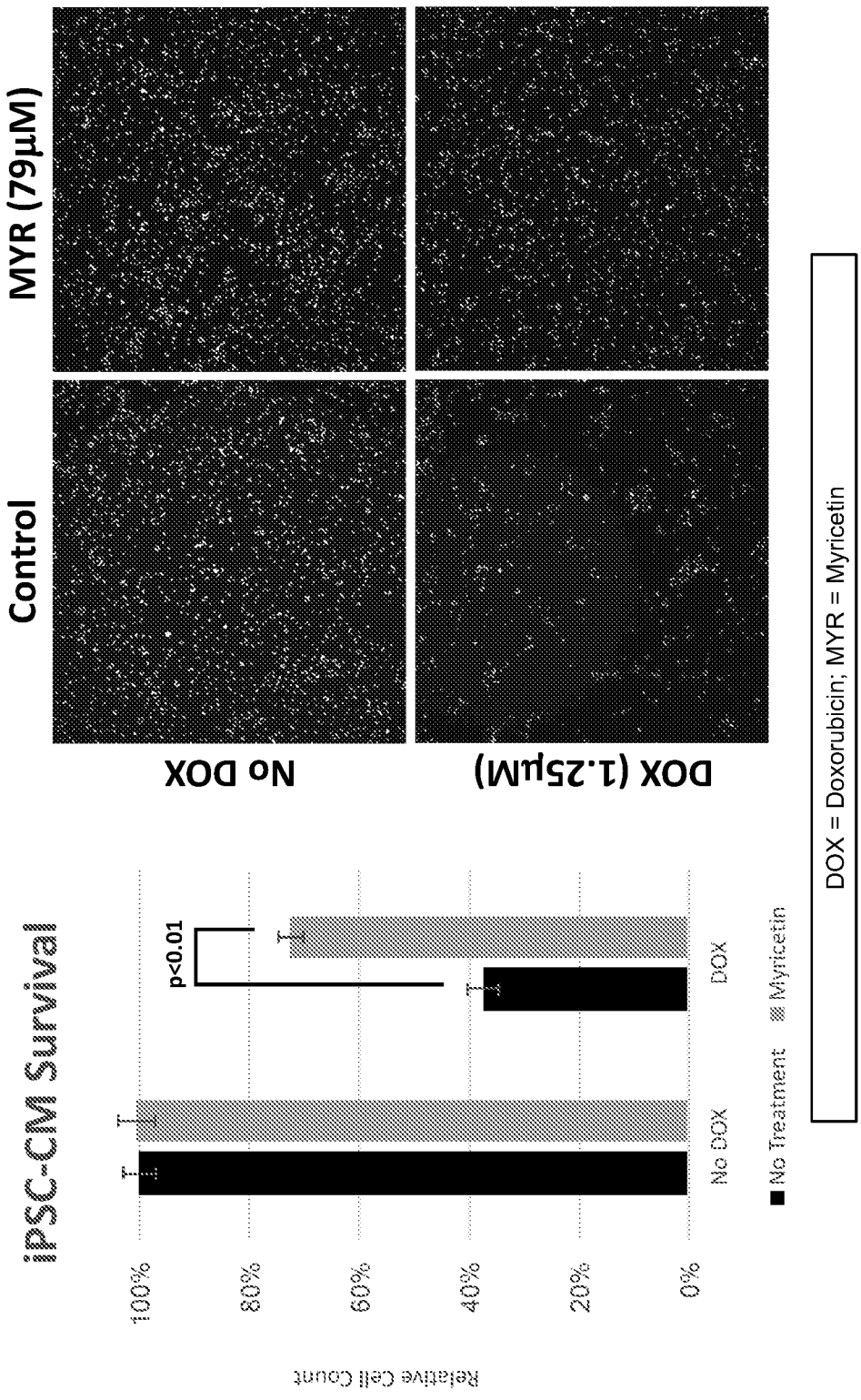

Control
MYR (79μM)
No DOX
DOX (1.25μM)
iPSC-CM Survival
Relative Cell Count
100%
80%
60%
40%
20%
0%
p<0.01
No DOX
DOX
No Treatment
Myricetin
DOX = Doxorubicin; MYR = Myricetin

Fig. 3

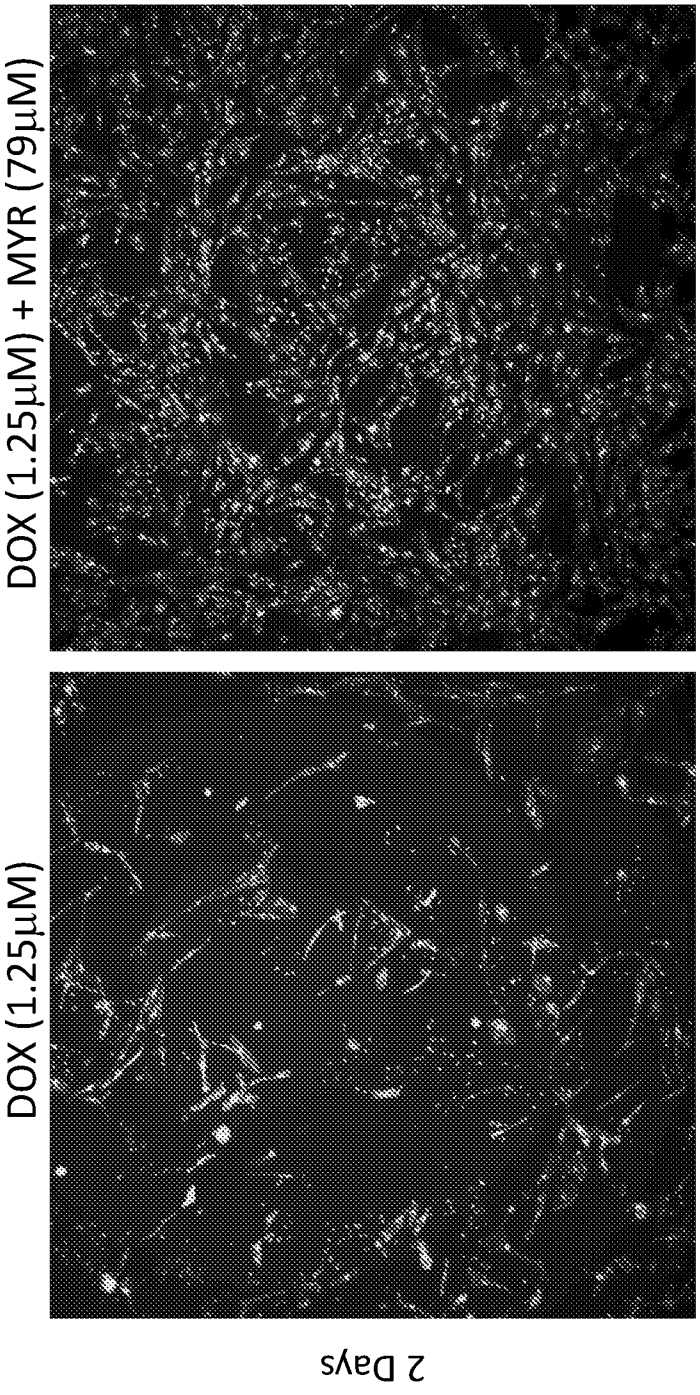


Fig. 4A

Fig. 4B

DOX = Doxorubicin; MYR = Myricetin

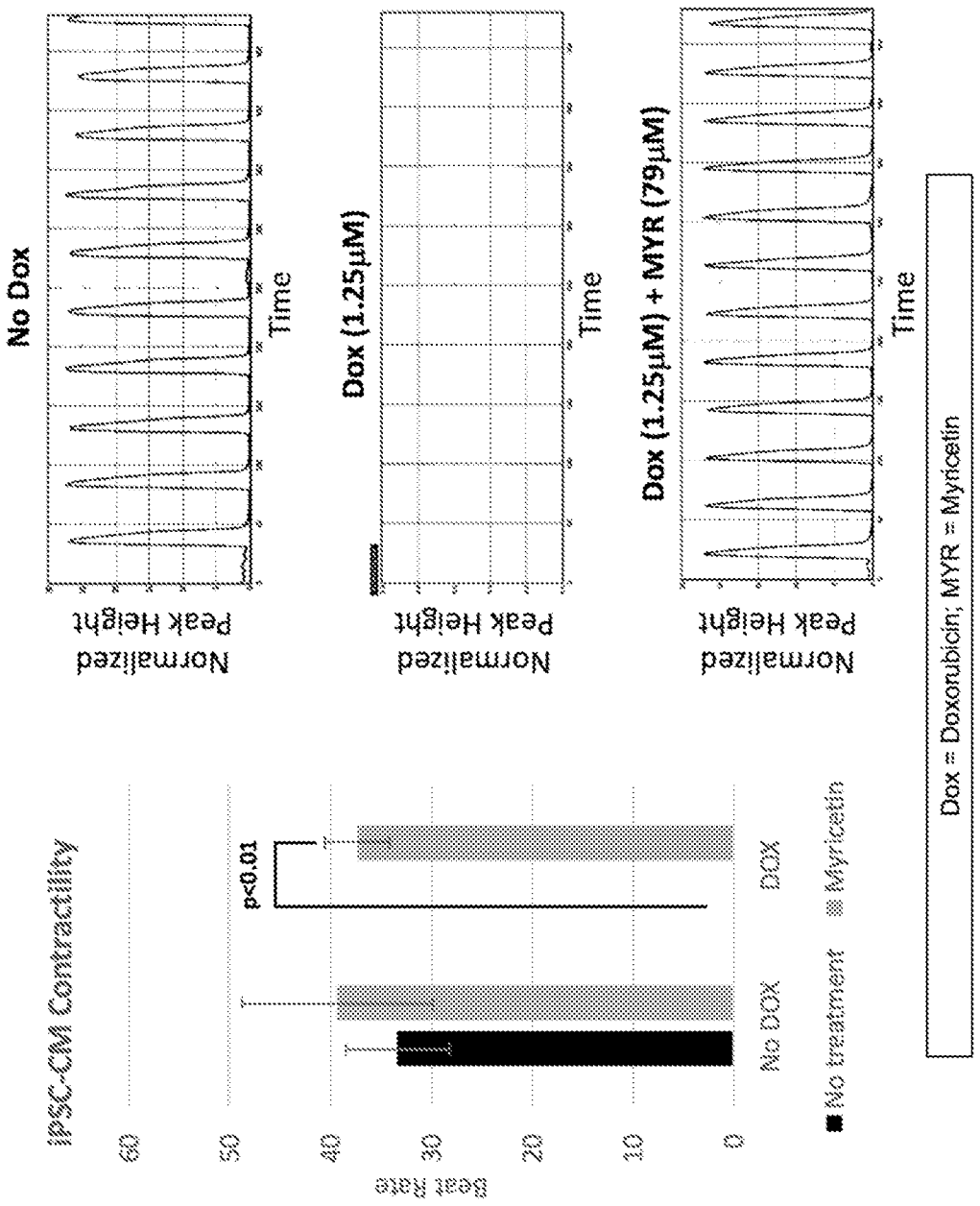

No Dox
Dox (1.25μM)
Dox (1.25μM) + MYR (79μM)
Normalized Peak Height
Time
IPSC-CM Contractility
Beat Rate
p<0.01
No DOX
DOX
No treatment
Myricetin
Dox = Doxorubicin; MYR = Myricetin

FIG. 5

		AVERAGE		STDEV	
	DOX Concentration (µM)	0	1.25	0	1.25
Total Cell Count	No Treatment	8543.50	3195.67	251.02	241.42
	Myricetin (79 µM)	8586.33	6187.67	285.98	194.28

Fig. 6A

	DOX Concentration (µM)	0	1.25	0	1.25
% Cells	No Treatment	100.00%	37.40%	2.94%	2.83%
	Myricetin (79 µM)	100.50%	72.43%	3.35%	2.27%

Fig. 6B

	DOX Concentration (µM)	0	1.25	0	1.25
Beat Rate (BPM)	DMSO	33.33	0.00	5.13	0.00
	Myricetin (79 µM)	39.33	37.33	9.45	3.21

Fig. 6C

DOX = Doxorubicin; MYR = Myricetin

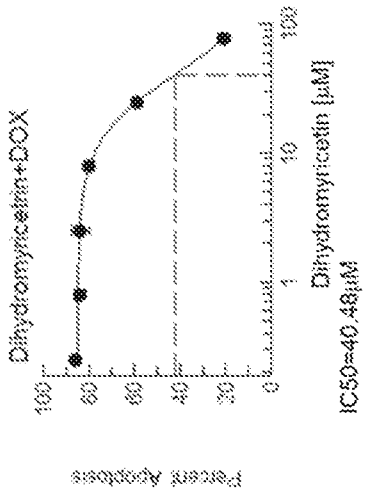


FIG. 7C

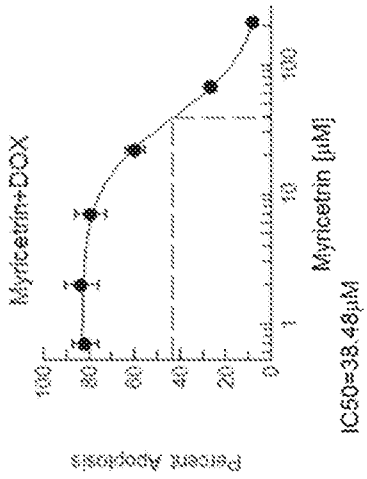


FIG. 7B

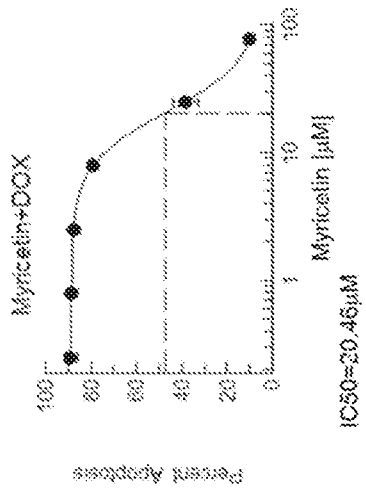


FIG. 7A

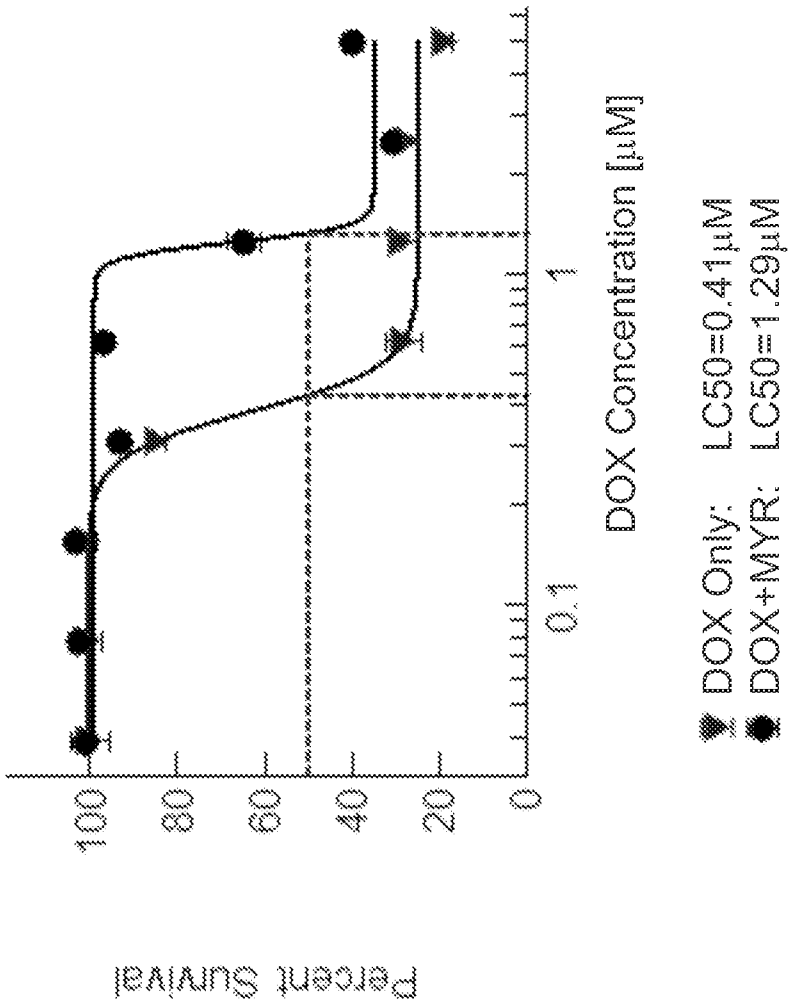

Percent Survival
100
80
60
40
20
0
0.1
1
DOX Concentration [μM]
DOX Only: LC50=0.41μM
DOX+MYR: LC50=1.29μM

Fig. 8

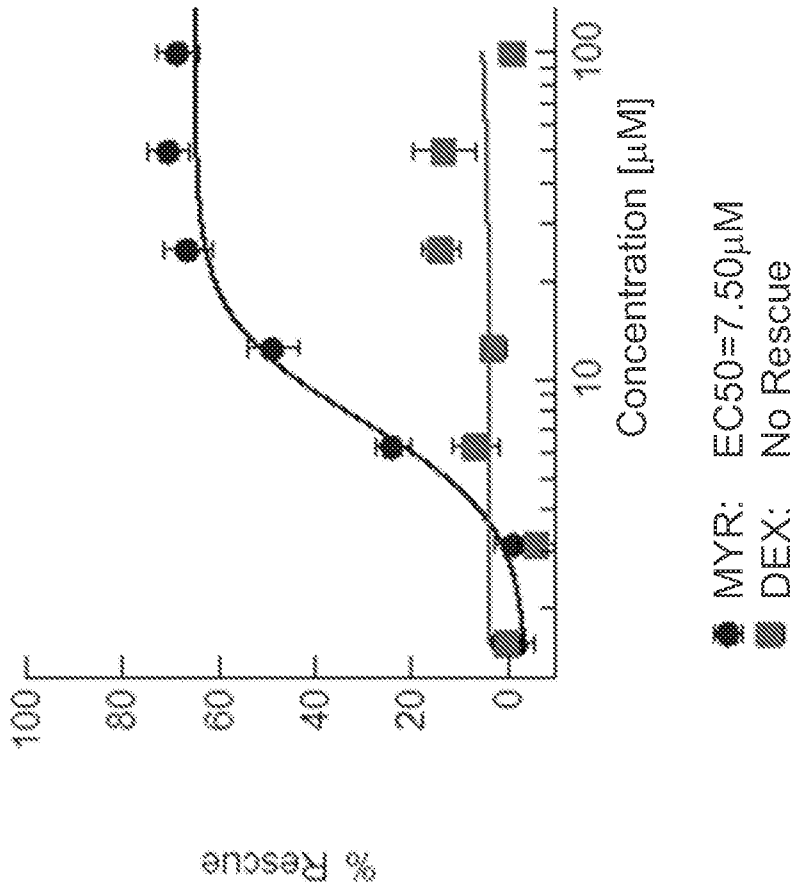

% Rescue
100
80
60
40
20
0
1
10
100
Concentration [µM]
MYR: EC50=7.50µM
DEX: No Rescue

Fig. 9

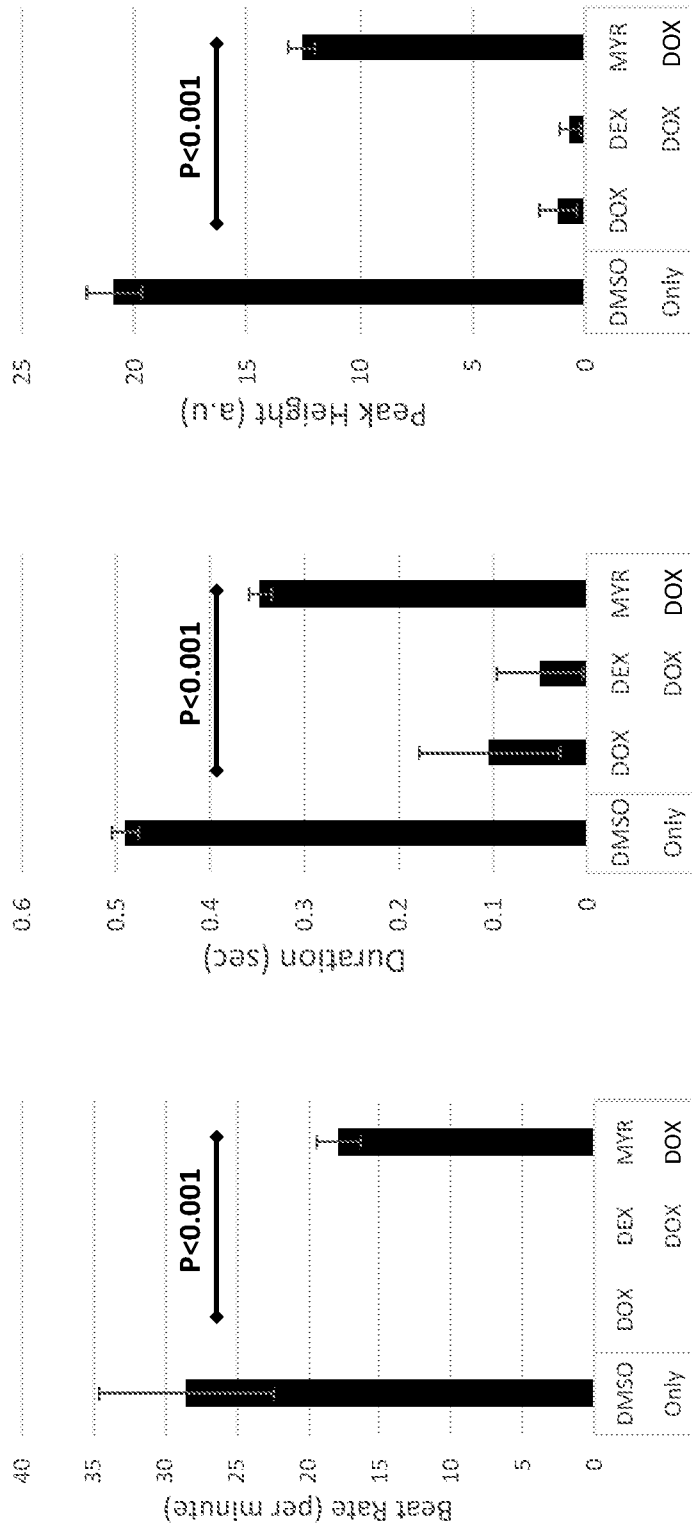

Peak Height (a.u)
25
20
15
10
5
0
P<0.001
DMSO
Only
DOX
DEX
DOX
MYR
DOX
Duration (sec)
0.6
0.5
0.4
0.3
0.2
0.1
0
P<0.001
DMSO
Only
DOX
DEX
DOX
MYR
DOX
Beat Rate (per minute)
40
35
30
25
20
15
10
5
0
P<0.001
DMSO
Only
DOX
DEX
DOX
MYR
DOX

Fig. 10

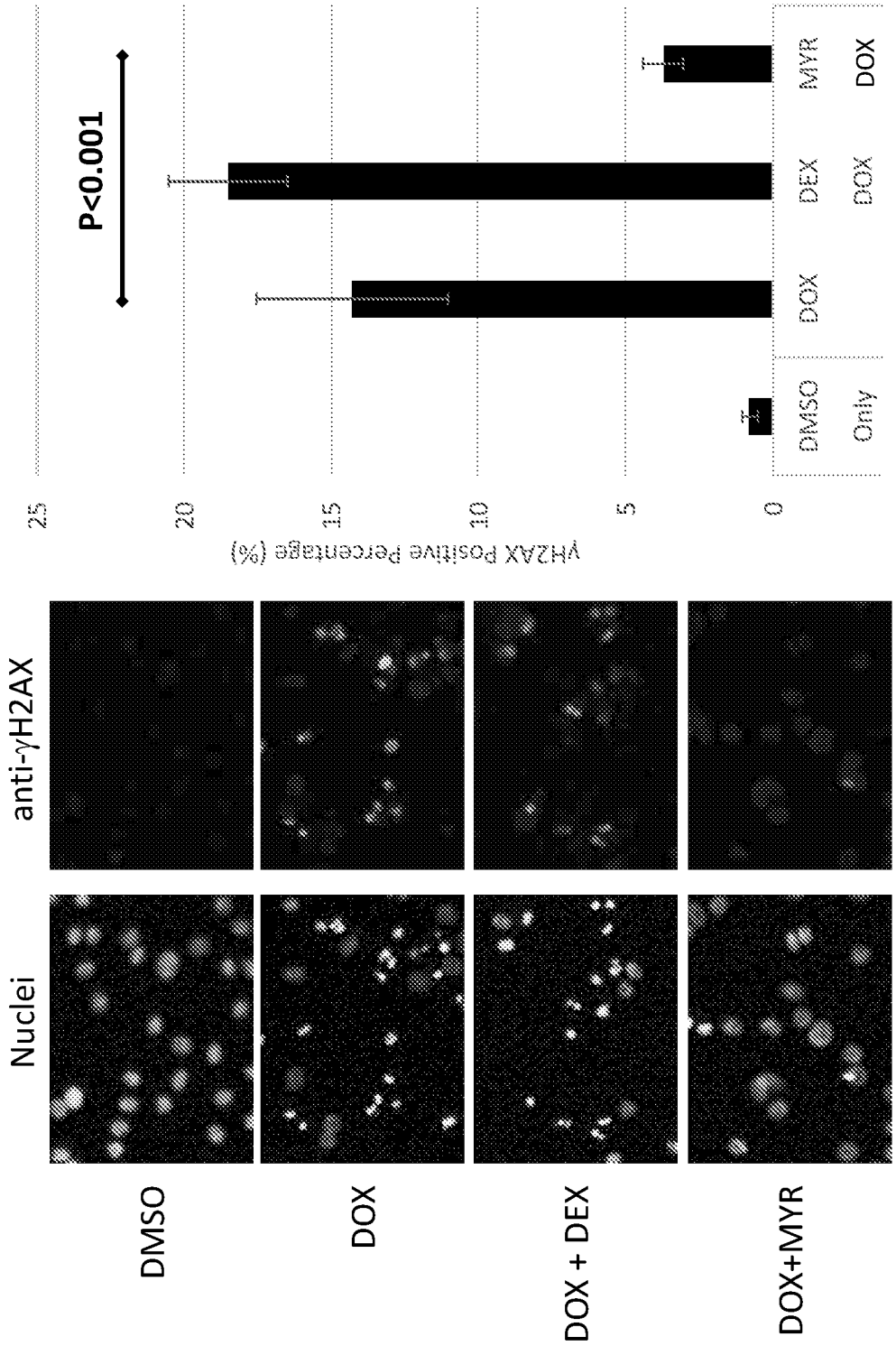

DMSO
DOX
DOX + DEX
DOX+MYR
Nuclei
anti-γH2AX
γH2AX Positive Percentage (%)
0
5
10
15
20
25
P<0.001
DMSO Only
DOX
DEX DOX
MYR DOX

Fig. 11

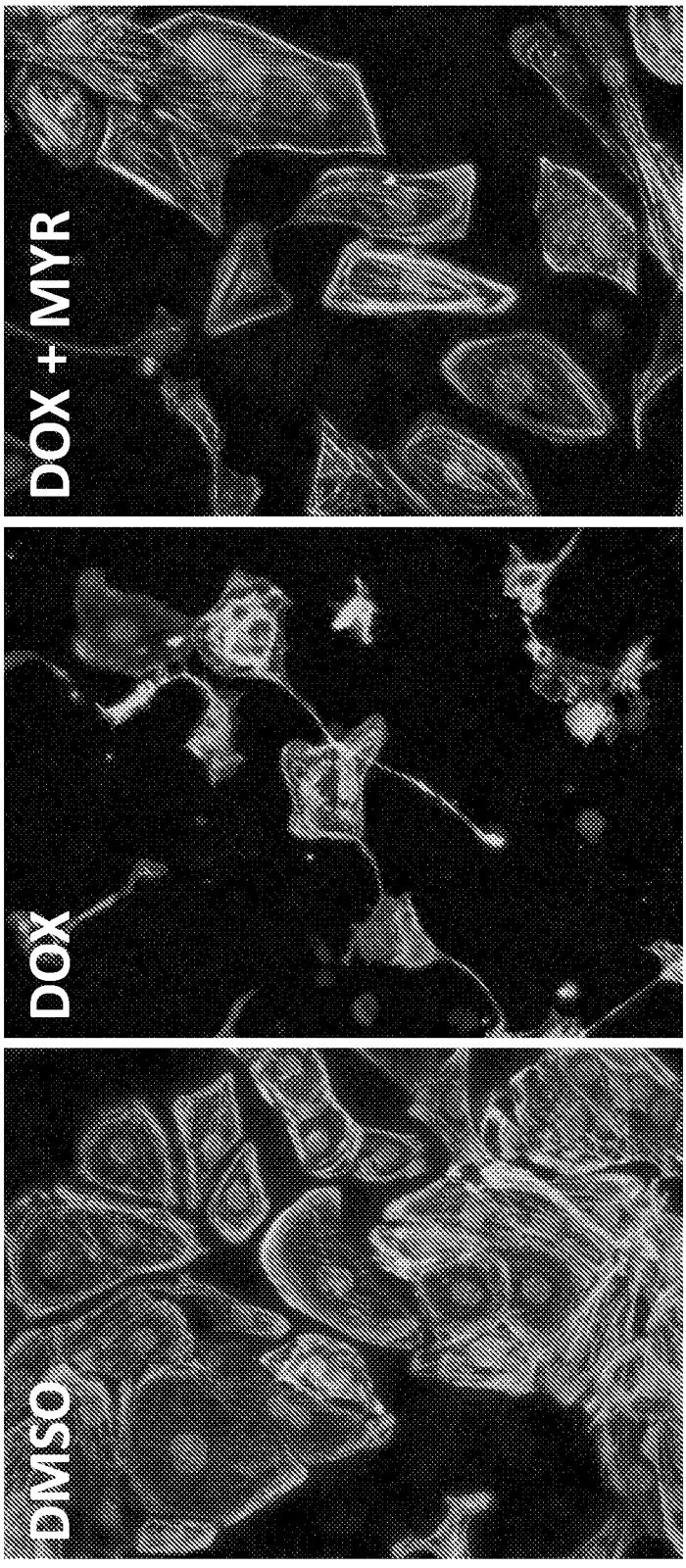
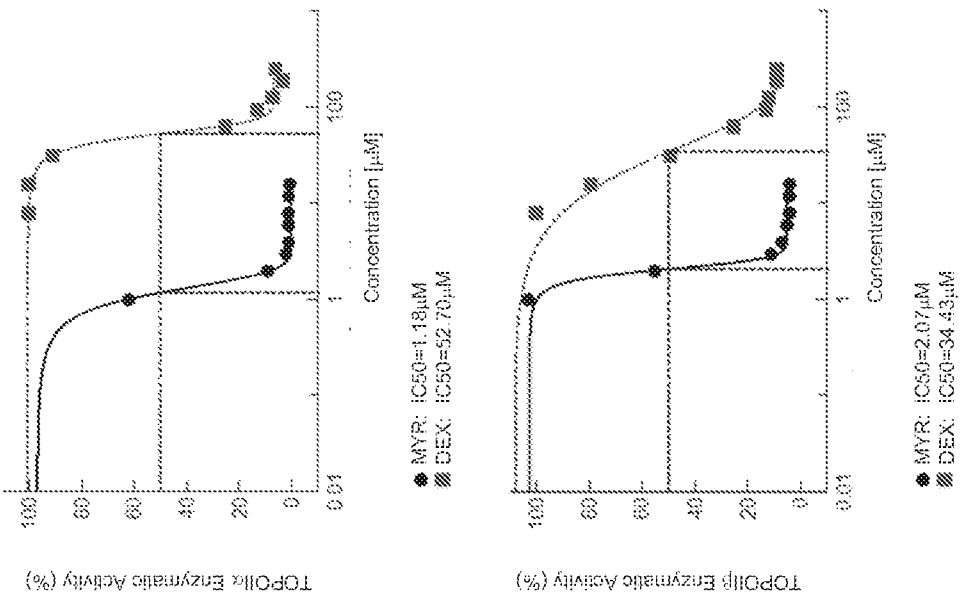


Fig. 12


TOPOIIα Enzymatic Activity (%)
100
80
60
40
20
0
0.01
1
100
Concentration [μM]
MYR: IC50=1.18μM
DEX: IC50=52.70μM
TOPOIIβ Enzymatic Activity (%)
100
80
60
40
20
0
0.01
1
100
Concentration [μM]
MYR: IC50=2.07μM
DEX: IC50=34.43μM

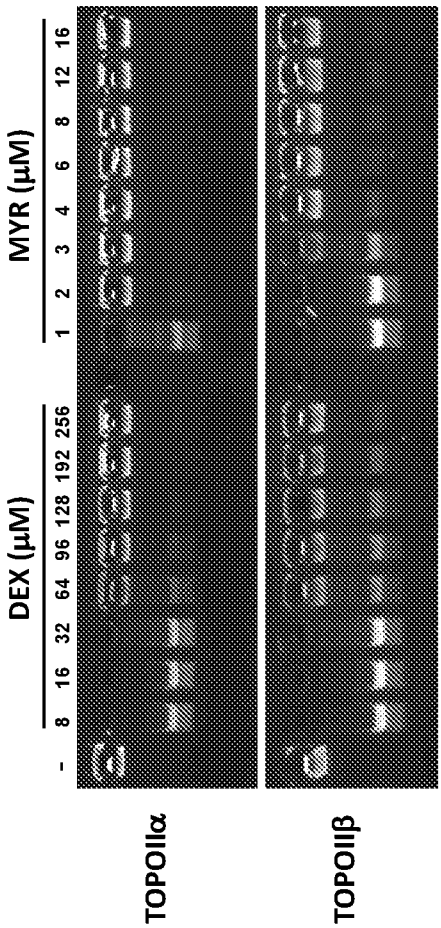

DEX (μM)
- 8 16 32 64 96 128 192 256
MYR (μM)
1 2 3 4 6 8 12 16
(a)
(b)
TOPOIIα
TOPOIIβ

Fig. 13

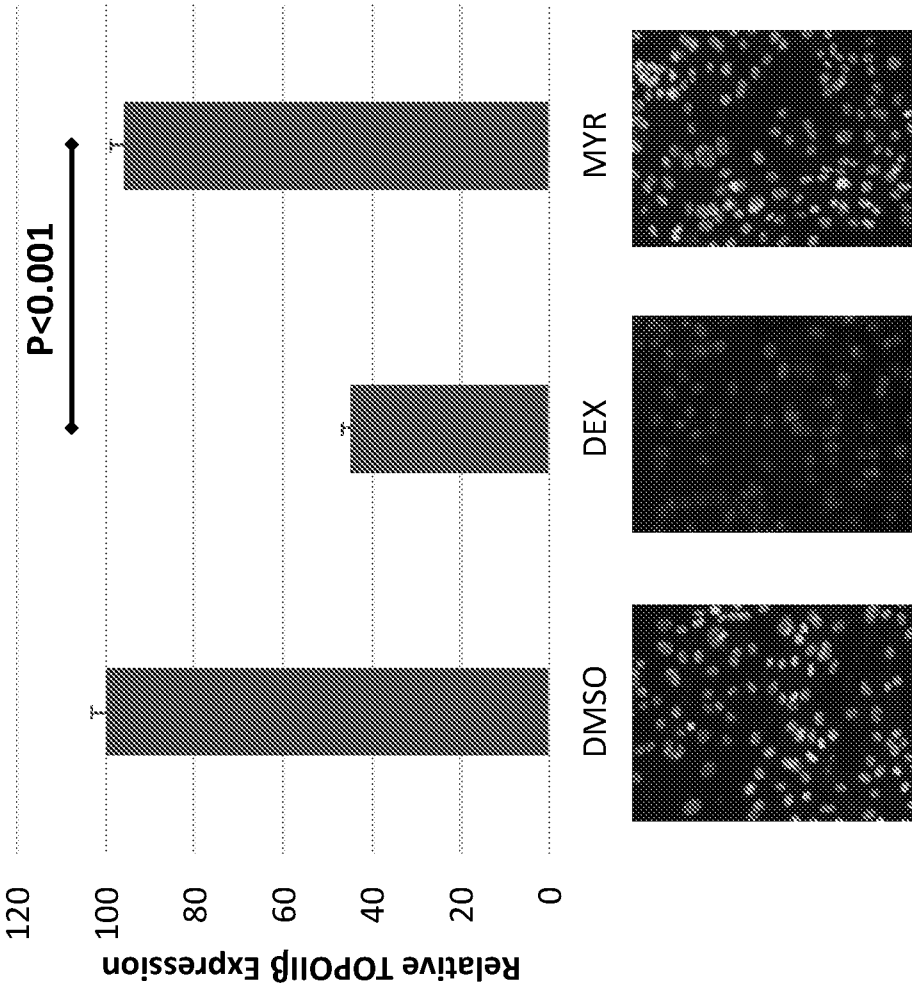

Relative TOPOIIβ Expression
120
100
80
60
40
20
0
P<0.001
DMSO
DEX
MYR

Fig. 14

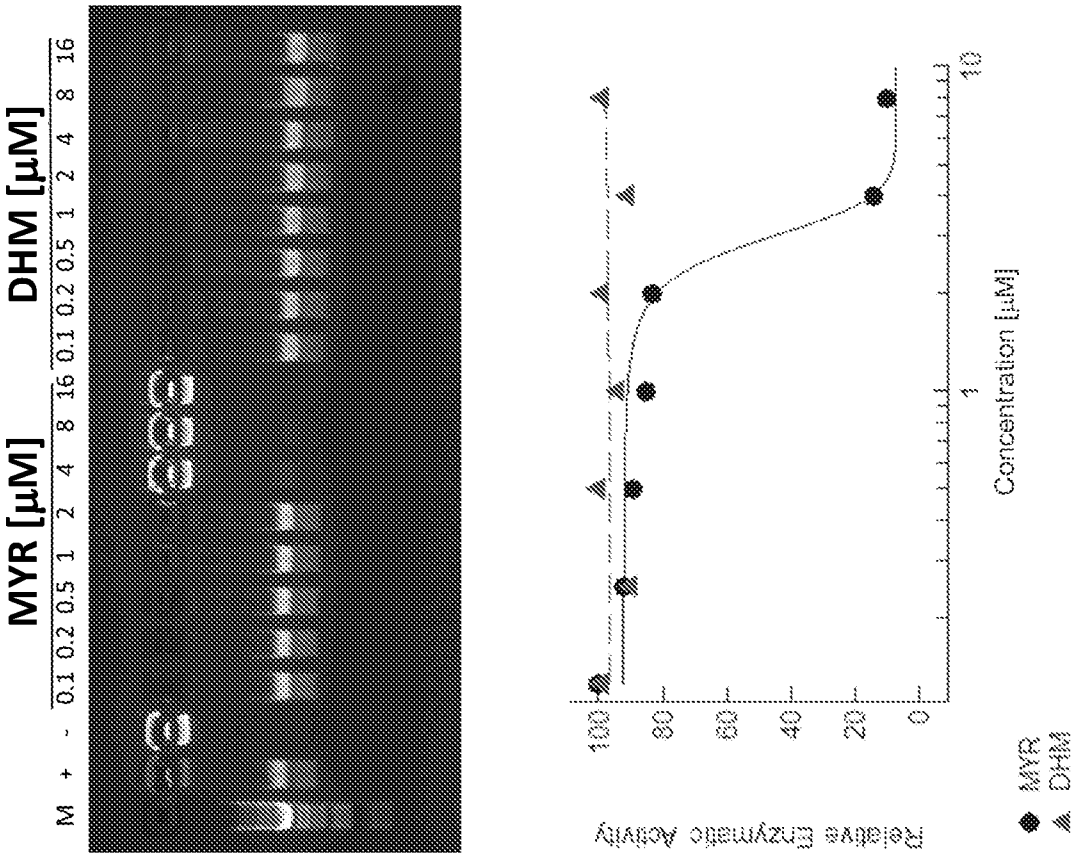

MYR [μM]
DHM [μM]
M + - 0.1 0.2 0.5 1 2 4 8 16 0.1 0.2 0.5 1 2 4 8 16
(a)
Relative Enzymatic Activity
100
80
60
40
20
0
1
10
Concentration [μM]
MYR
DHM
(b)

Fig. 15

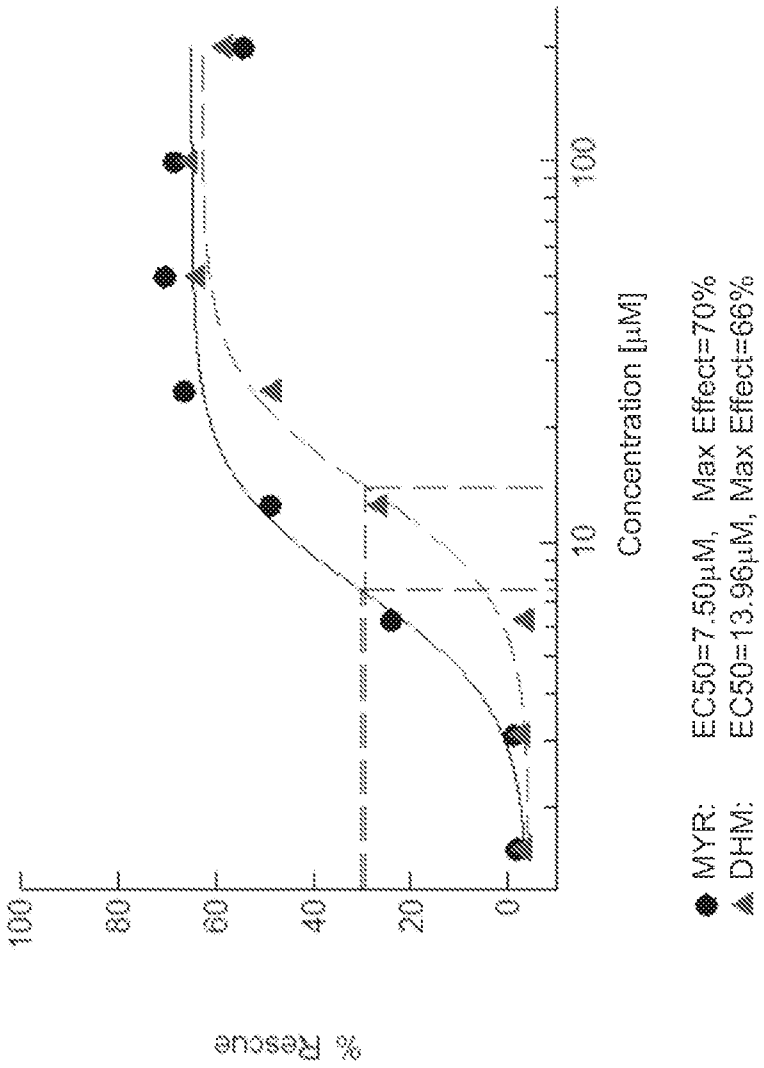

% Rescue
100
80
60
40
20
0
1
10
100
Concentration [µM]
MYR: EC50=7.50µM, Max Effect=70%
DHM: EC50=13.96µM, Max Effect=66%

Fig. 16

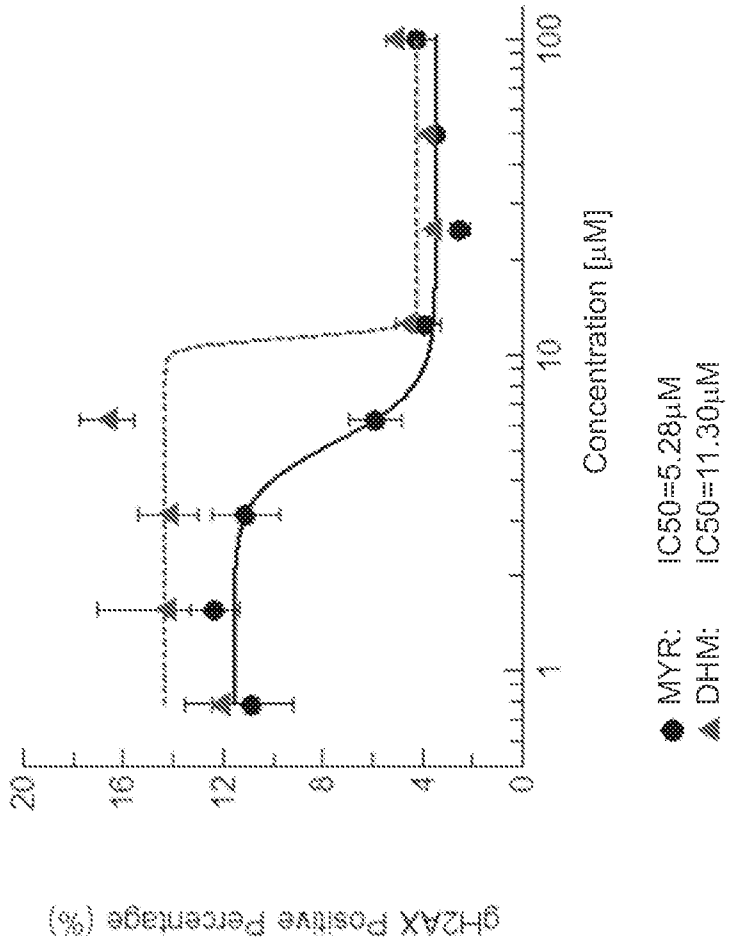

γH2AX Positive Percentage (%)
0
4
8
12
16
20
1
10
100
Concentration [μM]
MYR: IC50=5.28μM
DHM: IC50=11.30μM

Fig. 17

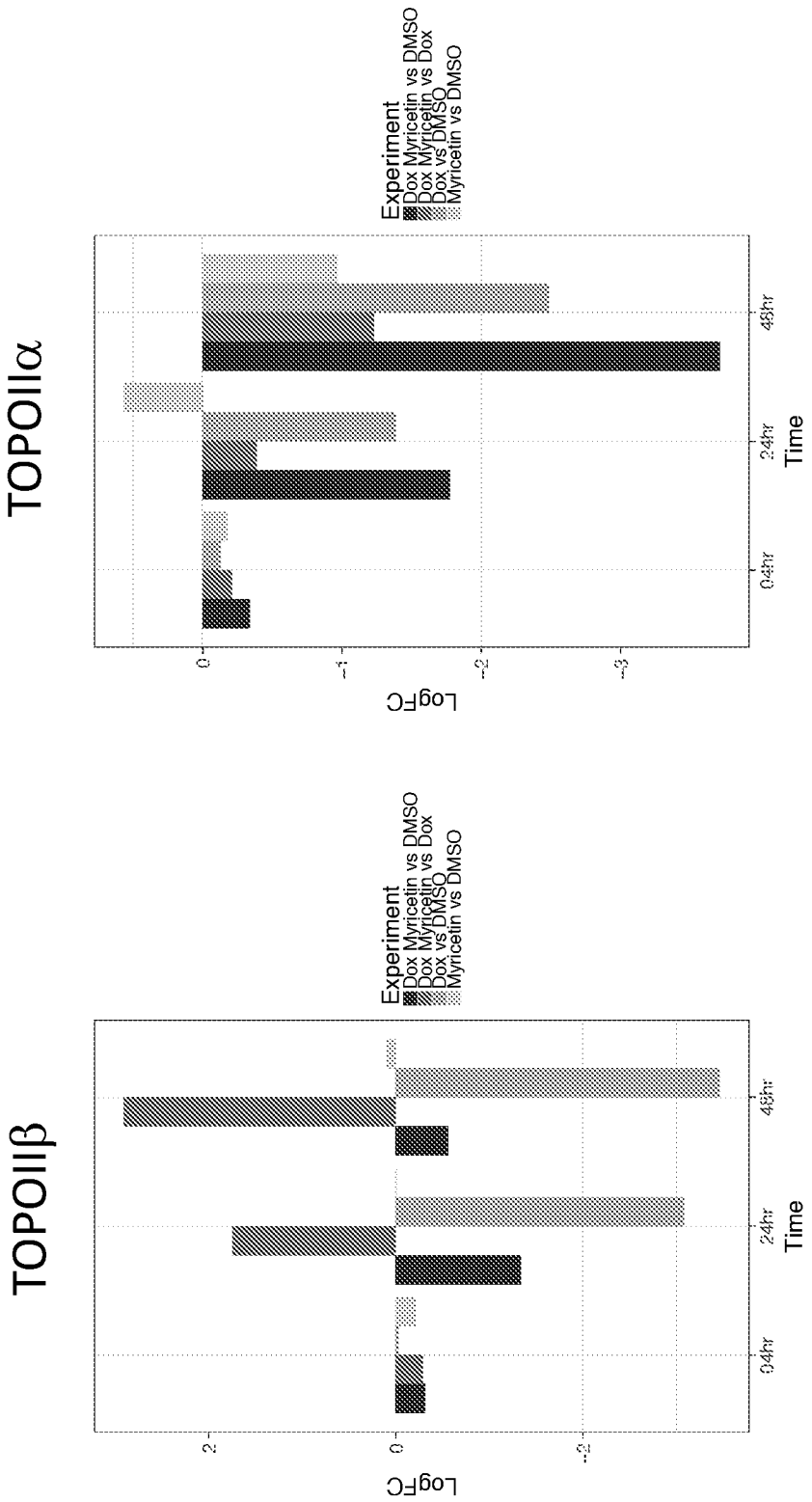

TOPOIIα
TOPOIIβ
Experiment
Dox Myricetin vs DMSO
Dox Myricetin vs Dox
Dox vs DMSO
Myricetin vs DMSO
LogFC
Time
0hr
24hr
48hr

Fig. 18

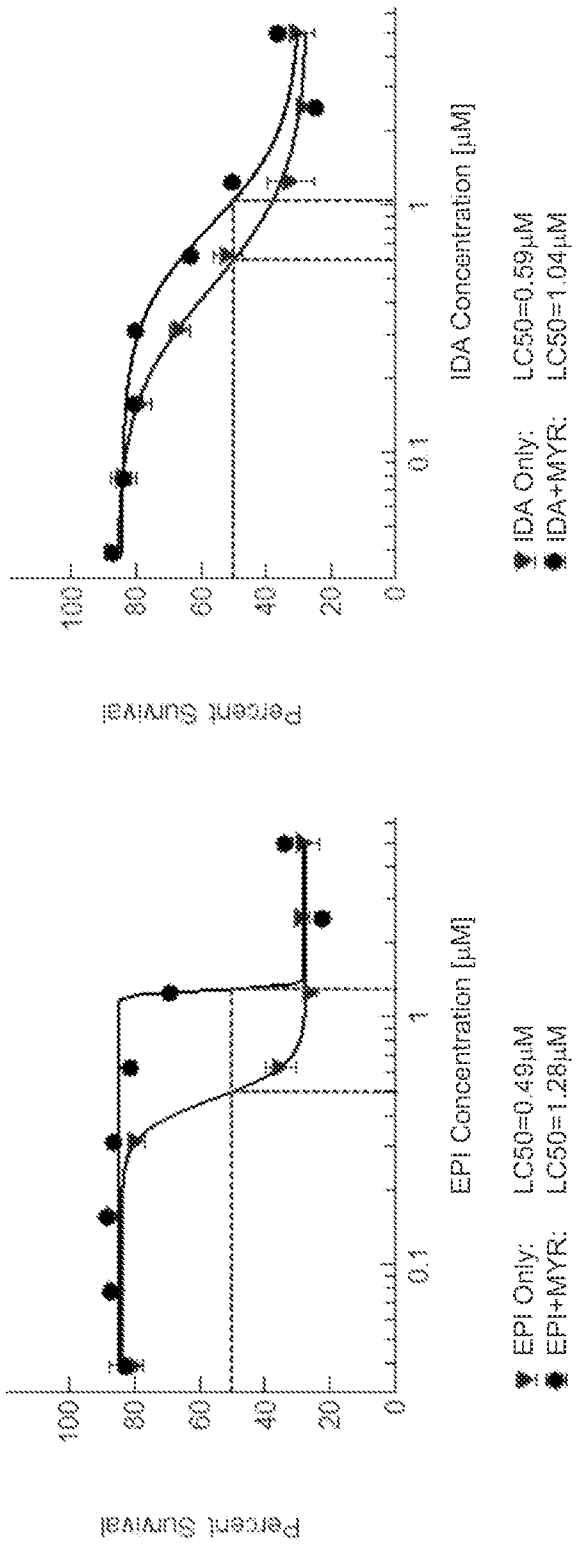

Percent Survival
100
80
60
40
20
0
0.1
1
EPI Concentration [µM]
EPI Only: LC50=0.49µM
EPI+MYR: LC50=1.28µM
Percent Survival
100
80
60
40
20
0
0.1
1
IDA Concentration [µM]
IDA Only: LC50=0.59µM
IDA+MYR: LC50=1.04µM

Fig. 19

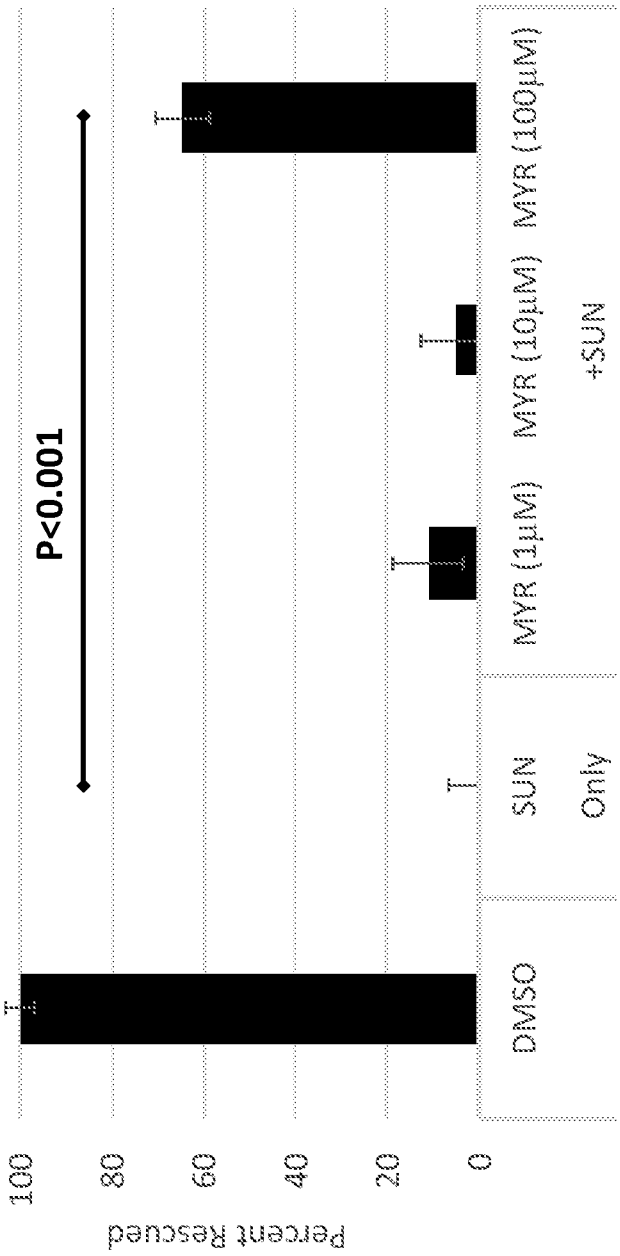

Percent Rescued
100
80
60
40
20
0
P<0.001
DMSO
SUN Only
MYR (1μM)
MYR (10μM)
MYR (100μM)
+SUN

Fig. 20

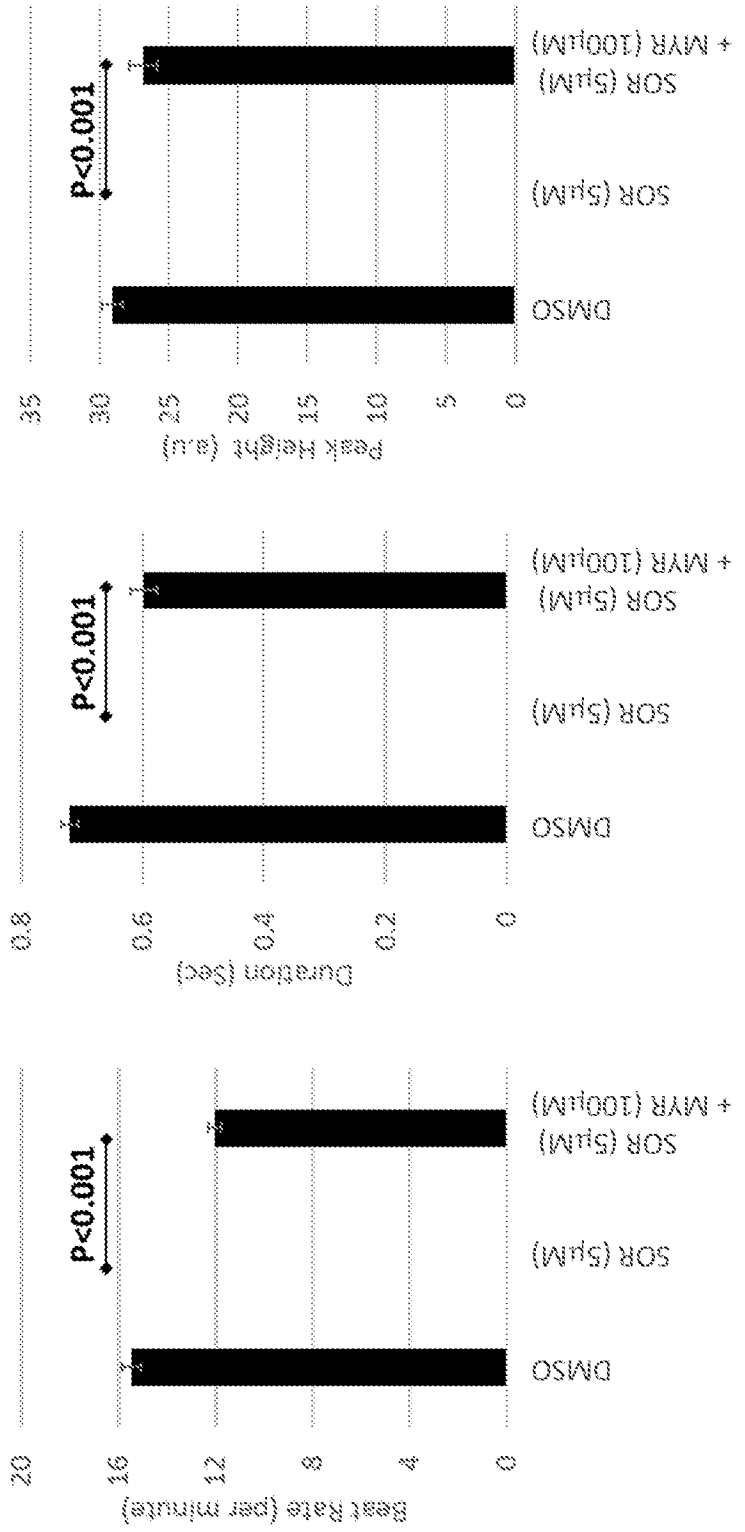

Peak Height (a.u.)
35
30
25
20
15
10
5
0
P<0.001
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)
Duration (Sec)
0.8
0.6
0.4
0.2
0
P<0.001
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)
Beat Rate (per minute)
20
16
12
8
4
0
P<0.001
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)

Fig. 21

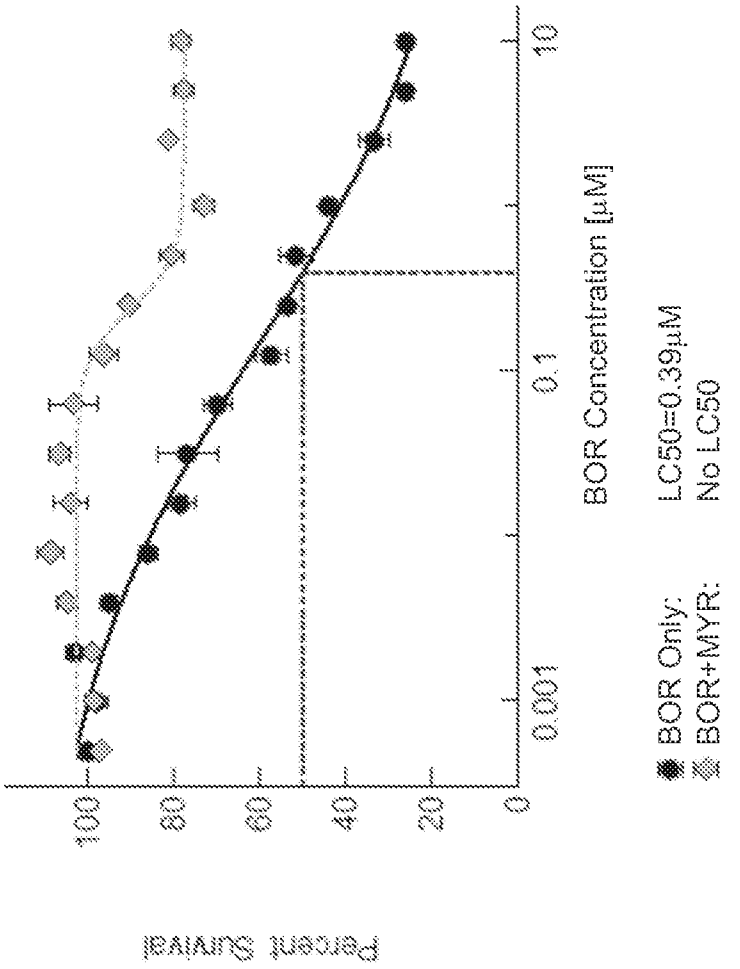

Percent Survival
100
80
60
40
20
0
0.001
0.1
10
BOR Concentration [μM]
BOR Only: LC50=0.39μM
BOR+MYR: No LC50

Fig. 22

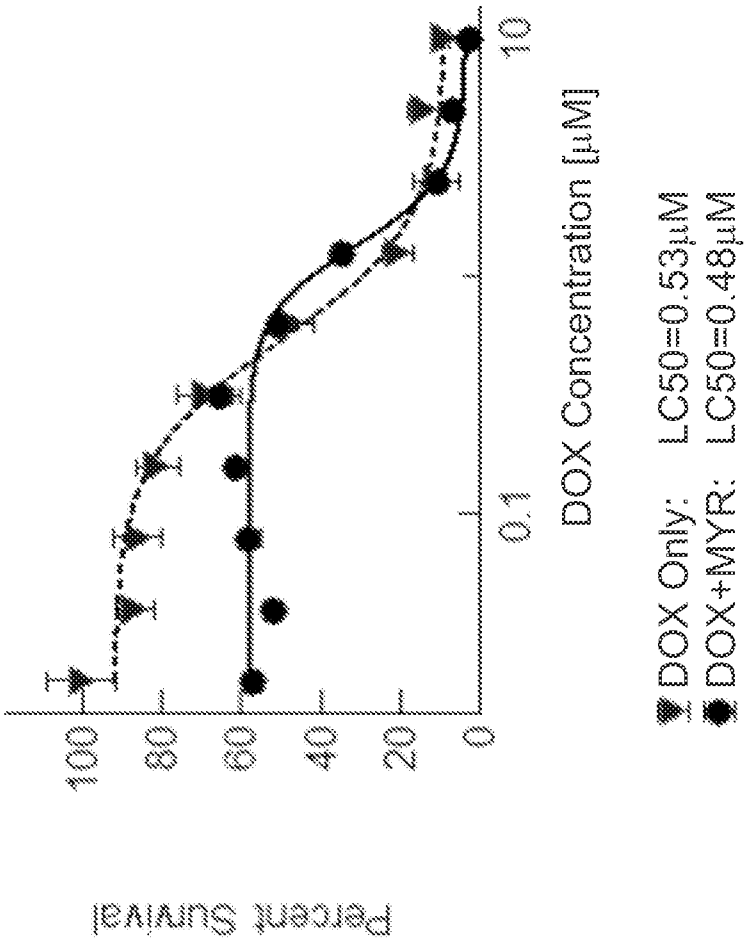

Percent Survival
100
80
60
40
20
0
0.1
1
10
DOX Concentration [μM]
DOX Only: LC50=0.53μM
DOX+MYR: LC50=0.48μM

Fig. 23

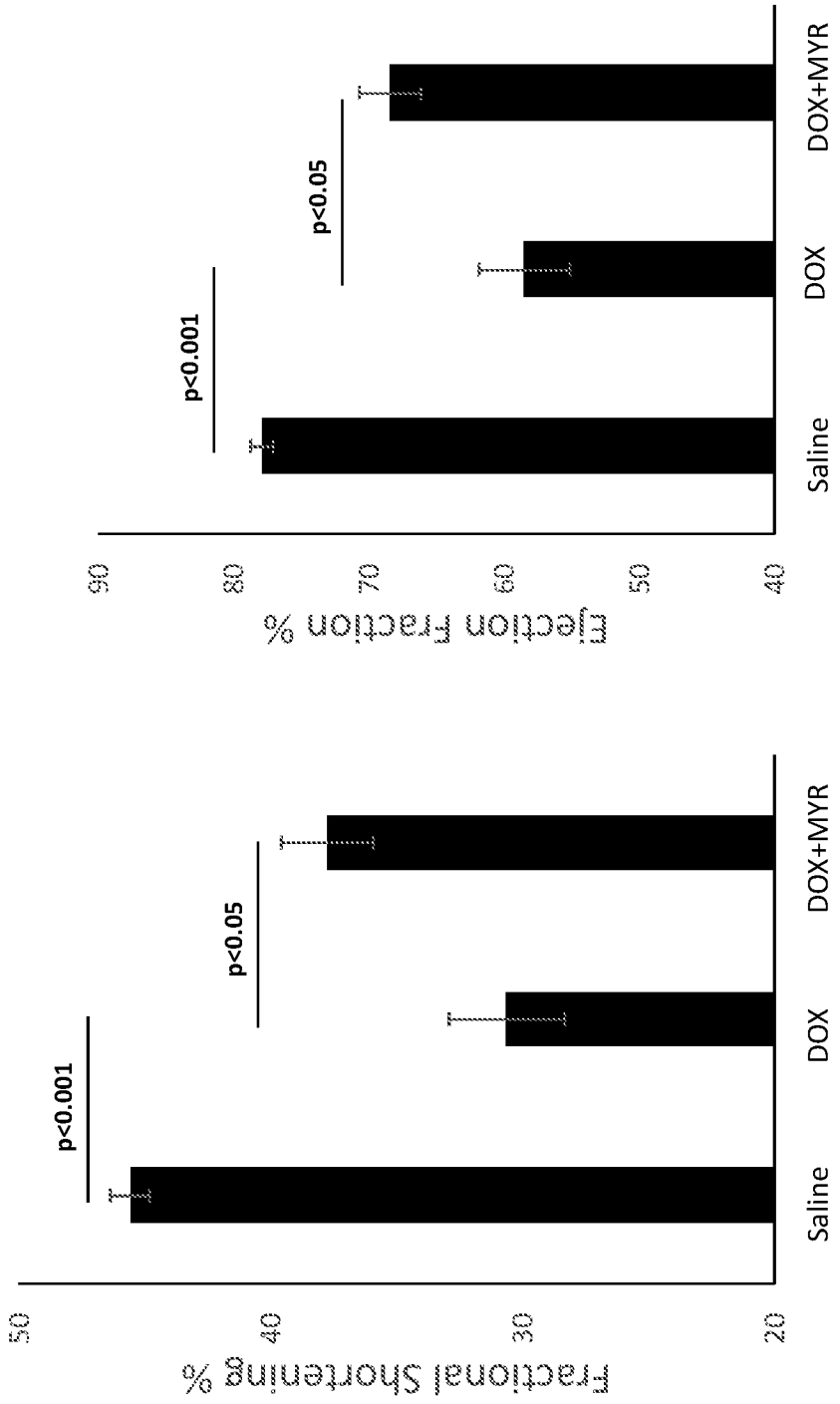

Fractional Shortening %
50
40
30
20
Saline
DOX
DOX+MYR
p<0.001
p<0.05
Ejection Fraction %
90
80
70
60
50
40
Saline
DOX
DOX+MYR
p<0.001
p<0.05

Fig. 24

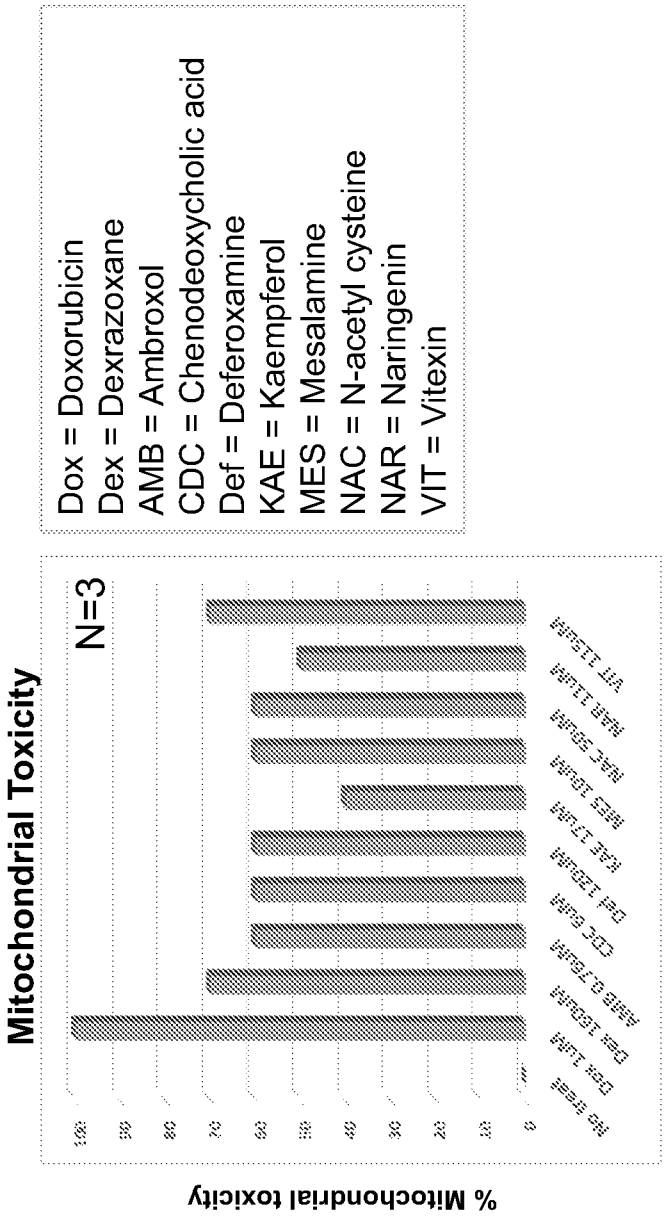

Dox = Doxorubicin
Dex = Dexrazoxane
AMB = Ambroxol
CDC = Chenodeoxycholic acid
Def = Deferoxamine
KAE = Kaempferol
MES = Mesalamine
NAC = N-acetyl cysteine
NAR = Naringenin
VIT = Vitexin
Mitochondrial Toxicity
N=3
% Mitochondrial toxicity
No treat
Dox 1uM
Dex 100uM
AMB 0.78uM
CDC 6uM
Def 120uM
KAE 17uM
MES 10uM
NAC 50uM
NAR 11uM
VIT 15uM

Fig. 25

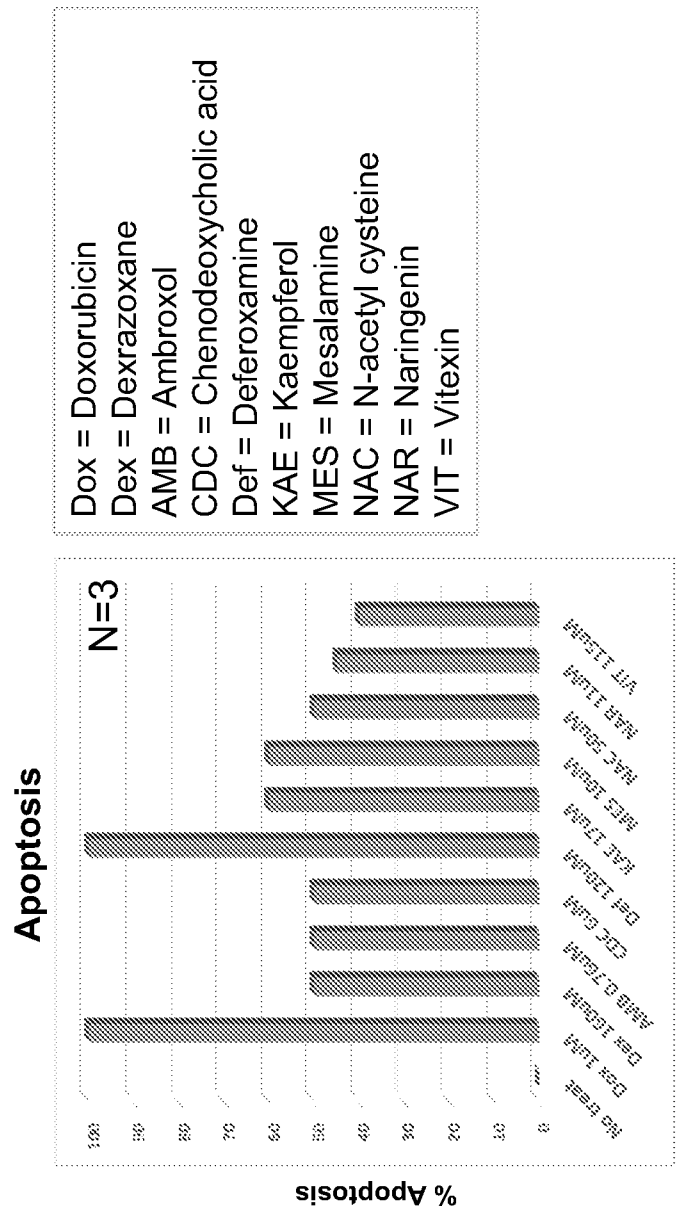

Dox = Doxorubicin
Dex = Dexrazoxane
AMB = Ambroxol
CDC = Chenodeoxycholic acid
Def = Deferoxamine
KAE = Kaempferol
MES = Mesalamine
NAC = N-acetyl cysteine
NAR = Naringenin
VIT = Vitexin
Apoptosis
N=3
% Apoptosis
No treat
Dox 1uM
Dex 100uM
AMB 0.375uM
CDC 0uM
Def 150uM
KAE 37.5uM
MES 150uM
NAC 250uM
NAR 31.3uM
VIT 15.6uM

Fig. 26

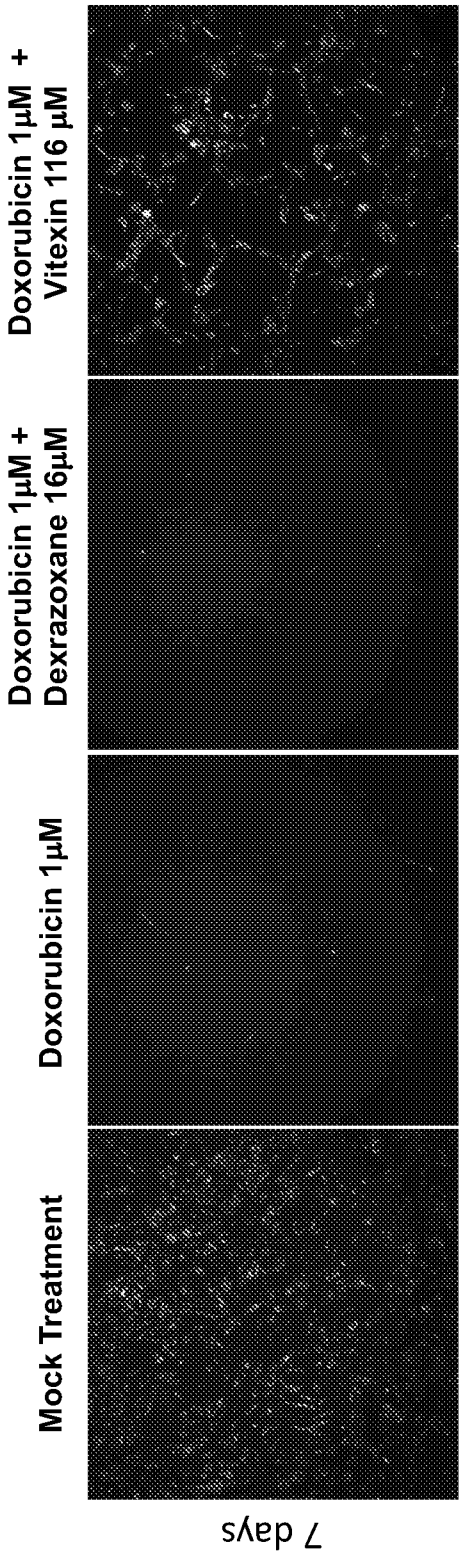


Fig. 27A Fig. 27B Fig. 27C Fig. 27D

Fig. 27

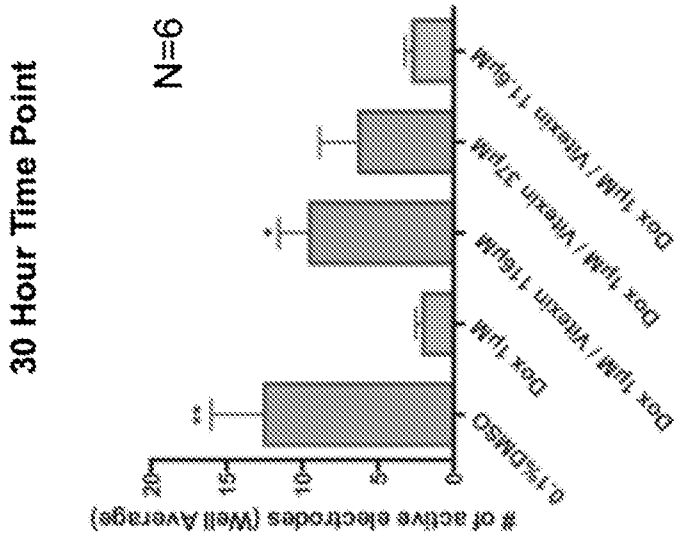


FIG. 28B

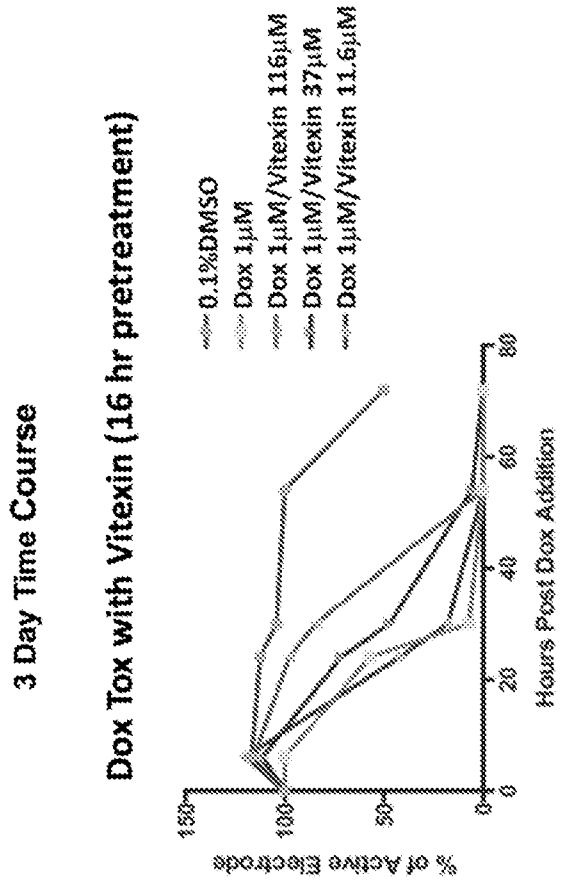


FIG. 28A

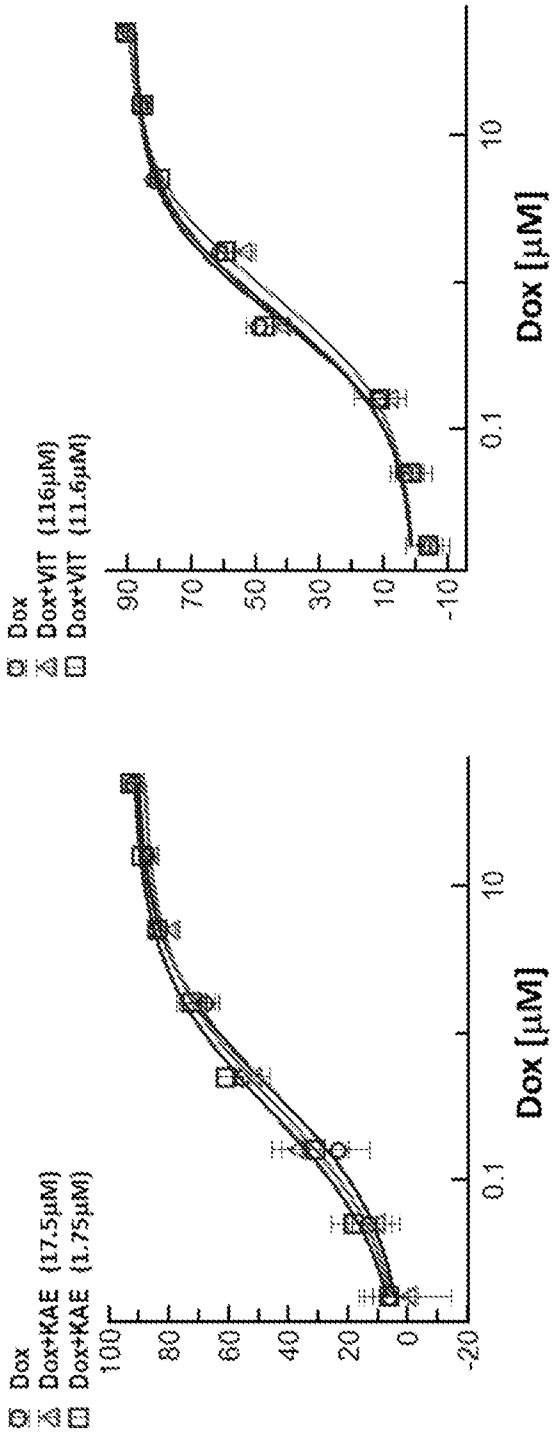


FIG. 29A

FIG. 29B

Dox = Doxorubicin; KAE = Kaempferol; VIT=Vitexin

Fig. 29

INTERNATIONAL SEARCH REPORT

International application No.:
PCT/US 17/16582

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/16, A61K 31/4704 (2017.01)
CPC - A61K 9/1635, A61K 9/205, A61K 9/2027

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)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2001/080855 A1 (Geron Corporation) 01 November 2001 (01.11.2001); Abstract, p4, p10, p17	1-7, 9-20
X	US 2013/0095124 A1 (Szathmary et al.) 18 April 2013 (18.04.2013); para[0004], para[0005], para[0018], para[0024], para[0073], para[0082]	1-5, 8
X	WO 2015/138186 A2 (Wake Forest University Health Sciences) 17 September 2015 (17.09.2015); p10, p23, p28, p44	1, 36, 115-116
X --- Y	Du et al. 'Quercetin greatly improved therapeutic index of doxorubicin against 4T1 breast cancer by its opposing effects on HIF-1a in tumor and normal cells', Cancer Chemotherapy and Pharmacology, 26 May 2009 (26.05.2009), Vol.65, page227-287; p277, p279, p284	38-49, 66-78, 80-87, 89-91,96-97, 108-112 --- 50-65, 79, 88, 92-95, 98-107
X	Sadzuka et al. 'Protective effect of flavonoids on doxorubicin-induced cardiotoxicity', Toxicology Letters, 20 May 1998 (20.05.1998), Vol.92, page1-7; p2, p5	113
X --- Y	Tiwari et al. 'Cardioprotective potential of myricetin in isoproterenol-induced myocardial infarction in wistar rats', Phytotherapy Research, 23 March 2009 (23.03.2009), Vol.23, page1361-1366; Abstract, p1361	114 --- 79, 88
Y	Lipshultz et al. 'Chronic Progressive Cardiac Dysfunction Years After Doxorubicin Therapy for Childhood Acute Lymphoblastic Leukemia, Pediatric Oncology, April 2005, Vol.23, page2629-2636; Title	50, 92

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
16 May 2017	25 MAY 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/16582

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Udhrain et al. 'Pegylated liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma', International Journal of Nanomedicine, September 2007, Vol.2, page 345-352; p351	51-52, 93-94
Y A	Menna et al. 'Cardiotoxicity of Antitumor Drugs', Chemical Research in Toxicology, 01 April 2008 (01.04.2008), Vol.21, page 978-989; p980, p984, p985	53-58, 60-63, 98-103, 105-107 76-77, 84-85
Y	Nowis et al. 'Cardiotoxicity of the Anticancer Therapeutic Agent Bortezomib', The American Journal of Pathology, 16 December 2010 (16.12.2010); Vol.176, page2658-2668; Title	59, 104
Y	Swain et al. 'Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer', Journal of Clinical Oncology, April 1997, Vol.15, page1318-1332; Title	64-65
Y A	Swain et al. 'Congestive heart failure in patients treated with doxorubicin', Cancer, 19 May 2003 (19.05.2003), Vol.97, page2869-2879; p2869	95 47, 89
A	Batra et al. 'Anti-cancer potential of flavonoids: recent trends and future Perspectives' , 3 Biotech, 12 February 2013 (12.02.2013), Vol.3, page439-459; p439, p442	1-7
A	Lopez-Lazaro et al. 'The dietary flavonoids myricetin and fisetin act as dual inhibitors of DNA topoisomerases I and II in cells', Mutation Research, 16 December 2009 (16.12.2009), Vol.696, page41-47; Title	15
A	Manuela et al. 'The Kinase Inhibitors Sunitinib (Sutent) and Sorafenib (Nexavar) Differentially Affect Reactivity of NK Cells Against Renal Cell Cancer', Blood, 2007, page1-3; Abstract	1-5
A	Bandele et al. 'Bioflavonoids as Poisons of Human Topoisomerase IIa and IIB', Biochemistry, 26 April 2007 (26.04.2007), Vol.46, page6097-6108; p6100, p6105	71-72, 78, 87
A	Cantero et al. 'Topoisomerase II inhibition and high yield of endoreduplication induced by the flavonoids luteolin and quercetin', Mutagenesis, 01 September 2006 (01.09.2006), Vol.21, page321-326; Abstract	86
A	US 2002/0147353 A1 (Vijgh et al.) 10 October 2002 (10.10.2002); entire document	1-20, 36, 38-116
A	US 2009/0274746 A1 (Gupta et el.) 05 November 2009 (05.11.2009); entire document	1-20, 36, 38-116
A	US 2015/0018294 A1 (DeBenedetti et al.) 15 January 2015 (15.01.2015); entire document	1-20, 36, 38-116

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/16582

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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.: 21-35, 37
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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.:

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/16582

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 must be paid.

Group I: claims 1-20, 36 and 115-116, directed to a pharmaceutical composition and kit comprising an anticancer agent and a protective agent according to Formula 1.

Group II: claims 38-114, directed to a method for preventing, reducing, or eliminating cardiotoxicity induced by an anticancer agent or biologic agent in a subject, the method comprising: administering to the subject an effective amount of at least one protective agent according to Formula 1 and the anticancer agent or biologic agent, thereby preventing, reducing, or eliminating the cardiotoxicity induced by the chemotherapy drug or biologic agent in the subject.

The inventions listed as Groups I-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:

Special technical features:

The special technical features of Group II is a method for preventing, reducing, or eliminating cardiotoxicity induced by an anticancer agent or biologic agent in a subject, the method comprising: administering to the subject an effective amount of at least one protective agent according to Formula 1 and the anticancer agent or biologic agent, thereby preventing, reducing, or eliminating the cardiotoxicity induced by the chemotherapy drug or biologic agent in the subject., not required in Group I

Common Technical Feature:

Groups I-II share the technical feature of compound of formula I. However, these shared technical features do not represent a contribution over prior art, because the shared technical feature is being anticipated by WO 2001/080855 A1 to Geron Corporation (hereinafter 'Geron'). Geron discloses a pharmaceutical composition (Abstract "The methods, compounds and compositions of the invention may be employed alone, or in combination with other pharmacologically active agents in the treatment of conditions or diseases mediated by telomerase activity, such as in the treatment of cancer") comprising an anticancer agent (p17, ln24-25 "In addition, it will be appreciated that therapeutic benefits for treatment of cancer can be realized by combining a telomerase inhibitor of the invention with other anti-cancer agents, including other inhibitors of telomerase such as described in U.S. Patent Nos. 5,656,638, 5,760,062, 5,767,278, 5,770,613 and 5,863,936") and a protective agent according to Formula 1, wherein: X1 is O; R1, R2, and R3 are hydroxyl; R9 and R10 are H; R4,R7, and R8 are hydroxyl; W1 is O (p4, ln1-4 "In a first aspect, the present invention is based on the surprising finding that certain known 3 -substituted flavone compounds and 2 -substituted isoflavone compounds, as well as the new flavone and isoflavone derivatives disclosed herein, are effective in the inhibition of telomerase activity in cells", p10, "Table 1 Naturally occurring flavones", "Myricetin", see document entitled 'Anti-cancer potential of flavonoids: recent trends and future Perspectives' by Batra et al. (hereinafter 'Batra'), p439, Abstract "A positive correlation between flavonoids-rich diet (from vegetables and fruits) and lower risk of colon, prostate and breast cancers lead to a question that whether flavonoids mediate the protective effects as chemopreventive agents or can interact with different genes and proteins to play role in chemotherapy", p442, right col 2nd para "A huge number of epidemiological studies have been conducted to prove the protective effect of flavonoids against cancer").

As the shared technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups. Therefore, Groups I-II lack unity under PCT Rule 13.

Note: claims 21-35 and 37 are determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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(54)发明名称

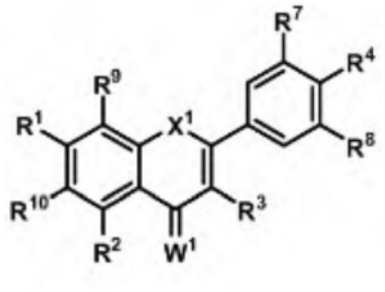
用于抵抗化学疗法诱导的心脏毒性的药物组合物和方法

(57)摘要

本公开提供了用于降低或消除心脏毒性尤其是由癌症治疗剂或其它疗法诱导的心脏毒性的方法和药物组合物。在一些情况下，所述方法和所述组合物预防或降低由蒽环类治疗引起的心脏毒性。本文提供的所述方法通常包括与施用抗癌药物或其它治疗联合施用保护剂，诸如杨梅素、五羟黄酮、洋槐黄素、ficetin、牡荆素、槲皮素、二氢洋槐黄素、山奈素、7,3',4',5'-四羟基黄酮和杨梅苷。这些方法可包括与右雷佐生组合施用保护剂。本文提供的所述组合物包括保护剂与不同保护剂或保护剂与癌症治疗剂(例如，蒽环类药物)的共同制剂。

CN 108697646 A

1.一种药物组合物,包含抗癌剂和根据式1的保护剂,



式 1

其中:

X^1为CR^5R^6、NR^5、O、S、C＝O或C＝S;

R^1、R^2、R^3、R^5、R^6、R^9和R^{10}各自独立地为烷基、烯基、炔基、烷氧基、酰基、酰氧基、羧酸、酯、胺、酰胺、碳酸根、氨基甲酸根、硝基、硫醚、硫酯、环烷基、杂烷基、杂环基、芳基或杂芳基,其中任一者是取代或未取代的,卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;

R^4、R^7和R^8为烷氧基、羟基或H;

W^1为O或S;或

其盐。

2.如权利要求1所述的药物组合物,其中

X^1为O或S;

R^1、R^2、R^3、R^9和R^{10}各自独立地为烷氧基、环烷基、杂环基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;

并且

R^4、R^7和R^8各自为烷氧基、羟基或H。

3.如权利要求2所述的药物组合物,其中

X^1为O;

R^1、R^2、R^3、R^9和R^{10}各自独立地为烷氧基、环烷基、杂环基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;

并且

R^4、R^7和R^8各自为烷氧基、羟基或H。

4.如权利要求3所述的药物组合物,其中

X^1为O;

R^1、R^2和R^3各自独立地为羟基或H;

R^9和R^{10}各自为环烷基、杂环基或H;

R^4为羟基;并且

R^7和R^8为甲氧基、羟基或H。

5.如权利要求4所述的药物组合物,其中

X^1为O;

R^1为羟基;

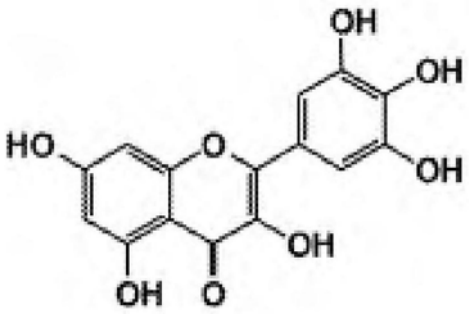
R^2和R^3各自独立地为羟基或H；

R^9和R^{10}为H；

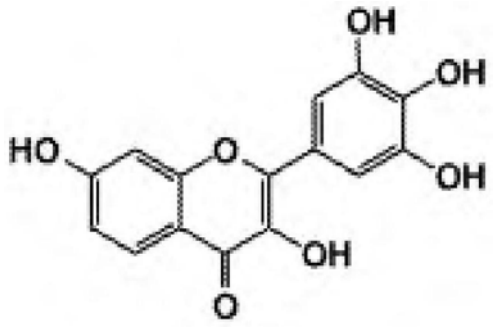
R^4为羟基；并且

R^7和R^8为羟基或H。

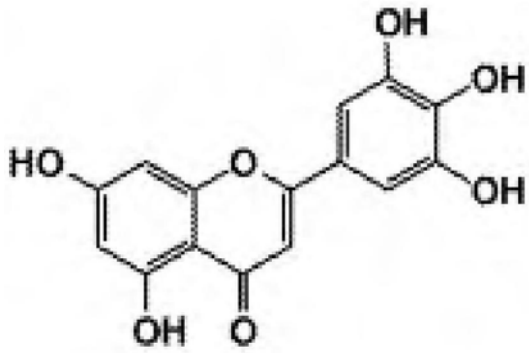
6.如权利要求5所述的药物组合物，其中所述保护剂是杨梅素并且是根据下式的化合物：



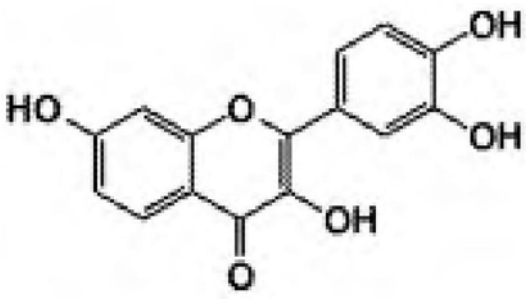
7.如权利要求5所述的药物组合物，其中所述保护剂是洋槐黄素并且是根据下式的化合物：



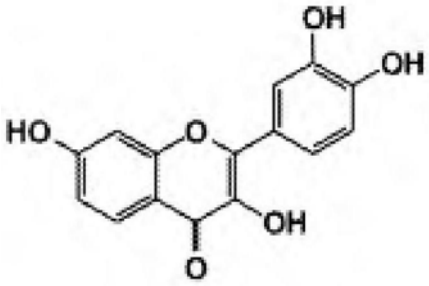
8.如权利要求5所述的药物组合物，其中所述保护剂是五羟黄酮并且是根据下式的化合物：



9.如权利要求5所述的药物组合物，其中所述保护剂是ficetin并且是根据下式的化合物：



或者



10.如权利要求1所述的药物组合物，其中所述保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷。

11.如权利要求1所述的药物组合物，其中所述保护剂是山奈素或槲皮素。

12.如权利要求1-11中任一项所述的药物组合物，其中所述药物组合物是片剂。

13.如权利要求1-11中任一项所述的药物组合物，其中所述药物组合物是液体组合物。

14.如权利要求1-11中任一项所述的药物组合物，其中所述药物组合物是胶囊、凝胶胶囊或脂质体。

15.如权利要求1所述的药物组合物，其中所述保护剂是拓扑异构酶IIα和β抑制剂。

16.如权利要求1所述的药物组合物，其中所述药物组合物包含至少10mg的至少一种保护剂。

17.如权利要求1所述的药物组合物，其中所述药物组合物包含至少50mg的至少一种保护剂。

18.如权利要求1所述的药物组合物，其中所述药物组合物包含至少100mg的所述保护剂。

19.如权利要求1所述的药物组合物，其中所述药物组合物包含至少200mg的至少一种保护剂。

20.如权利要求1所述的药物组合物，其中所述药物组合物包含在0.1mg至50mg之间的至少两种式1的保护剂。

21.如权利要求1-20中任一项所述的药物组合物，其中所述抗癌剂是蒽环类或其盐。

22.如权利要求21所述的药物组合物，其中所述蒽环类是柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。

23.如权利要求22所述的药物组合物，其中所述蒽环类是多柔比星。

24.如权利要求22所述的药物组合物，其中所述蒽环类是表柔比星。

25.如权利要求1-20中任一项所述的药物组合物，其中所述抗癌剂是蛋白激酶抑制剂。

26.如权利要求25所述的药物组合物，其中所述蛋白激酶抑制剂是阿法替尼、阿西替

尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。

27.如权利要求1-20中任一项所述的药物组合物，其中所述抗癌剂是硼替佐米。

28.如权利要求25所述的药物组合物，其中所述蛋白激酶抑制剂是酪氨酸激酶抑制剂。

29.如权利要求28所述的药物组合物，其中所述酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。

30.如权利要求29所述的药物组合物，其中所述酪氨酸激酶抑制剂是索拉非尼。

31.如权利要求29所述的药物组合物，其中所述酪氨酸激酶抑制剂是舒尼替尼。

32.如权利要求1-20中任一项所述的药物组合物，其中所述抗癌剂是生物剂。

33.如权利要求32所述的药物组合物，其中所述生物剂是抗体。

34.如权利要求33所述的药物组合物，其中所述抗体是曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。

35.如权利要求34所述的药物组合物，其中所述抗体是曲妥珠单抗。

36.如权利要求1所述的药物组合物，还包含第二保护剂，其中所述第二保护剂是右雷佐生。

37.一种治疗罹患癌症的受试者的方法，包括向所述受试者施用权利要求1-36中任一项所述的药物组合物。

38.一种预防、减轻或消除受试者中由抗癌剂或生物剂诱导的心脏毒性的方法，所述方法包括：向所述受试者施用有效量的至少一种根据式1的保护剂和所述抗癌剂或所述生物剂，从而预防、降低或消除由化学疗法药物或生物剂在所述受试者中引起的所述心脏毒性。

39.如权利要求38所述的方法，其中在施用所述至少一种根据式1的保护剂之前将所述抗癌剂或所述生物剂施用于所述受试者。

40.如权利要求38所述的方法，其中在施用所述至少一种根据式1的保护剂之后将所述抗癌剂或所述生物剂施用于所述受试者。

41.如权利要求38所述的方法，其中向所述受试者同时施用所述抗癌剂或所述生物剂和所述至少一种根据式1的保护剂。

42.如权利要求38所述的方法，其中所述抗癌剂或所述生物剂和所述至少一种保护剂被配制在一种液体组合物中。

43.如权利要求38所述的方法，其中所述抗癌剂或所述生物剂和所述至少一种保护剂被配制在片剂中。

44.如权利要求38所述的方法，其中所述至少一种保护剂经口施用。

45.如权利要求38所述的方法，其中所述至少一种保护剂经静脉内施用。

46.如权利要求38所述的方法，其中所述受试者罹患癌症。

47.如权利要求46所述的方法，其中所述受试者是人受试者。

48.如权利要求46所述的方法，其中所述癌症是膀胱癌、骨癌、脑肿瘤、乳腺癌、食道癌、结直肠癌、白血病、肝癌、肺癌、淋巴瘤、骨髓瘤、卵巢癌、前列腺癌、肉瘤、胃癌或甲状腺癌。

49.如权利要求48所述的方法，其中所述癌症是乳腺癌。

50.如权利要求48所述的方法，其中所述癌症是白血病。

51.如权利要求48所述的方法，其中所述癌症是肉瘤。

52.如权利要求51所述的方法，其中所述肉瘤是卡波西氏肉瘤。

53.如权利要求38所述的方法，其中所述抗癌剂是蛋白激酶抑制剂。

54.如权利要求53所述的方法，其中所述蛋白激酶抑制剂是阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。

55.如权利要求53所述的方法，其中所述蛋白激酶抑制剂是酪氨酸激酶抑制剂。

56.如权利要求55所述的方法，其中所述酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。

57.如权利要求56所述的方法，其中所述酪氨酸激酶抑制剂是索拉非尼。

58.如权利要求56所述的方法，其中所述酪氨酸激酶抑制剂是舒尼替尼。

59.如权利要求38所述的方法，其中所述抗癌剂是蛋白酶体抑制剂(例如，硼替佐米)。

60.如权利要求38所述的方法，其中所述抗癌剂是生物剂。

61.如权利要求60所述的方法，其中所述生物剂是抗体。

62.如权利要求61所述的方法，其中所述抗体是曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。

63.如权利要求62所述的方法，其中所述抗体是曲妥珠单抗。

64.如权利要求38所述的方法，还包括施用第二保护剂。

65.如权利要求64所述的方法，其中所述第二保护剂是右雷佐生。

66.如权利要求38所述的方法，其中在施用所述抗癌剂或所述生物剂之前至少24小时将所述至少一种根据式1的保护剂施用于所述受试者。

67.如权利要求38所述的方法，其中在施用所述抗癌剂或所述生物剂之前至少1、2、3、4、5、6、6、7、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或48小时将所述至少一种根据式1的保护剂施用于所述受试者。

68.如权利要求38所述的方法，其中在施用所述抗癌剂或所述生物剂的同时将所述至少一种根据式1的保护剂施用于所述受试者。

69.如权利要求38所述的方法，其中在施用所述抗癌剂或所述生物剂之后至少1、2、3、4、5、6、6、7、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或48小时将所述至少一种根据式1的保护剂施用于所述受试者。

70.如权利要求38所述的方法，其中将所述至少一种根据式1的保护剂以至少1:5单位的所述抗癌剂与所述保护剂的摩尔比施用于所述受试者。

71.如权利要求38所述的方法，其中所述至少一种保护剂是拓扑异构酶IIα或β抑制剂。

72.如权利要求71所述的方法，其中所述至少一种保护剂是拓扑异构酶IIα和拓扑异构酶β抑制剂。

73.如权利要求38所述的方法，其中所述抗癌剂是蒽环类或其盐。

74.如权利要求73所述的方法，其中所述蒽环类是柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。

75.如权利要求74所述的方法，其中所述蒽环类是多柔比星。

76.如权利要求74所述的方法，其中所述蒽环类是表柔比星。

77.如权利要求74所述的方法，其中所述蒽环类是伊达比星。

78.如权利要求74所述的方法，其中所述至少一种保护剂是拓扑异构酶IIα或β抑制剂。

79.如权利要求38所述的方法，其中所述至少一种保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷。

80.一种治疗癌症和预防由一种或多种抗癌剂引起的心脏毒性的方法，所述方法包括：

(a)向罹患癌症的受试者施用化学疗法药物或生物剂并且接收已知在所述受试者中引起心脏毒性的所述化学疗法药物或所述生物剂;以及

(b)向所述受试者施用有效量的根据式1的保护剂或其药学上可接受的盐，其中所述保护剂预防、减轻或消除所述受试者中的心脏毒性。

81.如权利要求80所述的方法，其中所述化学疗法药物是蒽环类或其盐。

82.如权利要求81所述的方法，其中所述蒽环类是柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。

83.如权利要求82所述的方法，其中所述蒽环类是多柔比星。

84.如权利要求82所述的方法，其中所述蒽环类是表柔比星。

85.如权利要求82所述的方法，其中所述蒽环类是伊达比星。

86.如权利要求80所述的方法，其中所述保护剂是拓扑异构酶II抑制剂。

87.如权利要求86所述的方法，其中所述保护剂是拓扑异构酶IIα或β抑制剂。

88.如权利要求80所述的方法，其中所述保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷。

89.如权利要求80所述的方法，其中所述受试者是人受试者。

90.如权利要求80所述的方法，其中所述癌症是膀胱癌、骨癌、脑肿瘤、乳腺癌、食道癌、结直肠癌、白血病、肝癌、肺癌、淋巴瘤、骨髓瘤、卵巢癌、前列腺癌、肉瘤、胃癌或甲状腺癌。

91.如权利要求90所述的方法，其中所述癌症是乳腺癌。

92.如权利要求90所述的方法，其中所述癌症是白血病。

93.如权利要求90所述的方法，其中所述癌症是肉瘤。

94.如权利要求93所述的方法，其中所述肉瘤是卡波西氏肉瘤。

95.如权利要求80所述的方法，其中所述人受试者具有心脏病状或具有患心脏病状的病史。

96.如权利要求80所述的方法，其中所述心脏毒性包括心脏组织损伤、电生理异常、线

粒体毒性、细胞凋亡和氧化应激。

97.如权利要求96所述的方法，其中所述心脏毒性是心脏组织损伤。

98.如权利要求80所述的方法，其中所述化学疗法药物是蛋白激酶抑制剂。

99.如权利要求98所述的方法，其中所述蛋白激酶抑制剂是阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。

100.如权利要求80所述的方法，其中所述蛋白激酶抑制剂是酪氨酸激酶抑制剂。

101.如权利要求100所述的方法，其中所述酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。

102.如权利要求101所述的方法，其中所述酪氨酸激酶抑制剂是索拉非尼。

103.如权利要求101所述的方法，其中所述酪氨酸激酶抑制剂是舒尼替尼。

104.如权利要求80所述的方法，其中所述抗癌剂是蛋白酶体抑制剂(例如，硼替佐米)。

105.如权利要求80所述的方法，其中所述生物剂是抗体。

106.如权利要求105所述的方法，其中所述抗体是曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。

107.如权利要求106所述的方法，其中所述抗体是曲妥珠单抗。

108.如权利要求80所述的方法，其中在施用所述保护剂之后所述受试者具有减小的QTc间期。

109.如权利要求80所述的方法，其中向所述受试者同时施用所述化学疗法药物和所述式1的保护剂。

110.如权利要求80所述的方法，其中向所述受试者依次施用所述化学疗法药物和所述式1的保护剂。

111.如权利要求110所述的方法，其中在施用所述化学疗法药物之前将所述保护剂施用于所述受试者。

112.如权利要求110所述的方法，其中在施用所述化学疗法药物之后将所述保护剂施用于所述受试者。

113.一种治疗或预防受试者器官损伤的方法，包括：向所述受试者施用有效量的式1的保护剂，所述受试者接收包含蒽环类、酪氨酸激酶抑制剂或曲妥珠单抗中的至少一者的抗癌剂。

114.一种预防受试者器官损伤或心力衰竭的方法，包括：向受试者施用有效量的保护剂，所述保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷。

115.一种药盒，包含：

a.保护剂，所述保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',

5'-四羟基黄酮和杨梅苷;以及

b.化学疗法药物或生物剂,

其中所述化学疗法药物或所述生物剂引起心脏毒性。

116.如权利要求115所述的药盒,还包含右雷佐生。

用于抵抗化学疗法诱导的心脏毒性的药物组合物和方法

[0001] 相关申请的交叉引用

[0002] 本申请要求于2016年2月4日提交的美国临时申请序列号62/291,480以及于2016年6月9日提交的美国临时申请序列号62/348,102的优先权,它们中的每一者据此全文以引用方式并入本文以用于所有目的。

背景技术

[0003] 心脏毒性和充血性心力衰竭是肿瘤疗法的严重副作用,最显著的是那些包含蒽环类的肿瘤疗法,每年有超过一百万的癌症患者和半数儿童癌症患者施用蒽环类。在用靶向蛋白激酶的蛋白激酶抑制剂和基于抗体的生物剂治疗的患者中也观察到不良的心脏副作用。通过限制蒽环类的最大剂量以及通过改变其施用方案已实现了心力衰竭率的一定降低,但所有这些措施严重限制了这些抗癌剂的治疗潜力。癌症药物的心脏毒性也可使那些先前存在心脏病状的患者无法接受治疗。

[0004] 蒽环类通常是一类具有蒽结构核心的化合物。它们通常是高效的化疗剂并因此用于治疗许多癌症,包括白血病、淋巴瘤、乳腺癌、子宫癌、卵巢癌、膀胱癌和肺癌,并且经常用于儿童癌症治疗方案中。一些蒽环类药物包括多柔比星、柔红霉素、伊达比星和表柔比星。虽然确切的机制还未能得到验证,但据报道蒽环类通过抑制DNA和RNA合成、通过氧化还原循环促进自由基形成、通过铁促进超氧化物转化为羟基自由基、抑制拓扑异构酶(例如,拓扑异构酶IIα和/或IIβ)以及从开放染色体区域驱逐组蛋白而起作用。

[0005] 使用蒽环类的常见副作用与心脏毒性有关,其是剂量依赖性的并且也可由累积暴露导致。在一些情况下,心脏毒性可由蒽环类的代谢期间通过氧化还原循环形成的有毒活性氧所致以及由拓扑异构酶II的抑制引起的双链DNA断裂形成所致。活性氧(ROS)可激活凋亡通路,导致癌细胞和正常细胞死亡。心肌细胞可对氧化应激敏感。心脏线粒体可易于受到蒽环类和蒽环类铁络合物的损伤,这两种化合物对线粒体内膜中以高浓度存在的二价阴离子磷脂心磷脂具有高亲和力。

[0006] 包括小分子和生物抑制剂的一些蛋白激酶抑制剂也可导致心脏毒性。蛋白激酶抑制剂是抑制蛋白激酶活性的一大类化合物,并且可用于癌症治疗。酪氨酸激酶调节多种细胞功能,包括细胞生长(例如,表皮生长因子(“EGFR”)),并且失调可导致某些形式的癌症。此类酪氨酸蛋白激酶的抑制可通过使用结合给定蛋白激酶的ATP袋的小分子来完成,从而阻止其催化靶蛋白的磷酸化。小分子可通过以下方式引起心脏毒性:(1)选择性地抑制也在心脏细胞中发挥作用的激酶(例如,靶向副作用);(2)靶向同一通路中的多种激酶(例如,影响非靶向激酶);以及(3)抑制在心脏功能中起作用的非激酶靶标;小分子也可通过不同机制引起心脏毒性。先前已经报道了TKI抑制剂诸如甲磺酸伊马替尼(Gleevec®)、尼罗替尼(Tasigna®)、索拉非尼(Nexavar®)、舒尼替尼(Sutent®)和达沙替尼(Sprycel®)的心脏毒性(Chu等人,Lancet(2007)370:2011-2019;Xu等人,Hematol Rev.(2009)Marl;1(1):e4;Kerketla等人,Nature Medicine(2006)12:908-916)。

[0007] 蛋白激酶活性也可受到生物药物诸如针对受体蛋白激酶的单克隆抗体的抑制。这

些治疗剂可通过防止受体蛋白激酶激活而发挥效力并且通常能够高特异性结合细胞表面抗原。几种单克隆抗体靶向在心脏功能中发挥重要作用的受体蛋白激酶，并因此可导致心脏毒性。曲妥珠单抗和贝伐单抗是可引起心脏毒性(例如，由心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激导致的心力衰竭)的单克隆抗体的实例。已知蛋白酶体抑制剂化学疗法化合物(例如，硼替佐米)也与心脏毒性和心力衰竭有关。

[0008] 目前，双二氧代哌嗪右雷佐生(DEX)是唯一批准用于降低接受抗癌剂的癌症患者的心脏毒性和心力衰竭发生率的药物。尽管有临床效果，但DEX仅被批准用于治疗已接受300-500mg/m^{2}累积剂量的如多柔比星或表柔比星的蒽环类的转移性乳腺癌患者。DEX未被批准用于儿童和青少年，尤其令人沮丧的是，发现有报道称在蒽环类治疗的幼儿的癌后预后期心力衰竭的发生率较高。此外，有限的适应症批准和使用也证实了DEX的缺点，包括干扰蒽环类抗肿瘤的效力、诱导继发性恶性肿瘤以及引起血液和骨髓疾病。

[0009] 鉴于许多癌症疗法对心脏功能产生严重影响，对于开发一种有效的预防、减轻或消除由蒽环类、蛋白激酶抑制剂(例如，酪氨酸激酶抑制剂)、蛋白酶体抑制剂和其它癌症治疗剂引起的心脏毒性的药物存在明确的临床需求。尤其重要的是开发能够预防或降低癌症药物诱导的心脏毒性而不显著干扰癌症药物的抗癌作用的药物。同样重要的是开发不会引起严重副作用诸如中性粒细胞减少症、心脏问题恶化或继发性恶性肿瘤风险增加的心脏保护药物。这些潜在药物将不仅仅通过防止癌症患者潜在的心脏损伤而且还通过使化学疗法剂量优化以实现最大的抗癌作用而显著改善现有的癌症治疗。

附图说明

[0010] 图1总体描绘了通过向患者共同施用癌症治疗剂和保护剂来降低患者中癌症治疗剂诱导的心脏毒性的方法。

[0011] 图2总体描绘了癌症治疗剂、右雷佐生(DEX)和保护剂的共同施用。

[0012] 图3描绘了处理3天后模拟处理、多柔比星(DOX)、杨梅素或多柔比星和杨梅素的共同施用对人诱导多能干细胞衍生的心肌细胞(iPSC-CM)的细胞存活率的作用。

[0013] 图4A至图4B描绘了处理2天后多柔比星(DOX)(图4A)或多柔比星和杨梅素的共同施用(图4B)对人诱导多能干细胞衍生的心肌细胞(iPSC-CM)中线粒体健康的作用。

[0014] 图5描绘了处理3天后模拟处理、多柔比星或多柔比星和杨梅素的共同施用对人诱导多能干细胞衍生的心肌细胞(iPSC-CM)的收缩性的作用。

[0015] 图6A至6C描绘了提供图3所示实验的原始数据(图6A)或归一化数据(图6B)或图5所示实验的原始数据(图6C)的图表。

[0016] 图7A至图7C描绘了处理3天后浓度递增的杨梅素(图7A)、杨梅苷/杨梅苷(图7B)或二氢杨梅素(图7C)对人诱导多能干细胞衍生的心肌细胞(iPSC-CM)中多柔比星诱导的细胞凋亡的作用。

[0017] 图8是示出72小时内随着多柔比星的浓度递增杨梅素(MYR;100μM)对多柔比星(DOX)诱导的心脏毒性的保护作用的图线。Y轴，细胞存活率百分比；X轴，DOX的递增浓度。

[0018] 图9是示出浓度递增(X-轴)的杨梅素(MYR;圆形)和右雷佐生(DEX;方形)对用0.5μM多柔比星(DOX)处理的人诱导多能干细胞衍生的心肌细胞的挽救百分比的图线。

[0019] 图10描绘了模拟处理、DOX(0.5μM)、DOX加DEX(100μM)或DOX加MYR(100μM)处理48

小时后杨梅素对心肌细胞中多柔比星(DOX)诱导的收缩性异常的保护作用,以搏动率(每分钟;左图)、持续时间(秒;中图)和峰高度(任意单位;右图)示出。

[0020] 图11描绘了处理48小时后测量的杨梅素(MYR)对用DMSO、单独DOX(0.5μM)、DOX加DEX(100μM)或DOX加MYR(100μM)处理的人iPSC衍生的心肌细胞中DOX诱导的DNA双链断裂的作用,以在每种条件下定量的γH2AX-阳性细胞百分比(右图)和细胞的代表性图像(左图)示出。

[0021] 图12描绘了杨梅素(MYR)对多柔比星(DOX)诱导的肌节破坏的作用,针对模拟处理(DMSO;左图)、单独DOX(0.5μM;中图)或DOX加MYR(100μM;右图)处理以代表性图像示出。

[0022] 图13描绘了与右雷佐生(DEX)的抑制作用相比杨梅素(MYR)对拓扑异构酶IIα和β(TOPOIIα和TOPOIIβ)的抑制作用。

[0023] 图14描绘了杨梅素(MYR)和右雷佐生(DEX)对TOPOIIβ蛋白降解的作用,以图线(上图)和代表性图像(下图)示出。

[0024] 图15描绘了杨梅素(MYR)和二氢杨梅素(DHM)对拓扑异构酶IIβ(TOPOIIβ)酶促抑制及其相对效力的作用,如脱色凝胶(decatenation gel)(上图)和图线(下图)所示。

[0025] 图16是示出MYR和DHM抵抗DOX诱导的细胞死亡挽救心肌细胞的相对效力的图线。

[0026] 图17是示出MYR和DHM抵抗DOX诱导的双链断裂挽救心肌细胞的相对效力的图线。

[0027] 图18是示出MYR对TOPOIIα(右图)和TOPOIIβ(左图)的RNA表达水平的作用的图线,如在用单独的DOX或DOX加MYR处理的心肌细胞中所证实的。

[0028] 图19描绘了示出杨梅素(MYR)对保护心肌细胞免受表柔比星(EPI;左图)和伊达比星(IDA;右图)诱导的细胞毒性的效力的两幅图线。

[0029] 图20是示出杨梅素(MYR)对舒尼替尼(SUN)诱导的细胞死亡的作用的图线。

[0030] 图21是示出杨梅素(MYR)对索拉非尼(SOR)诱导的收缩性异常的作用的图线。

[0031] 图22是示出杨梅素(MYR)对硼替佐米(BOR)诱导的细胞死亡的作用的图线。

[0032] 图23是示出杨梅素(MYR)对DOX的抗癌活性无作用的图线。

[0033] 图24描绘了以缩短分数百分比(左图)和射血分数百分比(右图)测量的MYR在小鼠中对DOX诱导的收缩性异常的作用。

[0034] 图25描绘了DOX、DEX和各种保护剂对人诱导多能干细胞衍生的心肌细胞中线粒体毒性的作用。

[0035] 图26描绘了DOX、DEX和各种保护剂对人诱导多能干细胞衍生的心肌细胞中细胞凋亡的作用。

[0036] 图27A至图27D描绘了模拟处理(27A)、DEX(27B)、多柔比星和右雷佐生的共同施用(27C)或多柔比星和牡荆素的共同施用(27D)对人诱导多能干细胞衍生的心肌细胞中线粒体健康的作用。

[0037] 图28A至图28B描绘了在三天的时间段内(左图)或在第30小时的时间点上(右图)DOX或DOX与不同浓度的牡荆素(VIT)的共同施用对人诱导多能干细胞衍生的心肌细胞中电生理学活性的作用。

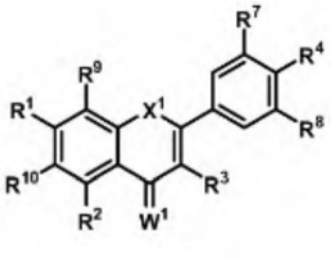
[0038] 图29A至图29B描绘了多柔比星与山奈素(KAE;左图)和多柔比星与牡荆素(VIT;右图)的共同施用对MDA-MB-231转移性乳腺癌细胞活性的作用。

发明内容

[0039] 本公开提供了用于在用蒽环类、蛋白激酶抑制剂和/或生物剂治疗的患者中保护心脏和预防心力衰竭的组合物、药盒和方法。通过使癌症患者在化学疗法中潜在的破坏性心力衰竭的风险最小化，常规癌症治疗可用本文所述的发明实现改善的效力和安全性。

[0040] 组合物在具有或不具有抗癌剂的情况下包括一种或多种保护剂。药盒通常包括一种或多种保护剂，有时也包括抗癌剂。方法包括降低、预防或消除由药物或包括癌症治疗在内的其它疗法诱导的心脏毒性的方法。

[0041] 在一些方面，本公开提供了包含根据式1的保护剂的药物组合物，

[0042] 

式 1

[0043] 其中：

[0044] X^1为CR^5R^6、NR^5、O、S、C＝O或C＝S；

[0045] R^1、R^2、R^3、R^5、R^6、R^9和R^{10}各自独立地为烷基、烯基、炔基、烷氧基、酰基、酰氧基、羧酸、酯、胺、酰胺、碳酸根、氨基甲酸根、硝基、硫醚、硫酯、环烷基、杂烷基、杂环基、单糖、芳基或杂芳基(其中任一者是取代或未取代的)、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H；

[0046] R^4、R^7和R^8为烷氧基、羟基或H；

[0047] W^1为O或S；或

[0048] 其盐。

[0049] 在某些方面，X^1可以为O或S；R^1、R^2、R^3、R^9和R^{10}各自可独立地为烷氧基、环烷基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H；并且R^4、R^7和R^8各自可独立地为烷氧基、羟基或H。

[0050] 在某些方面，X^1为O；R^1、R^2、R^3、R^9和R^{10}各自可独立地为烷氧基、环烷基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H；并且R^4、R^7和R^8各自可独立地为烷氧基、羟基或H。

[0051] 在其它方面，X^1为O；R^1和R^2各自可独立地为羟基或H；R^3、R^9和R^{10}各自可独立地为环烷基、杂环基、羟基或H；R^4为羟基；并且R^7和R^8各自可独立地为羟基或H。

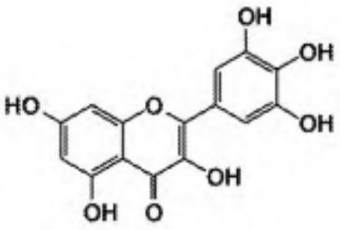
[0052] 在其它方面，X^1为O；R^1为羟基；R^2和R^3各自可独立地为羟基或H；R^9和R^{10}为H；R^4为羟基；并且R^7和R^8各自可独立地为羟基或H。

[0053] 在其它方面，X^1为O；R^1为羟基；R^2和R^3各自可独立地为羟基或H；R^9可以为杂环基或H；R^{10}为H；R^4可独立地为羟基或H；并且R^7和R^8各自可独立地为羟基或H。

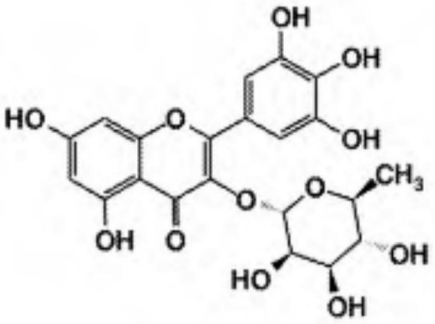
[0054] 在其它方面，X^1为O；R^1为羟基；R^2和R^9各自可独立地为羟基或H；R^3可以为环烷基、羟基或H；R^{10}为H；R4为羟基；并且R^7和R^8各自可独立地为羟基或H。在一个实施方案中，R^3的环烷

基可以为单糖。

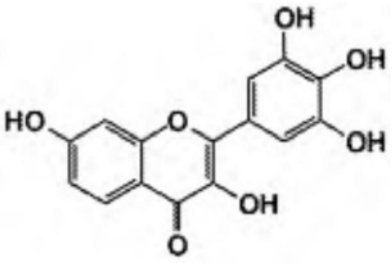
[0055] 在一些实施方案中,药物组合物可包含杨梅素并且是根据下式的化合物。

[0056] 

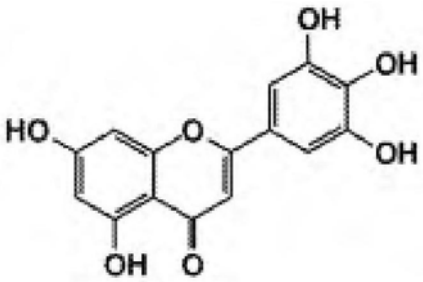
[0057] 在一些实施方案中,药物组合物可包含杨梅苷/杨梅苷并且是根据下式的化合物。

[0058] 

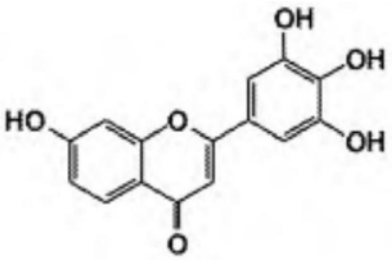
[0059] 在一些实施方案中,药物组合物可包含洋槐黄素并且是根据下式的化合物。

[0060] 

[0061] 在一些实施方案中,药物组合物可包含五羟黄酮并且是根据下式的化合物。

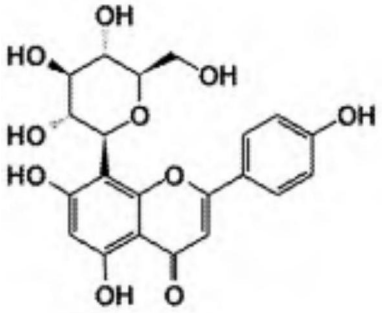
[0062] 

[0063] 在一些实施方案中,药物组合物可包含7,3',4',5'-四羟基黄酮并且是根据下式的化合物。

[0064] 

[0065] 在一些实施方案中,药物组合物包含ficetin。在一些实施方案中,药物组合物包含槲皮素。在一些实施方案中,药物组合物包含山奈素。在一些实施方案中,药物组合物内

的保护剂可以是具有以下结构的化合物：

[0066] 

[0067] 在具体实例中，药物组合物内的保护剂可以是牡荆素。

[0068] 在一些实施方案中，药物组合物可包括一种或多种化学疗法药物(抗癌剂)或一种或多种生物剂。在一些实施方案中，药物组合物可包括化学疗法药物。在一些实施方案中，药物组合物可包括一种或多种化学疗法药物(抗癌剂)和一种或多种选自杨梅素、五羟黄酮(5,7,3',4',5'-五羟基黄酮)、洋槐黄素、ficetin、牡荆素、7,3',4',5'-四羟基黄酮和杨梅苷的保护剂。

[0069] 在一些实施方案中，药物组合物可包含蒽环类或其盐。在一些实施方案中，蒽环类可以是柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。在一些实施方案中，蒽环类是多柔比星。在一些实施方案中，蒽环类是表柔比星。在一些实施方案中，蒽环类是伊达比星。

[0070] 在一些实施方案中，化学疗法药物可以是蛋白激酶抑制剂。在一些实施方案中，蛋白激酶抑制剂是阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉(vismodegib)。

[0071] 在一些实施方案中，化学疗法药物可以是蛋白酶体抑制剂。在具体实例中，蛋白酶体抑制剂可以是硼替佐米。

[0072] 在一些实施方案中，蛋白激酶抑制剂可以是酪氨酸激酶抑制剂。在一些实施方案中，例如，酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。在具体实例中，酪氨酸激酶抑制剂是索拉非尼。在另一个具体实例中，酪氨酸激酶抑制剂是舒尼替尼。

[0073] 在一些实施方案中，化学疗法药物可以是生物剂。在一些实施方案中，生物剂是抗体。在一些实施方案中，抗体可以是曲妥珠单抗美坦新偶联物(adotrastuzumabemtansine)、阿仑单抗、贝伐单抗、博纳吐单抗(blinatumomab)、本妥昔单抗(brentuximab vedotin)、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星(gemtuzumab ozogamicin)、替伊莫单抗(ibritumomab tiuxetan)、伊匹单抗、耐昔妥珠单抗(necitumumab)、纳武单抗(nivolumab)、奥比妥珠单抗(obinutuzumab)、奥法木单抗、帕尼单抗、派姆单抗(pembrolizumab)、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。在一个具体实例中，抗体是曲妥珠单抗。

[0074] 在一些实施方案中，药物组合物可以是液体组合物。在一些实施方案中，药物组合

物可以是胶囊剂、凝胶胶囊剂或脂质体。在一些实施方案中，药物组合物可以是片剂。

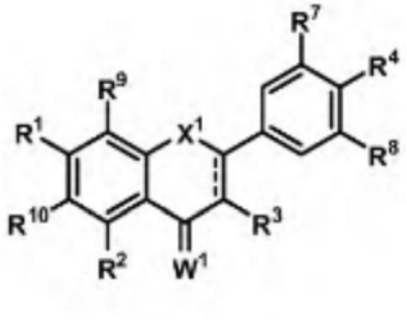
[0075] 在一些实施方案中，药物组合物还可包括右雷佐生作为附加保护剂。

[0076] 在一些实施方案中，药物组合物可包含至少1mg的一种或多种保护剂。在一些实施方案中，药物组合物可包含在0.1mg至200mg之间的一种或多种保护剂。在一些实施方案中，药物组合物可包含在0.1mg至300mg之间的一种或多种保护剂。

[0077] 在一些实施方案中，存在两种保护剂并且共同配制在一起。在一些实施方案中，两种保护剂可在药物组合物内作为不同实体存在。在一些实施方案中，药物组合物可包含化学疗法药物并且化学疗法药物与两种保护剂中的一者共同配制。

[0078] 在一些方面，本公开提供了药物组合物，其包含：(a)选自以下的保护剂：

[0079] 根据式2的化合物，

[0080] 

式2

[0081] 其中：

[0082] ----表示单键或双键；

[0083] X^1为CR^5R^6、NR^5、O、S、C＝O或C＝S；

[0084] R^1、R^2、R^3、R^5、R^6、R^9和R^{10}各自独立地为烷基、烯基、炔基、烷氧基、酰基、酰氧基、羧酸、酯、胺、酰胺、碳酸根、氨基甲酸根、硝基、硫醚、硫酯、环烷基、杂烷基、杂环基、芳基或杂芳基(其中任一者是取代或未取代的)、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H；R^4、R^7和R^8为羟基；

[0085] W^1为O或S；

[0086] 或其盐；以及

[0087] (b)化学疗法药物或生物剂。

[0088] 在一些实施方案中，药物组合物可包含抗癌剂或化学疗法药物。在一些实施方案中，保护剂选自杨梅素、五羟黄酮、洋槐黄素、ficetin、牡荆素、二氢洋槐黄素(dihydrorobinetin)、7,3',4',5'-四羟基黄酮和杨梅苷。

[0089] 在一些实施方案中，药物组合物可包含一种或多种保护剂。在一些实施方案中，药物组合物可包含杨梅素。在一些实施方案中，药物组合物可包含杨梅苷。在一些实施方案中，药物组合物可包含洋槐黄素。在一些实施方案中，药物组合物可包含二氢洋槐黄素。在一些实施方案中，药物组合物可包含牡荆素。在一些实施方案中，药物组合物可包含五羟黄酮。在一些实施方案中，药物组合物包含槲皮素。在一些实施方案中，药物组合物包含山奈素。

[0090] 在一些实施方案中，药物组合物包含蒽环类或其盐。在一些实施方案中，蒽环类是

柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。在一些实施方案中，蒽环类是多柔比星。在一些实施方案中，蒽环类是表柔比星。在一些实施方案中，蒽环类是伊达比星。

[0091] 在一些实施方案中，化学疗法药物可以是蛋白激酶抑制剂。在一些实施方案中，蛋白激酶抑制剂是阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。

[0092] 在一些实施方案中，化学疗法药物是蛋白酶体抑制剂。在具体实例中，蛋白酶体抑制剂是硼替佐米。

[0093] 在一些实施方案中，蛋白激酶抑制剂是酪氨酸激酶抑制剂。在一些实施方案中，酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。在具体实例中，酪氨酸激酶抑制剂是索拉非尼。在另一个具体实例中，酪氨酸激酶抑制剂是舒尼替尼。

[0094] 在一些实施方案中，化学疗法药物是生物剂。在一些实施方案中，生物剂是抗体。在一些实施方案中，抗体是曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。在具体实例中，抗体是曲妥珠单抗。在具体实例中，抗体是贝伐单抗。

[0095] 在一些实施方案中，药物组合物可包含至少1、2、3、4、5、6、7、8、9、10、20、30、40、50、60、70、80、90、100、110、120、130、140、150、160、170、180、190、200、210、220、230、240、250、260、270、280、290或300mg的一种或多种保护剂。

[0096] 在一些实施方案中，药物组合物可包含在0.1mg至50mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至10mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至20mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至30mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至40mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至50mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至100mg之间的保护剂。在一些实施方案中，药物组合物可包含在1mg至200mg之间的保护剂。在一些实施方案中，药物组合物可包含在40mg至300mg之间的保护剂。在一些实施方案中，药物组合物可包含在50mg至400mg之间的保护剂。

[0097] 在一些实施方案中，药物组合物可包含化学疗法药物；并且化学疗法药物和保护剂在药物组合物内混合。

[0098] 在一些实施方案中，药物组合物包含化学疗法药物，其中该化学疗法药物的剂量为至少0.1mg。在一些实施方案中，药物组合物包含化学疗法药物，其中该化学疗法药物的剂量在0.01mg至50mg之间。在一些实施方案中，药物组合物包含化学疗法药物，其中该化学疗法药物的剂量在0.01mg至100mg之间。在一些实施方案中，药物组合物包含化学疗法药物，其中该化学疗法药物的剂量在0.01mg至200mg之间。

[0099] 在一些实施方案中，药物组合物包含至少50mg剂量的生物剂。在一些实施方案中，药物组合物包含剂量在0.1mg至100mg之间的生物剂。在一些实施方案中，药物组合物包含剂量在0.1mg至200mg之间的生物剂。

[0100] 在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为1:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为2:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为3:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为4:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为5:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为6:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为7:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为8:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为9:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为10:1。在一些实施方案中，药物组合物包含化学疗法药物，并且保护剂与化学疗法药物的摩尔比至少为20:1。在一些实施方案中，药物组合物包含化学疗法药物，并且其中保护剂与化学疗法药物的摩尔比至少为100:1。在一些实施方案中，药物组合物包含化学疗法药物，并且其中保护剂与化学疗法药物的摩尔比至少为1:2。在一些实施方案中，药物组合物包含化学疗法药物，并且其中保护剂与化学疗法药物的摩尔比至少为1:3。在一些实施方案中，药物组合物包含化学疗法药物，并且其中保护剂与化学疗法药物的摩尔比至少为1:4。在一些实施方案中，药物组合物包含化学疗法药物，并且其中保护剂与化学疗法药物的摩尔比至少为1:5。

[0101] 本公开提供了用于向受试者施用本文公开的药物组合物中的任一种的方法。在一些方面，一般来讲，本公开提供了用于预防、降低或消除心脏毒性或心力衰竭的方法。在一些方面，本公开提供了用于预防、减轻或消除受试者中由化学疗法药物或生物剂诱导的心脏毒性的方法，该方法包括：向受试者施用一种或多种根据式1的保护剂，从而预防、降低或消除受试者中由化学疗法药物或生物剂引起的心脏毒性。在一些情况下，药物组合物包含选自诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷的化合物。

[0102] 在一些方面，本公开提供了用于预防、减轻或消除受试者中由化学疗法药物或生物剂诱导的心脏毒性的方法，该方法包括：向受试者施用至少一种根据式1或式2的保护剂，从而预防、降低或消除受试者中由化学疗法药物或生物剂引起的心脏毒性。

[0103] 在一些实施方案中，在向受试者施用一种或多种根据式1或式2的保护剂之前将化学疗法药物或生物剂施用于受试者。

[0104] 在一些实施方案中，在向受试者施用至少两种式1或式2的保护剂之后将化学疗法药物或生物剂施用于受试者。

[0105] 在一些方面，本公开提供了用于治疗癌症的方法，该方法包括：(a)向受试者施用化学疗法药物或生物剂，其中该受试者患有癌症并且该化学疗法药物或生物剂能够在受试者中引起心脏毒性；以及(b)将至少一种根据式1或式2的保护剂施用于受试者，其中该保护

剂预防、减轻或消除受试者的心脏毒性。

[0106] 在一些实施方案中，受试者是罹患癌症的人。在一些实施方案中，癌症是膀胱癌、骨癌、脑肿瘤、乳腺癌、食道癌、胃肠癌、白血病、肝癌、肺癌、淋巴瘤、骨髓瘤、卵巢癌、前列腺癌、肉瘤、胃癌或甲状腺癌。

[0107] 在一些实施方案中，在施用保护剂之前，受试者具有心脏病状或具有患心脏病状的病史。在一些实施方案中，保护剂的施用降低受试者经受由化学疗法药物或生物剂诱导的心脏毒性的风险。在一些实施方案中，保护剂的施用降低受试者经受由化学疗法药物或生物剂诱导的心脏毒性的至少30％、40％、50％、60％、70％、80％、90％或95％的风险。在一些实施方案中，心脏毒性可包括心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激。在一些实施方案中，心脏毒性是心脏组织损伤。在一些实施方案中，心脏毒性是电生理异常。

[0108] 在一些实施方案中，本文所述方法中使用的化学疗法药物可包含蒽环类或其盐。在一些实施方案中，蒽环类是柔红霉素、多柔比星、表柔比星、伊达比星、米托蒽醌或戊柔比星。在一些实施方案中，蒽环类是多柔比星。在一些实施方案中，蒽环类是表柔比星。在一些实施方案中，蒽环类是伊达比星。

[0109] 在一些实施方案中，本文所述方法中使用的化学疗法药物是蛋白激酶抑制剂。在一些实施方案中，蛋白激酶抑制剂是阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。

[0110] 在一些实施方案中，蛋白激酶抑制剂是酪氨酸激酶抑制剂。在一些实施方案中，蛋白激酶抑制剂是酪氨酸激酶抑制剂。在一些实施方案中，酪氨酸激酶抑制剂选自索拉非尼、舒尼替尼、波舒替尼、吉非替尼、达沙替尼、达拉菲尼、维莫非尼、伊马替尼、拉帕替尼、甲磺酸盐和尼洛替尼。在具体实例中，酪氨酸激酶抑制剂是索拉非尼。在另一个具体实例中，酪氨酸激酶抑制剂是舒尼替尼。

[0111] 在一些方面，化学疗法药物是蛋白酶体抑制剂。在一个具体实例中，蛋白酶体抑制剂是硼替佐米。

[0112] 在一些实施方案中，本文所述方法中使用的生物剂可以是抗体。在一些实施方案中，抗体是曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。

[0113] 在一个具体实例中，抗体是曲妥珠单抗。在一些实施方案中，根据本文所述方法的受试者在施用保护剂后具有减小的QTc间期。在一些情况下，保护剂选自诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷。在一个具体实例中，保护剂是杨梅素。

[0114] 在一些实施方案中，向受试者同时施用化学疗法药物和式1或式2的保护剂。在一些实施方案中，向受试者依次施用化学疗法药物和保护剂。在一些实施方案中，在施用化学

疗法药物之前将保护剂施用于受试者。在一些实施方案中，在施用化学疗法药物之后将保护剂施用于受试者。

[0115] 在一些实施方案中，可施用至少两种式1或式2的保护剂。例如，该至少两种保护剂可选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷。

[0116] 在一些实施方案中，一种或多种保护剂可还包含右雷佐生。

[0117] 本公开提供了用于治疗或预防受试者器官损伤的方法，该方法包括：向具有器官损伤的受试者施用一种或多种选自诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷的保护剂，从而治疗或预防受试者的器官损伤。

[0118] 本公开还提供了药盒。在一些方面，本公开提供了一种药盒，其包括：(a)选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷的保护剂；以及(b)化学疗法药物或生物剂。

[0119] 在一些方面，本公开提供了一种药盒，其包括：(a)选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮和杨梅苷的保护剂；(b)化学疗法药物或生物剂；以及(c)右雷佐生。在一些实施方案中，保护剂是杨梅素。

具体实施方式

[0120] 某些癌症药物(例如，蒽环类、蛋白激酶抑制剂)和其它疗法可对患者造成心脏毒性。例如，当诸如多柔比星之类的药物通过拓扑异构酶II酶切割DNA而嵌入DNA从而有效地阻止TOPOIIα或β将切割后的链连接在一起时，发生蒽环类诱导的心脏毒性。

[0121] 本公开提供了可预防、降低或消除此类心脏毒性并且还可预防、降低或消除由心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激引起的器官损伤的药物组合物和方法。本文提供的许多组合物和方法涉及与一种或多种癌症治疗剂联合施用的特定保护剂，由此降低癌症治疗将引起或加重患者心脏毒性事件的风险。本文所述的保护剂包括诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷以及/或者其衍生物或盐。在一些情况下，保护剂可以是类黄酮。在一些情况下，保护剂可与不同的保护剂组合施用。在一些情况下，保护剂可以组合(诸如包括右雷佐生和另一种保护剂的组合)的形式施用。

[0122] 本公开可使癌症患者(包括心脏健康患者和先前存在心脏病状的患者)能够接受期望剂量的疗法(例如，蒽环类或其盐)，而不会由于心脏毒性风险而显著改变剂量方案。本公开的另一个优势是它可使较大的患者群体(诸如先前存在心脏病状或具有年龄限制的某些患者)能够接受给定的疗法。此外，降低或预防心脏毒性可使癌症患者避免必须服用药物来治疗心脏病状。总之，本文提供的优势可有助于促进患者获得更好的治疗效果。

[0123] 本文提供的药物组合物和方法(包括使用方法)整体涉及降低、消除或预防由化疗药物、生物剂或放射疗法引起的心脏毒性；它们也可用于减少或消除由电生理异常、线粒体毒性、细胞凋亡或氧化应激引起的器官损伤。图1描绘了本文提供的方法的一些实施方案的总体示意图。上图示出了向患者[120]施用的癌症治疗剂[110]，诸如化疗药物、生物剂或放射疗法，该患者产生心脏毒性，然后随时间逐渐降低癌症治疗剂的服用剂量[130]。因此，在不存在保护剂[140]的情况下与癌症治疗剂[110]的施用相关联的心脏毒性可限制有资格

接受治疗的患者群体。在下图中，向患者[151]共同施用癌症治疗剂[110]与保护剂[140]，例如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷，该患者经受降低的心脏毒性或者完全无心脏毒性[160]，从而使患者能够耐受给药方案。尽管描绘了用于癌症治疗剂和保护剂的单独载体，但在一些情况下，癌症治疗剂和保护剂被共同配制在一起。癌症治疗剂[110]与保护剂[140]的共同施用可使较大的患者群体[150]能够接受癌症治疗，包括健康患者和先前存在心脏病状的患者[152,153]。

[0124] 图2还描绘了本文提供的实施方案的总体示意图。上图示出了向患者[230]共同施用癌症治疗剂[210](例如，化疗药物、生物剂或放射疗法)和右雷佐生[220]，随后该患者随时间经受一定的心脏毒性[240]，在不存在保护剂[250]的情况下癌症治疗剂[210]和右雷佐生[220]的共同施用可限制有资格接受治疗的患者群体。在下图中，向患者[261]施用癌症治疗剂[210]、右雷佐生[220]和保护剂[250](诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷)，该患者经受降低的心脏毒性或完全无心脏毒性[270]。在该实施方案中，保护剂[250]与癌症治疗剂[210]和右雷佐生[220]的共同施用可增强右雷佐生的活性，以预防、缓解或消除患者[261]的心脏毒性，由此使更大的患者群体[260]能够接受治疗，包括没有心脏病状和先前存在心脏病状的患者[262,263]，在一些情况下，保护剂、右雷佐生和/或癌症治疗剂单独施用；在一些情况下，它们同时施用或作用共同制剂施用。一般来讲，本文提供的共同制剂和方法可降低化疗药物、生物剂或放射疗法在患者中引起的心脏毒性。

[0125] 本文提供的组合物可包括两种或更多种保护剂的共同制剂。例如，该共同制剂可包含诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和右雷佐生。在一些情况下，组合物可包括保护剂(例如，诸如杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷)与某种癌症治疗剂(例如，化疗药物或生物剂)的共同制剂。在一些情况下，本文提供了包含至少两种保护剂(或保护剂和癌症治疗剂)作为单独组分的药盒，通常连同有使用说明。

[0126] 方法

[0127] 本文提供用于向患者(尤其是癌症患者)施用可降低、消除或预防由癌症治疗剂(例如，化疗药物、生物剂或放射疗法)引起的心脏毒性的药物组合物的方法。本文提供的方法还包括使用本文提供的至少一种组合物治疗患者的癌症。在一些情况下，患者心脏可以是健康的；在一些情况下，患者具有患心脏病状的风险。

[0128] 本文提供的方法通常包括向患者施用包含本文所述的至少一种保护剂或至少一种保护剂和癌症治疗剂(例如，蒽环类药物、蛋白激酶抑制剂、生物剂或放射疗法)的药物组合物。保护剂和癌症治疗剂可与不同的心脏保护剂(例如，右雷佐生)组合。在一些情况下，保护剂和癌症治疗剂可共同配制，其中它们在同一药物组合物(例如，片剂、胶囊剂、脂质体、液体或蒸气)内混合；在一些情况下，它们作为不同实体存在。

[0129] 主题

[0130] 本文公开的方法和组合物通常用于预防、降低、治疗或消除受试者中癌症治疗剂诱导的心脏毒性。受试者可以是任何人类患者，尤其是癌症患者、具有患癌风险的患者或具有家族或个人癌症史的患者。在一些情况下，患者处于癌症治疗的特定阶段。例如，可将本文所述的药物组合物施用于患有早期或晚期癌症的人类患者，以降低由癌症治疗剂引起的

心脏毒性。

[0131] 癌症患者可具有任何类型的癌症。癌症的实例可包括但不限于肾上腺癌、肛门癌、基底细胞癌、胆管癌、膀胱癌、血癌、骨癌、脑肿瘤、乳腺癌、支气管癌、心血管系统癌症、宫颈癌、结肠癌、结直肠癌、消化系统癌症、内分泌系统癌症、子宫内膜癌、食道癌、眼癌、胆囊癌、胃肠肿瘤、肾癌、造血系统恶性肿瘤、喉癌、白血病、肝癌、肺癌、淋巴瘤、黑素瘤、间皮瘤、肌肉系统癌症、骨髓增生异常综合征(MDS)、骨髓瘤、鼻腔癌、鼻咽癌、神经系统癌症、淋巴系统癌症、口腔癌、口咽癌、骨肉瘤、卡波西氏肉瘤、卵巢癌、胰腺癌、阴茎癌、垂体瘤、前列腺癌、直肠癌、肾盂癌、生殖系统癌症、呼吸系统癌症、肉瘤、唾液腺癌、骨骼系统癌症、皮肤癌、小肠癌、胃癌、睾丸癌、咽喉癌、胸腺癌、甲状腺癌、肿瘤、泌尿系统癌症、子宫癌、阴道癌或外阴癌。术语“淋巴瘤”可指任何类型的淋巴瘤，包括B细胞淋巴瘤(例如，弥漫性大B细胞淋巴瘤、滤泡淋巴瘤、小淋巴细胞淋巴瘤、套细胞淋巴瘤、边缘区B细胞淋巴瘤、伯基特淋巴瘤、淋巴浆细胞淋巴瘤、毛细胞白血病或原发性中枢神经系统淋巴瘤)或T细胞淋巴瘤(例如，前体T淋巴母细胞淋巴瘤或外周T细胞淋巴瘤)。术语“白血病”可指任何类型的白血病，包括急性白血病或慢性白血病。白血病的类型包括急性骨髓性白血病、慢性粒细胞白血病、急性淋巴细胞白血病、急性未分化白血病或慢性淋巴细胞白血病。在一些情况下，癌症患者不具有特定类型的癌症。例如，在一些情况下，患者可患有不是乳腺癌的癌症。

[0132] 癌症的实例包括引起实体瘤的癌症以及不引起实体瘤的癌症。此外，本文提及的任何癌症可以是原发性癌症(例如，以其首先开始生长的身体部位命名的癌症)或继发性或转移性癌症(例如，源自身体的另一部分的癌症)。

[0133] 具有患癌风险的患者可能因某种特定病状(诸如癌前病状)而具有风险。癌前病状包括但不限于光化性角化病、巴雷特食管病(Barrett’s esophagus)、萎缩性胃炎、原位导管癌、先天性角化不良、缺铁性吞咽困难、扁平苔藓、口腔黏膜下纤维化、日光性弹力组织变性、宫颈异常、白斑症和红斑症。在一些情况下，患者可由于细胞或组织发育异常(例如，细胞数量异常改变、细胞形状异常改变、细胞大小异常改变或细胞色素沉着异常改变)而具有患癌风险。

[0134] 具有患癌风险的患者可以是暴露于致癌物质的患者。此类患者可包括暴露于已知或可能的致癌物(例如，乙酰醛、石棉或烟草制品)的患者或暴露于电离辐射(例如，γ辐射、β辐射、X辐射或紫外辐射)的患者。在一些情况下，具有患癌风险的患者由于家族癌症史而具有风险。

[0135] 本文公开的方法和组合物还可用于预防、降低或消除具有癌症病史的患者的心脏毒性，尤其是已经施用具有心脏毒性作用的癌症治疗剂(例如，蒽环类药物、蛋白激酶抑制剂、蛋白酶体抑制剂或生物剂)的患者。具有癌症病史的患者的实例包括但不限于缓解患者、完全缓解患者、复发性癌症患者或复发性癌症患者。

[0136] 本文公开的方法和组合物通常用于已经施用或正在施用心脏毒性诱导剂(例如，癌症治疗剂)的患者。心脏毒性诱导剂的非限制性实例在本文其它地方有所描述，并且可包括癌症治疗剂、化疗药物、蒽环类(例如，多柔比星、表柔比星和伊达比星)、蛋白激酶抑制剂(例如，酪氨酸激酶抑制剂)、生物剂(例如，曲妥珠单抗)或放射疗法以及任何已知会引起心脏毒性的癌症治疗。在一些实例中，将本文公开的药物组合物施用于先前暴露于已知具有心脏毒性作用的癌症治疗剂的癌症患者，以降低与患者当前癌症治疗方案相关联的心脏毒

性的风险。在一些情况下，将药物组合物施用于癌症患者以减少或抵消先前暴露于癌症治疗剂或药物或引起心脏毒性的其它试剂的累积作用。在一些实例中，可将包含杨梅素和蒽环类的药物共同制剂施用于还具有由先前的癌症治疗剂引起的扩张型心肌病的前列腺癌患者。在另一个实例中，可将包含牡荆素的药物共同制剂施用于同时正在用蒽环类治疗的肺癌患者。在又一个实例中，可将包含洋槐黄素的药物共同制剂施用于乳腺癌患者。在又一个实例中，可将包含五羟黄酮的药物共同制剂施用于卡波西氏肉瘤患者。在又一个实例中，可将包含ficetin的药物共同制剂施用于乳腺癌患者。在又一个实例中，可将包含7,3',4',5'-四羟基黄酮的药物共同制剂施用于乳腺癌患者。在又一个实例中，可将包含杨梅苷的药物共同制剂施用于乳腺癌患者。在又一个实例中，可将包含杨梅素和蒽环类的药物共同制剂施用于还具有由先前的癌症治疗剂引起的扩张型心肌病的肝癌患者。在又一个实例中，可将包含杨梅素和多柔比星的药物共同制剂施用于肉瘤癌症患者。

[0137] 在一些情况下，本文的方法和组合物可用于缓解非癌症治疗剂引起的心脏毒性。因此，患者可具有由癌症非特异性药物(诸如蛋白激酶抑制剂)诱导的心脏毒性或具有这种风险。此类患者可具有诸如神经或心脏疾病之类的病状。在一些情况下，该病状可以是可用蛋白激酶抑制剂治疗的病状。

[0138] 在一些情况下，患者可具有器官损伤或具有器官损伤的风险。例如，患者可具有由心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激引起的器官损伤(或具有器官损伤风险)。对于此类患者，本文提供的方法和组合物可减少或消除由心脏组织损伤、电生理异常、线粒体毒性、凋亡或氧化应激引起的器官损伤。

[0139] 在一些情况下，通过本文所述的任何方法或组合物治疗的患者可患有心脏疾病或具有心脏疾病家族史。心脏疾病的实例包括但不限于致心律失常性心肌病、动脉疾病、Brugada综合征、先天性心脏病、扩张型心肌病、心悸、心脏瓣膜病、高血压性心脏病、肥厚型心肌病、长QT综合征、风湿性心脏病或脉管疾病。在一些情况下，心脏疾病由心脏毒性剂(例如，蒽环类)引起。例如，心脏疾病可由本文提到的任何心脏毒性剂引起。在一个具体实例中，可将包含杨梅素和多柔比星的药物共同制剂施用于还患有肥厚型心肌病的乳腺癌患者。在另一个实例中，可将一种或多种选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷的化合物的共同制剂施用于经受由先前施用的化学疗法药物引起的心脏毒性的癌症患者。

[0140] 通过本文所述的任何方法或组合物治疗的患者可以是任何年龄并且可以是成人、婴儿或儿童。在一些情况下，患者为0、1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49、50、51、52、53、54、55、56、57、58、59、60、61、62、63、64、65、66、67、68、69、70、71、72、73、74、75、76、77、78、79、80、81、82、83、84、85、86、87、88、89、90、91、92、93、94、95、96、97、98或99岁或在其中的范围内(例如，在2至20岁之间、在20至40岁之间或在40至90岁之间)。可受益的特定类别的患者是40岁以上的患者。另一类可受益的患者是可具有慢性心脏症状的儿科患者。此外，通过本文所述的任何方法或组合物治疗的患者可以是男性或女性。

[0141] 也可将本文公开的任何组合物施用于非人受试者，诸如实验室动物或农场动物。非人受试者的非限制性实例包括狗、山羊、豚鼠、仓鼠、小鼠、猪、非人灵长类动物(例如，大

猩猩、猿、猩猩、狐猴、或狒狒)、大鼠、绵羊、牛或斑马鱼。

[0142] 药物施用

[0143] 本文提供的公开内容描述了通过向患者施用一种或多种式1、式2的保护剂或其衍生物或盐来预防、降低或消除患者中癌症治疗剂诱导的心脏毒性的方法。本文的公开内容还描述了通过向患者施用一种或多种选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷(或其衍生物或盐)的保护剂来预防、降低或消除癌症治疗剂诱导的心脏毒性的方法。本文提供的公开内容还描述了向受试者施用癌症治疗剂的方法,其中该受试者患有癌症并且该癌症治疗剂能够在受试者中引起心脏毒性和器官损伤,以及施用一种或多种选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷(或其衍生物或盐)的保护剂的方法,其中该保护剂预防、降低或消除受试者的心脏毒性。

[0144] 本文公开的方法可还包括向患者施用右雷佐生(或其衍生物或盐)和根据式1、式2的保护剂或其衍生物或盐的组合;该组合剂可作为共同制剂施用或单独施用。在一些方面,该方法包括向患者施用右雷佐生(或其衍生物或盐)和杨梅苷(或其衍生物或盐)的组合;该组合剂可作为共同制剂施用或单独施用。

[0145] 本文公开的方法可还包括向患者施用包含右雷佐生(或其衍生物或盐)和选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷(或其衍生物或盐)的保护剂的组合的组合剂;该组合剂可作为共同制剂施用或单独施用。

[0146] 保护剂可以式1或式2的化合物的任何组合的形式施用于受试者或患者。在一些情况下,仅向受试者或患者施用一种保护剂(例如,杨梅素或其衍生物或盐)。在一些情况下,仅向受试者或患者施用一种保护剂(例如,杨梅苷或其衍生物或盐)。在一些情况下,仅向受试者或患者施用一种保护剂(例如,牡荆素或其衍生物或盐)。在一些情况下,仅向受试者或患者施用一种保护剂(例如,洋槐黄素或其衍生物或盐)。在一些情况下,仅向受试者或患者施用一种保护剂(例如,五羟黄酮或其衍生物或盐)。在一些情况下,仅向受试者或患者施用一种保护剂(例如,7,3',4',5'-四羟基黄酮或其衍生物或盐)。在一个具体实例中,可向本文所述的受试者或患者施用治疗有效剂量的杨梅素(或其衍生物或盐)。在另一个实例中,可向本文所述的受试者或患者施用治疗有效剂量的洋槐黄素(或其衍生物或盐)。在又一个实例中,可向本文所述的受试者或患者施用治疗有效剂量的牡荆素(或其衍生物或盐)。

[0147] 在一些情况下,向受试者施用两种选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和右雷佐生(或其衍生物或盐)的保护剂。在向患者施用两种或更多种保护剂的情况下,保护剂可作为不同实体施用或共同制剂施用。例如,可向经受心脏毒性的患者施用治疗有效的杨梅素和洋槐黄素的共同制剂、杨梅素和右雷佐生的共同制剂或本文所述的其它共同制剂。两种或更多种保护剂可同时或依次施用。在一些情况下,两种或更多种保护剂可以特定顺序依次施用。例如,可向患者首先施用杨梅素,然后施用右雷佐生,或者可首先施用右雷佐生,然后施用杨梅素。

[0148] 在一些情况下,可向患者施用抗癌剂(例如,化疗药物、生物剂、蛋白激酶抑制剂、放射疗法)(或其它治疗剂)和一种或多种式1或式2的保护剂(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素和杨梅苷)。在向患者施用

癌症治疗剂(或其它治疗剂)和至少两种保护剂的情况下,癌症治疗剂(或其它治疗剂)和至少两种保护剂(或其衍生物或盐)可作为共同制剂以任何组合的形式施用。例如,可向患者施用保护剂和化疗药物的共同制剂或者包含一种或多种化疗药物和至少两种保护剂的共同制剂。

[0149] 在一些情况下,可同时向患者或受试者施用一种或多种保护剂(或其衍生物或盐)和一种或多种癌症治疗剂(或其它治疗剂)。例如,该方法可包括同时向患者施用作为单独实体的保护剂和化学疗法。

[0150] 在一些情况下,可向患者或受试者依次施用式1或式2的一种或多种保护剂(或其衍生物或盐)和一种或多种癌症治疗剂(或其它治疗剂)。例如,保护剂可在施用癌症治疗剂(或其它治疗剂)之前施用。例如,可向癌症患者施用治疗有效剂量的杨梅素以预防心脏毒性,随后施用化疗药物(例如,多柔比星)。在另一个实例中,可向癌症患者施用治疗有效剂量的杨梅苷以预防心脏毒性,随后施用化疗药物(例如,多柔比星)。在又一个实例中,可向癌症患者施用治疗有效剂量的牡荆素以预防心脏毒性,随后施用化疗药物(例如,多柔比星)。在另一个实例中,可向癌症患者施用治疗有效剂量的洋槐黄素以预防心脏毒性,随后施用化疗药物(例如,多柔比星)。在另一个实例中,可向癌症患者施用治疗有效剂量的五羟黄酮以预防心脏毒性,随后施用化疗药物(例如,多柔比星)。在其它实例中,在施用一种或多种式1或式2的保护剂之前向将癌症治疗剂(或其它治疗剂)施用于患者或受试者。在一些情况下,在接受癌症治疗(或其它治疗)之前向患者施用一种或多种保护剂,然后在癌症治疗之后施用一种或多种保护剂。

[0151] 在依次施用的情况下,在一种或多种保护剂和一种或多种癌症治疗剂(或其它治疗剂)的施用之间可存在延迟周期。例如,保护剂可在施用癌症治疗剂或其它治疗剂之前的数分钟、数小时、数天或数周(例如,在施用癌症治疗剂之前的至少5分钟、至少10分钟、至少30分钟、至少1小时、至少2小时、至少3小时、至少4小时、至少5小时、至少6小时、至少7小时、至少8小时、至少9小时、至少10小时、至少1天、至少2天、至少3天、至少5天、至少1周、至少2周、至少3周、至少4周、至少2个月、至多2个月、至多1个月、至多3周、至多2周、至多1周、至多6天、至多5天、至多4天、至多3天、至多2天、至多1天、至多12个小时、至多6小时、至多4小时、至多3小时、至多2小时或至多1小时)施用。在一些情况下,已在癌症治疗之前至少1天向患者施用了保护剂。在一些情况下,已在癌症治疗之前至多1天施用了保护剂。在一些情况下,在癌症治疗之后至多2小时内施用保护剂。在一些情况下,在癌症治疗之后至多4小时内施用保护剂。在一些情况下,在癌症治疗之后至多6小时内施用保护剂。在一些情况下,在癌症治疗之后至多12小时内施用保护剂。在一些情况下,在癌症治疗之后至多1天内施用保护剂。在一些情况下,在癌症治疗之后至多2天内施用保护剂。在一些情况下,在癌症治疗之后至多3天内施用保护剂。在一些情况下,在癌症治疗之后至多4天内施用保护剂。在一些情况下,在癌症治疗之后至多5天内施用保护剂。

[0152] 每当向患者施用本文所述的剂量方案的抗癌剂时,可向患者施用本公开的化合物(例如,式1的保护剂)。例如,每当在患者预定给药抗癌剂之前24小时内可向患者施用保护剂。在一些情况下,每当在患者预定给药抗癌剂之前48小时内可向患者施用保护剂。在一些情况下,每当患者给药抗癌剂时可同时向患者施用保护剂。在一些情况下,每当患者给药抗癌剂时可在癌症治疗后至少24小时内向患者施用保护剂。

[0153] 本公开的化合物可通过具有类似用途的试剂的任何可接受的给药模式施用，例如通过皮肤、口腔、局部、皮内、鞘内、静脉内、皮下、肌内、关节内、脊柱内或脊髓、鼻腔、硬膜外、直肠、阴道或鼻内/跨粘膜途径。最合适的途径取决于待治疗病状的性质和严重程度。本公开化合物的途径可以是皮下、皮内和经皮注射。本公开化合物的施用途径可以是舌下施用。本公开化合物的施用途径可以是静脉内施用。在一个具体实例中，可向患者经口施用本文提供的药物组合物。在另一个具体实例中，可向患者静脉内(经由例如注射或输注)施用包含本文提供的保护剂的药物组合物。在另一个具体实例中，可向患者肌内施用包含本文提供的保护剂的药物组合物。在一个具体实例中，可向患者鼻内施用包含本文提供的保护剂的药物组合物。

[0154] 药物组合物(例如，用于经口施用或用于注射、输注、皮下递送、肌内递送、腹膜内递送、舌下递送或其它方法)可呈液体形式。例如，液体药物组合物可包括以下中的一种或多种：无菌稀释剂，诸如水、盐溶液(优选生理盐水)、林格溶液、等渗氯化钠、可用作溶剂或悬浮介质的不挥发性油、聚乙二醇、甘油、丙二醇或其它溶剂；抗菌剂；抗氧化剂；螯合剂；缓冲剂和用于调节张力的试剂，诸如氯化钠或右旋糖。肠胃外组合物可被封装在由玻璃或塑料制成的安瓿、一次性注射器或多剂量小瓶中。优选使用生理盐水，并且可注射药物组合物优选是无菌的。在另一个实施方案中，为了治疗眼科病状或疾病，可以滴眼剂的形式将液体药物组合物施用于眼睛。液体药物组合物可经口递送。

[0155] 对于经口制剂，本文所述的至少一种化合物或试剂可单独使用或与合适的添加剂(并且如果需要，与稀释剂、缓冲剂、润湿剂、防腐剂、着色剂和调味剂)组合制成片剂、粉剂、颗粒剂或胶囊剂而使用。可用缓冲剂配制化合物，以保护化合物免受胃环境的低pH的影响和/或提供肠溶衣。药物组合物中所含的化合物可配制成用调味剂(例如，液体、固体或半固体制剂)和/或用肠溶衣经口递送。在一些情况下，本公开的化合物可被溶解和包封(例如，在脂质体或可生物降解的聚合物中)，或以涂有适当的无毒脂质的微晶形式使用。在一些情况下，本公开的化合物可被溶解和包封在脂质体、胶束或两者中。

[0156] 包含本文所述的任何一种化合物或药剂的药物组合物可配制用于持续释放或缓慢释放(也称为定时释放或控制释放)。此类组合物通常可使用众所周知的技术来制备，并且通过例如经口、直肠、皮内或皮下植入或通过在期望靶点植入来施用。持续释放制剂可含有分散在载体基质中的化合物和/或包含在由速率控制膜包围的贮存器内。这种制剂中使用的赋形剂是生物相容的，并且也可以是可生物降解的；优选地，该制剂提供相对恒定水平的活性组分释放。赋形剂的非限制性实例包括水、醇、甘油、壳聚糖、海藻酸盐、软骨素、维生素E、矿物油和二甲基亚砜(DMSO)。持续释放制剂中所含化合物的量取决于植入部位、释放速率和预计持续时间以及待治疗或预防病状、疾病或失调的性质。

[0157] 本文提供的公开内容还描述了通过向患者施用一种或多种式1或式2的保护剂来预防、降低或消除受试者器官损伤的方法。用于预防、降低或消除受试者器官损伤的式1或式2的保护剂可包括但不限于杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、或杨梅苷(或其衍生物或盐)。具体地讲，器官损伤可由心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激引起，从而导致心力衰竭。例如，可向正经受癌症治疗剂诱导的心力衰竭的患者施用包含式1的化合物(即保护剂)的药物组合物，其中通过施用药物组合物来防止进一步心力衰竭。

[0158] 本文所述的药物方法和组合物预防、降低或消除患者中癌症治疗剂引起的心脏毒性。因此，本文提供的方法和组合物使患者(例如，心脏健康患者、具有心脏疾病的患者)能够接受更高剂量的治疗，而不会由于心脏毒性风险而显著改变剂量方案。在一些情况下，向患者施用本文的药物组合物可包括向患者施用日剂量大于0.1mg/m^2、1mg/m^2、2mg/m^2、3mg/m^2、4mg/m^2、5mg/m^2、6mg/m^2、7mg/m^2、8mg/m^2、9mg/m^2、10mg/m^2、11mg/m^2、12mg/m^2、13mg/m^2、14mg/m^2、15mg/m^2、16mg/m^2、17mg/m^2、18mg/m^2、19mg/m^2、20mg/m^2、21mg/m^2、22mg/m^2、23mg/m^2、24mg/m^2、25mg/m^2、26mg/m^2、27mg/m^2、28mg/m^2、29mg/m^2、30mg/m^2、31mg/m^2、32mg/m^2、33mg/m^2、34mg/m^2、35mg/m^2、36mg/m^2、37mg/m^2、38mg/m^2、39mg/m^2、40mg/m^2、41mg/m^2、42mg/m^2、43mg/m^2、44mg/m^2、45mg/m^2、46mg/m^2、47mg/m^2、48mg/m^2、49mg/m^2、50mg/m^2、100mg/m^2、150mg/m^2、200mg/m^2、300mg/m^2、350mg/m^2、400mg/m^2、450mg/m^2、500mg/m^2、750mg/m^2、1000mg/m^2、1250mg/m^2、1500mg/m^2、1750mg/m^2或2000mg/m^2的化疗药物(例如，蒽环类，多柔比星或其衍生物或盐)。

[0159] 在一些情况下，向患者施用本文的药物组合物可包括向患者施用日剂量约0.1mg/m^2、0.2mg/m^2、0.3mg/m^2、0.4mg/m^2、0.5mg/m^2、0.6mg/m^2、0.7mg/m^2、0.8mg/m^2、0.9mg/m^2、1mg/m^2、1.1mg/m^2、1.2mg/m^2、1.3mg/m^2、1.4mg/m^2、1.5mg/m^2、1.6mg/m^2、1.7mg/m^2、1.8mg/m^2、1.9mg/m^2、2mg/m^2、2.1mg/m^2、2.2mg/m^2、2.3mg/m^2、2.4mg/m^2、2.5mg/m^2、2.6mg/m^2、2.7mg/m^2、2.8mg/m^2、2.9mg/m^2、3mg/m^2、3.1mg/m^2、3.2mg/m^2、3.3mg/m^2、3.4mg/m^2、3.5mg/m^2、3.6mg/m^2、3.7mg/m^2、3.8mg/m^2、3.9mg/m^2、4mg/m^2、4.1mg/m^2、4.2mg/m^2、4.3mg/m^2、4.4mg/m^2、4.5mg/m^2、4.6mg/m^2、4.7mg/m^2、4.8mg/m^2、4.9mg/m^2、5mg/m^2、5.1mg/m^2、5.2mg/m^2、5.3mg/m^2、5.4mg/m^2、5.5mg/m^2、5.6mg/m^2、5.7mg/m^2、5.8mg/m^2、5.9mg/m^2、6mg/m^2、6.1mg/m^2、6.2mg/m^2、6.3mg/m^2、6.4mg/m^2、6.5mg/m^2、6.6mg/m^2、6.7mg/m^2、6.8mg/m^2、6.9mg/m^2、7mg/m^2、7.1mg/m^2、7.2mg/m^2、7.3mg/m^2、7.4mg/m^2、7.5mg/m^2、7.6mg/m^2、7.7mg/m^2、7.8mg/m^2、7.9mg/m^2、8mg/m^2、8.1mg/m^2、8.2mg/m^2、8.3mg/m^2、8.4mg/m^2、8.5mg/m^2、8.6mg/m^2、8.7mg/m^2、8.8mg/m^2、8.9mg/m^2、9mg/m^2、9.1mg/m^2、9.2mg/m^2、9.3mg/m^2、9.4mg/m^2、9.5mg/m^2、9.6mg/m^2、9.7mg/m^2、9.8mg/m^2、9.9mg/m^2、10mg/m^2、11mg/m^2、12mg/m^2、13mg/m^2、14mg/m^2、15mg/m^2、16mg/m^2、17mg/m^2、18mg/m^2、19mg/m^2、20mg/m^2、21mg/m^2、22mg/m^2、23mg/m^2、24mg/m^2、25mg/m^2、26mg/m^2、27mg/m^2、28mg/m^2、29mg/m^2、30mg/m^2、31mg/m^2、32mg/m^2、33mg/m^2、34mg/m^2、35mg/m^2、36mg/m^2、37mg/m^2、38mg/m^2、39mg/m^2、40mg/m^2、41mg/m^2、42mg/m^2、43mg/m^2、44mg/m^2、45mg/m^2、46mg/m^2、47mg/m^2、48mg/m^2、49mg/m^2、50mg/m^2、51mg/m^2、52mg/m^2、53mg/m^2、54mg/m^2、55mg/m^2、56mg/m^2、57mg/m^2、58mg/m^2、59mg/m^2、60mg/m^2、61mg/m^2、62mg/m^2、63mg/m^2、64mg/m^2、65mg/m^2、66mg/m^2、67mg/m^2、68mg/m^2、69mg/m^2、70mg/m^2、71mg/m^2、72mg/m^2、73mg/m^2、74mg/m^2、75mg/m^2、76mg/m^2、77mg/m^2、78mg/m^2、79mg/m^2、80mg/m^2、81mg/m^2、82mg/m^2、83mg/m^2、84mg/m^2、85mg/m^2、86mg/m^2、87mg/m^2、88mg/m^2、89mg/m^2、90mg/m^2、91mg/m^2、92mg/m^2、93mg/m^2、94mg/m^2、95mg/m^2、96mg/m^2、97mg/m^2、98mg/m^2、99mg/m^2或100mg/m^2的生物剂。

[0160] 在一些情况下，向患者施用本文的药物组合物可包括向患者施用日剂量约0.1mg/m^2、1mg/m^2、2mg/m^2、3mg/m^2、4mg/m^2、5mg/m^2、6mg/m^2、7mg/m^2、8mg/m^2、9mg/m^2、10mg/m^2、11mg/m^2、12mg/m^2、13mg/m^2、14mg/m^2、15mg/m^2、16mg/m^2、17mg/m^2、18mg/m^2、19mg/m^2、20mg/m^2、21mg/m^2、22mg/m^2、23mg/m^2、24mg/m^2、25mg/m^2、26mg/m^2、27mg/m^2、28mg/m^2、29mg/m^2、30mg/m^2、31mg/m^2、32mg/m^2、33mg/m^2、34mg/m^2、35mg/m^2、36mg/m^2、37mg/m^2、38mg/m^2、39mg/m^2、40mg/m^2、41mg/m^2、42mg/m^2、43mg/m^2、44mg/m^2、45mg/m^2、46mg/m^2、47mg/m^2、48mg/m^2、49mg/m^2、50mg/m^2、51mg/

m^2、52mg/m^2、53mg/m^2、54mg/m^2、55mg/m^2、56mg/m^2、57mg/m^2、58mg/m^2、59mg/m^2、60mg/m^2、61mg/m2、62mg/m^2、63mg/m^2、64mg/m^2、65mg/m^2、66mg/m^2、67mg/m^2、68mg/m^2、69mg/m^2、70mg/m^2、71mg/m^2、72mg/m^2、73mg/m^2、74mg/m^2、75mg/m^2、76mg/m^2、77mg/m^2、78mg/m^2、79mg/m^2、80mg/m^2、81mg/m^2、82mg/m^2、83mg/m^2、84mg/m^2、85mg/m^2、86mg/m^2、87mg/m^2、88mg/m^2、89mg/m^2、90mg/m^2、90mg/m^2、95mg/m^2、100mg/m^2、110mg/m^2、120mg/m^2、130mg/m^2、140mg/m^2、150mg/m^2、160mg/m^2、170mg/m^2、180mg/m^2、190mg/m^2、200mg/m^2、300mg/m^2、400mg/m^2、500mg/m^2的式1或式2的保护剂药物(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和/或其衍生物或盐)。

[0161] 本文所述的保护剂的日固定剂量或保护剂组合的总体剂量可含有大于0.1mg、1mg、2mg、3mg、4mg、5mg、6mg、7mg、8mg、9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、150mg、200mg、300mg、350mg、400mg、450mg、500mg、750mg或更高的保护剂(或其任何衍生物或盐)。在一些情况下,该一种或多种保护剂选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和/或其衍生物或盐。在一个具体实例中,向患者施用药物组合物可包括施用化学疗法药物(例如,多柔比星)与至少10mg杨梅素的共同制剂。在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的杨梅素(或其任何衍生物或盐)。

[0162] 在另一个实例中,向患者施用药物组合物可包括施用化学疗法药物(例如,多柔比星)与至少10mg的杨梅苷的共同制剂。在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、

13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的杨梅苷(或其任何衍生物或盐)。

[0163] 在又一个实例中,向患者施用药物组合物可包括施用化学药物(例如,多柔比星)与至少10mg牡荆素的共同制剂。在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的牡荆素(或其任何衍生物或盐)。

[0164] 在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的洋槐黄素(或其任何衍生物或盐)。

[0165] 在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、

18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的五羟黄酮(或其任何衍生物或盐)。

[0166] 在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的7,3',4',5'-四羟基黄酮(或其任何衍生物或盐)。

[0167] 在一些情况下,向患者施用本文的药物组合物可包括向患者施用0.1mg、0.2mg、0.3mg、0.4mg、0.5mg、0.6mg、0.7mg、0.8mg、0.9mg、1mg、1.1mg、1.2mg、1.3mg、1.4mg、1.5mg、1.6mg、1.7mg、1.8mg、1.9mg、2mg、2.1mg、2.2mg、2.3mg、2.4mg、2.5mg、2.6mg、2.7mg、2.8mg、2.9mg、3mg、3.1mg、3.2mg、3.3mg、3.4mg、3.5mg、3.6mg、3.7mg、3.8mg、3.9mg、4mg、4.1mg、4.2mg、4.3mg、4.4mg、4.5mg、4.6mg、4.7mg、4.8mg、4.9mg、5mg、5.1mg、5.2mg、5.3mg、5.4mg、5.5mg、5.6mg、5.7mg、5.8mg、5.9mg、6mg、6.1mg、6.2mg、6.3mg、6.4mg、6.5mg、6.6mg、6.7mg、6.8mg、6.9mg、7mg、7.1mg、7.2mg、7.3mg、7.4mg、7.5mg、7.6mg、7.7mg、7.8mg、7.9mg、8mg、8.1mg、8.2mg、8.3mg、8.4mg、8.5mg、8.6mg、8.7mg、8.8mg、8.9mg、9mg、9.1mg、9.2mg、9.3mg、9.4mg、9.5mg、9.6mg、9.7mg、9.8mg、9.9mg、10mg、11mg、12mg、13mg、14mg、15mg、16mg、17mg、18mg、19mg、20mg、21mg、22mg、23mg、24mg、25mg、26mg、27mg、28mg、29mg、30mg、31mg、32mg、33mg、34mg、35mg、36mg、37mg、38mg、39mg、40mg、41mg、42mg、43mg、44mg、45mg、46mg、47mg、48mg、49mg、50mg、100mg、500mg、750mg或1g日剂量的ficetin(或其任何衍生物或盐)。

[0168] 本文所述的药物方法和组合物预防、降低或消除患者中癌症治疗剂引起的心脏毒性。因此,本文提供的方法和组合物使患者能够更频繁地接受治疗,而不会由于心脏毒性风险而显著改变剂量方案。本文提供的药物组合物内的化疗药物、生物剂或保护剂的日剂量可以每天一次或多次剂量施用于患者。在一些情况下,化疗药物的日剂量可以单次剂量施用。在一些情况下,化疗药物的日剂量可分成每天1、2、3、4、5、6、7、8、9或10次剂量。例如,化疗药物(例如,多柔比星)的日剂量可分成每天3次剂量。在一些情况下,化疗药物的日剂量可分成每小时至少1、2、3、4、5、6、7、8、9、10、15、20、25、30、35、40、45、50、55或60次输注。在一些情况下,包含化疗药物的组合物的每次输注可持续至少5分钟、10分钟、15分钟、20分钟、25分钟、30分钟、35分钟、40分钟、45分钟、50分钟、55分钟、1小时、1.5小时、2小时、2.5小时、3小时、3.5小时、4小时、4.5小时、5小时、5.5小时或6小时。在一些情况下,生物剂的日剂量可以单次剂量施用。在一些情况下,生物剂的日剂量可分成每天1、2、3、4、5、6、7、8、9、10、

11、12、13、14、15、16、17、18、19、20、21、22、23或24次剂量。例如，生物剂(例如，贝伐单抗)的日剂量可分成每天3次剂量。在一些情况下，生物剂的日剂量可分成每小时至少1、2、3、4、5、6、7、8、9、10、15、20、25、30、35、40、45、50、55或60次输注。在一些情况下，包含生物剂的组合物的每次输注可持续至少5分钟、10分钟、15分钟、20分钟、25分钟、30分钟、35分钟、40分钟、45分钟、50分钟、55分钟、1小时、1.5小时、2小时、2.5小时、3小时、3.5小时、4小时、4.5小时、5小时、5.5小时或6小时。在一些情况下，保护剂的日剂量可以单次剂量施用。在一些情况下，保护剂的日剂量可分成每天1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23或24次剂量。例如，保护剂(例如，杨梅素)的日剂量可分成每天3次剂量。在一些情况下，保护剂的日剂量可分成每小时至少1、2、3、4、5或6次输注。在一些情况下，包含一种或多种保护剂的组合物的每次输注可持续至少5分钟、10分钟、15分钟、20分钟、25分钟、30分钟、35分钟、40分钟、45分钟、50分钟、55分钟、1小时、1.5小时、2小时、2.5小时、3小时、3.5小时、4小时、4.5小时、5小时、5.5小时或6小时。

[0169] 可每天一次或多次向患者施用本文所述的药物组合物。在一些情况下，可每天一次向患者施用药物组合物。在一些情况下，可每天至少2次、3次、4次、5次、6次、7次、8次、9次、10次、11次、12次、13次、14次、15次、16次、17次、18次、19次、20次、21次、22次、23次或24次向患者施用药物组合物。例如，可每天3次向患者施用药物组合物。

[0170] 可持续一天或多天向患者施用本文所述的药物组合物。在一些情况下，可持续一天向患者施用药物组合物。在一些情况下，可持续至少2天、3天、4天、5天、6天、1周、2周、3周、1个月、2个月、3个月、4个月、5个月、6个月、7个月、8个月、9个月、10个月、11个月、1年、2年、3年、4年、5年、6年、7年、8年、9年、10年、20年、30年、40年或50年向患者施用药物组合物。例如，可持续至少1年的周期向癌症患者施用多柔比星和杨梅素的药物共同制剂。在一些情况下，可连续两天或更多天向患者施用药物组合物。在一些情况下，可持续两天或更多非连续天向患者施用药物组合物。例如，可连续4天每天向患者施用药物组合物。在另一个实例中，可在第1天、第3天、第7天和第15天向患者施用药物组合物。在一些情况下，当在一段时间内向患者施用药物组合物时，一天施用于患者的剂量可不同于随后一天施用于患者的剂量。例如，可在第一天向患者施用5mg药物组合物，并在随后一天施用10mg药物组合物。

[0171] 本文所述的药物组合物可随时间有效。在一些情况下，药物组合物可持续一天或更多天有效。在一些情况下，药物组合物的效力持续较长的时间段。在一些情况下，药物组合物的效力可大于2天、3天、4天、5天、6天、1周、2周、3周或1个月。

[0172] 本文提供的方法可还包括向患者施用作为本文所述任何药物组合物的一部分的右雷佐生(或其任何衍生物或盐)。此类方法允许向患者施用含有至少一种保护剂和右雷佐生的药物组合物，其中与单独施用右雷佐生相比，至少一种保护剂和右雷佐生的共制剂可提供更大的保护作用。在一些情况下，本文所述的任何药物组合物的施用可使患者库中心脏毒性的可能性降低多达1％、2％、3％、4％、5％、6％、8％、9％、10％、11％、12％、13％、14％、15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％、30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％、50％、51％、52％、53％、54％、55％、56％、57％、58％、59％、60％、61％、62％、63％、64％、65％、66％、67％、68％、69％、70％、71％、72％、73％、74％、75％、76％、77％、78％、79％、80％、81％、82％、83％、84％、85％、86％、87％、88％、

89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％。例如，如果施用右雷佐生的患者库中80％的患者可能会出现心脏毒性，则向患者施用杨梅素和右雷佐生的共同制剂可使心脏毒性的可能性降低75％，使得20％的患者可能会出现心脏毒性。这种更大的保护作用还可使更大的患者群体(包括先前存在心脏病状的患者)能够接受癌症治疗剂(例如，多柔比星)，否则它们将被排除在外。在一些情况下，右雷佐生可共同配制在药物组合物中，其中其在药物组合物内混合或作为不同实体存在。在一些情况下，癌症治疗剂、保护剂和右雷佐生可同时施用。在一些情况下，癌症治疗剂、保护剂和右雷佐生可依次施用。在一个实例中，可每天至少一次以单剂量向癌症患者施用化疗药物、右雷佐生和杨梅素的共同制剂。在另一个实例中，可向癌症患者施用右雷佐生，随后施用杨梅素。

[0173] 施用的药物组合物内右雷佐生(或其任何衍生物或盐)的剂量可大于0.1mg/m^2、1mg/m^2、2mg/m^2、3mg/m^2、4mg/m^2、5mg/m^2、6mg/m^2、7mg/m^2、8mg/m^2、9mg/m^2、10mg/m^2、11mg/m^2、12mg/m^2、13mg/m^2、14mg/m^2、15mg/m^2、16mg/m^2、17mg/m^2、18mg/m^2、19mg/m^2、20mg/m^2、21mg/m^2、22mg/m^2、23mg/m^2、24mg/m^2、25mg/m^2、26mg/m^2、27mg/m^2、28mg/m^2、29mg/m^2、30mg/m^2、31mg/m^2、32mg/m^2、33mg/m^2、34mg/m^2、35mg/m^2、36mg/m^2、37mg/m^2、38mg/m^2、39mg/m^2、40mg/m^2、41mg/m^2、42mg/m^2、43mg/m^2、44mg/m^2、45mg/m^2、46mg/m^2、47mg/m^2、48mg/m^2、49mg/m^2、50mg/m^2、51mg/m^2、52mg/m^2、53mg/m^2、54mg/m^2、55mg/m^2、56mg/m^2、57mg/m^2、58mg/m^2、59mg/m^2、60mg/m^2、61mg/m^2、62mg/m^2、63mg/m^2、64mg/m^2、65mg/m^2、66mg/m^2、67mg/m^2、68mg/m^2、69mg/m^2、70mg/m^2、71mg/m^2、72mg/m^2、73mg/m^2、74mg/m^2、75mg/m^2、76mg/m^2、77mg/m^2、78mg/m^2、79mg/m^2、80mg/m^2、81mg/m^2、82mg/m^2、83mg/m^2、84mg/m^2、85mg/m^2、86mg/m^2、87mg/m^2、88mg/m^2、89mg/m^2、90mg/m^2、91mg/m^2、92mg/m^2、93mg/m^2、94mg/m^2、95mg/m^2、96mg/m^2、97mg/m^2、98mg/m^2、99mg/m^2、100mg/m^2、150mg/m^2、200mg/m^2、300mg/m^2、350mg/m^2、400mg/m^2、450mg/m^2、500mg/m^2、750mg/m^2、1g/m^2、5g/m^2、10g/m^2或更高。在一个具体实例中，向患者施用药物组合物可包括施用式1或式2的保护剂(例如，杨梅素)与50mg/m^2右雷佐生的共同制剂。

[0174] 在一些情况下，向患者施用本文所述的药物组合物可包括施用约0.1mg/kg、0.2mg/kg、0.3mg/kg、0.4mg/kg、0.5mg/kg、0.6mg/kg、0.7mg/kg、0.8mg/kg、0.9mg/kg、1mg/kg、1.5mg/kg、2mg/kg、2.5mg/kg、3mg/kg、3.5mg/kg、4mg/kg、4.5mg/kg、5mg/kg、6mg/kg、7mg/kg、8mg/kg、9mg/kg、10mg/kg、11mg/kg、12mg/kg、13mg/kg、14mg/kg、15mg/kg、16mg/kg、17mg/kg、18mg/kg、19mg/kg、20mg/kg、21mg/kg、22mg/kg、23mg/kg、24mg/kg、25mg/kg、26mg/kg、27mg/kg、28mg/kg、29mg/kg、30mg/kg、31mg/kg、32mg/kg、33mg/kg、34mg/kg、35mg/kg、36mg/kg、37mg/kg、38mg/kg、39mg/kg、40mg/kg、41mg/kg、42mg/kg、43mg/kg、44mg/kg、45mg/kg、46mg/kg、47mg/kg、48mg/kg、49mg/kg、50mg/kg、51mg/kg、52mg/kg、53mg/kg、54mg/kg、55mg/kg、56mg/kg、57mg/kg、58mg/kg、59mg/kg、60mg/kg、61mg/kg、62mg/kg、63mg/kg、64mg/kg、65mg/kg、66mg/kg、67mg/kg、68mg/kg、69mg/kg、70mg/kg、71mg/kg、72mg/kg、73mg/kg、74mg/kg、75mg/kg、76mg/kg、77mg/kg、78mg/kg、79mg/kg、80mg/kg、81mg/kg、82mg/kg、83mg/kg、84mg/kg、85mg/kg、86mg/kg、87mg/kg、88mg/kg、89mg/kg、90mg/kg、90mg/kg、95mg/kg、100mg/kg、110mg/kg、120mg/kg、130mg/kg、140mg/kg、150mg/kg、160mg/kg、170mg/kg、180mg/kg、190mg/kg、200mg/kg、300mg/kg、400mg/kg、500mg/kg的式1或式2的保护剂。在一些方面，保护剂可选自杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷及其衍生物或盐。在一个实施方案中，向患者静脉内施用

0.5mg/kg、1mg/kg、1.5mg/kg、2mg/kg、2.5mg/kg、3mg/kg、3.5mg/kg、4mg/kg、4.5mg/kg、5mg/kg、6mg/kg、7mg/kg、8mg/kg、9mg/kg、10mg/kg、11mg/kg、12mg/kg、13mg/kg、14mg/kg、15mg/kg、16mg/kg、17mg/kg、18mg/kg、19mg/kg、20mg/kg、21mg/kg、22mg/kg、23mg/kg、24mg/kg、25mg/kg、26mg/kg、27mg/kg、28mg/kg、29mg/kg、30mg/kg、31mg/kg、32mg/kg、33mg/kg、34mg/kg、35mg/kg、36mg/kg、37mg/kg、38mg/kg、39mg/kg、40mg/kg、41mg/kg、42mg/kg、43mg/kg、44mg/kg、45mg/kg、46mg/kg、47mg/kg、48mg/kg、49mg/kg、50mg/kg、60mg/kg、70mg/kg、80mg/kg、90mg/kg或100mg/kg、150mg/kg或200mg/kg的保护剂。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少10分钟向患者施用剂量在约0.5mg/kg至约50mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少10分钟向患者施用剂量在约0.5mg/k至约100mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少30分钟向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少1小时向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少2小时向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少4小时向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少6小时向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)后6小时内向患者静脉内施用剂量在约0.5mg/kg至约200mg/kg之间的杨梅素。在一个实施方案中，在施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少0.5、1、2、3、4、5或6小时向患者经口施用剂量在0.5mg/kg至200mg/kg之间的杨梅素。

[0175] 在一些方面，在患者被诊断患有癌症之后首次施用蒽环类(例如，多柔比星、表柔比星或伊达比星)之前至少4小时向患者例如静脉内或经口施用剂量在约0.5mg/kg至约200mg/kg之间的保护剂(例如，杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素或杨梅苷)。

[0176] 患者反应

[0177] 本文提供的方法和组合物预防、降低或消除患者中由化疗药物、生物剂或放射疗法引起的心脏毒性。此外，向患者施用本文公开的药物组合物还可预防、降低或消除癌症治疗剂诱导的器官损伤(例如，由心脏组织损伤、电生理异常、线粒体毒性、细胞凋亡或氧化应激引起的器官损伤)。

[0178] 本文公开的方法和组合物通常可降低患者的心脏毒性。心脏毒性的实例可包括但不限于线粒体毒性、细胞凋亡、电生理异常(例如，QT延长)、机械性故障(例如，心脏射血分数减少)、氧化应激、心脏组织损伤(例如，由氧化应激、线粒体引起的损伤或由活性氧通量增加引起的损伤)以及对任何不是心脏的器官(例如，肝脏、肾脏或胰腺)的细胞毒性损伤。

[0179] 线粒体毒性可指降低给定细胞、组织、器官或生物体内活性线粒体数量的任何损伤。在一些情况下，可使用体外测定来测量线粒体毒性。一种可用于测量线粒体毒性的此类方法是通过将细胞共暴露于：(1)指示细胞核的细胞渗透性荧光染料和(2)四甲基罗丹明甲

基酯(TMRM)，即被活性线粒体吸收的细胞渗透性荧光染料。线粒体毒性可被计算为TMRM阳性细胞与细胞核总数的比例。如通过体外测定所测量的，与未处理的对照相比，在心肌细胞中癌症治疗剂诱导的线粒体毒性可大于1%、2%、3%、4%、5%、6%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、21%、22%、23%、24%、25%、26%、27%、28%、29%、30%、31%、32%、33%、34%、35%、36%、37%、38%、39%、40%、41%、42%、43%、44%、45%、46%、47%、48%、49%、50%、51%、52%、53%、54%、55%、56%、57%、58%、59%、60%、61%、62%、63%、64%、65%、66%、67%、68%、69%、70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%。例如、与未处理的对照相比，将心肌细胞暴露于1微摩尔多柔比星至少48小时可引起100%的线粒体毒性。本文所述的药物方法和组合物通常降低癌症治疗剂诱导的线粒体毒性。如通过体外测定所测量的，与在不存在保护剂的情况下暴露于癌症治疗剂的心肌细胞相比，将心肌细胞暴露于本文所述的任何药物组合物可使线粒体毒性降低多达100%、99%、98%、97%、96%、95%、94%、93%、92%、91%、90%、89%、88%、87%、86%、85%、84%、83%、82%、81%、80%、79%、78%、77%、76%、75%、74%、73%、72%、71%、70%、69%、68%、67%、66%、65%、64%、63%、62%、61%、60%、59%、58%、57%、56%、55%、54%、53%、52%、51%、50%、49%、48%、47%、46%、45%、44%、43%、42%、41%、40%、39%、38%、37%、36%、35%、34%、33%、32%、31%、30%、29%、28%、27%、26%、25%、24%、23%、22%、21%、20%、19%、18%、17%、16%、15%、14%、13%、12%、11%、10%、9%、8%、7%、6%、5%、4%、3%、2%或1%。例如，与将心肌细胞暴露于1微摩尔的多柔比星相比，将心肌细胞暴露于1微摩尔的多柔比星和115微摩尔的杨梅素的共同制剂至少48小时可使线粒体毒性降低30%。

[0180] 细胞凋亡可指细胞经历程序性细胞死亡的过程。经历细胞凋亡的细胞内可检测变化包括但不限于细胞色素C自线粒体的易位、线粒体功能减弱、膜结构变化、蛋白水解活性增加和DNA断裂。在一些情况下，可使用体外测定来测量细胞凋亡。一种可用于测量细胞凋亡的此类方法是通过将细胞共暴露于：(1)指示细胞核的细胞渗透性荧光染料以及(2)CellEvent胱天蛋白酶3/7检测试剂，即唯一存在于凋亡细胞中的活化胱天蛋白酶3的荧光底物。细胞凋亡百分比可被计算为CellEvent阳性细胞与细胞核总数的比例。如通过体外测定所测量的，与未处理的对照相比，在心肌细胞中癌症治疗剂诱导的细胞凋亡可大于1%、2%、3%、4%、5%、6%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、21%、22%、23%、24%、25%、26%、27%、28%、29%、30%、31%、32%、33%、34%、35%、36%、37%、38%、39%、40%、41%、42%、43%、44%、45%、46%、47%、48%、49%、50%、51%、52%、53%、54%、55%、56%、57%、58%、59%、60%、61%、62%、63%、64%、65%、66%、67%、68%、69%、70%、71%、72%、73%、74%、75%、76%、77%、78%、79%、80%、81%、82%、83%、84%、85%、86%、87%、88%、89%、90%、91%、92%、93%、94%、95%、96%、97%、98%或99%。例如，与未处理的对照相比，将心肌细胞暴露于1微摩尔多柔比星至少48小时可引起100%的细胞凋亡。

[0181] 本文所述的药物方法和组合物通常可减少癌症治疗剂诱导的细胞凋亡。如通过体外测定所测量的，与在不存在保护剂的情况下暴露于癌症治疗剂的心肌细胞相比，将心肌

细胞暴露于本文所述的任何药物组合物可使细胞凋亡减少多达100％、99％、98％、97％、96％、95％、94％、93％、92％、91％、90％、89％、88％、87％、86％、85％、84％、83％、82％、81％、80％、79％、78％、77％、76％、75％、74％、73％、72％、71％、70％、69％、68％、67％、66％、65％、64％、63％、62％、61％、60％、59％、58％、57％、56％、55％、54％、53％、52％、51％、50％、49％、48％、47％、46％、45％、44％、43％、42％、41％、40％、39％、38％、37％、36％、35％、34％、33％、32％、31％、30％、29％、28％、27％、26％、25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％或1％。例如，与将心肌细胞暴露于1微摩尔多柔比星相比，将心肌细胞暴露于1微摩尔的多柔比星和115微摩尔的杨梅素的共同制剂至少48小时可使线粒体毒性降低30％。

[0182] 电生理异常可指其中离子通过生物组织的流动被破坏的任何损伤。例如，向癌症患者施用化疗药物(例如，多柔比星)可引起急性心肌梗塞，其中离子不能再流经受损的心脏组织，从而导致传导阻滞。在一些情况下，电生理异常可包括QT间期延长，并且可使用体内测定来测量。QT间期可用于描述心电图中Q波开始和T波结束之间的时间。QT延长可指示心室复极化延迟，并且可使心脏易于发生早期后去极化(EAD)，从而导致折返性心律失常(例如，尖端扭转)。QT间期也可取决于心动周期(RR)的长度，即一个QRS波群起始到下一个QRS波群起始之间的时间量。经校正的QT(QTc)间期可用于表示考虑到周期长度经校正的QT间期。Bazett公式(QTc＝QT/√RR)、Fridericia公式(QTc＝QT/^{3}√RR)或回归分析法(QTc＝QT+0.154(1-RR))都可用于从QT间期计算QTc间期。

[0183] 在不存在保护剂的情况下，向患者施用化疗药物、生物剂或放射疗法可引起超过患者基线QTc间期的QTc延长。患者的基线QTc间期是在患者施用任何药物之前测量的QTc间期。例如，在不存在保护剂的情况下向患者施用化疗药物(例如，多柔比星)可使QTc延长超过患者基线QTc间期40毫秒(ms)。在一些情况下，向患者施用化疗药物、生物剂或放射疗法(尤其是在不存在保护剂的情况下)可使QTc延长超过患者基线QTc间期至少1ms、2ms、3ms、4ms、5ms、6ms、7ms、8ms、9ms、10ms、15ms、20ms、25ms、30ms、35ms、40ms、45ms、50ms、55ms、60ms、65ms、70ms、75ms、80ms、85ms、90ms、95ms或100ms。

[0184] 本文所述的任何药物组合物的施用可限制患者经受的超过患者基线QTc间期的癌症治疗剂诱导的QTc延长。例如，向患者施用化疗药物(例如，多柔比星)和保护剂(例如，杨梅素)的共同制剂可使QTC延长小于5ms。在一些情况下，本文描述的药物组合物可使QTc延长超过患者基线QTc间期小于100ms、95ms、90ms、85ms、80ms、75ms、70ms、65ms、60ms、55ms、50ms、45ms、40ms、35ms、30ms、25ms、20ms、15ms、10ms、9ms、8ms、7ms、6ms、5ms、4ms、3ms、2ms或1ms。

[0185] 在一些情况下，电生理异常还可包括电活性降低，并且可使用体外测定来测量。多电极阵列(MEA)是包含多个平面导电电极的装置，细胞(例如，心肌细胞)可接触这些电极。尽管从MEA测量的电记录的大小和形状可取决于若干因素(例如，细胞均匀性、细胞与电极之间的接触、MEA的几何形状)，但可通过电极测量时间变化来提供关于接触细胞的电活性信息(例如，活性电极的百分比、场电位持续时间和搏动率)。

[0186] 如通过体外测定所测量的，在不存在保护剂的情况下将心肌细胞暴露于化疗药物、生物剂或放射疗法可引起活性电极(例如，能够测量接触细胞的某些电活性的电极)的

百分比暂时降低。例如，与时间零点相比，将心肌细胞暴露于1微摩尔多柔比星至少24小时可引起活性电极数量减少50％。在一些情况下，在不存在保护剂(例如，杨梅素)的情况下将心肌细胞暴露于癌症治疗剂(例如，多柔比星)可使活性电极数量减少多达100％、99％、98％、97％、96％、95％、94％、93％、92％、91％、90％、89％、88％、87％、86％、85％、84％、83％、82％、81％、80％、79％、78％、77％、76％、75％、74％、73％、72％、71％、70％、69％、68％、67％、66％、65％、64％、63％、62％、61％、60％、59％、58％、57％、56％、55％、54％、53％、52％、51％、50％、49％、48％、47％、46％、45％、44％、43％、42％、41％、40％、39％、38％、37％、36％、35％、34％、33％、32％、31％、30％、29％、28％、27％、26％、25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％或1％。在一个具体实例中，将心肌细胞暴露于1μM多柔比星至少24小时可引起活性电极数量减少50％。

[0187] 本文描述的药物方法和组合物通常减少癌症治疗剂诱导的电生理异常(例如，活性电极数量减少)。如通过体外测定所测量的，与在不存在保护剂的情况下暴露于癌症治疗剂的心肌细胞相比，将心肌细胞暴露于本文所述的任何药物组合物可使活性电极的数量减少小于1％、2％、3％、4％、5％、6％、8％、9％、10％、11％、12％、13％、14％、15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％、30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％、50％、51％、52％、53％、54％、55％、56％、57％、58％、59％、60％、61％、62％、63％、64％、65％、66％、67％、68％、69％、70％、71％、72％、73％、74％、75％、76％、77％、78％、79％、80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％。例如，将心肌细胞暴露于1μM多柔比星和100μM杨梅素的共同制剂至少24小时可使活性电极数量减少少于5％。

[0188] 本文所述的药物方法和组合物通常降低患者在施用癌症治疗剂时将经历心脏毒性的风险。在一些情况下，本文所述的药物方法和组合物可使患者心脏毒性的风险降低100％、99％、98％、97％、96％、95％、94％、93％、92％、91％、90％、89％、88％、87％、86％、85％、84％、83％、82％、81％、80％、79％、78％、77％、76％、75％、74％、73％、72％、71％、70％、69％、68％、67％、66％、65％、64％、63％、62％、61％、60％、59％、58％、57％、56％、55％、54％、53％、52％、51％、50％、49％、48％、47％、46％、45％、44％、43％、42％、41％、40％、39％、38％、37％、36％、35％、34％、33％、32％、31％、30％、29％、28％、27％、26％、25％、24％、23％、22％、21％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％或1％。在一些情况下，本文公开的药物方法和组合物可使患者心脏毒性的风险降低大于10％、11％、12％、13％、14％、15％、16％、17％、18％、19％、20％、21％、22％、23％、24％、25％、26％、27％、28％、29％、30％、31％、32％、33％、34％、35％、36％、37％、38％、39％、40％、41％、42％、43％、44％、45％、46％、47％、48％、49％、50％、51％、52％、53％、54％、55％、56％、57％、58％、59％、60％、61％、62％、63％、64％、65％、66％、67％、68％、69％、70％、71％、72％、73％、74％、75％、76％、77％、78％、79％、80％、81％、82％、83％、84％、85％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％或99％。例如，如果在不存在保护剂(例如，杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、杨梅苷和/或其衍生物或盐))

的情况下施用化疗药物(例如,多柔比星、表柔比星或伊达比星)时患者具有90％的经受QT延长的风险,则在单独施用保护剂或与化疗药物作为共同制剂施用时,患者可经受降低50％的QT延长风险,使得患者QT间期延长的风险为45％。例如,在一个具体实施方案中,在施用化疗药物(例如,多柔比星、表柔比星或伊达比星))之前至少30分钟、1小时、2小时、3小时、4小时、5小时或6小时向患者静脉内施用剂量在约0.5mg/kg和约100mg/kg之间的保护剂(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四氢黄酮、杨梅苷和/或其衍生物或盐),其中QT延长风险与未接收保护剂的对照组相比降低至少30％、40％、50％、60％、70％、80％或90％。

[0189] 蒽环类诱导的心脏毒性对收缩性的作用还可通过测量收缩功能指标-缩短分数(FS)和射血分数(EF)来评估。蒽环类诸如多柔比星可对收缩特性产生深远影响。然而,施用了式1或式2的保护剂(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和/或衍生物或盐)的患者可经历显著降低的例如多柔比星诱导的心脏毒性,如通过FS和EF的显著改善观察到的。例如,与已用蒽环类治疗但没有与保护剂一起给药的对照组相比,杨梅素可挽救患者中至少30％、40％、50％、60％、70％、80％、90％的蒽环类诱导的FS和EF异常。

[0190] 当提及数值或数值范围时,术语"约"意指所提及的数值或数值范围是实验可变性内(或实验统计误差内)的近似值,并且因此该数值或数值范围可在例如所述数值或数值范围的1％和10％之间变化。

[0191] 术语"治疗有效量"通常可指在向需要化合物或其它疗法的个体施用时足以预防、减少、治疗或消除病状或其风险的此类化合物或其它疗法的最小量(或剂量)。在一些情况下,术语"治疗有效量"可指施用于受试者时足以具有预防作用的化合物或其它疗法的量。治疗有效量可以变化;例如,其可根据受试者的状况、受试者的体重和年龄、疾病病状的严重程度、施用方式等而变化,所有这些可由本领域的普通技术人员来确定。

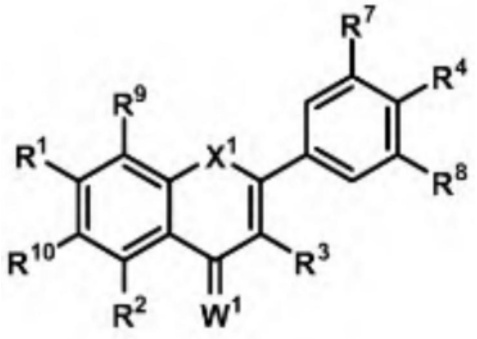
[0192] 组合物

[0193] 本文公开的药物组合物可包含式1或式2中公开的保护剂(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、十五烷酸、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和/或衍生物或盐)。该药物组合物可包含一种或多种任意组合的保护剂、两种或更多种任意组合的保护剂、三种或更多种任意组合的保护剂或者四种或更多种任意组合的保护剂。在一些情况下,药物组合物可以是至少两种保护剂(例如,杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、杨梅苷(杨梅苷)、右雷佐生和/或其衍生物或盐)的共同制剂或者至少一种保护剂和癌症治疗剂(例如,化疗药物、生物剂、蛋白激酶抑制剂或放射疗法)的共同制剂。药物组合物内的保护剂可降低、消除或预防癌症治疗剂引起的心脏毒性。另外,药物组合物内的保护剂还可减少、消除或预防由癌症治疗剂引起的器官损伤。在一个实例中,本公开提供了包含杨梅素和右雷佐生的共同制剂。在另一个实例中,本公开提供了包含化疗药物多柔比星和杨梅素的共同制剂。

[0194] 在一些情况下,组合物中的至少一种保护剂可以是类黄酮或其衍生物。一般来讲,类黄酮可以是具有由两个苯基和一个杂环组成的15-碳骨架的任何化合物。类黄酮可属于以下类型的化合物中的任一种,包括但不限于:表氧化玉米黄质(anthroxanthin)、黄烷酮、黄酮醇、二氢黄酮醇、黄烷、花青素、生物类黄酮、异类黄酮、异黄酮、异黄烷、异黄烷醇、异黄

烷或新类黄酮。类黄酮的非限制性实例包括阿亚黄素、刺苞菊苷、二氢大豆苷元、dihydroobavatin、野鸢尾黄素、异去氢淫羊藿素、异苦参酮、异黄腐醇、栀子黄素、黄羽扇豆魏特酮、甲氧基葛根素、葛根素芹菜糖苷、杨梅素、杨梅苷(杨梅苷)、二氢杨梅素、吡罗糖苷、山奈素、槲皮素、当药黄素、二甲基芹菜苷元、五羟黄酮、ficetin、洋槐黄素、二氢洋槐黄素、7,3',4',5'-四氢氧基黄酮,5,7,3',4',5'-五羟基黄酮或黄花夹竹桃黄酮。在一个实例中,本文公开的药物组合物可包含黄酮,诸如7,3',4',5'-四羟基黄酮和五羟黄酮。在另一个实例中,本文公开的药物组合物可包含黄酮醇,诸如杨梅素、ficetin、洋槐黄素、槲皮素和山奈素。在另一个实例中,本文公开的药物组合物可包含杨梅苷。在另外的实例中,本文公开的药物组合物可包含黄烷酮醇,诸如二氢杨梅素和二氢洋槐黄素。在又一个实例中,本文公开的药物组合物可包含右雷佐生和类黄酮杨梅素的共同制剂。具体地讲,类黄酮杨梅素可通过改变丙酮酸脱氢酶激酶(PDK4)(一种可调节心脏组织中酶活性的蛋白质)的活性来调节心脏中的线粒体毒性。

[0195] 在一些情况下,本文所述的药物组合物可包含根据式1的化合物,

[0196] 

式1

[0197] 其中:

[0198] X^1为CR^5R^6、NR^5、O、S、C=O或C=S;

[0199] R^1、R^2、R^3、R^5、R^6、R^9和R^{10}各自独立地为烷基、烯基、炔基、烷氧基、酰基、酰氧基、羧酸、酯、胺、酰胺、碳酸根、氨基甲酸根、硝基、硫醚、硫酯、环烷基、杂烷基、杂环基、单糖、芳基或杂芳基(其中任一者是取代或未取代的)、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;

[0200] R^4、R^7和R^8为烷氧基、羟基或H;

[0201] W^1为O或S;或

[0202] 其盐。

[0203] 在某些方面,X^1可以为O或S;R^1、R^2、R^3、R^9和R^{10}各自可独立地为烷氧基、环烷基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;并且R^4、R^7和R^8各自可以为烷氧基、羟基或H。

[0204] 在某些方面,X^1可以为O;R^1、R^2、R^3、R^9和R^{10}各自可独立地为烷氧基、环烷基、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H;并且R^4、R^7和R^8各自可以为烷氧基、羟基或H。

[0205] 在其它方面,X^1可以为O;R^1和R^2各自可独立地为羟基或H;R^3、R^9和R^{10}各自可以为环烷基、杂环基、羟基或H;R^4可以为羟基;R^7和R^8可以为羟基或H。

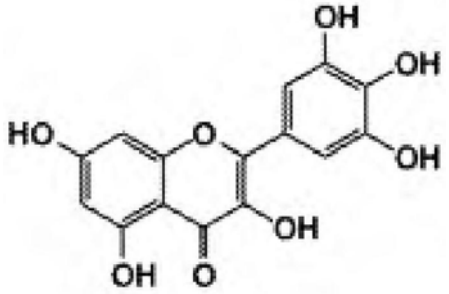
[0206] 在其它方面,X^1可以为O;R^1可以为羟基;R^2和R^3各自可独立地为羟基或H;R^9和R^{10}可

以为H;R^4可以为羟基;R^7和R^8可以为羟基或H。

[0207] 在其它方面,X^1为O;R^1为羟基;R^2和R^3各自可独立地为羟基或H;R^9可以为杂环基或H;R^{10}为H;R^4可独立地为羟基或H;并且R^7和R^8各自可独立地为羟基或H。

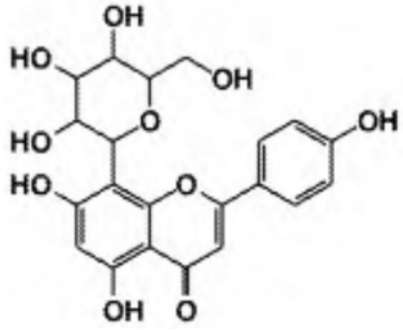
[0208] 在其它方面,X^1为O;R^1为羟基;R^2和R^9各自可独立地为羟基或H;R^3可以为环烷基、羟基或H;R^{10}为H;R4为羟基;并且R^7和R^8各自可独立地为羟基或H。在一个实施方案中,R^3的环烷基可以为单糖。

[0209] 在具体实例中,该化合物可以具有下式:

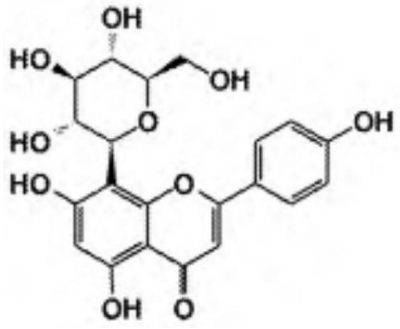
[0210] 

[0211] 在具体实例中,该化合物可以是杨梅素。在一个具体实例中,该化合物可以是洋槐黄素。在一个具体实例中,该化合物可以是五羟黄酮。在一个具体实例中,该化合物可以是7,3',4',5'-四羟基黄酮。在一个具体实例中,该化合物可以是ficetin。在一个具体实例中,该化合物可以是山奈素。在一个具体实例中,该化合物可以是槲皮素。

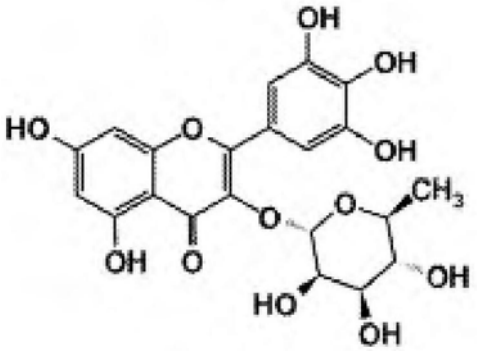
[0212] 在具体实例中,药物组合物内的保护剂可以是具有以下结构的化合物:

[0213] 

[0214] 在具体实例中,药物组合物内的保护剂可以是牡荆素,其中牡荆素具有以下结构:

[0215] 

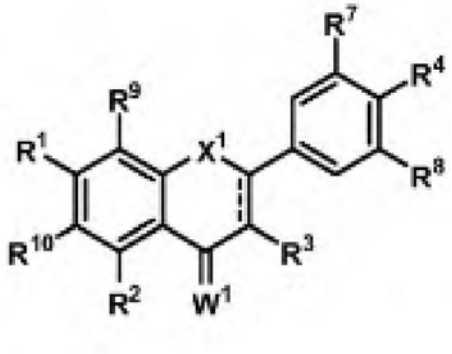
[0216] 在具体实例中,该化合物可以是根据式1的化合物,其中R^1为羟基,R^2为羟基,R^3为单糖,R^4为羟基,R^7为羟基,R^8为羟基,R^9为H,R^{10}为H,X^1为O,并且W^1为O或其盐。在具体实例中,该化合物可以具有下式:

[0217] 

[0218] 在具体实例中，该化合物可以是杨梅苷/杨梅苷。

[0219] 在一些情况下，单糖可以是天然或非天然的糖分子。单糖的非限制性实例包括葡萄糖、右旋糖、果糖、半乳糖甘露糖、核糖、脱氧核糖、D-阿洛糖、L-阿洛糖、D-阿卓糖、L-阿卓糖、D-岩藻糖、L-岩藻糖、D-古洛糖、L-古洛糖、D-山梨糖、D-塔格糖、D-阿拉伯糖、L-阿拉伯糖、D-来苏糖、L-来苏糖、鼠李糖、D-核糖、核糖、sucroribulose、D-木糖、D-赤藓糖、L-赤藓糖、赤藓酮糖、D-苏糖和L-苏糖。

[0220] 在一些情况下，本文所述的药物组合物可包含根据式2的化合物，

[0221] 

式 2

[0222] 其中：

[0223] X^1为CR^5R^6、NR^5、O、S、C＝O或C＝S；

[0224] ----- 表示单键或双键；

[0225] R^1、R^2、R^3、R^5、R^6、R^9和R^{10}各自独立地为烷基、烯基、炔基、烷氧基、酰基、酰氧基、羧酸、酯、胺、酰胺、碳酸根、氨基甲酸根、硝基、硫醚、硫酯、环烷基、杂烷基、杂环基、单糖、芳基或杂芳基(其中任一者是取代或未取代的)、卤素、羟基、巯基、硝基、亚硝基、氰基、叠氮基或H；

[0226] R^4、R^7和R^8为羟基；

[0227] W^1为O或S；

[0228] 或其盐。

[0229] 在一个具体实例中，式2的药物组合物可包含二氢洋槐黄素。

[0230] 在一些情况下，本文所述药物组合物内的癌症治疗剂可以是化疗药物(例如，蒽环类、蛋白激酶抑制剂和蛋白酶体抑制剂)。通常，化疗药物可以是可在患者或受试者中引起心脏毒性的药物。蒽环类的非限制性实例可包括柔红霉素、多柔比星、表柔比星、伊达比星、

米托蒽醌或戊柔比星。蛋白激酶抑制剂的非限制性实例可包括酪氨酸激酶抑制剂、阿法替尼、阿西替尼、波舒替尼、卡博替尼、卡非佐米、色瑞替尼、考比替尼、克唑替尼、达拉菲尼、达沙替尼、厄洛替尼、依维莫司、吉非替尼、依鲁替尼、伊马替尼、拉帕替尼、乐伐替尼、尼洛替尼、尼达尼布、奥希替尼、帕博西尼、帕唑帕尼、哌加他尼、普纳替尼、瑞戈非尼、鲁索替尼、西罗莫司、索拉非尼、舒尼替尼、托法替尼、托法替尼、替西罗莫司、曲美替尼、凡德他尼、维莫非尼或维莫德吉。引起心脏毒性的酪氨酸激酶抑制剂的非限制性实例包括达沙替尼、伊马替尼、拉帕替尼、甲磺酸盐、尼洛替尼、索拉非尼和舒尼替尼。蛋白酶体抑制剂的非限制性实例包括硼替佐米。

[0231] 在一些情况下，本文公开的药物组合物可包含蒽环类(例如，多柔比星)和式1或式2化合物(例如，杨梅素、牡荆素、洋槐黄素、五羟黄酮、ficetin、7,3',4',5'-四羟基黄酮、二氢洋槐黄素、杨梅苷和/或其衍生物或盐)。例如，药物组合物包含多柔比星和杨梅素的共同制剂。在另一个实例中，本文公开的药物组合物可包含蛋白激酶抑制剂或蛋白酶体抑制剂(例如，阿法替尼或硼替佐米)和杨梅素的共同制剂。在另一个实例中，本文公开的药物组合物可包含酪氨酸激酶抑制剂和保护剂的共同制剂。在一个实施方案中，本文公开的药物组合物可包含舒尼替尼和杨梅素的共同制剂。在另一个实例中，本文公开的药物组合物可包含索拉非尼和杨梅素的共同制剂。

[0232] 在一些情况下，本文所述的药物组合物内的癌症治疗剂可以是生物剂(例如，抗体)。生物剂的非限制性实例包括曲妥珠单抗美坦新偶联物、阿仑单抗、贝伐单抗、博纳吐单抗、本妥昔单抗、卡妥索单抗、西妥昔单抗、吉妥珠单抗奥佐米星、替伊莫单抗、伊匹单抗、耐昔妥珠单抗、纳武单抗、奥比妥珠单抗、奥法木单抗、帕尼单抗、派姆单抗、帕妥珠单抗、雷莫芦单抗、利妥昔单抗、托西莫单抗-I131或曲妥珠单抗。例如，本文公开的药物组合物可包含贝伐单抗和杨梅素的共同制剂。例如，本文公开的药物组合物可包含曲妥珠单抗和杨梅素的共同制剂。

[0233] 本公开的化合物或其药学上可接受的盐可含有一个或多个不对称中心，并因此可产生对映体、非对映体和其它立体异构形式，其就绝对立体化学被定义为相对于氨基酸的(R)-或(S)-或者(D)-或(L)-构型。本发明意在包括所有这些可能的异构体以及它们的外消旋和光学纯形式。“立体异构体”是指由通过相同的键结合的相同原子组成但具有不可互换的不同三维结构的化合物。本公开设想了各种立体异构体及其混合物，并且包括“对映体”，其是指其分子是彼此不可重叠的镜像的两种立体异构体。光学活性(+)和(-)的(R)-和(S)-或(D)-和(L)-异构体可使用手性合成子或手性试剂来制备，或者使用常规技术例如色谱和分级结晶来拆分。用于制备/分离单个对映体的常规技术包括从合适的光学纯前体手性合成或者使用例如手性高压液相色谱法(HPLC)拆分外消旋体(或盐或衍生物的外消旋体)。当本文所述的化合物含有烯属双键或其它几何不对称中心时，除非另有说明，否则该化合物包括E和Z几何异构体。

[0234] 当需要时，本公开化合物的(R)-和(S)-异构体(如果存在)可通过本领域技术人员已知的方法来拆分，例如通过形成可通过例如结晶分离的非对映异构体盐或络合物；通过形成可通过例如结晶、气-液或液相色谱法分离的非对映异构体衍生物；通过使一种对映体与对映体特异性试剂选择性反应(例如酶促氧化或还原反应)，然后分离经修饰的和未修饰的对映体；或者通过手性环境中(例如在手性载体上(诸如在具有结合的手性配体的二氧化

硅上)或在存在手性溶剂的情况下))的气液或液相色谱。另选地,可通过使用光学活性剂、底物、催化剂或溶剂的不对称合成或通过不对称转化将一种对映体转化为另一种对映体来合成特定的对映体。

[0235] 化合物可以其对映体纯形式给药。在一些实例中,化合物具有大于约50%、60%、70%、80%、90%、95%、96%、97%、98%或99%的对映体过量。化合物可以其非对映异构纯形式给药。在一些实例中,该化合物具有大于约50%、60%、70%、80%、90%、95%、96%、97%、98%或99%的非对映异构体过量。

[0236] 可使用Cahn-Ingold-Prelog顺序规则来定义立体中心。化合物可具有R构型的立体中心。化合物可具有S构型的立体中心。

[0237] 一些化合物可表现出多态性。应当理解,本公开涵盖本公开化合物的具有本文所述有用性质的任何外消旋、光学活性、多晶型或立体异构形式或其混合物,如何制备光学活性形式是本领域众所周知的(例如,通过重结晶技术拆分外消旋形式、通过光学活性原料合成、通过手性合成或者通过使用手性固定相的色谱分离)。

[0238] 在某些具体实施方案中,可向受试者施用超过一种本公开的化合物。在一些实施方案中,本公开的两种化合物组合可协同地或相加地起作用,并且任一化合物的使用量与单独施用时相比可较小。

[0239] 在某些实施方案中,本文公开的化合物和/或其药物组合物可与其它治疗剂组合使用。本文公开的化合物和/或其药物组合物和治疗剂可相加或更优选地协同地起作用。在一些实施方案中,本文公开的化合物和/或其药物组合物与另一种治疗剂的施用同时施用。例如,本文公开的化合物和/或其药物组合物可与另一种治疗剂一起施用。在其它实施方案中,本文公开的化合物和/或其药物组合物在施用其它治疗剂之前或之后施用。

[0240] 本公开的化合物或其药学上可接受的盐通常以治疗有效量施用。实际施用的化合物的量可由医生或护理员根据相关情况确定,包括待治疗的病状、所选择的施用途径、所施用的化合物及其相对活性、年龄、体重、个体患者的反应、患者症状的严重程度等。

[0241] 本公开还提供了本文所述的任何化合物的盐。术语“盐”是指衍生自本领域众所周知的各种有机和无机抗衡离子的盐。盐包括例如酸加成盐和碱加成盐。加入到化合物中以形成酸加成盐的酸可以是有机酸或无机酸。可衍生盐的无机酸包括例如盐酸、氢溴酸、硫酸、硝酸、磷酸等。可衍生盐的有机酸包括例如乙酸、丙酸、乙醇酸、丙酮酸、草酸、马来酸、丙二酸、琥珀酸、富马酸、酒石酸、柠檬酸、苯甲酸、肉桂酸、扁桃酸、甲磺酸、乙磺酸、对甲苯磺酸、水杨酸等。加入到化合物中以形成碱加成盐的碱可以是有机碱或无机碱。在一些情况下,盐可以是金属盐。在一些情况下,盐可以是铵盐。可衍生盐的无机碱包括例如钠、钾、锂、铵、钙、镁、铁、锌、铜、锰、铝等。可衍生盐的有机碱包括例如伯胺、仲胺和叔胺、取代胺(包括天然存在的取代胺)、环状胺、碱性离子交换树脂等。

[0242] 可通过向本文所述的化合物中添加酸来产生酸加成盐。在一些情况下,可以是有机酸。在一些情况下,可以是无机酸。合适的酸的非限制性实例包括盐酸、氢溴酸、氢碘酸、硝酸、亚硝酸、硫酸、亚硫酸、磷酸、烟酸、异烟酸、乳酸、水杨酸、4-氨基水杨酸、酒石酸、抗坏血酸、龙胆酸、葡萄糖酸、葡糖醛酸、葡萄糖二酸、甲酸、苯甲酸、谷氨酸、泛酸、乙酸、丙酸、丁酸、富马酸、琥珀酸、柠檬酸、草酸、马来酸、羟基马来酸、甲基马来酸、乙醇酸、苹果酸、肉桂酸、扁桃酸、2-苯氧基苯甲酸、2-乙酰氧基苯甲酸、扑酸、苯乙酸、N-环己基氨基磺酸、甲磺

酸、乙磺酸、苯磺酸、对甲苯磺酸、2-羟基乙磺酸、乙烷-1,2-二磺酸、4-甲基苯磺酸、萘-2-磺酸、萘-1,5-二磺酸、2-磷酸甘油酸、3-磷酸甘油酸、葡萄糖-6-磷酸和氨基酸。合适的酸加成盐的非限制性实例包括盐酸盐、氢溴酸盐、氢碘酸盐、硝酸盐、亚硝酸盐、硫酸盐、亚硫酸盐、磷酸盐、磷酸氢盐、磷酸二氢盐、碳酸盐、碳酸氢盐、烟酸盐、异烟酸盐、乳酸盐、水杨酸盐、4-氨基水杨酸盐、酒石酸盐、抗坏血酸盐、龙胆酸盐、葡糖酸盐、葡糖醛酸盐、葡萄糖二酸盐、甲酸盐、苯甲酸盐、谷氨酸盐、泛酸盐、乙酸盐、丙酸盐、丁酸盐、富马酸盐、琥珀酸盐、柠檬酸盐、草酸盐、马来酸盐、羟基马来酸盐、甲基马来酸盐、乙醇酸盐、苹果酸盐、肉桂酸盐、扁桃酸盐、2-苯氧基苯甲酸盐、2-乙酰氧基苯甲酸盐、扑酸盐、苯乙酸盐、N-环己基氨基磺酸盐、甲磺酸盐、乙磺酸盐、苯磺酸盐、对甲苯磺酸盐、2-羟基乙磺酸盐、乙烷-1,2-二磺酸盐、4-甲基苯磺酸盐、萘-2-磺酸盐、萘-1,5-二磺酸盐、2-磷酸甘油酸盐、3-磷酸甘油酸盐、葡萄糖-6-磷酸盐和氨基酸盐。

[0243] 可通过向本文所述的化合物添加无机碱来产生金属盐。无机碱由与碱性抗衡离子(例如,氢氧化物,碳酸盐、碳酸氢盐或磷酸盐)配对的金属阳离子组成。金属可以是碱金属、碱土金属、过渡金属或主族金属。合适金属的非限制性实例包括锂、钠、钾、铯、铈、镁、锰、铁、钙、锶、钴、钛、铝、铜、镉和锌。合适的金属盐的非限制性实例包括锂盐、钠盐、钾盐、铯盐、铈盐、镁盐、锰盐、铁盐、钙盐、锶盐、钴盐、钛盐、铝盐、铜盐、镉盐和锌盐。可通过向本文所述的化合物添加氨或有机胺来产生铵盐。合适的有机胺的非限制性实例包括三乙胺、二异丙胺、乙醇胺、二乙醇胺、三乙醇胺、吗啉、N-甲基吗啉、哌啶、N-甲基哌啶、N-乙基哌啶、二苄胺、哌嗪、吡啶、吡唑、哌吡唑(pipyrrazole)、咪唑、吡嗪、哌吡嗪(pipyrazine)、乙二胺、N,N'-二苄基乙二胺、普鲁卡因、氯普鲁卡因、胆碱、二环己胺和N-甲基葡糖胺。合适的铵盐的非限制性实例可以是三乙胺盐、二异丙胺盐、乙醇胺盐、二乙醇胺盐、三乙醇胺盐、吗啉盐、N-甲基吗啉盐、哌啶盐、N-甲基哌啶盐、N-乙基哌啶盐、二苄基胺盐、哌嗪盐、吡啶盐、吡唑盐、哌吡唑盐、咪唑盐、吡嗪盐、哌吡嗪盐、乙二胺盐、N,N'-二苄基乙二胺盐、普鲁卡因盐、氯普鲁卡因盐、胆碱盐、二环己胺盐和N-甲基葡糖胺盐。

[0244] 术语“药学上可接受的载体”或“药学上可接受的赋形剂”包括任何和所有溶剂、分散介质、包衣、抗细菌剂和抗真菌剂、等渗剂和吸收延迟剂等。此类介质和试剂对于药物活性物质的用途在本领域是众所周知的。除非任何常规介质或试剂与活性成分不相容,否则考虑其在本公开的治疗组合物中的使用。还可将补充活性成分掺入到组合物中。

[0245] 术语“药学上可接受的赋形剂”旨在包括能够与化合物共同施用以促进其预期功能性能的赋形剂和载体。此类介质对于药物活性物质的用途在本领域是众所周知的。此类介质和载体的实例包括溶液、溶剂、分散介质、延迟剂、乳剂等。适用于与多结合化合物一起使用的任何其它常规载体也落入本公开的范围内。

[0246] 在制备本公开的组合物时,可用赋形剂稀释活性成分。合适的赋形剂的一些实例包括乳糖、葡萄糖、蔗糖、山梨糖醇、甘露醇、淀粉、阿拉伯树胶、磷酸钙、藻酸盐、黄蓍胶、明胶、硅酸钙、微晶纤维素、PEG、聚乙烯吡咯烷酮、纤维素、水、无菌盐水、糖浆和甲基纤维素。制剂可另外包括:润滑剂,诸如滑石、硬脂酸镁和矿物油;润湿剂;乳化剂和悬浮剂;防腐剂,诸如羟基苯甲酸甲酯和羟基苯甲酸丙酯;甜味剂;以及调味剂。本公开的组合物可被配制成以便在通过采用本领域已知的规程向患者施用后提供活性成分的快速、持续或延迟释放。

[0247] 在一些情况下,本文所述的药物组合物可包含可提供长期保存、填充含有效活性

成分的制剂、促进药物吸收、降低粘度、添加风味或增强药物组合物溶解度的赋形剂。赋形剂的非限制性实例可包括抗粘连剂、粘合剂(例如,蔗糖、乳糖、淀粉、纤维素、明胶或聚乙二醇)、包衣(例如,羟丙基甲基纤维素或明胶)、崩解剂、染料、风味剂(例如,薄荷、桃子、覆盆子、香草)、助流剂、润滑剂、防腐剂(例如,酸、酯、酚、汞化合物或铵化合物)、吸附剂或载体(例如,石油或矿物油)。

[0248] 制剂

[0249] 本文公开的药物组合物可以是任何类型的制剂,包括包含式1或式2的化合物的固体制剂。

[0250] 在一些情况下,固体制剂包含单独配制或与化疗药物或生物剂组合配制的至少0.01mg、0.1mg、1mg、2mg、3mg、4mg、5mg、6mg、7mg、8mg、9mg、10mg、20mg、30mg、40mg、50mg、60mg、70mg、80mg、90mg、100mg、150mg、200mg、250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg、850mg、900mg、950mg或1000mg的一种或多种式1或式2的保护剂。

[0251] 在一些情况下,固体制剂可包含至少0.1mg、1mg、2mg、3mg、4mg、5mg、6mg、7mg、8mg、9mg、10mg、20mg、30mg、40mg、50mg、60mg、70mg、80mg、90mg、100mg、150mg、200mg、250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg、850mg、900mg、950mg、1g、5g、10g、25g、50g或100g的一种或多种保护剂(例如,杨梅素和/或其衍生物或盐)。例如,本文所述的药物组合物可以是杨梅素(100mg剂量中的75g)和多柔比星(100mg剂量中的25mg)的100mg固体共同制剂。

[0252] 在一些情况下,固体制剂(或其它类型的制剂)可包含至少0.1mg、1mg、2mg、3mg、4mg、5mg、6mg、7mg、8mg、9mg、10mg、20mg、30mg、40mg、50mg、60mg、70mg、80mg、90mg、100mg、150mg、200mg、250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、650mg、700mg、750mg、800mg、850mg、900mg、950mg或1000mg右雷佐生。例如,本文所述的药物组合物可包含杨梅素(100mg剂量中的75mg)和右雷佐生(100mg剂量中的25mg)的100mg固体共同制剂。

[0253] 本文公开的药物组合物可以是液体制剂。在一些情况下,液体制剂可包含单独配制或与化疗药物或生物剂组合配制的至少0.1mg/ml、1mg/ml、2mg/ml、3mg/ml、4mg/ml、5mg/ml、6mg/ml、7mg/ml、8mg/ml、9mg/ml、10mg/ml、20mg/ml、30mg/ml、40mg/ml、50mg/ml、60mg/ml、70mg/ml、80mg/ml、90mg/ml、100mg/ml、150mg/ml、200mg/ml、250mg/ml、300mg/ml、350mg/ml、400mg/ml、450mg/ml、500mg/ml、550mg/ml、600mg/ml、650mg/ml、700mg/ml、750mg/ml、800mg/ml、850mg/ml、900mg/ml、950mg/ml或1000mg/ml浓度的一种或多种式1或式2的保护剂。例如,本文所述的药物组合物可包含100mg/mL浓度的保护剂杨梅素和50mg/mL浓度的多柔比星。

[0254] 在一些情况下,液体制剂可包含至少0.1mg/ml、1mg/ml、2mg/ml、3mg/ml、4mg/ml、5mg/ml、6mg/ml、7mg/ml、8mg/ml、9mg/ml、10mg/ml、20mg/ml、30mg/ml、40mg/ml、50mg/ml、60mg/ml、70mg/ml、80mg/ml、90mg/ml、100mg/ml、150mg/ml、200mg/ml、250mg/ml、300mg/ml、350mg/ml、400mg/ml、450mg/ml、500mg/ml、550mg/ml、600mg/ml、650mg/ml、700mg/ml、750mg/ml、800mg/ml、850mg/ml、900mg/ml、950mg/ml或1000mg/ml浓度的杨梅素或其衍生物或盐。例如,本文所述的药物组合物可包含100mg/mL浓度的杨梅素。

[0255] 在一些情况下,液体制剂可包含与一种或多种保护剂共同配制的至少0.1mg/ml、

1mg/ml、2mg/ml、3mg/ml、4mg/ml、5mg/ml、6mg/ml、7mg/ml、8mg/ml、9mg/ml、10mg/ml、20mg/ml、30mg/ml、40mg/ml、50mg/ml、60mg/ml、70mg/ml、80mg/ml、90mg/ml、100mg/ml、150mg/ml、200mg/ml、250mg/ml、300mg/ml、350mg/ml、400mg/ml、450mg/ml、500mg/ml、550mg/ml、600mg/ml、650mg/ml、700mg/ml、750mg/ml、800mg/ml、850mg/ml、900mg/ml、950mg/ml或1000mg/ml浓度的右雷佐生。

[0256] 在一些情况下，本文所述的药物组合物可包含至少2种保护剂。一种保护剂与至少一种其它保护剂的摩尔比可以是约1:1、约1:2、约1:3、约1:4、约1:5、约1:6、约1:7、约1:8、约1:9、约1:10、约1:20、约1:30、约1:40、约1:50、约1:60、约1:70、约1:80、约1:90、约1:100、约1:1,000、约1:10,000或约1:>10,000。

[0257] 在一些情况下，本文所述的药物组合物可包含癌症治疗剂(例如，化疗药物或生物剂)和至少一种保护剂。癌症治疗剂与至少一种其它保护剂的摩尔比可以是约>10,000:1、约10,000:1、约1,000:1、约100:1、约90:1、约80:1、约70:1、约60:1、约50:1、约40:1、约30:1、约20:1、约10:1、约9:1、约8:1、约7:1、约6:1、约5:1、约4:1、约3:1、约2:1、约1:1、约1:2、约1:3、约1:4、约1:5、约1:6、约1:7、约1:8、约1:9、约1:10、约1:20、约1:30、约1:40、约1:50、约1:60、约1:70、约1:80、约1:90、约1:100、约1:1,000、约1:10,000或约1:>10,000。

[0258] 药盒

[0259] 在一些情况下，本文公开的药物组合物可组装成药盒。在一些情况下，药盒可包含保护剂，其中保护剂可作为药盒内的不同实体存在或作为共同制剂存在。例如，药盒可包含一种或多种选自杨梅素、五羟黄酮、洋槐黄素、ficetin、牡荆素、二氢洋槐黄素、7,3',4',5'-四羟基黄酮、杨梅苷和右雷佐生的保护剂。在一些情况下，药盒可包含至少两种保护剂，其中两种保护剂可作为药盒内的不同实体存在或作为共同制剂存在。例如，药盒可包含至少两种选自杨梅素、五羟黄酮、洋槐黄素、ficetin、牡荆素、二氢洋槐黄素、7,3',4',5'-四羟基黄酮、杨梅苷和右雷佐生的保护剂。在具体实例中，药盒可包含杨梅素和右雷佐生的共同制剂。在一些情况下，药盒可包含癌症治疗剂和至少一种保护剂，其中癌症治疗剂和至少一种保护剂可作为药盒内的不同实体存在或作为共同制剂存在。例如，药盒可包含癌症治疗剂和杨梅素和/或其衍生物。例如，药盒可包含癌症治疗剂和洋槐黄素和/或其衍生物。例如，药盒可包含癌症治疗剂和二氢洋槐黄素和/或其衍生物。例如，药盒可包含癌症治疗和五羟黄酮和/或其衍生物。例如，药盒可包含癌症治疗和ficetin和/或其衍生物。例如，药盒可包含癌症治疗剂和7,3',4',5'-四羟基黄酮和/或其衍生物。

[0260] 在一个实施方案中，药盒可包含多柔比星和杨梅素的共同制剂。

[0261] 在一些情况下，药盒还可包含使用说明。药盒还可包含小瓶、管、针、包装或其它材料。

[0262] 提供具有单位剂量(通常为经口或注射剂量)的一种或多种本文所述化合物的药盒。此类药盒可包括含有单位剂量的容器、描述药物在疾病治疗中的用途和伴随益处的信息包装说明书以及任选地用于递送组合物的器具或装置。

[0263] 药盒可还包含适用于组合物施用的任何装置。例如，包含药物组合物的可注射制剂的药盒可包含适用于皮下施用的针和用于注射部位消毒的酒精擦拭物。

[0264] 在一些情况下，药盒可提供有说明。该说明可在药盒中提供，或可通过电子方式访问。该说明可提供关于如何使用本公开的组合物的信息。该说明还可提供关于如何使用本

公开的装置的信息。该说明可提供关于如何执行本公开的方法的信息。在一些情况下，该说明可提供给药信息。该说明可提供药物信息，诸如作用机制、药物配制、不良风险、禁忌症等。在一些情况下，该药盒由医师或医疗服务人员购买，用于在诊所或医院施用。在一些情况下，药盒由实验室购买并用于筛选候选化合物。

[0265] 实施例

[0266] 实施例1.杨梅素提供长期的心脏保护(细胞活性)

[0267] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天，在第3天更换培养基，然后进行实验。模拟处理、用1.25μM多柔比星处理、用杨梅素处理或用1.25μM多柔比星和杨梅素共同处理样品72小时。处理后，用Hoeschst 33342孵育样品以指示细胞核。使用INCell Analyzer2200对细胞进行成像，分析图像以对细胞总数进行定量并绘制为相对于对照进行归一化的总细胞百分比(左图)，其中每个数据点得自三个生物学重复。呈现每个样品的代表性图像(图3，右图)，其中Hoechst 33342信号的增加表示离子细胞活性增加。

[0268] 模拟处理、用1.25μM多柔比星处理、用杨梅素处理或者用1.25μM多柔比星和杨梅素共同处理心肌细胞72小时，随后进行染色以检测细胞总数(图3)。杨梅素是细胞活性的强效保护剂。与模拟处理对照相比，在不存在杨梅素的情况下用1.25μM多柔比星处理的心肌细胞表现出总细胞数减少62.6％，而用杨梅素和1.25μM多柔比星共同处理的心肌细胞表现出总细胞数减少27.57％。与模拟处理对照相比，与不存在多柔比星的情况下用杨梅素处理的心肌细胞表现出总细胞数没有显著差异。误差条表示标准偏差。呈现每个样品的代表性图像，其中Hoechst 33342信号的增加表示细胞活性增加。与模拟处理对照(图3，右：左上图)相比，在不存在杨梅素的情况下用1.25μM多柔比星处理的心肌细胞(图3，右：左下图)表现出Hoechst 33342信号减少，而用79μM杨梅素和1.25μM多柔比星共同处理的心肌细胞(图3，右：右下图)表现出更小的Hoechst 33342信号减少。与模拟处理对照相比，在不存在多柔比星的情况下用杨梅素处理的心肌细胞(图3，右：右上图)表现出Hoechst 33342信号没有显著差异。

[0269] 实施例2.处理2天后杨梅素对多柔比星诱导的心脏毒性(线粒体毒性)的作用

[0270] 通过将诱导多能干细胞分化成心肌细胞来制备人iPSC衍生的心肌细胞。细胞分化后培养4天，在第3天更换培养基，然后进行实验。用1.25μM多柔比星(图4A)处理或用1.25μM多柔比星和79μM杨梅素(图4B)共同处理心肌细胞2天。处理后，用四甲基罗丹明甲酯(TMRM)染料孵育样品以指示线粒体健康，并且用Hoechst 33342鉴定细胞核。使用INCell Analyzer2200对细胞进行成像。呈现每个样品的代表性图像，其中TMRM信号的减少指示线粒体毒性增加。如与在不存在杨梅素的情况下用1.25μM多柔比星处理的细胞相比(图4A)用1.25μM多柔比星和79μM杨梅素共同处理的细胞(图4B)中TMRM信号更大所指示的，杨梅素是防止多柔比星诱导的线粒体毒性的强效保护剂。

[0271] 实施例3.处理3天后杨梅素对多柔比星诱导的心脏毒性(收缩性)的作用

[0272] 如上所述制备人iPSC衍生的心肌细胞。模拟处理、用1.25μM多柔比星处理、用79μM杨梅素处理或用1.25μM多柔比星和79μM杨梅素共同处理样品72小时。处理后，使用Pulse捕获搏动心肌细胞的视频，并进行分析以从细胞收缩图中定量搏动率(图5；左图)，其中每个数据点得自三个生物学重复。呈现每个样品细胞收缩的代表性图像(图5；右图)。杨梅素是

细胞收缩性的强效保护剂。经模拟处理的心肌细胞以每分钟33.33次搏动进行收缩，而在不存在杨梅素的情况下用1.25μM多柔比星处理的心肌细胞的收缩完全受到抑制。用杨梅素处理或用杨梅素和1.25μM多柔比星共同处理的心肌细胞分别以每分钟39.33次搏动或37.33次搏动进行收缩。图6A至图6C描绘了提供图3所示实验的原始数据(图6A)或归一化数据(图6B)或图5所示实验的原始数据(图6C)的图表。

[0273] 实施例4.处理3天后各种类黄酮和黄酮对多柔比星诱导的心脏毒性(细胞凋亡)的作用

[0274] 如上所述制备心肌细胞。用1μM的多柔比星与杨梅素(图7A)、杨梅苷(图7B)或二氢杨梅素(图7C)共同处理细胞3天。处理后，用CellEvent染料孵育样品以指示凋亡阳性细胞，并用第二染料鉴定细胞核。使用INCell Analyzer2200对细胞进行成像，然后分析图像以定量凋亡细胞的百分比。数据来自两个独立的筛选组，其中每个数据点得自三个重复。

[0275] 用多柔比星与杨梅素(图7A)、杨梅苷(图7B)或二氢杨梅素(图7C)共同处理的心肌细胞表现出抗细胞凋亡的保护作用，其中半数最小抑制浓度(IC50；例如，诱导50％细胞凋亡的药物浓度)分别为20.46μM、38.48μM、40.48μM。

[0276] 实施例5.杨梅素降低心肌细胞中DOX的细胞毒性

[0277] 为了评估MYR对DOX诱导的细胞毒性的作用，模拟处理(三角形)或用100μM杨梅素(MYR；圆形)和浓度递增的多柔比星(DOX)处理人iPSC衍生的心肌细胞72小时，然后用指示线粒体健康的染料(TMRM，Life Technologies)和指示细胞核的染料(Hoechst33342，Life Technologies)孵育。使用INCell Analyzer2200(GE)对细胞进行成像。对健康细胞的总数进行计数并绘制为模拟处理对照的百分比。对于iPSC心肌细胞，50％细胞被杀死的致死浓度(LC50)从模拟处理下的0.41μM变为MYR处理条件下的1.29μM(图8)。数据来自多个独立的筛选组，其中每个数据点得自三个重复。(n＝3)。Y轴：细胞存活率百分比；X轴：DOX的递增浓度(图8)。

[0278] 实施例6.杨梅素防止心肌细胞中DOX诱导的细胞死亡

[0279] 为了测量防止心肌细胞中DOX诱导的细胞死亡的挽救率，将杨梅素的保护作用直接与右雷佐生(DEX；标准护理)的保护作用进行比较。用0.5μM多柔比星和浓度递增的杨梅素(MYR，圆形)或右雷佐生(DEX，正方形)处理人iPSC衍生的心肌细胞。处理72小时后，用指示线粒体健康的染料(TMRM，Life Technologies)和指示细胞核的染料(Hoechst33342，Life Technologies)孵育细胞。使用INCell Analyzer2200(GE)对细胞进行成像。对健康细胞的总数进行计数并绘制为多柔比星治疗对照的百分比。MYR的半数最大有效浓度(EC50)为7.50μM(图9)。相反，DEX没有表现出任何挽救DOX诱导的细胞毒性的显著作用。(n＝3)。

[0280] 实施例7.杨梅素防止心肌细胞中DOX诱导的收缩性异常和DNA双链断裂

[0281] 为了评估杨梅素对心脏细胞收缩性的保护作用，通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天，在第3天更换培养基，然后进行实验。然后用DMSO、DOX(0.5μM)、DOX加DEX(100μM)或DOX加MYR(100μM)处理人iPSC衍生的心肌细胞。处理48小时后，用Pulse(Cellogy)捕获搏动心肌细胞的视频。DOX处理诱导心肌细胞收缩性异常，如搏动、持续时间和峰高减少所证实的。与DEX相比，MYR显著纠正了这种收缩性异常(图10)。数据来自多个独立的实验组，其中每个数据点得自6个样品(n＝6)。使用学生T检验(未配对，双尾)来确定差异的显著性。

[0282] 为了确定MYR是否防止细胞中DOX诱导的DNA双链断裂，用DMSO、DOX(0.5μM)、DOX加DEX(100μM)或DOX加MYR(100μM)处理人iPSC衍生的心肌细胞。处理48小时后，用抗γH2AX抗体(EMD Millipore)对细胞进行免疫染色以检测双链断裂。然后使用INCell Analyzer2200(GE)对细胞进行成像，并且定量每种条件下的γH2Ax阳性细胞百分比。尽管DEX在测试的心脏细胞中几乎没有或没有表现出对DOX诱导的双链断裂的保护作用，但MYR赋予对DOX相关的DNA损伤的显著保护作用(图11)。学生T检验(未配对，双尾;n＝6)。

[0283] 实施例8.MYR防止DOX诱导的肌节破坏

[0284] DOX诱导的细胞死亡通常表现为心肌细胞组织(例如，肌节)结构的严重破坏。为了评估MYR对DOX诱导的肌节破坏的保护作用，用DMSO、DOX(0.5μM)或DOX加MYR(100μM)处理人iPSC衍生的心肌细胞。处理72小时后，用抗心脏肌钙蛋白T抗体(Abcam)对细胞进行免疫染色，以显示心脏细胞中的肌节组织。如图12所示，MYR赋予心肌细胞抗DOX诱导的肌节破坏的显著保护作用，从而表明MYR对DOX诱导的细胞死亡的保护作用通过心肌细胞的结构完整性充分表现出来。

[0285] 实施例9.杨梅素是强效的TOPOIIα和β抑制剂

[0286] 为了深入了解杨梅素(MYR)和右雷佐生(DEX)的心脏保护分子机制，评估了这两种化合物对拓扑异构酶II(即TOPOIIα和β)(DOX的明显靶标)的作用。

[0287] 用一个酶单位的TOPOIIα或TOPOIIβ酶(Inspiralis)以及各种浓度的MYR或DEX在37℃下孵育200ng动基体DNA(kDNA)30分钟。然后在1％琼脂糖凝胶上分离反应物以使脱色的DNA可见(底部条带)。通过测量条带的相对强度来定量催化抑制的效率。

[0288] MYR和DEX在浓度分别为1.18μM和52.70μM时表现出对TOPOIIα酶活性50％的抑制(IC50)(图13;n＝3)。MYR和DEX对TOPOIIβ酶活性的IC50分别为2.07μM和34.43μM(图13;n＝3)。这些数据表明，对于两种拓扑异构酶IIα和β，MYR是一种比DEX显著更有效的抑制剂。

[0289] 实施例10.与DEX不同，MYR不诱导TOPOII蛋白降解

[0290] 为了进一步区分MYR与DEX的分子机制，并且还为了确定在上述脱色测定中观察到的MYR对TOPOII的抑制作用是否是由于TOPOII蛋白降解引起的，用DMSO、DEX(100μM)或MYR(100μM)处理人iPSC衍生的心肌细胞24小时，并用抗拓扑异构酶IIβ抗体(BD Biosciences)进行免疫染色。

[0291] 使用INCell Analyzer2200(GE)对细胞进行成像，并且对拓扑异构酶IIβ蛋白水平进行定量。使用学生T检验(未配对，双尾)来确定差异的显著性。

[0292] 如图14所示，用DEX处理导致iPSC-CM中TOPOIIβ显著消失，而MYR对拓扑异构酶IIβ蛋白水平没有作用(图14)(n＝3)。结果证实了这一假设:DEX可对拓扑异构酶IIβ(TOPOIIβ)的稳定性产生不利作用，这可导致这些酶从心脏细胞中消耗掉，从而有效地防止蒽环类对这些酶的毒性作用而产生的DNA损伤。这些结果还证实，MYR赋予对蒽环类诱导的毒性的保护机制是完全独立的，并且可与DEX的机制区别开来。此外，在拓扑异构酶抑制中观察到的MYR的作用不是由于DOX对心脏细胞的负面作用导致的TOPOIIβ蛋白降解或该酶的消耗。可得出结论，抑制拓扑异构酶II活性(尤其是在不影响TOPOII酶的稳定性的情况下)是MYR赋予心脏保护作用的重要因素。

[0293] 实施例11.DHM和DHR都不抑制TOPOIIα或TOPOIIβ

[0294] 由于MYR赋予对DOX诱导的毒性的心脏保护作用独立于DEX，因此进一步研究以确

定其它类黄酮化合物对拓扑异构酶II活性是否具有如MYR的相似作用。

[0295] 首先，测试MYR(黄酮醇)和二氢杨梅素(二氢黄酮醇)对拓扑异构酶II酶功能的抑制作用。除了在类黄酮支架的主C环中存在单键之外，二氢杨梅素(DHM)共享类似的化学结构。

[0296] 用一个酶单位的TOPOIIβ和不同浓度的MYR(圆形)或DHM(三角形)在37℃下孵育200ng动基体DNA(kDNA)30分钟(图15)。然后在1％琼脂糖凝胶上分离反应物以使脱色的DNA可见(底部条带)，并且通过测量条带的相对强度来定量催化抑制的效率。令人惊讶的是，DHM不抑制TOPOIIβ酶活性(n＝3)(图15)或TOPOIIα酶活性，即使在极端浓度(>200μM)下也是如此。

[0297] 此外，关于DHM的这一结果在二氢洋槐黄素(DHR)和洋槐黄素(ROB)的单独实验中也得到证实，其中DHR与DHM一样对这些拓扑异构酶没有显示出抑制活性，而洋槐黄素与MYR一样对TOPOIIβ和TOPOIIα两者均显示出高水平的抑制作用。这些数据指示，黄酮/类黄酮支架的C环上的结构差异在TOPOII抑制中起重要作用。

[0298] 实施例12.MYR在保护DOX诱导的细胞死亡方面的效力是DHM的2倍

[0299] 接下来，将MYR赋予心脏保护作用的能力与DHM的能力直接进行比较，因为这两种化合物在其结构和TOPOII抑制活性方面显示出独特的性质。通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天，在第3天更换培养基，然后进行实验。用0.5μM多柔比星和浓度递增的杨梅素(MYR，圆形)或二氢杨梅素(DHM，三角形)处理人iPSC衍生的心肌细胞。处理72小时后，用指示线粒体健康和细胞核的染料孵育细胞。然后对细胞进行成像并对健康细胞总数进行计数，并且如上所述绘制为多柔比星处理对照的百分比。

[0300] 如图16所示，MYR在保护DOX诱导的细胞死亡方面表现出的效力是DHM的2倍，因为MYR和DHM的半数最大有效浓度(EC50)分别为7.50μM和13.96μM。(n＝3)基于这些结果，得出结论：黄酮/类黄酮支架的C环上的双键通过赋予对拓扑异构酶II的抑制作用而增强心脏保护特性的效力。

[0301] 这些观察随后通过DOX诱导的DNA双链断裂测定进行。用0.5μM多柔比星和浓度递增的MYR(圆形)或DHM(三角形)处理人心肌细胞。处理48小时后，用抗γH2AX抗体(EMD Millipore)对细胞进行免疫染色以检测DNA双链断裂。使用INCell Analyzer2200(GE)对细胞进行成像，并且定量每种条件下的γH2AX阳性细胞百分比。与其细胞死亡拯救率一致，MYR在保护DOX诱导的双链断裂方面的效力是DHM的2倍。使DOX诱导的双链断裂降低50％(IC50)的MYR和DHM浓度分别为5.28μM和11.30μM(图17)。(n＝3)

[0302] 为了研究杨梅素对暴露于多柔比星的心肌细胞的作用，在仅用DOX处理、仅用杨梅霉素处理和DOX加杨梅素处理的细胞中测定mRNA表达水平。令人惊讶的是，虽然杨梅素本身对TOPOIIβmRNA表达没有任何作用，但DOX在第24小时和48小时时显著抑制TOPOIIβ表达(图18)。然而，在存在杨梅素的情况下，杨梅素使TOPOIIβ表达恢复至接近正常水平，从而有效地防止DOX引起的任何转录改变(图18)。该数据表明DOX和杨梅素对TOPOIIβ表达似乎有协同作用。相对于TOPOIIα的表达，DOX随着时间推移缓慢地抑制TOPOIIα的表达。然而，在存在DOX的情况下，杨梅素进一步抑制TOPOIIα，表明杨梅素对这些拓扑异构酶II在分子和细胞水平上具有不同的作用。杨梅素和DOX对TOPOIIα的组合下调作用大于单独用DOX观察到的作用。

[0303] 实施例13.MYR类似物的心脏保护特性

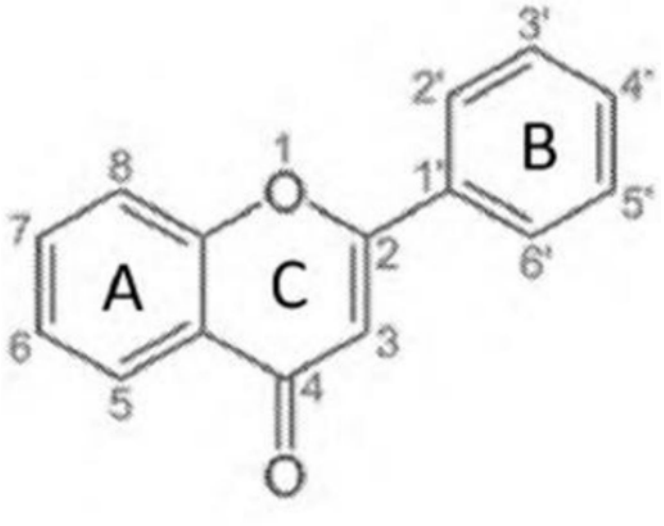
[0304] 为了进一步研究结构(例如,黄酮/类黄酮支架)与生物活性(例如,心脏保护、TOPOII抑制等)之间的关系,鉴定了一组与黄杨素相关的另外的类黄酮化合物并测试其活性。

[0305] I.具有通过TOPOII抑制介导的心脏保护特性的类黄酮的鉴定

[0306] 当诸如多柔比星之类的药物通过拓扑异构酶II酶切割DNA而嵌入DNA从而有效地防止TOPOIIα或β将切割后的DNA链连接在一起时,发生蒽环类诱导的心脏毒性。因此,基于类黄酮通过拓扑异构酶IIα和β(TOPOIIα和TOPOIIβ)抑制介导的心脏保护特性提出工作假设。

[0307] 对MYR支架的羟基取代基进行系统研究以确定生物活性。目的是探索MYR周围的化学空间以鉴定在各个位置哪些取代基(例如,羟基、烷氧基或杂环)是必需的,并且确定哪种或哪些化学结构是产生心脏保护的基本组分。

[0308] 对于生物活性,如上所述使用脱色测定来评估TOPOIIα和TOPOIIβ抑制。采用多柔比星处理的人iPSC衍生的心肌细胞来测量这些类似物对心肌细胞的保护作用。

[0309] 

黄酮/类黄酮支架

[0310] 从裸黄酮开始,鉴定了在3、5、7、3'、4'或5'位存在或缺失羟基取代基的48种杨梅素类似物化合物(杨梅素是存在全部六个羟基取代基的化合物)。除了48种杨梅素类似物之外,还有缺少黄酮B环的色酮以及在C环中均缺乏双键的二氢杨梅素和二氢洋槐黄素(DHR)。

[0311] 由于取代基可类似于牡荆素在A环上的8位和/或6位以及在C环上的2'位和/或6'位掺入到黄酮支架中,还设想了羟基、烷氧基、烷基和杂环基、卤化物以用于分析。研究还包括除了MYR支架(式1)上存在的羟基取代基之外的化学部分,诸如3、5、7、3'、4'和/或5'位上的烷氧基(尤其是甲氧基)、烷基(甲基)、杂环或卤化物。

[0312] 这项研究使得能够确定基于MYR支架的终点活性所需的最小结构。在针对心脏保护生物学活性(例如,TOPOIIβ抑制和DNA双链断裂)而选择的在3、5、7、3'、4'、5'位上具有特定组合的羟基的化合物中,发现在3、5、7、3'、4'、5'位上存在或缺失特定组合的取代基的某些化合物组对于产生心脏保护作用和降低的细胞毒性的生物学性质是关键的。

[0313] 表1

ID	化合物名称	iPSC-CM最大保护作用(%)	EC50 (μM)	毒性	挽救特性	TOPOIIβ抑制	TOPOIIα抑制
1	3 5 7 3' 4' 5'-六羟基黄酮(杨梅素)	78	14.48	-	++++	+++	+++
2	3 7 3' 4' 5'-五羟基黄酮(洋槐黄素)	64	12.62	-	++++	+++	+++
3	5 7 3'4'5'-五羟基黄酮(五羟黄酮)	56	17.19	*	+++	+++	+++
4	3 5 7 3'4'-五羟基黄酮(槲皮素)	58	20.5	*	++	+++	+++
5	3,7,3',4'-四羟基黄酮(漆黄素)	36	16.32	*	++	+++	+++
6	7,3',4',5'-四羟基黄酮	71	17.13	-	+++	-	-
7	3,5,7,4'-四羟基黄酮(山柰素)	46	26.01	-	++	-	-
8	3',4',5'-三羟基黄酮	64	43.01	-	+	-	-
9	5,7,3',4'-四羟基黄酮(木犀草素)	62	9.67	*	+++	-	-
10	3,7,4'-三羟基黄酮(5-去羟山柰素)	27	3.26	*	+	-	-
11	7,3',4'-三羟基黄酮	24	6.25	*	+	-	-
12	3,3',4'-三羟基黄酮	16	6.43	*	+	-	-
13	5,7,4'-三羟基黄酮(芹黄素)	†	-	-	-	N/A	N/A
14	3',4'-二羟基黄酮	†	-	*	-	N/A	N/A
15	7,4'-二羟基黄酮	†	-	*	-	N/A	N/A
16	3,4'-二羟基黄酮	†	-	*	-	N/A	N/A
17	4'-羟基黄酮	†	-	-	-	N/A	N/A
18	3,7,3'-三羟基黄酮	†	-	*	-	N/A	N/A
19	3,5,7-三羟基黄酮	†	-	*	-	N/A	N/A
20	3,7-二羟基黄酮	†	-	*	-	N/A	N/A
21	7,3'-二羟基黄酮	†	-	*	-	N/A	N/A
22	3,3'-二羟基黄酮	†	-	*	-	N/A	N/A
23	5,7-二羟基黄酮	†	-	*	-	N/A	N/A
24	7-羟基黄酮	†	-	*	-	N/A	N/A
25	3-羟基黄酮	†	-	*	-	N/A	N/A
26	3',5'-二羟基黄酮	†	-	*	-	N/A	N/A
27	3'-羟基黄酮	†	-	*	-	N/A	N/A
28	黄酮	†	-	*	-	N/A	N/A

[0314]

[0315]

29	色酮	†	-	-	-	N/A	N/A
30	二氢洋槐黄素	**53**	**14.02**	-	+++	-	-
31	3'-O-甲基杨梅素	**76**	**58.7**	-	+	-	-
32	4'-O-甲基杨梅素	**68**	**48.6**	-	+	-	-
33	3',5'-O-二甲基杨梅素	†	-	*	-	-	-
34	3',4',5'-O-三甲基杨梅素	†	-	*	-	-	-
35	3',4',5'-O-三甲基洋槐黄素	†	-	*	-	-	-
36	7,3',4',5'-O-四甲基洋槐黄素	†	-	*	-	-	-
37	3,7,3',4',5'-O-五甲基洋槐黄素	†	-	*	-	-	-
38	7-羟基-4-色酮	†	-	*	-	-	-

[0316] +化合物表现出对相应生物特性的积极作用

[0317] -化合物表现出对相应生物特性的负面作用

[0318] †初始筛查时化合物在10μM或100μM下没有表现出>30％的最大保护作用。

[0319] *化合物在100μM下表现出细胞毒性

[0320] N/A,由于化合物表现出细胞毒性以及无心脏保护活性而没有进行实验

[0321] 对于产生TOPOIIβ抑制和心脏保护作用的羟基取代基的最低要求

[0322] 如上表1所示,TOPOIIβ抑制剂(1-5)的共同特征允许这一推论:为了使类黄酮化合物抑制TOPOIIβ,在3、7、3'和4'位需要羟基取代基。唯一的例外是不含3-羟基取代基的五羟黄酮(3);所有其它四种TOPOIIβ抑制剂在3、7、3'和4'位具有羟基取代基。此外,上表1中的心脏保护化合物(1-12)的共同特征允许另外的推论:B环上的4'羟基取代基可能是基本特征,连同在3、7和3'位的其它三个羟基取代基中的两个(其中优选在7位的羟基)以产生心脏保护活性;唯一的例外是在3位和7位上不具有羟基但在B环上的3'、4'和5'位具有全部三个羟基取代基的化合物8。此外,考虑到所测试化合物的毒性(参见表1),可推断出这一趋势:在B环上具有全部三个3'、4'和5'羟基取代基的心脏保护化合物(1-12)在浓度小于100μM时不表现出毒性作用,而仅在3'位和4'位具有羟基取代基的那些心脏保护化合物在浓度小于100μM时确实表现出毒性作用。同样,这种趋势的一个例外是五羟黄酮(3),其在浓度小于100μM时表现出一些毒性作用,尽管该化合物在B环上含有全部三个羟基取代基。在仅在B环上具有4'羟基取代基的两种心脏保护化合物(山奈素7和5-去羟山奈素10)中,山奈素在低于100μM时显示出无毒性作用,而5-去羟山奈素在低于100μM的浓度下表现出毒性作用。基于这一分析,得出以下结论:

[0323] (1)对于心脏保护,需要B环上的4'羟基取代基连同以下中的一者:(a)3、7和3'位上的三个羟基取代基中的两个(优选7位),或者(b)B环上3'、4'和5'位的全部三个羟基取代基;

[0324] (2)对于心脏毒性,B环上的3'、4'和5'羟基取代基优于B环上的3'和4'羟基取代基,以缓解浓度低于100μM时的毒性作用;或者仅B环上的4'羟基连同A/C环体系上的所有三

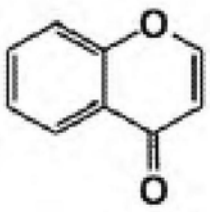
个3、5和7羟基取代基;以及

[0325] (3)对于TOPOIIβ抑制,需要3、7、3'和4'位上的全部四个羟基取代基。五羟黄酮(3)不遵循这些要求,是一个异常。

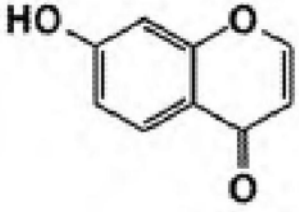
[0326] 对B环的分析

[0327] 从表1所列出的化合物中显而易见的是,B环上的4'位需要羟基取代基以产生心脏保护作用。在通过初始筛选的十二种化合物(1-12)中,它们中的所有都具有4'-羟基取代基。此外,在未通过初始筛选的十六种化合物(13-28)中,有十一种(18-28)缺乏4'-羟基取代基。剩余的未通过初始筛选的五种4'-羟基化合物(13-17)具有最少的取代,例如,化合物17仅具有4'-羟基或化合物14、15和16仅具有连同4'-羟基的一个其它羟基取代基。化合物13仅具有上述3、7和3'位置组中所需羟基取代基中的一个;因此,它也不满足心脏保护活性的最低要求。总之,B环4'位上的羟基取代基的存在是类黄酮化合物产生心脏保护作用的必要但非充分条件。这种结构要求强烈暗示在保护剂的B环上的4'羟基与生物靶标复合之间存在氢键。

[0328] 1.色酮相关化合物

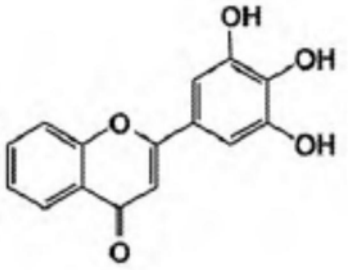


色酮(CHR)

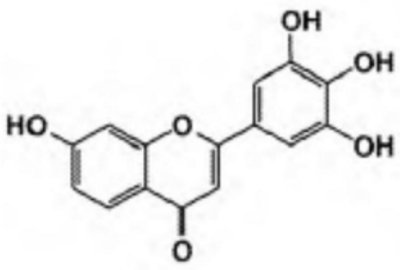


7-羟基-4-色酮

[0329]



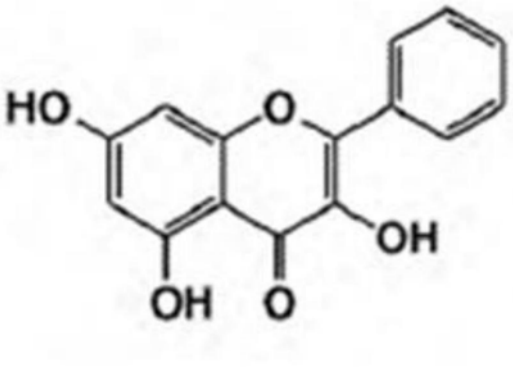
3',4',5'-三羟基黄酮



7,3',4',5'-四羟基黄酮

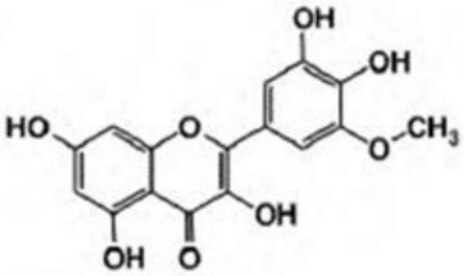
[0330] 各自完全缺乏黄酮支架B环的色酮(29)和7-羟基-4-色酮(38)在心脏保护方面均显示无积极作用。这两种化合物都不赋予TOPOIIβ或α抑制作用(表1)。此外,7-羟基-4-色酮在100μM时表现出高水平的细胞毒性。将这两种无B环化合物与对应的三取代B环黄酮化合物(分别为8和6)相比较,得出结论:B环的存在是心脏保护所必需的。

[0331] 接下来,在具有B环但缺乏全部B环取代基的3,5,7-三羟基黄酮中进一步探索从7-羟基-4-色酮得到的观察结果。3,5,7-三羟基黄酮既不表现出心脏保护作用也不表现出TOPOII抑制,并且显示出广泛的细胞毒性,从而表明要产生心脏保护活性在B环上需要一个或多个部分。

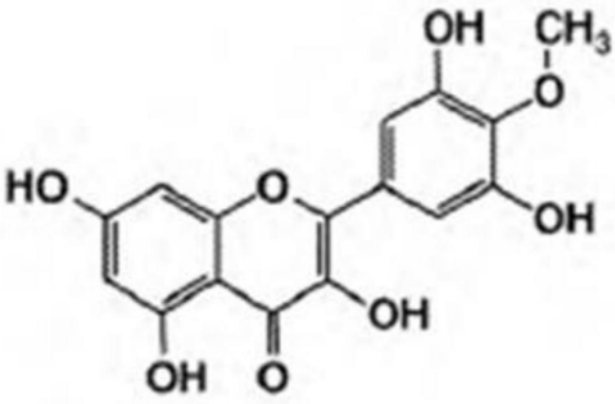
[0332] 

3,5,7-三羟基黄酮

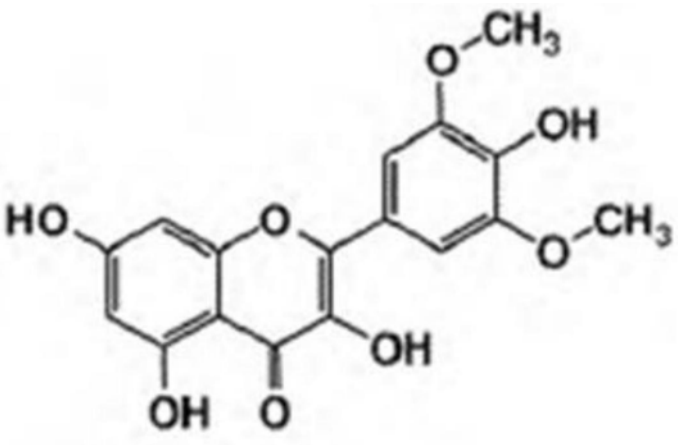
[0333] 2.B环上的甲氧基取代基



3'-O-甲基杨梅素

[0334] 

4'-O-甲基杨梅素

[0335] 

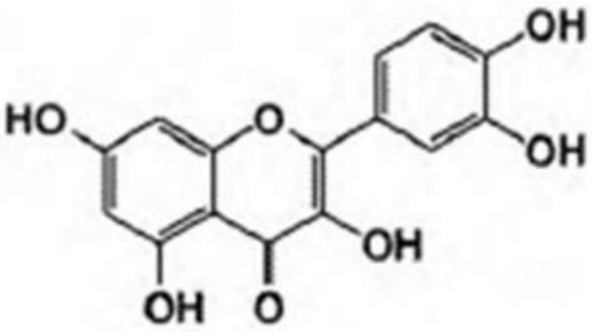
3',5'-O-二甲基杨梅素

[0336] 由于B环似乎是产生生物活性所必需的组分,因此测试了具有B环的化合物的活性,其中B环具有羟基和/或甲氧基的各种位置组合。

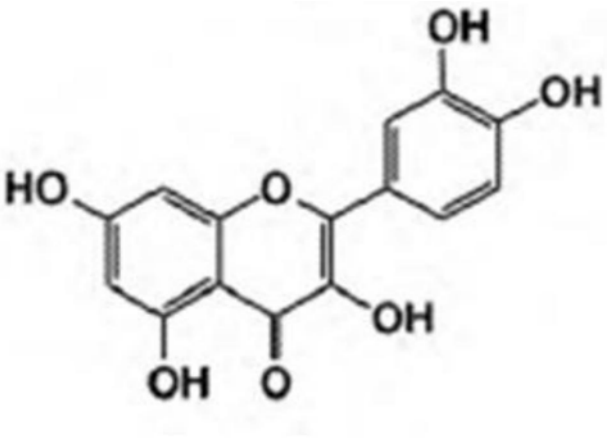
[0337] 在3'位具有甲氧基的3'-O-甲基杨梅素不能抑制TOPOII酶,但赋予心脏保护作用而不显示广泛的细胞毒性。然而,其表现出显著的心脏保护效力损失(EC50,～59μM)。类似地,在4'位具有甲氧基的4'-O-甲基杨梅素赋予心脏保护作用而不具有TOPOII抑制作用。与MYR的心脏保护效力相比,该化合物表现出心脏保护效力的损失(EC50,～48.7μM)。这表明,在B环的3'或4'位存在单个甲氧基取代基是产生心脏保护作用的重要因素。在4位缺少甲氧基取代基但在B环的3'和5'位具有甲氧基的3',5'-O-二甲基杨梅素不显示出心脏保护作用也不显示出TOPOIIα和TOPOIIβ抑制作用,其证实了这一观察结果。该化合物还表现出显著的细胞毒性。还测试了在3'、4'和5'位具有多个甲氧基取代的其它化合物的心脏保护作用和TOPOII抑制作用。例如,3',4',5'-O-三甲基杨梅素、3',4',5'-O-三甲基洋槐黄素、3,7,3',4',5'-O-五甲基洋槐黄素、7,3',4',5'-O-四甲基洋槐黄素中的所有完全不显示出心脏保护作用或TOPOII抑制作用。所有这些化合物在100μM时表现出增加的细胞毒性水平。

[0338] 因此,用甲氧基代替4'或3'羟基显著降低效力并且导致TOPOII抑制作用完全损失。此外,因为甲氧基取代基从B环轻微增大和扩展化合物,据推测具有从B环延伸的较大取代基(即使处于边缘水平)也可对TOPOII酶与化合物之间的相互作用构成空间位阻。因此,B环上(3'、4'、5'位)的羟基似乎是产生心脏保护作用的关键组分,并且可在TOPOII酶抑制中起重要作用。

[0339] 3.槲皮素和山奈素

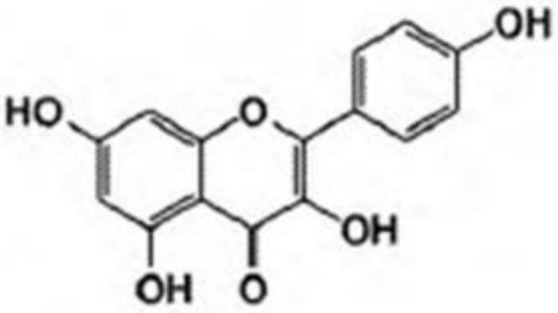
[0340] 

[0341] 或者

[0342] 

槲皮素

[0343] 槲皮素赋予心脏保护作用并且表现出TOPOII抑制作用。然而，在浓度为100μM时观察到高水平的对心肌细胞的一般细胞毒性。

[0344] 

山奈素

[0345] 山奈素在100μM时表现出中等水平的心脏保护作用而无一定水平的细胞毒性，但对TOPOIIα或TOPOIIβ没有表现出任何抑制作用。然而，山奈素表现出降低的效力并且未能实现最大50％的挽救率。

[0346] 从该数据推断：如在槲皮素中观察到的，移除3’(或5’)羟基可不一定导致TOPOII抑制作用的损失，但导致细胞毒性增加和效力降低。然而，这些数据导致这一结论：从B环移除3’、4’或5’(尤其是4’位)羟基导致效力显著降低和/或TOPOII抑制作用的损失。

[0347] 总之，用烷氧基(例如，甲氧基)代替一个或两个3’、4’或5’羟基使得化合物具有细胞毒性。从MYR支架中移除3’位和5’位羟基(如在山奈素中观察到的)或者移除3’位或5’位羟基(如在槲皮素中观察到的)可降低心脏保护效力并且使化合物具有细胞毒性。然而，如在3,5,7-三羟基黄酮中观察到的，移除B环上的所有羟基导致心脏保护和TOPOII抑制作用的完全损失，并且引起严重的细胞毒性。此外，B环的4’羟基似乎是导致具有TOPOII抑制的心脏保护作用效力增加和最小细胞毒性的增强物理属性所必需的。

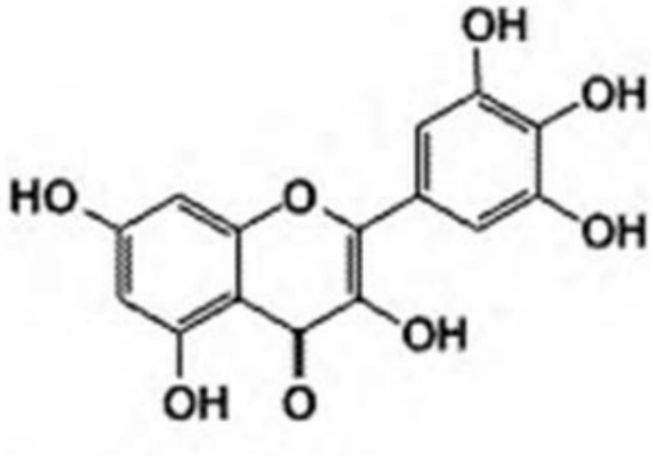
[0348] 因此，如杨梅素和洋槐黄素所证实的，B环的优选取代基为在所有3’、4’和5’位的-OH，以确保效力和最小毒性。

[0349] A环和C环分析

[0350] 评估黄酮-类黄酮支架的杂二环A/C环体系上的取代基的心脏保护活性。基于对B

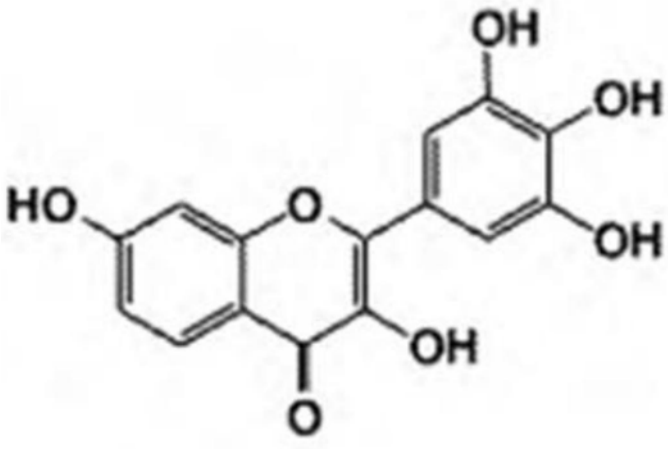
环进行的观察，测试了一组在B环上具有羟基与在A-C环的3、5、7位具有各种组合的-OH的化合物。

[0351] 1.杨梅素、洋槐黄素和五羟黄酮

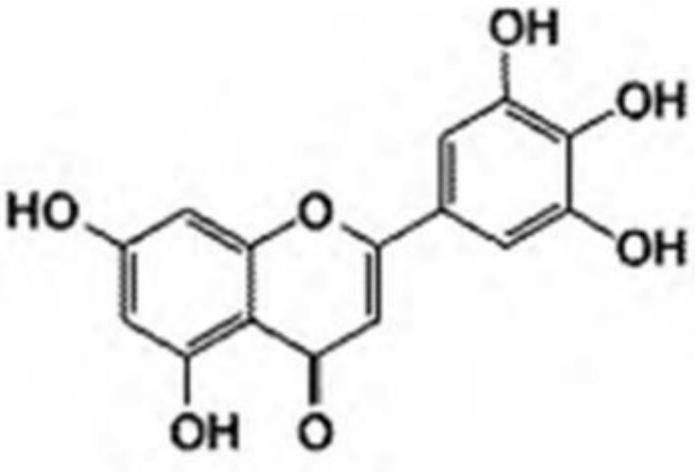


杨梅素(MYR)

[0352]

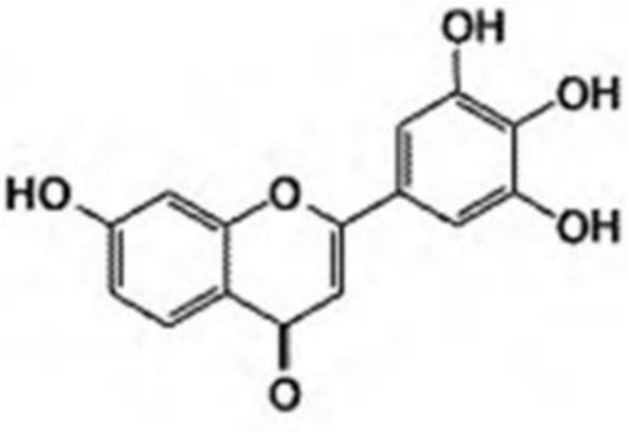


洋槐黄素(ROB)

[0353] 

五羟黄酮(TRI)

[0354] MYR(3,5,7,3',4',5'-六羟基黄酮)和ROB(3,7,3',4',5'-五羟基黄酮)在小于10μM时显示出水平相当的心脏保护作用以及TOPOIIβ和TOPOIIα抑制作用,其中EC50约为10-20μM。类似地,在3位缺少-OH的五羟黄酮(5,7,3',4',5'-五羟基黄酮)在100μM时也显示出心脏保护和TOPOII抑制作用以及低水平的细胞毒性。此外,在A/C环体系的3位和5位都缺少-OH的7,3',4',5'-四羟基黄酮显示出心脏保护作用,但不抑制TOPOII酶。

[0355] 

7,3',4',5'-四羟基黄酮

[0356] 然而,3',4',5'-三羟基黄酮和其它在7位不具有-OH的化合物在小于30μM时未显示出心脏保护或TOPOII抑制的效力。这些数据表明,A环的7位上的羟基(-OH)可能是心脏保护的必需但不充分条件,因为3位和/或5位上的至少一个-OH基团可极大地增强这些化合物的心脏保护活性(例如,效力和/或TOPOII抑制)。因此,A/C环(3位、7位)体系中的羟基对于心脏保护和TOPOII抑制起重要作用,前提条件是B环上存在3'、4'、5'羟基。尤其是A环上7位的-OH对于该活性似乎是关键的。

[0357] 实施例14.MYR对抗癌剂的保护作用

[0358] 1.蒽环类

[0359] MYR防止表柔比星诱导的细胞死亡和伊达比星诱导的细胞死亡

[0360] 表柔比星和伊达比星是与患者心力衰竭有关的蒽环类。除了上述多柔比星之外,

还测试了MYR对表柔比星和伊达比星诱导的心脏损伤的作用。如图19所示，模拟处理(三角形)或用100μM的MYR处理(圆形)和用浓度递增的表柔比星或伊达比星处理人iPSC衍生的心肌细胞72小时，然后如上所述用指示线粒体健康和细胞核的染料孵育。对细胞进行成像并对健康细胞的总数进行计数并绘制为模拟处理对照的百分比。

[0361] 50％细胞被表柔比星杀死的致死浓度(LC50)从模拟处理下的0.49μM变为MYR处理条件下的1.28μM，表明MYR有效地防止心肌细胞中表柔比星诱导的细胞死亡(图19，左图)。(n＝3)

[0362] 类似地，伊达比星的LC50从模拟处理下的0.59μM变为MYR处理条件下的1.04μM，表明MYR也防止伊达比星诱导的细胞死亡(图19，右图)。(n＝3)

[0363] 2.蛋白激酶和蛋白酶体抑制剂

[0364] MYR防止硼替佐米、舒尼替尼和索拉非尼诱导的细胞死亡

[0365] 心脏毒性可由通过各种抗癌剂引起的氧化还原循环形成的有毒活性氧(ROS)导致。活性氧(ROS)可激活凋亡通路，导致癌细胞和正常细胞死亡。心肌细胞可对氧化应激尤其敏感，并且心脏线粒体可易于被如蒽环类、TKI或蛋白酶体抑制剂的癌症药物破坏。根据上面呈现的数据，假设本文描述的MYR及其类似物保护心脏细胞的能力可以是多方面的：(1)如在蒽环类中通过与心脏细胞中的TOPOII酶相互作用起保护作用；以及(2)独立于TOPOII的分子机制发挥作用(例如，ROS螯合、促进线粒体完整性)。为了确定MYR是否赋予对非蒽环类药物的心脏保护作用，测试该化合物在保护心脏细胞免受蛋白激酶抑制剂诱导的细胞毒性方面的能力。

[0366] 舒尼替尼和索拉非尼是酪氨酸激酶拮抗剂，用于治疗包括白血病和肉瘤的各种癌症。然而，据报道舒尼替尼和索拉非尼在患者中引起如心力衰竭等的不良事件。酪氨酸激酶是负责激活参与信号转导通路的许多蛋白质的酶类。这些蛋白质通过磷酸化被激活，这是已知的TKI进行靶向抑制的一个步骤。

[0367] 硼替佐米是用于治疗多发性骨髓瘤和淋巴瘤的蛋白酶体抑制剂。在一些癌症中，通常破坏癌细胞的蛋白质被过早地分解。硼替佐米中断这一过程，从而允许这些蛋白质破坏分裂的癌细胞。

[0368] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天，在第3天更换培养基，然后进行实验。随后用DMSO、舒尼替尼(10μM)或舒尼替尼加浓度递增的MYR(1至100μM)处理人干细胞衍生的心肌细胞72小时，然后用指示线粒体健康和细胞核的染料孵育。对细胞进行成像以及对健康细胞的总数进行计数，并且绘制为舒尼替尼处理对照的百分比。MYR显示对心肌细胞中舒尼替尼诱导的细胞死亡起保护作用(图20)。(n＝3)。同样，MYR成功纠正了5μM索拉非尼处理的心肌细胞中超过80％的心脏功能异常(图21)。用100μM杨梅素处理也挽救了硼替佐米诱导的心脏毒性(图22)。这些数据表明，MYR能够防止蛋白激酶抑制剂诱导的心肌细胞死亡。

[0369] 实施例15.不干扰多柔比星的抗癌活性

[0370] 双二氧哌嗪右雷佐生(DEX)是唯一可用于降低接收抗癌剂的癌症患者的心力衰竭发生率的药物。尽管DEX有临床效果，但会产生若干副作用，诸如干扰蒽环类的抗肿瘤效力、诱导继发性恶性肿瘤以及引起血液和骨髓疾病。这些局限性严重限制了其用于某些癌症患者。

[0371] 研究MYR的作用以确定该化合物是否具有与在DEX中观察到的那些缺点类似的缺点。模拟处理或用100μM MYR和浓度递增的多柔比星处理乳腺癌细胞(MDA-MB-231)72小时(图23)。然后进行细胞活性测定(CellTiter-Glo,Promega)。通过Synergy HT(Biotek)酶标仪记录发光量并绘制为模拟处理对照的百分比。据观察,模拟处理(0.53μM)与用MYR处理(0.48μM)之间的细胞活性(LC50)基本上没有差异,表明MYR不干扰多柔比星的抗癌活性(图23)(n=3)。

[0372] 实施例16.抗DOX诱导毒性的心脏保护作用的体内验证

[0373] 在从美国杰克逊实验室获得的9-10周龄的C57BL/6小鼠中建立蒽环类诱导的急性心脏毒性模型。将动物分成三组:盐水处理(n=8),多柔比星处理(n=16)或多柔比星+MYR处理(n=17)。通过单一腹膜内注射施用多柔比星(20mg/kg)、MYR(40mg/kg)和盐水。MYR在多柔比星处理前30分钟施用。在整个研究过程中,每天监测动物的总体健康状况。在处理前4天,用异氟烷(约1.0%)麻醉小鼠,并使用VevoLAZR成像系统(VisualSonics Inc.,Toronto,Canada)进行经胸超声心动图检查以获得基线测量值,然后在处理后第5天重复。在靠近乳头肌的二维短轴视图中获取左心室(LV)的M-模式图像。离线制作收缩和舒张期间心内膜组织的描记线。然后使用这些数据计算整体指示收缩功能的缩短分数(FS)和射血分数(EF)。

[0374] 在研究过程中,盐水组的收缩特性没有改变。相反,多柔比星处理对收缩特性有深远影响。在该组中,FS和EF随时间($P<0.001$)分别显著降低15%和19%。MYR处理显著降低了多柔比星诱导的心脏毒性($P<0.05$),如观察到的FS和EF分别提高了7%和10%(图24)。在浓度是多柔比星的2倍时,MYR引发对多柔比星引起的异常的52%的FS挽救率和49%的EF挽救率(图24)。

[0375] 实施例17.各种保护剂(包括牡荆素)对多柔比星诱导的有关线粒体毒性的心脏毒性的作用

[0376] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。模拟处理、用1μM多柔比星处理或用1μM多柔比星和指定药物处理样品48小时。处理后,用指示线粒体健康的四甲基罗丹明甲酯(TMRM)染料和鉴定细胞核的第二染料孵育样品。使用INCell Analyzer2200对细胞进行成像,并通过CellProfiler分析图像以定量TMRM阴性细胞的百分比。呈现来自两个独立筛选组的保护剂的代表性数据,其中每个数据点从三个生物学重复获得。通过重新校准基于模拟处理样品(0%线粒体毒性)和1μM多柔比星处理样品(100%线粒体毒性)的数据进行数据归一化。

[0377] 模拟处理(未处理)、用1μM多柔比星('Dox 1μM')处理或用1μM多柔比星和指定药物处理心肌细胞,随后进行染色以检测线粒体健康(图25)。与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,暴露于17μM山奈素("KAE17μM")的心肌细胞表现出至少60%的线粒体毒性降低。与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,暴露于0.76μM氨溴索('AMB 0.76μM')、10μM美沙拉嗪('MES 10μM')或50μM N-乙酰半胱氨酸('NAC 50μM')的心肌细胞表现出至少40%的线粒体毒性降低;与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,暴露于160μM右雷佐生('Dex 160μM')或115μM牡荆素('VIT 115μM')的心肌细胞表现出至少30%的线粒体毒性降低。

[0378] 实施例18.各种保护剂(包括牡荆素)对多柔比星诱导的心脏毒性(细胞凋亡)的作用

[0379] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。模拟处理、用1μM多柔比星处理或用1μM多柔比星和指定药物处理样品48小时。处理后,用指示凋亡阳性细胞的TUNEL染料和鉴定细胞核的第二染料孵育样品。使用INCell Analyzer2200对细胞进行成像,并且通过CellProfiler分析图像以定量凋亡阳性细胞的百分比。呈现来自两个独立筛选组的保护剂的代表性数据,其中每个数据点从三个生物学重复获得。通过重新校准基于模拟处理样品(0%凋亡)和1微摩尔多柔比星处理样品(100%凋亡)的数据进行数据归一化。

[0380] 模拟处理('未处理')、用1μM多柔比星('Dox 1μM')处理或用1μM多柔比星和指定药物共同处理心肌细胞,随后进行染色以检测细胞凋亡(图26)。与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,用115μM牡荆素('VIT 115μM')处理的心肌细胞表现出至少60%的细胞凋亡减少;与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,暴露于160μM右雷佐生('Dex 160μM')、0.76μM氨溴索('AMB 0.76μM')或50μM N-乙酰半胱氨酸('NAC 50μM)的心肌细胞表现出至少50%的细胞凋亡减少;与在不存在保护剂('Dox 1μM')的情况下用多柔比星处理的心肌细胞相比,暴露于17μM山奈素('KAE 17μM')或10μM美沙拉嗪('MES 10μM')的心肌细胞表现出至少40%的细胞凋亡减少。

[0381] 实施例19.牡荆素提供长期的心脏保护(线粒体健康)

[0382] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。模拟处理(图27A)、用1μM多柔比星处理(图27B)、用1μM多柔比星和16μM右雷佐生共同处理(图27C)或用1μM多柔比星和116μM右雷佐生共同处理(图27D)样品7天。处理后,用指示线粒体健康的四甲基罗丹明甲酯(TMRM)染料孵育样品。使用INCell Analyzer2200对细胞进行成像,并通过CellProfiler分析图像以定量TMRM阴性细胞的百分比。呈现每个样品的代表性图像,其中TMRM信号的损失表示线粒体毒性。

[0383] 与模拟处理的心肌细胞(图27A)相比,暴露于多柔比星(图27B)或用多柔比星和右雷佐生共同处理(图27C)的心肌细胞表现出线粒体毒性增加,如通过TMRM阳性细胞的显著减少所指示的。与暴露于多柔比星(图27B)或多柔比星和右雷佐生(图27C)的心肌细胞相比,用多柔比星和牡荆素处理(图27D)的心肌细胞表现出改善的长期线粒体保护。

[0384] 实施例20.牡荆素提供剂量依赖性心脏保护(电生理学活性)

[0385] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。用0.1%DMSO模拟处理、用1μM多柔比星处理或用1μM多柔比星和各种浓度的牡荆素(例如,11.6μM、37μM或116μM)共同处理样品。处理后,持续72小时测量每个样品中活性电极的百分比。对活性电极的百分比进行定量,并在时间进程中作图(图28A)。在处理30小时后,对每孔中活性电极的平均数进行定量并作图(图28B,n=6,标准偏差显示为误差条)。

[0386] 在不存在牡荆素的情况下暴露于1μM多柔比星的心肌细胞表现出在药物处理(时间零点)24小时后活性电极数量减少约50%,并且在药物处理30小时后相对于时间零点活性电极数量减少约95%(图28A)。共暴露于多柔比星和牡荆素的心肌细胞表现出活性电极

百分比的剂量依赖性增加(图28A)。药物处理24小时后,共暴露于1μM多柔比星和11.6μM、37μM或116μM牡荆素的心肌细胞分别表现出约50%、约25%或约0%的活性电极数量减少。在处理30小时后,与在不存在牡荆素的情况下暴露于1μM多柔比星的样品(约2个活性电极)(图28B)相比,共暴露于1μM多柔比星和116μM牡荆素的样品表现出具有统计学上显著更高的活性电极平均数(约10个活性电极)。

[0387] 实施例21.保护剂不抑制多柔比星介导的乳腺癌细胞死亡

[0388] 在进行实验之前,将MDA-MB-231细胞(转移性乳腺癌)培养1天。用浓度递增(例如0μM、0.016μM、0.05μM、0.16μM、0.5μM、1.6μM、5μM、16μM或50μM)的多柔比星或用浓度递增的多柔比星和指定保护剂处理样品72小时。随后用CellTiter-Glo试剂裂解细胞以鉴定具有代谢活性的(例如,活的)细胞,其中从裂解细胞悬浮液中测得的发光量与存在于培养物中的活细胞数量成正比。通过测量发光量的减少对细胞死亡百分比进行定量。使用XLFit进行曲线拟合。用来自三个重复的平均值作图,并且标准偏差显示为误差条。

[0389] 与在不存在保护剂的情况下用多柔比星处理的细胞相比,用浓度递增的多柔比星和右雷佐生、氨溴索、山奈素(图29A)、美沙拉嗪、N-乙酰半胱氨酸或牡荆素(图29B)共同处理的MDA-MB-231细胞没有显示出细胞死亡百分比上的显著差异。这些结果表明,如通过体外测定所测量的,本文所述的药物组合物不赋予对MDA-MB-231乳腺癌细胞的保护益处。

[0390] 实施例22.保护剂不抑制多柔比星介导的肺癌细胞死亡

[0391] 在进行实验之前,将A549细胞(肺癌)培养1天。用浓度递增的(例如,0μM、0.016μM、0.05μM、0.16μM、0.5μM、1.6μM、5μM、16μM或50μM)的多柔比星或用浓度递增的多柔比星和指定药物处理样品72小时。随后用CellTiter-Glo试剂裂解细胞以鉴定具有代谢活性的(例如,活的)细胞,其中从裂解细胞悬浮液中测得的发光量与存在于培养物中的活细胞数量成正比。通过测量发光量的减少对细胞死亡百分比进行定量。使用XLFit进行曲线拟合。用来自三个重复的平均值作图,并且标准偏差显示为误差条。

[0392] 与在不存在保护剂的情况下用多柔比星处理的细胞相比,用浓度递增的多柔比星和右雷佐生、氨溴索、山奈素、美沙拉嗪、N-乙酰半胱氨酸或牡荆素共同处理的A549细胞没有显示出细胞死亡百分比上的显著差异。这些结果表明,如通过体外测定所测量的,本文所述的药物组合物不赋予对A549肺癌细胞的保护益处。

[0393] 实施例23.各种保护剂(包括牡荆素)对电生理学的急性毒性

[0394] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。用0.1%DMSO模拟处理或用浓度递增的指定药物处理样品至少20分钟。作为对照,心肌细胞用hERG钾通道阻滞剂E4031处理。处理后,使用MEA测量每个样品的搏动周期和场电位持续时间(FPD)。

[0395] 与对照样品相比,在较低的药物浓度下,暴露于右雷佐生、氨溴索、鹅去氧胆酸、去铁胺、N-乙酰半胱氨酸、柚皮素或牡荆素的心肌细胞没有表现出搏动周期或场电位持续时间上的显著差异。在较高浓度下,暴露于鹅去氧胆酸或柚皮素的心肌细胞表现出急性药物毒性导致的搏动中止。

[0396] 实施例24.各种保护剂(包括牡荆素)对电生理学的长期毒性

[0397] 通过将诱导多能干细胞分化成心肌细胞来制备细胞样品。细胞分化后培养4天,在第3天更换培养基,然后进行实验。用0.1%DMSO模拟处理或用各种浓度的指定药物处理样

品。处理后，持续至少5天测量每个样品中活性电极的百分比。对活性电极的百分比进行定量，并在时间进程中作图。

[0398] 相对于模拟处理样品，暴露于氨溴索、山奈素、美沙拉嗪或牡荆素的心肌细胞没有显示出活性电极数量可察觉的减少。暴露于临床批准的心脏保护剂右雷佐生的心肌细胞表现出长期的剂量依赖性心脏毒性作用。在处理约2天后，暴露于167μM或500μM右雷佐生的心肌细胞分别显示出约25％或50％的活性电极数量减少。在处理约3天后，暴露于167μM或500μM右雷佐生的心肌细胞分别表现出约50％或100％的活性电极数量减少。

[0399] 实施例25.通过经口施用含多柔比星和牡荆素的丸剂治疗患有心脏病的乳腺癌患者

[0400] 一名具有心脏病史的患者被诊断患有乳腺癌。由于心力衰竭的风险增加，所以患者不能接受已知会诱导心脏毒性的多柔比星的标准治疗方案。作为替代，护理员施用多柔比星(10mg)和牡荆素(100mg)的共同制剂。进行超声心动图检查并测量血流速度以确定该治疗是否对患者产生心脏毒性作用。患者没有显示出心脏功能异常的指示。因为没有表现出心脏毒性，所以患者能够在接下来的几周接受更高剂量的治疗。随后对该患者进行组织活检，其未显示有乳腺癌的指示。

[0401] 实施例26.通过静脉内施用多柔比星、右雷佐生和牡荆素治疗肝癌患者

[0402] 一名患者被诊断患有肝癌。护理员向患者施用多柔比星(5mg/mL)和右雷佐生(50mg/mL)的共同制剂。进行心电图检查以确定右雷佐生是否成功减轻患者的心脏毒性作用。患者出现20ms的QT延长。为了增强右雷佐生的活性，护理员向患者施用多柔比星(5mg/mL)和牡荆素(100mg/mL)的共同制剂。治疗后，进行心电图检查，患者未表现出QT延长的迹象。患者能够在数周后继续接受治疗，之后进行组织活检以确认肝癌已被根除。

[0403] 实施例27.通过经口施用含多柔比星和杨梅素的丸剂治疗具有心动过缓的肺癌患者

[0404] 一名患者被诊断患有II期肺癌，并出现心动过缓。由于心力衰竭的风险增加，该患者不能接受已知会影响心脏收缩和引起心动过缓的多柔比星的标准治疗方案。作为替代，护理员施用多柔比星(10mg)和杨梅素(100mg)的共同制剂。进行心电图检查以监测患者的心率。患者没有显示出心脏功能异常的指示。因为没有表现出心脏毒性，所以患者能够在接下来的几周接受更高剂量的治疗。肺癌降级至1期，并且通过手术成功移除癌症。随访时，进行组织活检并且没有显示出癌症迹象。

[0405] 实施例28.通过静脉内施用含多柔比星、右雷佐生和杨梅素的溶液治疗肝癌患者

[0406] 一名患者被诊断患有肝癌。护理员向患者施用多柔比星(5mg/mL)和右雷佐生(50mg/mL)的共同制剂。进行心电图检查以确定右雷佐生是否成功减轻患者的心脏毒性作用。患者出现20ms的QT延长。为了增强右雷佐生的活性，护理员向患者施用多柔比星(5mg/mL)和杨梅苷(50mg/mL)的共同制剂。治疗后，进行心电图检查，患者未表现出QT延长的迹象。患者能够在数周后继续接受治疗，之后进行组织活检以确认肝癌已被根除。

[0407] 实施例29.通过经口施用含杨梅素的丸剂治疗具有心动过缓的肺癌患者

[0408] 一名患者被诊断患有II期肺癌，并出现心动过缓。由于心力衰竭的风险增加，该患者不能接受已知会影响心脏收缩和引起心动过缓的多柔比星的标准治疗方案。作为替代，护理员在施用多柔比星(10mg)前24小时施用杨梅素(100mg)。进行心电图检查以监测患者

的心率。患者没有显示出心脏功能异常的指示。因为没有表现出心脏毒性，所以患者能够在接下来的几周接受更高剂量的治疗。肺癌降级至1期，并且通过手术成功移除癌症。随访时，进行组织活检并且显示没有癌症迹象。

[0409] 实施例30.通过静脉内施用含多柔比星、右雷佐生和杨梅素的溶液治疗肝癌患者

[0410] 一名患者被诊断患有肝癌。护理员向患者施用多柔比星(5mg/mL)和右雷佐生(50mg/mL)的共同制剂。进行心电图检查以确定右雷佐生是否成功减轻患者的心脏毒性作用。患者出现20ms的QT延长。为了增强右雷佐生的活性，护理员在静脉内施用(5mg/mL)和(100mg/mL)多柔比星之前24小时向患者施用杨梅素(100mg)。治疗后，进行心电图检查，患者未表现出QT延长的迹象。患者能够在数周后继续接受治疗，之后进行组织活检以确认肝癌已被根除。

[0411] 对于本领域技术人员而言显而易见的是，可在不脱离本发明的精神和范围的情况下对本发明作出许多修改和变型。本文所述的具体实施方案仅以举例的方式提供，并且本发明仅受所附权利要求的条款以及此类权利要求所授权的等同物的全部范围的限制。此类修改旨在落入所附权利要求的范围内。

[0412] 本文所引用的所有参考文献、专利和非专利全文以引用方式并入本文并且用于所有目的，其程度如同各个单独的出版物或专利或专利申请被具体地和单独地指示其全文以引用方式并入本文以用于所有目的。

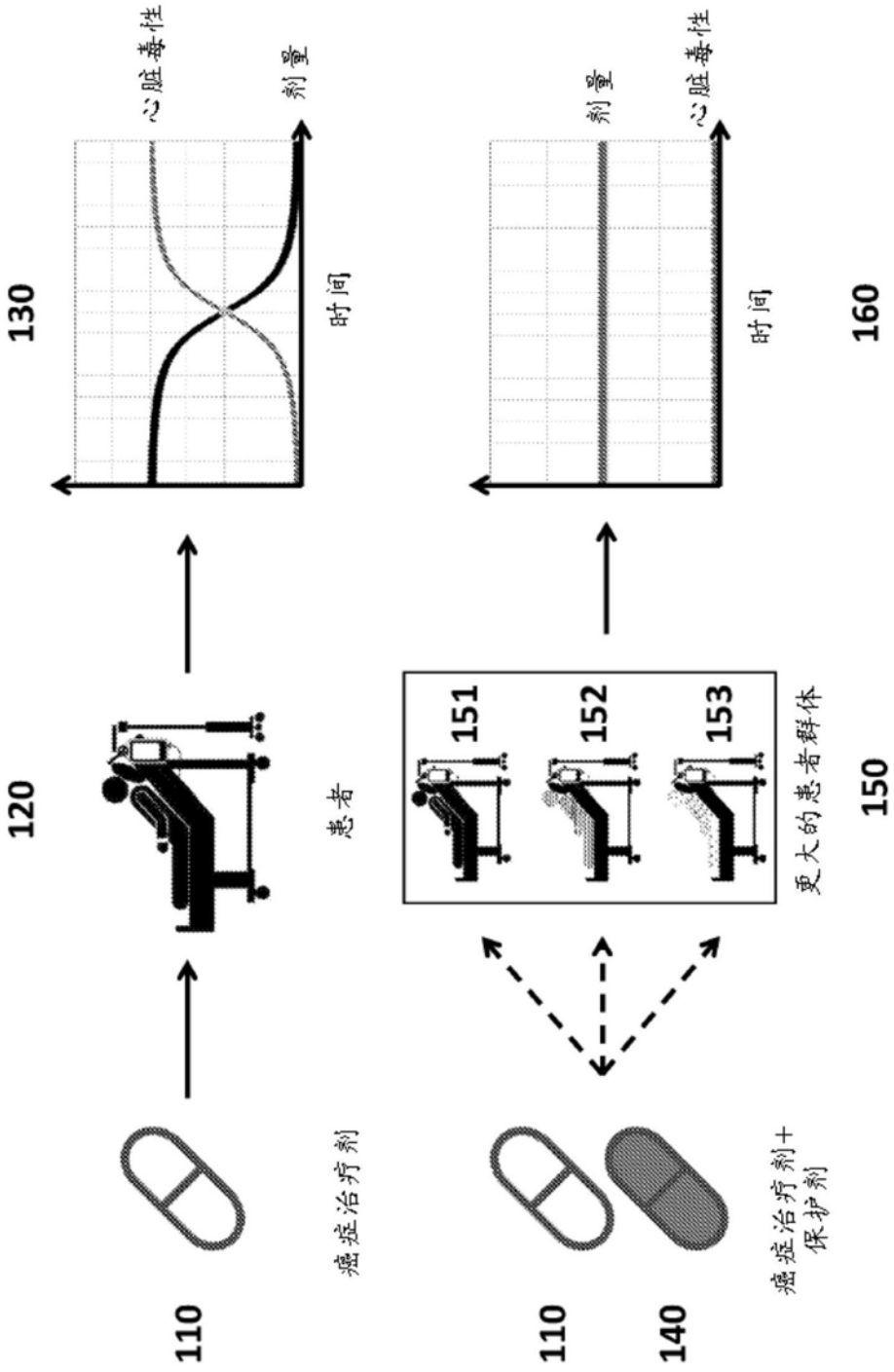

心脏毒性
剂量
时间
130
120
患者
癌症治疗剂
110
151
152
153
更大的患者群体
150
剂量
心脏毒性
时间
160
110
140
癌症治疗剂+
保护剂

图1

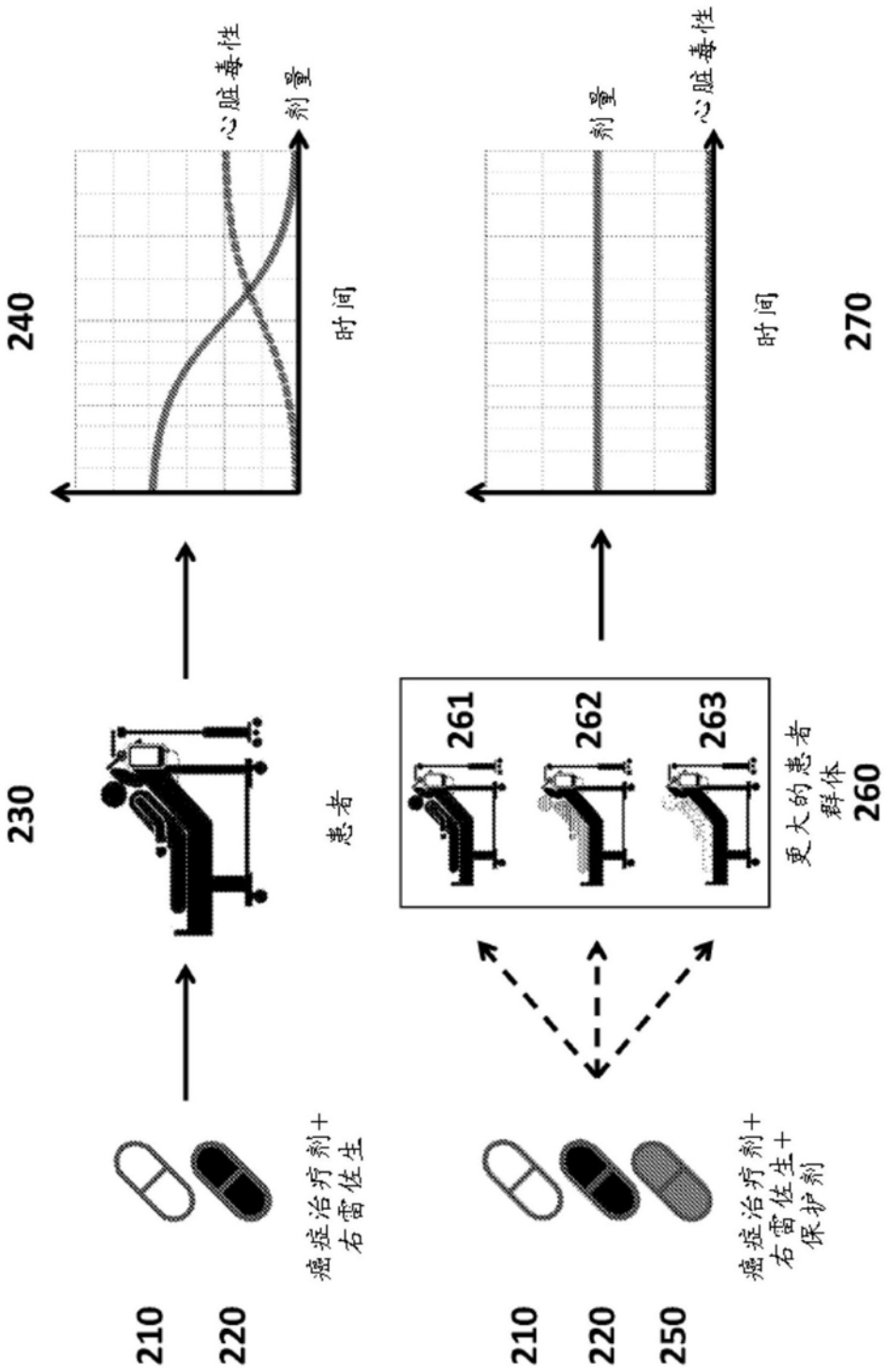

210
220
癌症治疗剂+
右雷佐生
230
患者
240
心脏毒性
剂量
时间
210
220
250
癌症治疗剂+
右雷佐生+
保护剂
261
262
263
更大的患者
群体
260
剂量
心脏毒性
时间
270

图2

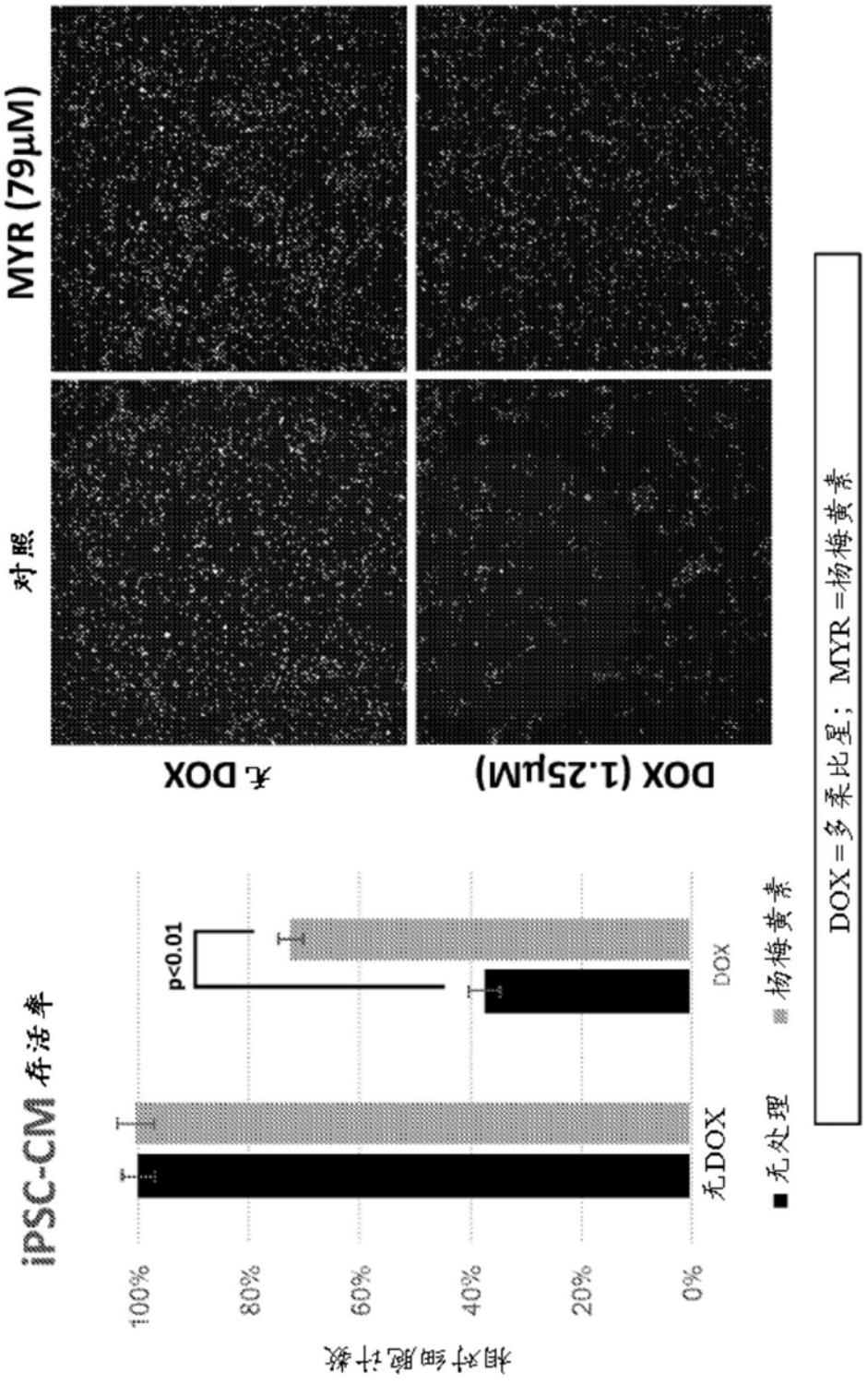
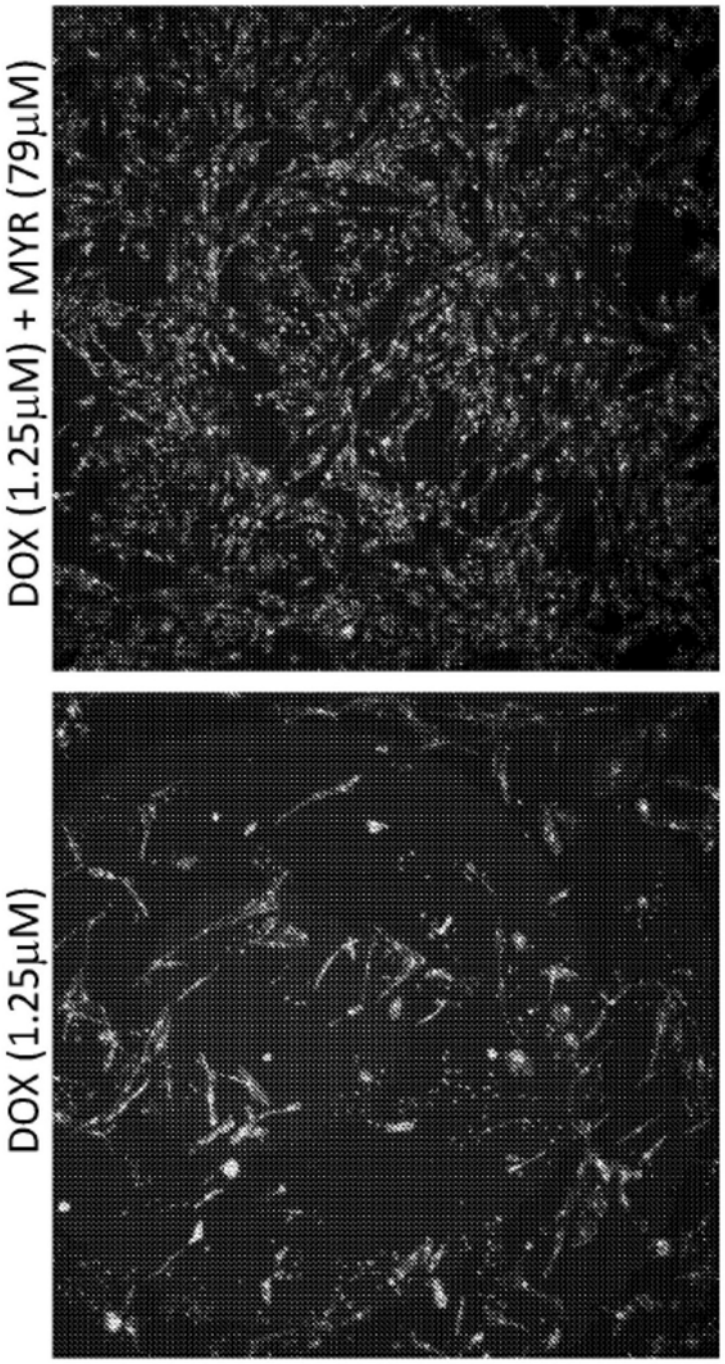

MYR (79μM)
对照
无DOX
DOX (1.25μM)
DOX = 多柔比星； MYR = 杨梅黄素
iPSC-CM 存活率
相对细胞计数
100%
80%
60%
40%
20%
0%
p<0.01
无DOX
DOX
无处理
杨梅黄素

图3



2天

DOX＝多柔比星； MYR＝杨梅黄素

图 4A 图 4B

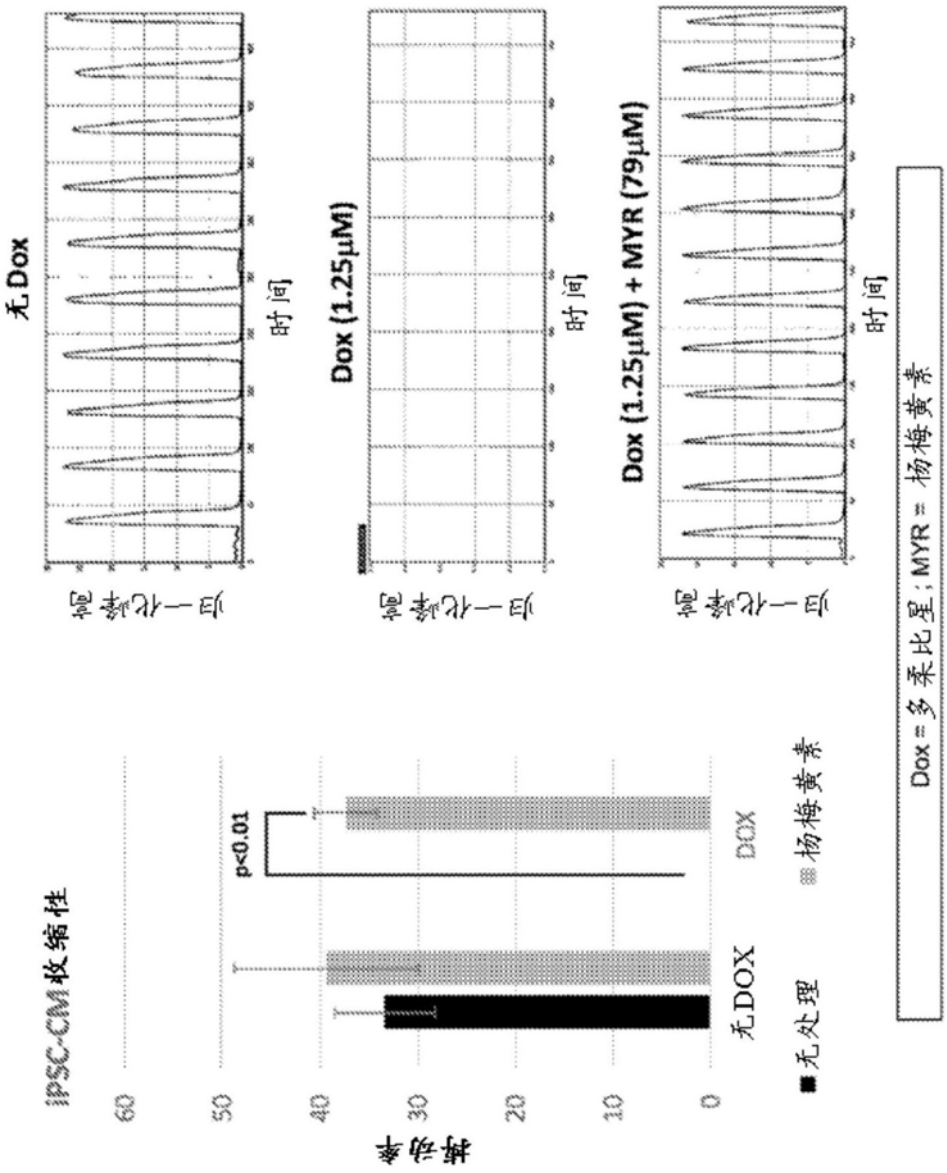

无Dox
Dox (1.25μM)
Dox (1.25μM) + MYR (79μM)
归一化峰高
时间
Dox = 多柔比星；MYR = 杨梅黄素
IPSC-CM收缩性
搏动率
p<0.01
无DOX
DOX
无处理
杨梅黄素

图5

图 6A

	DOX浓度(μM)	平均值 0	平均值 1.25	标准差 0	标准差 1.25
总细胞计数	无处理	8543.50	3195.67	251.02	241.42
	杨梅黄素 (79 μM)	8586.33	6187.67	285.98	194.28

图 6B

	DOX浓度(μM)	0	1.25	0	1.25
细胞%	无处理	100.00%	37.40%	2.94%	2.83%
	杨梅黄素 (79 μM)	100.50%	72.43%	3.35%	2.27%

图 6C

	DOX浓度(μM)	0	1.25	0	1.25
搏动率(BPM)	DMSO	33.33	0.00	5.13	0.00
	杨梅黄素 (79 μM)	39.33	37.33	9.45	3.21

DOX＝多柔比星;MYR＝杨梅黄素

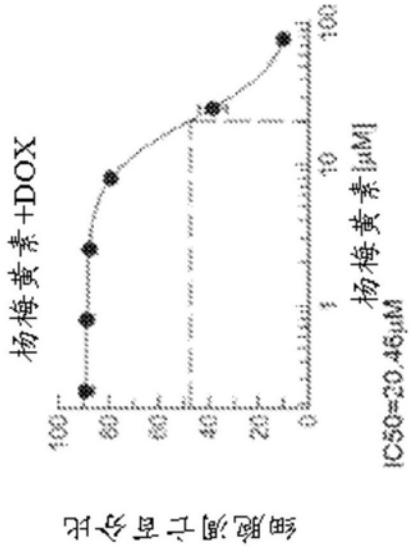

杨梅黄素+DOX
细胞凋亡百分比
杨梅黄素[μM]
IC50=20.46μM
0
20
40
60
80
100
1
10
100

图7A

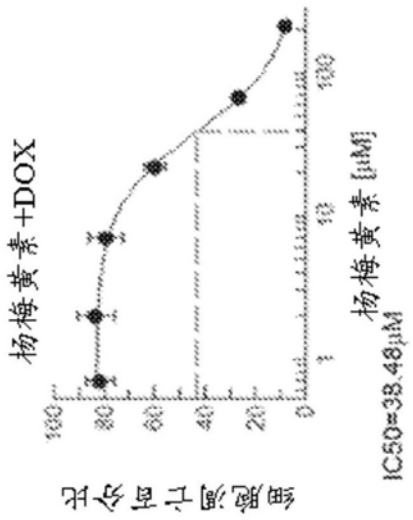

杨梅黄素+DOX
细胞凋亡百分比
杨梅黄素[μM]
IC50=38.48μM
0
20
40
60
80
100
1
10
100

图7B

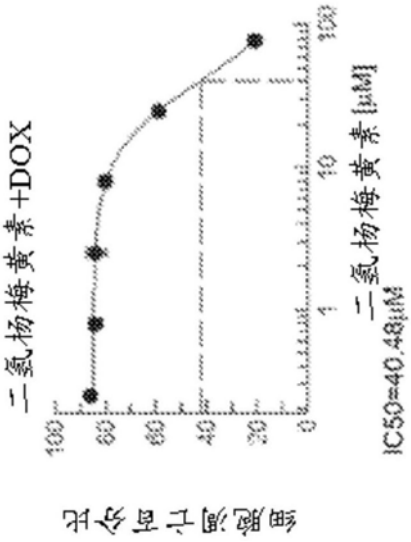

二氢杨梅黄素+DOX
细胞凋亡百分比
100
80
60
40
20
0
0.1
1
10
100
二氢杨梅黄素[μM]
IC50=40.48μM

图7C

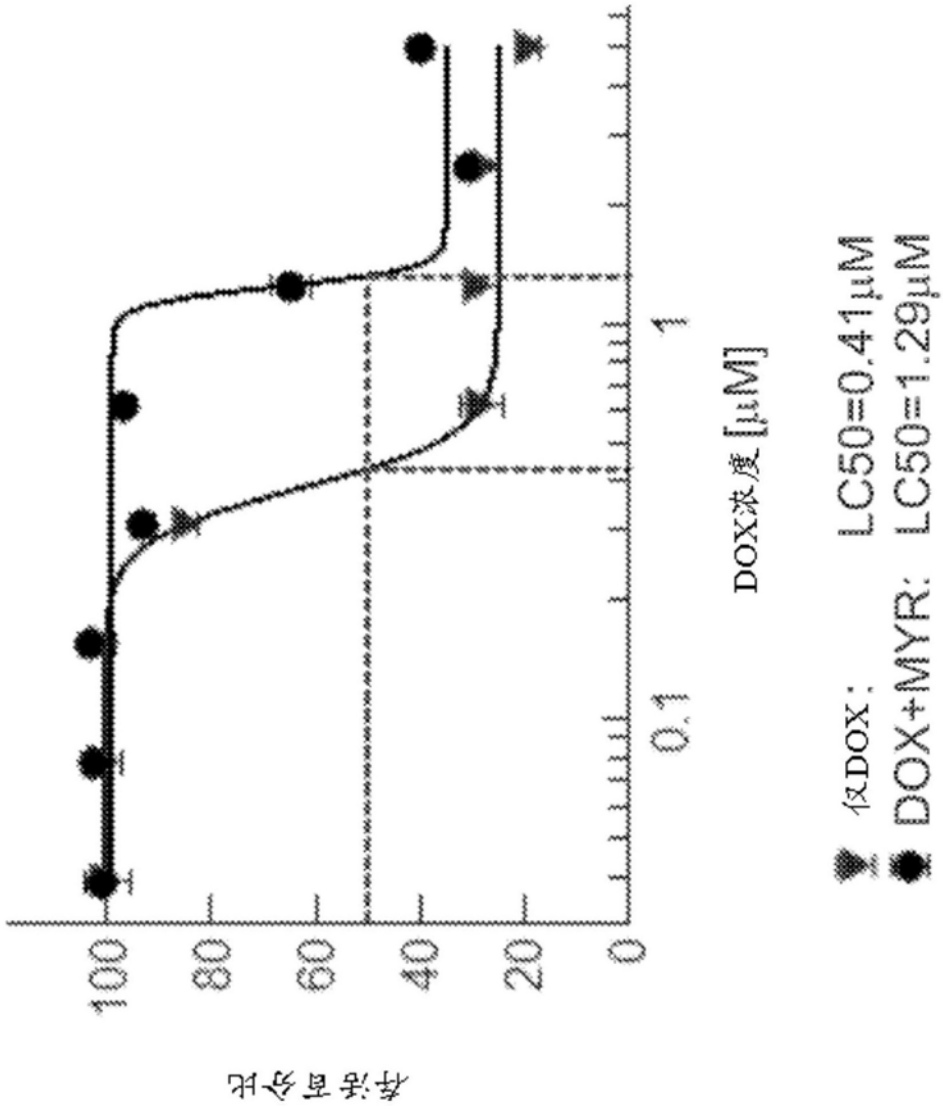

存活百分比
100
80
60
40
20
0
0.1
1
DOX浓度[μM]
仅DOX: LC50=0.41μM
DOX+MYR: LC50=1.29μM

图8

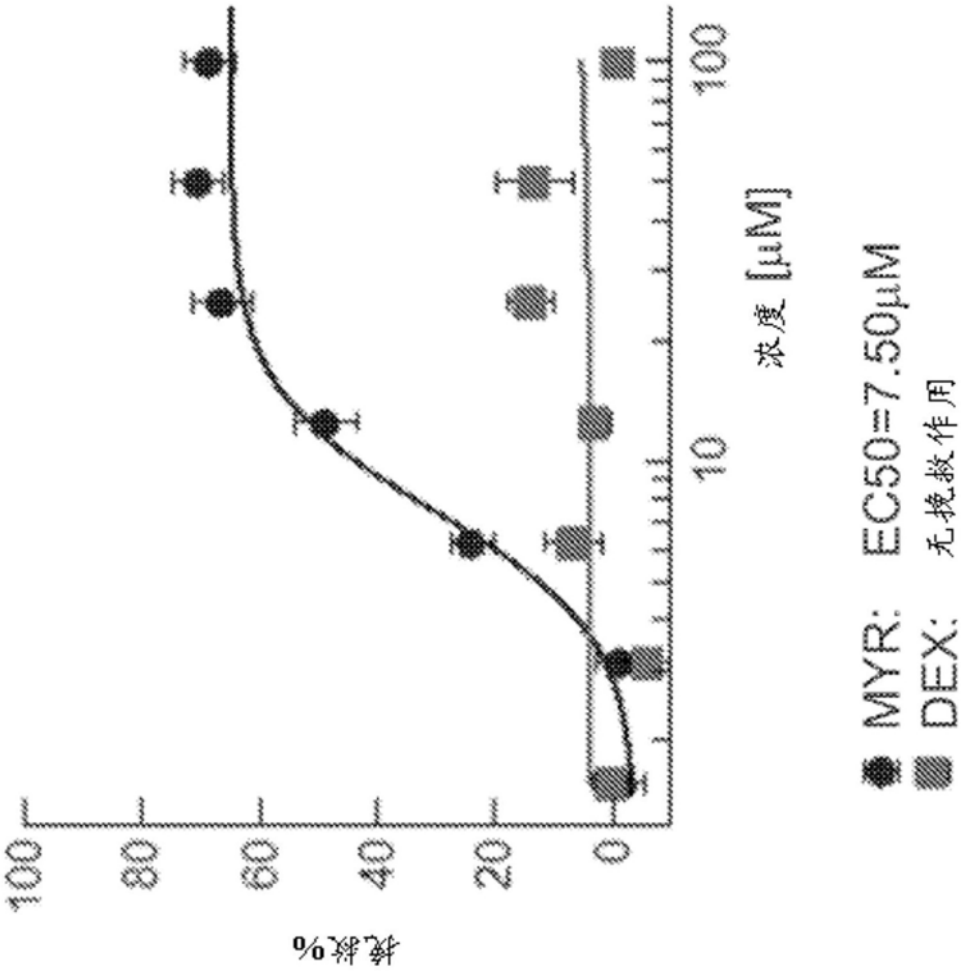


图9

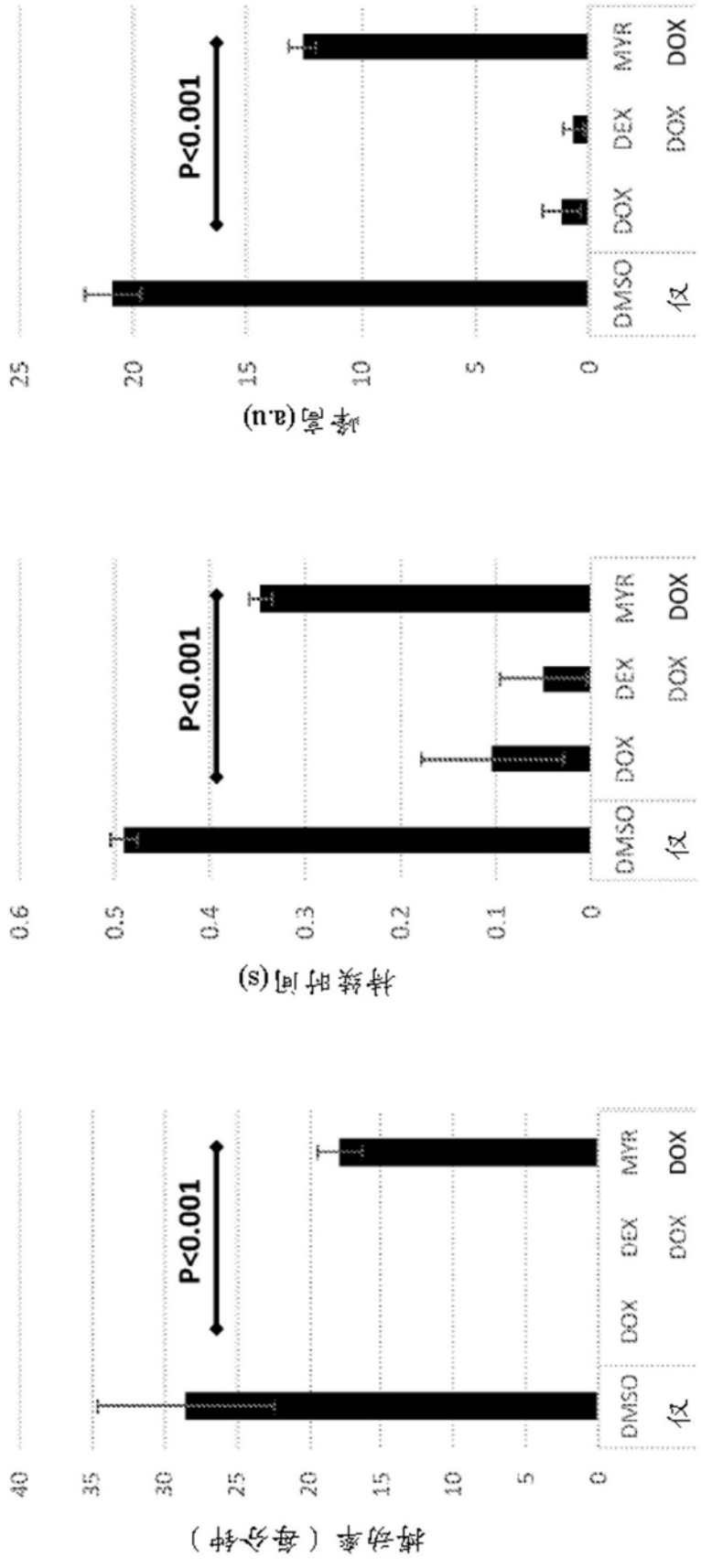

峰高(a.u)
25
20
15
10
5
0
P<0.001
仅
DMSO
DOX
DEX
DOX
MYR
DOX
持续时间(s)
0.6
0.5
0.4
0.3
0.2
0.1
0
P<0.001
仅
DMSO
DOX
DEX
DOX
MYR
DOX
搏动率（每分钟）
40
35
30
25
20
15
10
5
0
P<0.001
仅
DMSO
DOX
DEX
DOX
MYR
DOX

图10

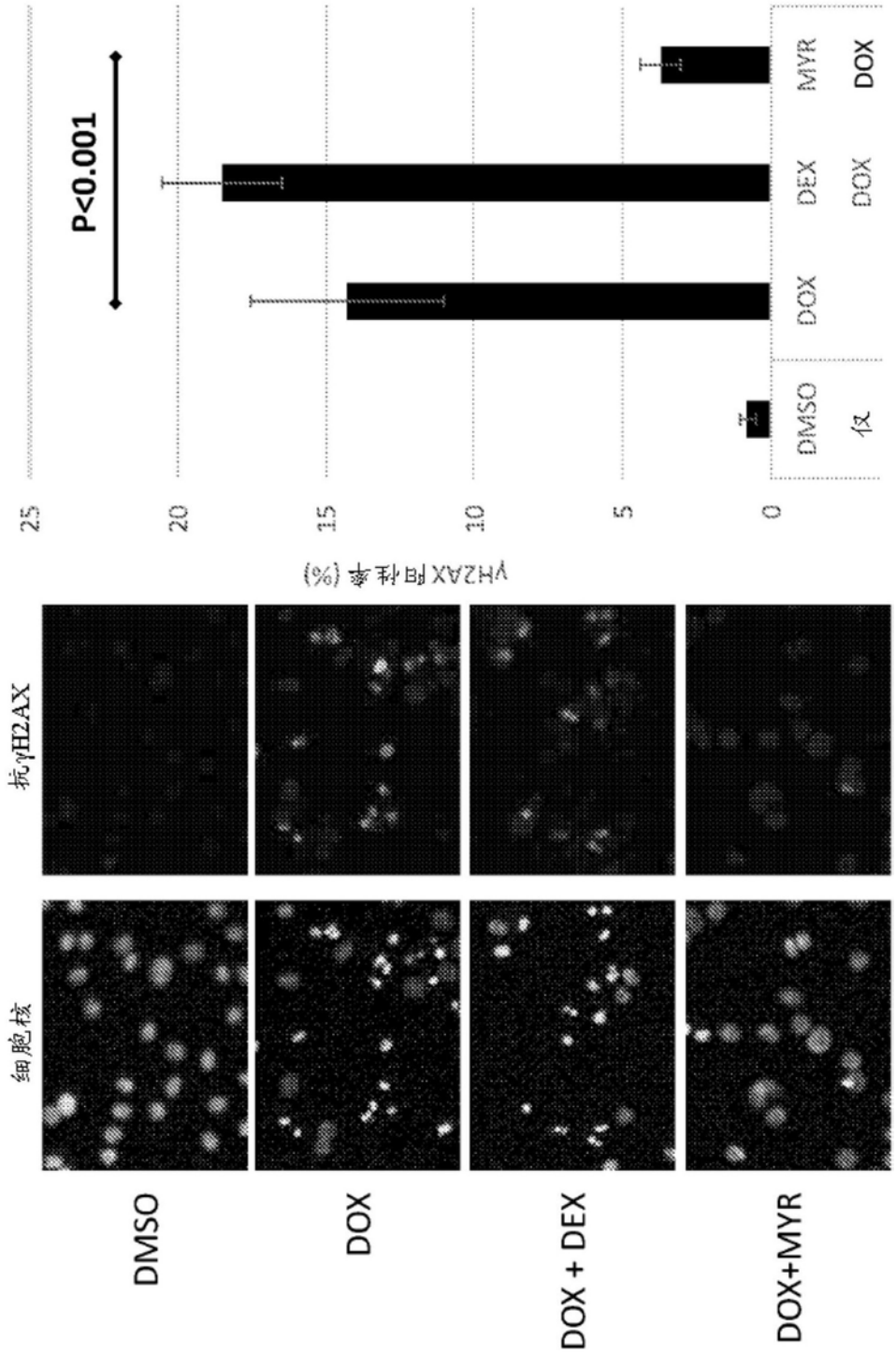

P<0.001
γH2AX阳性率 (%)
25
20
15
10
5
0
DMSO
仅
DOX
DEX
DOX
MYR
DOX
抗γH2AX
细胞核
DMSO
DOX
DOX + DEX
DOX+MYR

图11

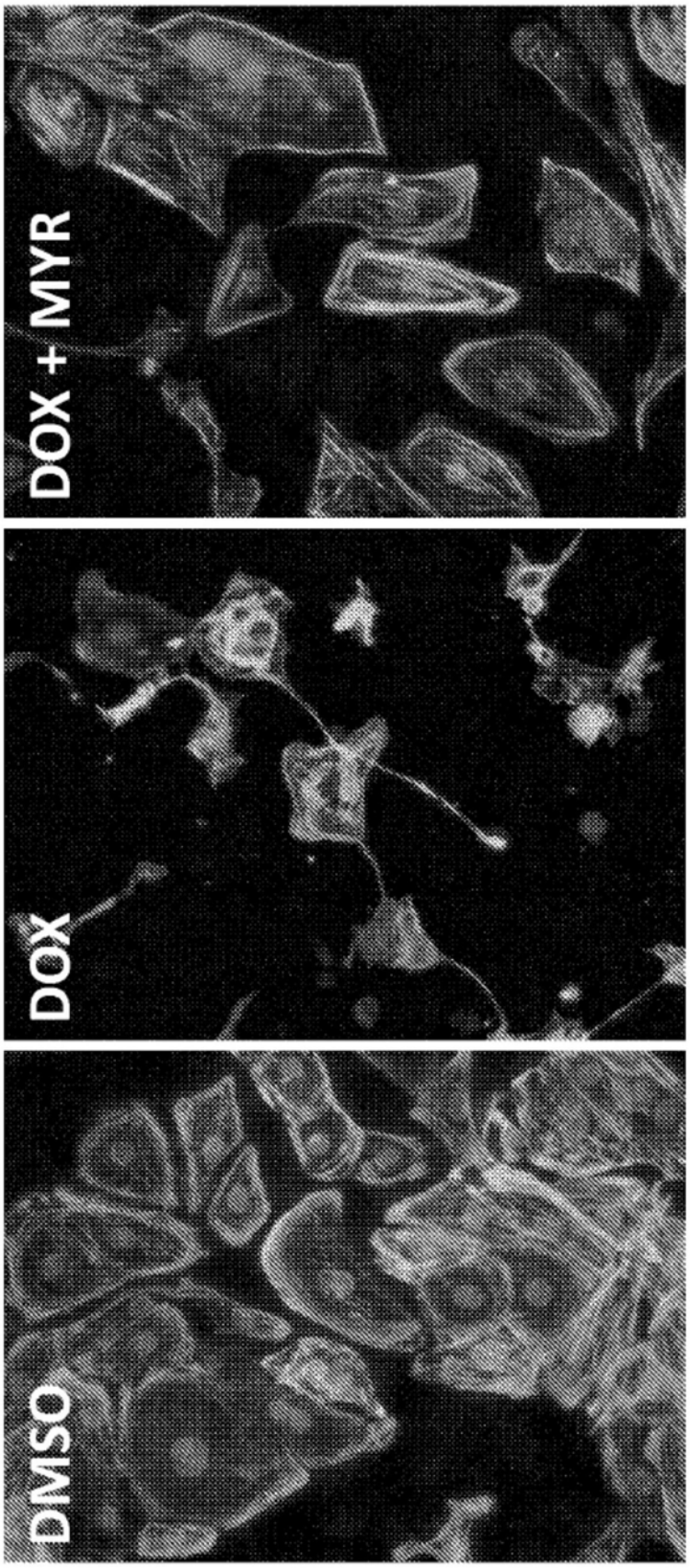


图12

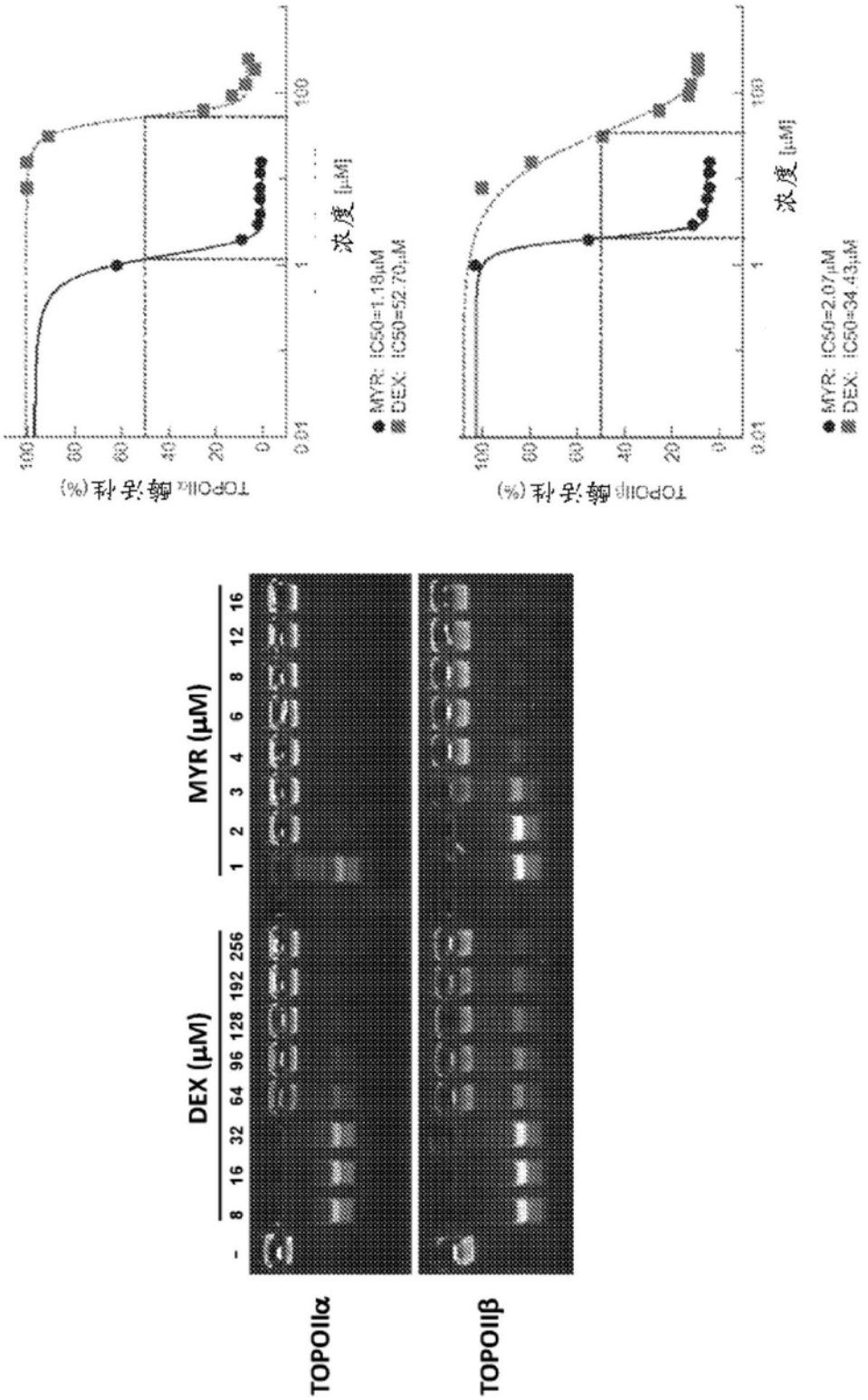

TOPOIIα 酶活性(%)
浓度 [μM]
MYR: IC50=1.18μM
DEX: IC50=52.70μM
TOPOIIβ 酶活性(%)
浓度 [μM]
MYR: IC50=2.07μM
DEX: IC50=34.43μM
DEX (μM)
MYR (μM)
TOPOIIα
TOPOIIβ

图13

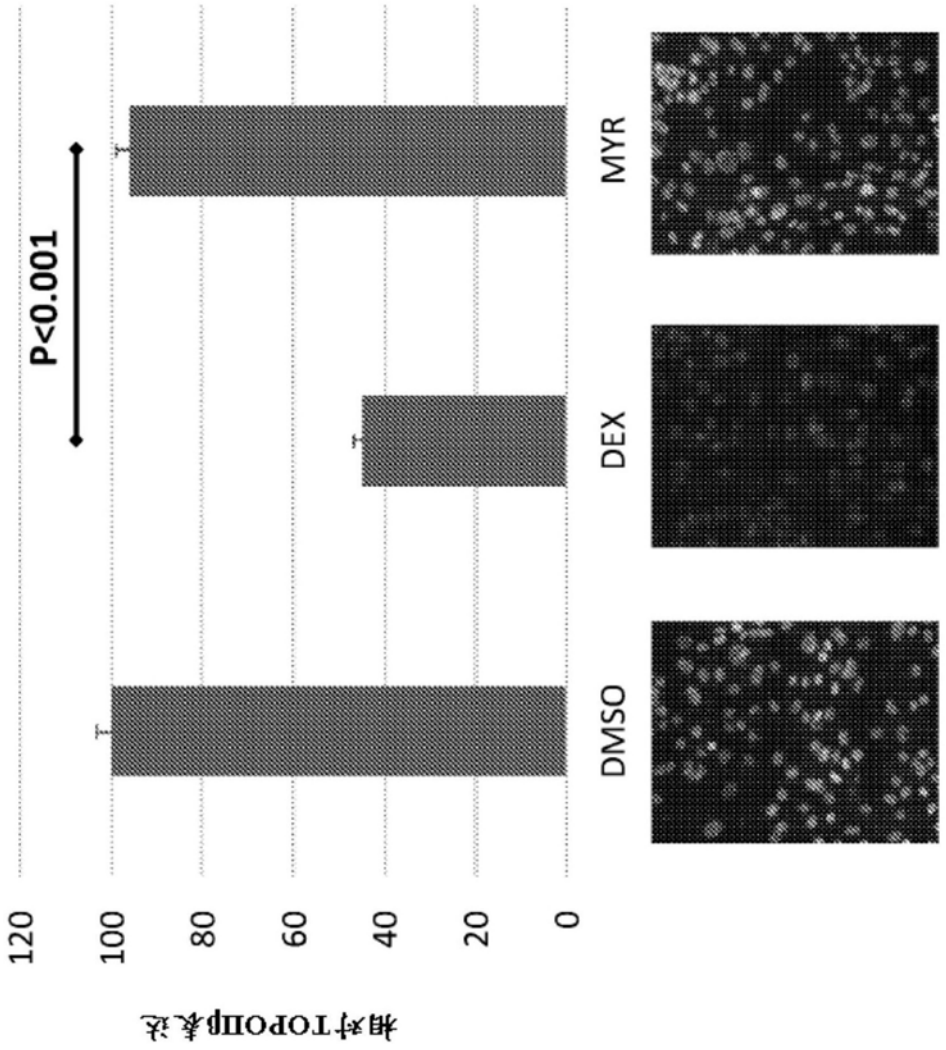


图14

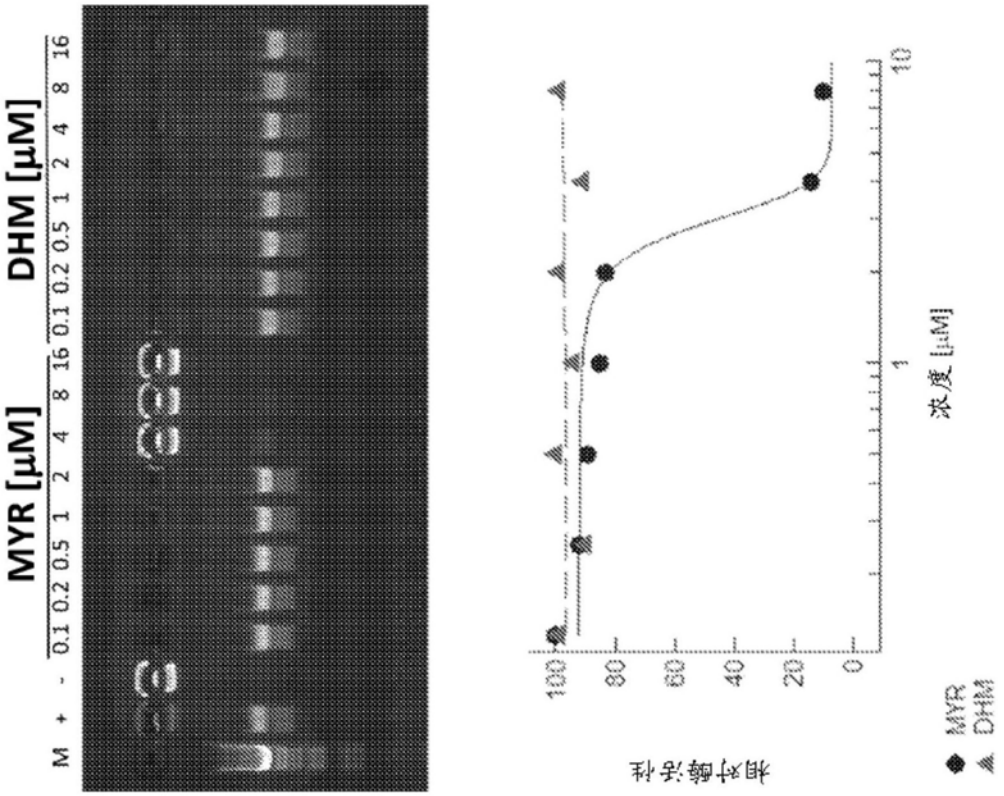

MYR [μM]
DHM [μM]
M + - 0.1 0.2 0.5 1 2 4 8 16 0.1 0.2 0.5 1 2 4 8 16
相对酶活性
浓度 [μM]
MYR
DHM

图15

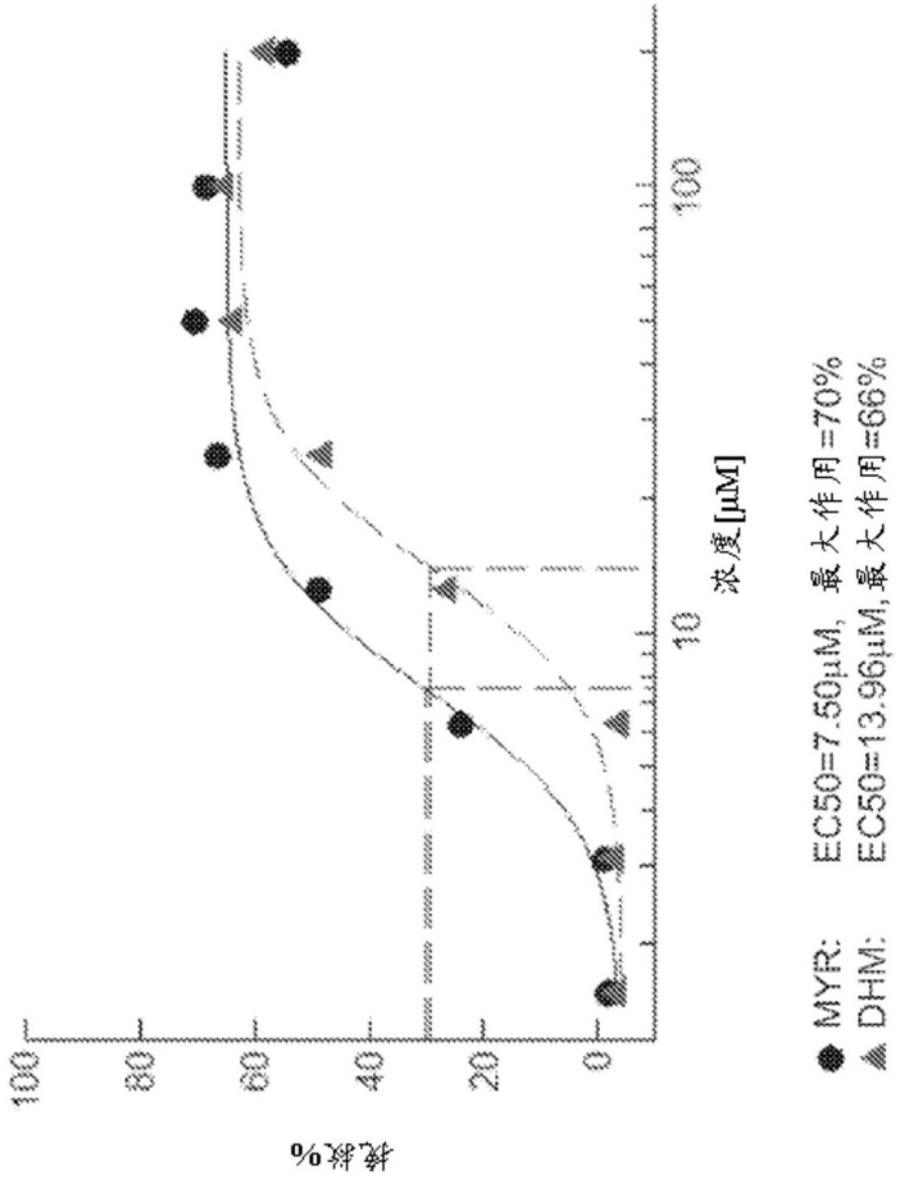

挽救%
100
80
60
40
20
0
10
100
浓度[μM]
● MYR: EC50=7.50μM, 最大作用=70%
▲ DHM: EC50=13.96μM, 最大作用=66%

图16

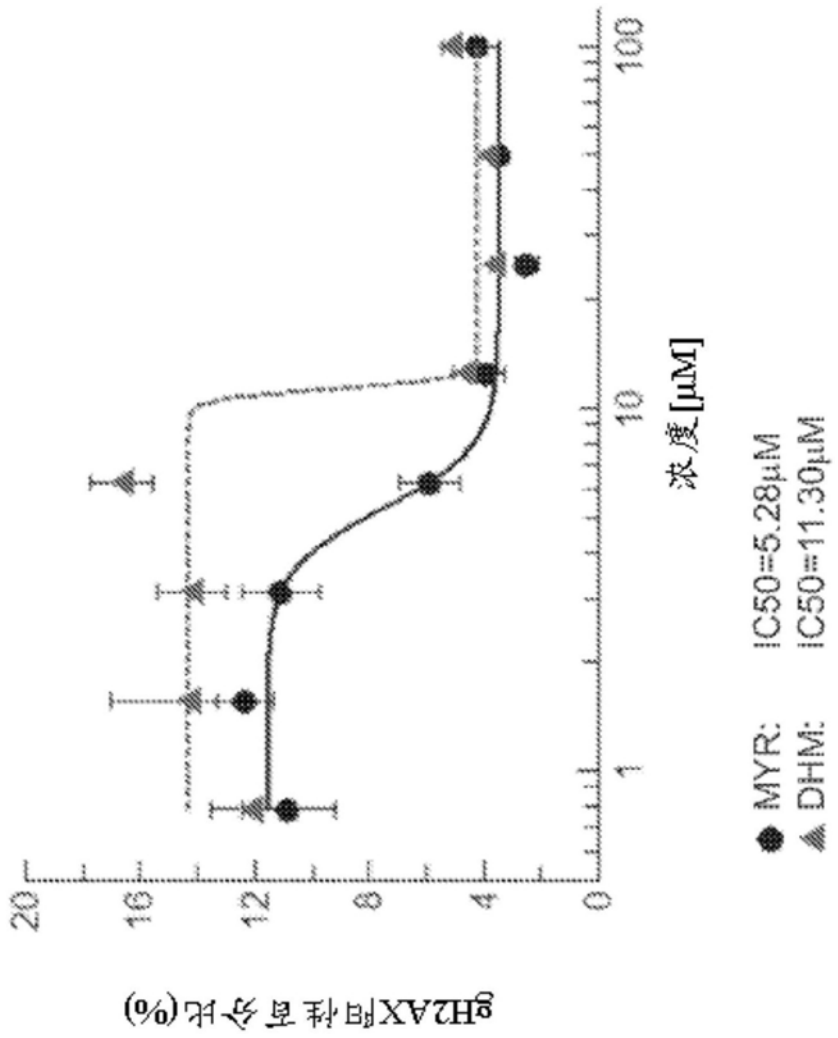

γH2AX阳性百分比(%)
0
4
8
12
16
20
1
10
100
浓度[μM]
MYR: IC50=5.28μM
DHM: IC50=11.30μM

图17

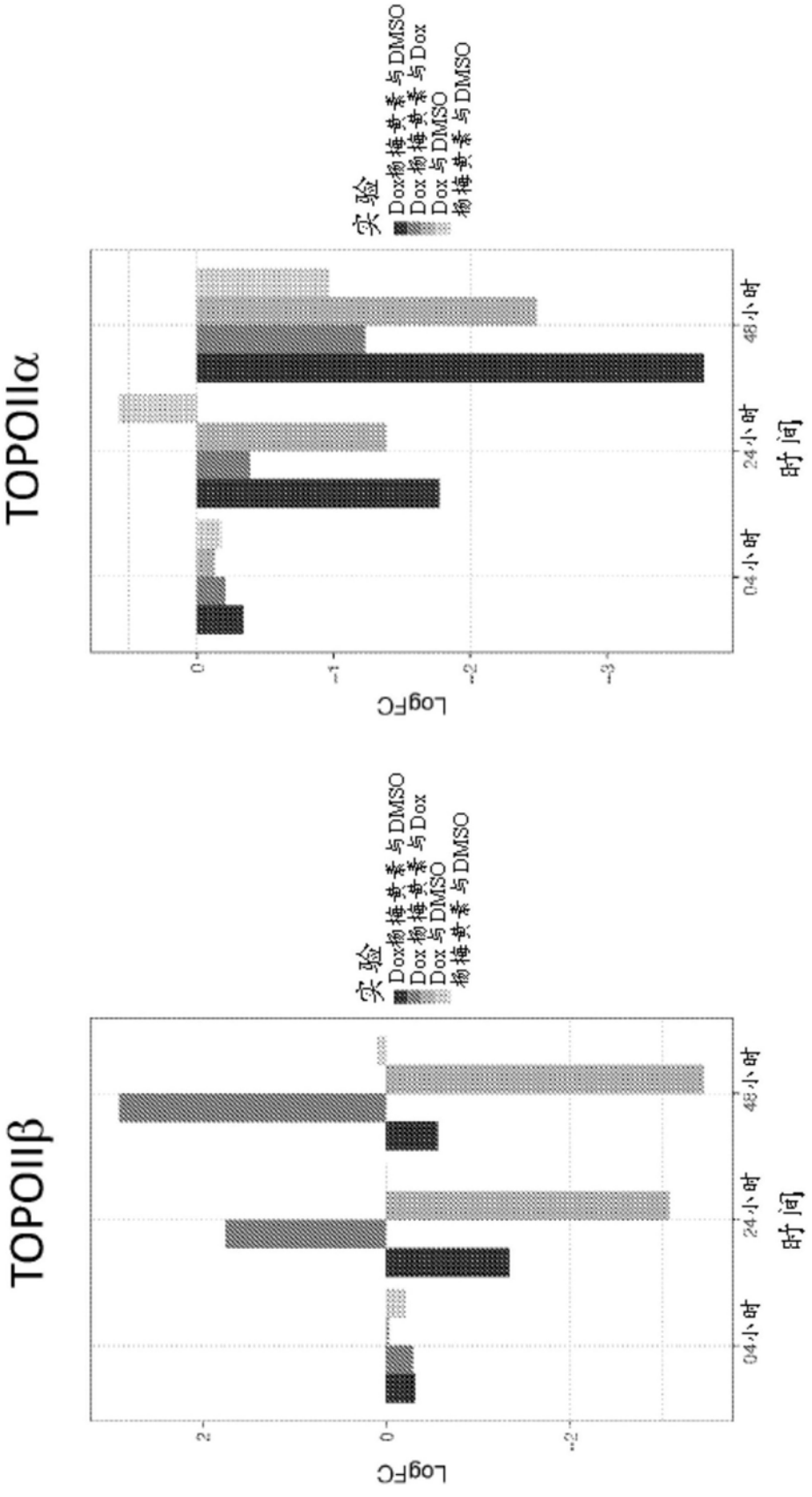
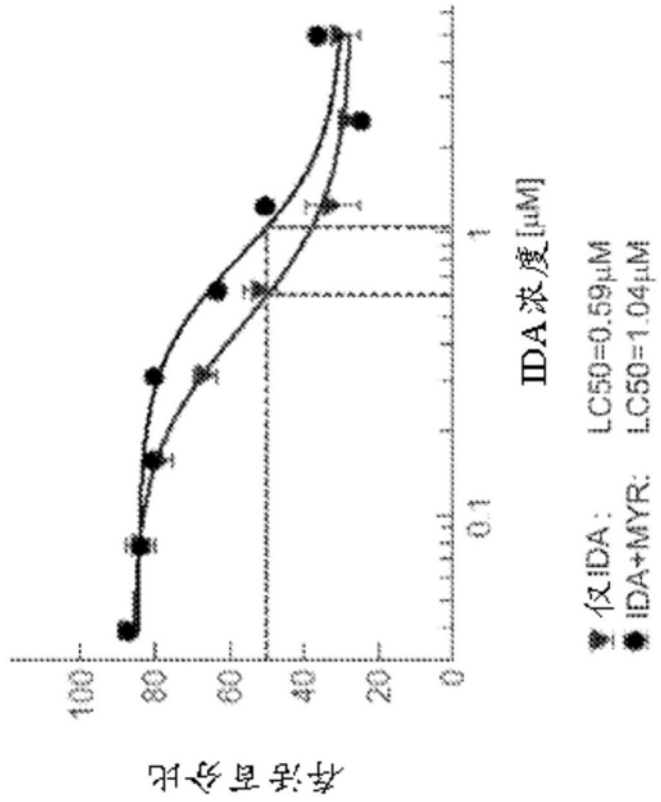

TOPOIIα
实验
Dox柳梅黄素与DMSO
Dox柳梅黄素与Dox
Dox与DMSO
柳梅黄素与DMSO
LogFC
0
-1
-2
-3
04小时
24小时
48小时
时间
TOPOIIβ
实验
Dox柳梅黄素与DMSO
Dox柳梅黄素与Dox
Dox与DMSO
柳梅黄素与DMSO
LogFC
2
0
-2
04小时
24小时
48小时
时间

图18


存活百分比
100
80
60
40
20
0
0.1
1
IDA浓度[μM]
仅IDA： LC50=0.59μM
IDA+MYR： LC50=1.04μM

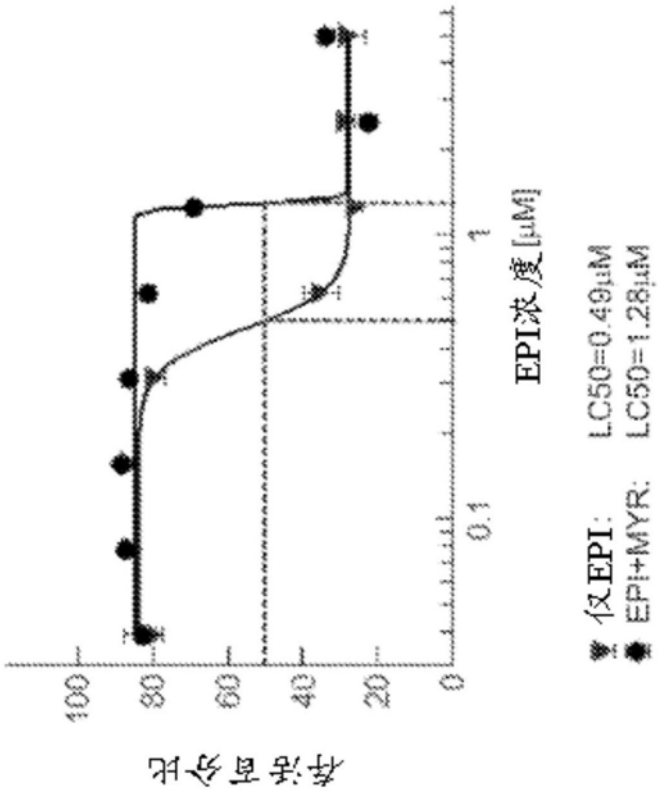

存活百分比
100
80
60
40
20
0
0.1
1
EPI浓度[μM]
仅EPI： LC50=0.49μM
EPI+MYR： LC50=1.28μM

图19

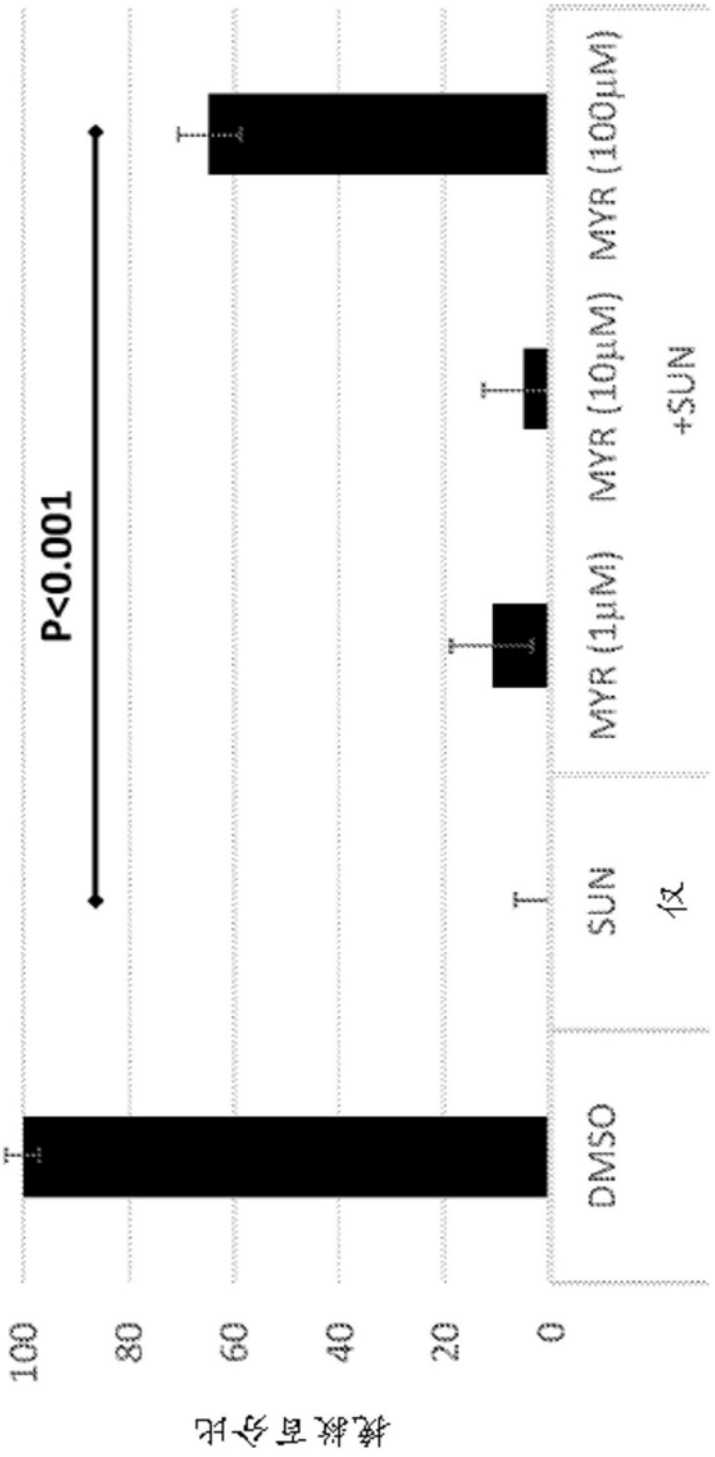

P<0.001
DMSO
SUN
仅
MYR (1μM)
MYR (10μM)
MYR (100μM)
+SUN
0
20
40
60
80
100
拔救百分比

图20

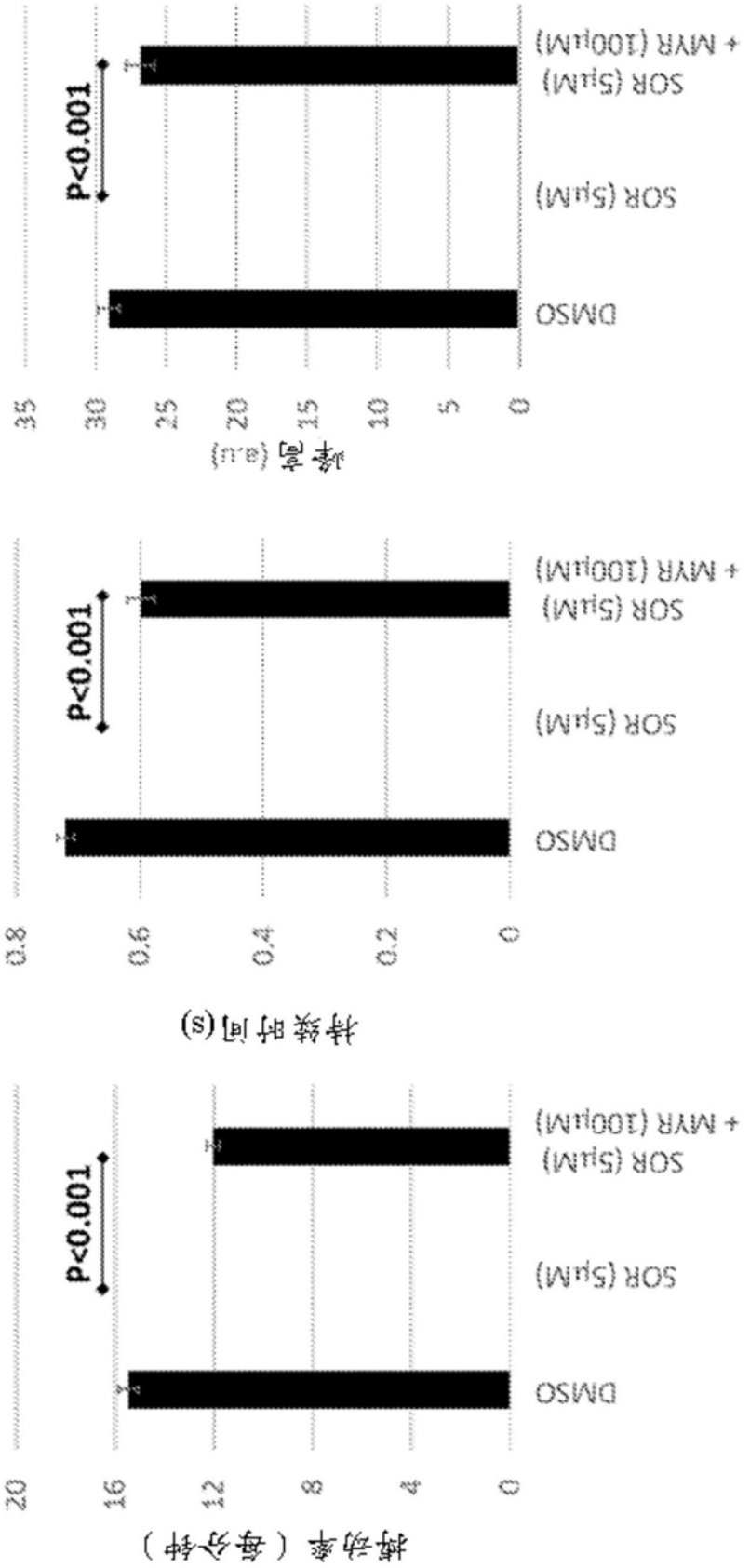

峰高 (a.u)
0
5
10
15
20
25
30
35
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)
P<0.001
持续时间 (s)
0
0.2
0.4
0.6
0.8
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)
P<0.001
搏动率 （每分钟）
0
4
8
12
16
20
DMSO
SOR (5μM)
SOR (5μM) + MYR (100μM)
P<0.001

图21

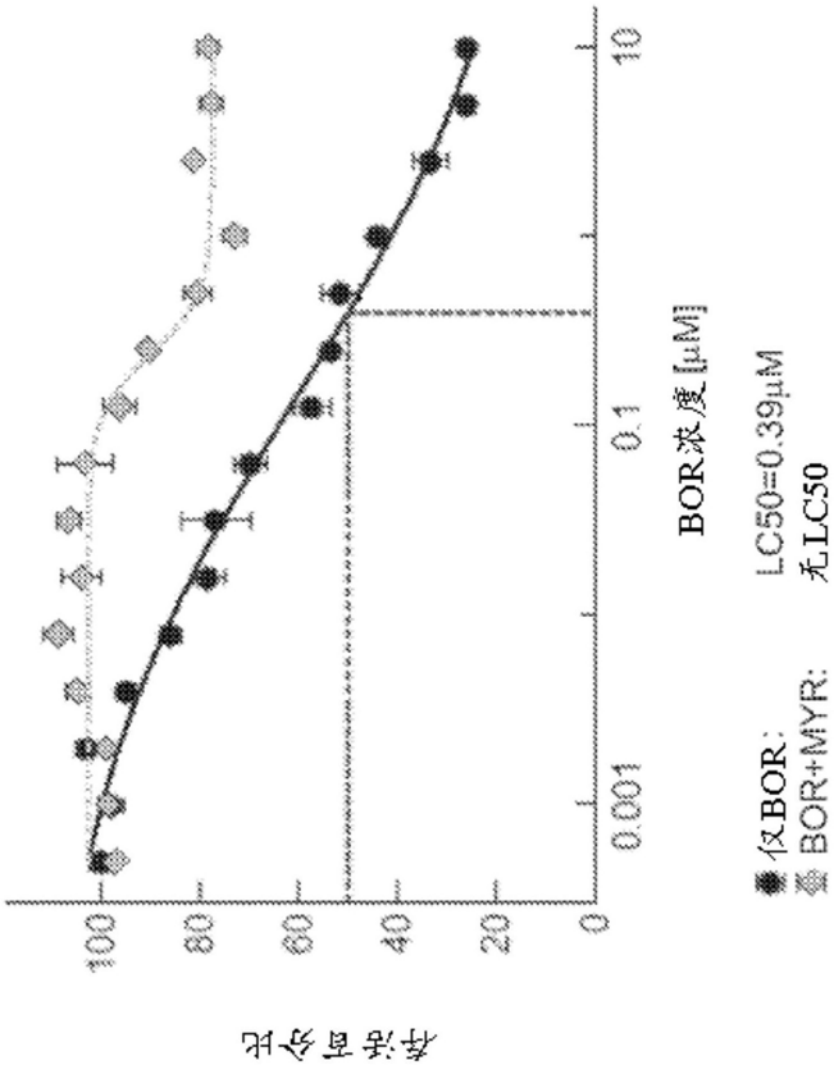

存活百分比
100
80
60
40
20
0
0.001
0.1
10
BOR浓度[μM]
仅BOR:
BOR+MYR:
LC50=0.39μM
无LC50

图22

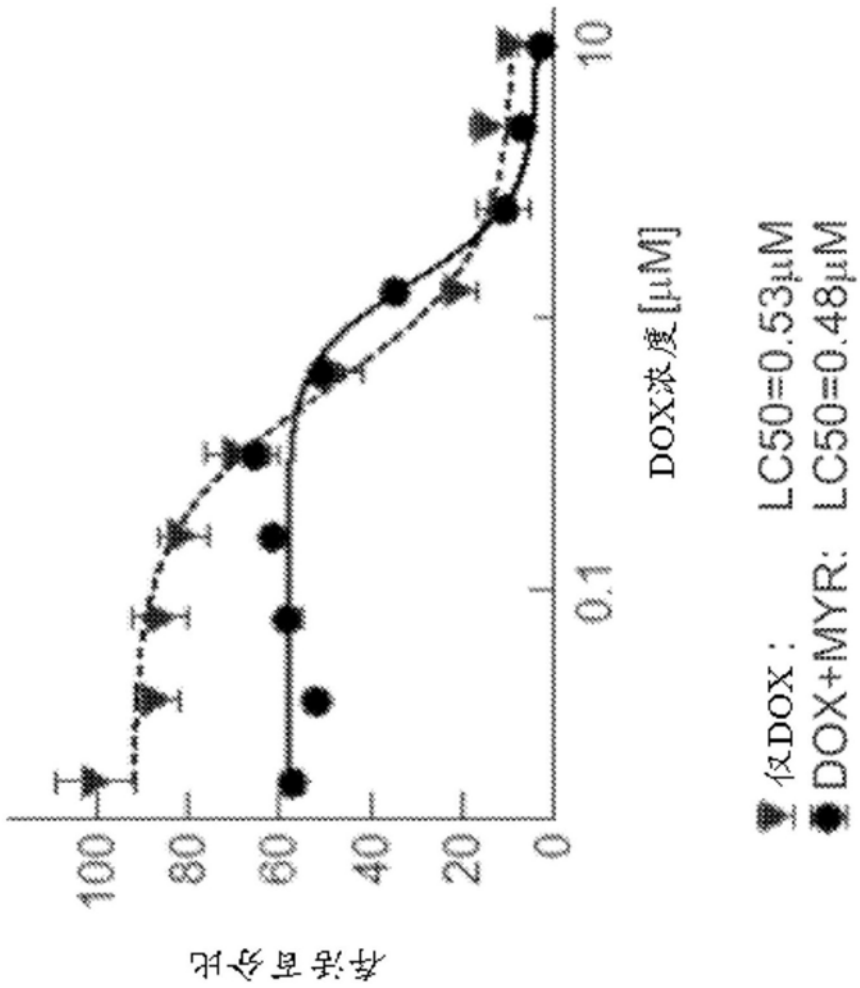

存活百分比
100
80
60
40
20
0
0.1
10
DOX浓度[μM]
仅DOX： LC50=0.53μM
DOX+MYR: LC50=0.48μM

图23

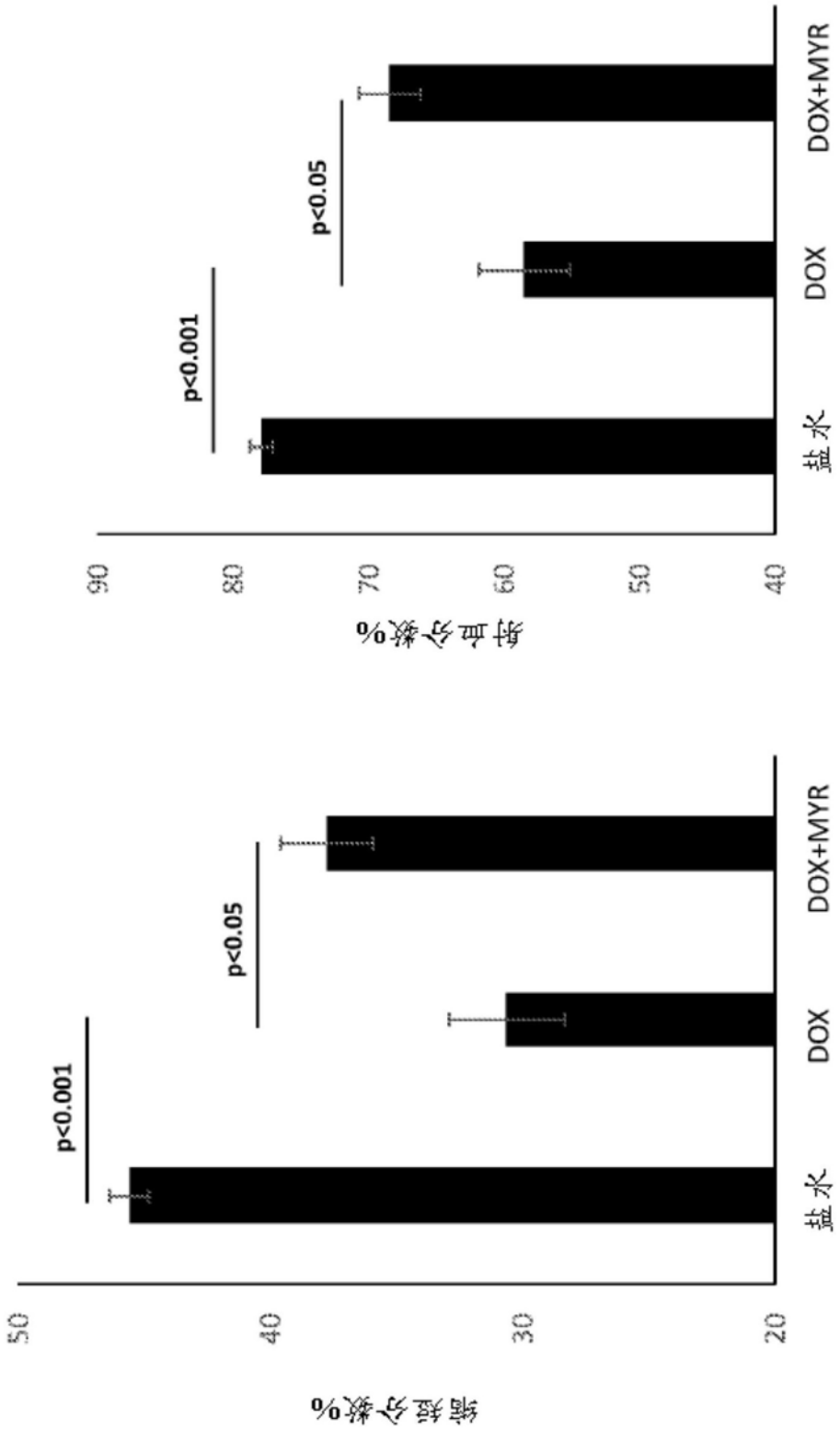

缩短分数%
50
40
30
20
p<0.001
p<0.05
盐水
DOX
DOX+MYR
射血分数%
90
80
70
60
50
40
p<0.001
p<0.05
盐水
DOX
DOX+MYR

图24

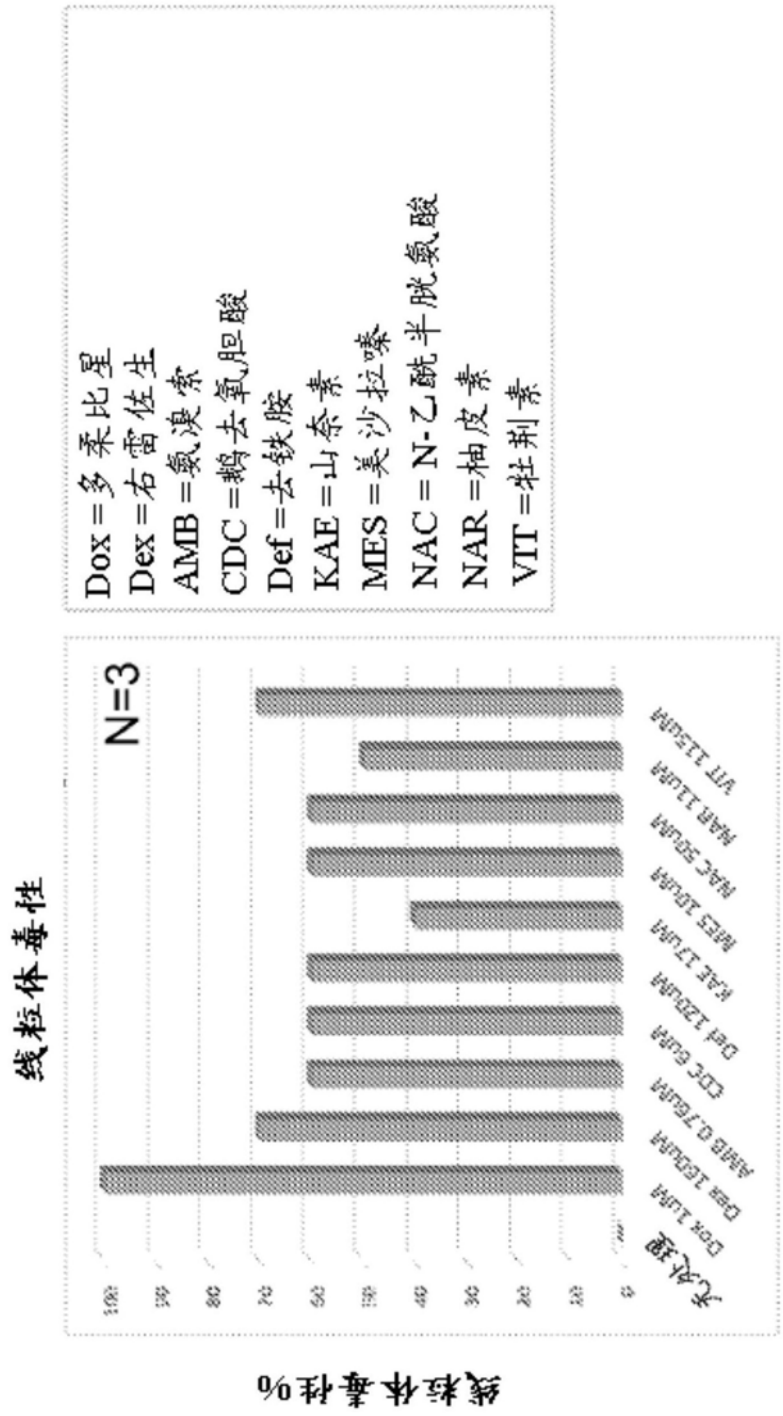

Dox=多柔比星
Dex=右雷佐生
AMB=氨澳索
CDC=鹅去氧胆酸
Def=去铁胺
KAE=山奈素
MES=美沙拉嗪
NAC=N-乙酰半胱氨酸
NAR=柚皮素
VIT=维生素
N=3
线粒体毒性
线粒体毒性%
100
90
80
70
60
50
40
30
20
10
0
对照
Dox 1uM
Dex 100uM
AMB 0.7uM
CDC 6uM
Def 120uM
KAE 17uM
MES 10uM
NAC 50uM
NAR 1uM
VIT 15uM

图25

Dox = 多柔比星
Dex = 右雷佐生
AMB = 氨澳索
CDC = 鹅去氧胆酸
Def = 去铁胺
KAE = 山奈素
MES = 美沙拉嗪
NAC = N-乙酰半胱氨酸
NAR = 柚皮素
VIT = 牡荆素
N=3
细胞凋亡
细胞凋亡%
无处理
Dox 1uM
Dex 100uM
AMB 0.75uM
CDC 6uM
Def 120uM
KAE 17uM
MES 10uM
NAC 50uM
NAR 1uM
VIT 15uM

图26

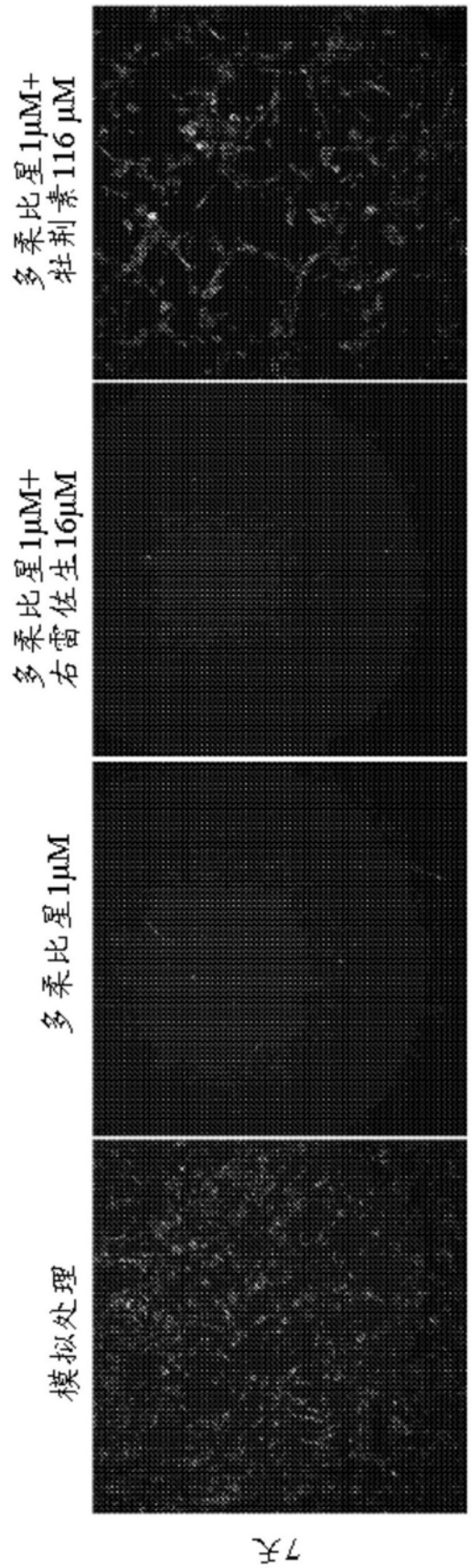

模拟处理
多柔比星1μM
多柔比星1μM+
右雷佐生16μM
多柔比星1μM+
壮荆素116 μM
图 27A
图 27B
图 27C
图 27D

图27

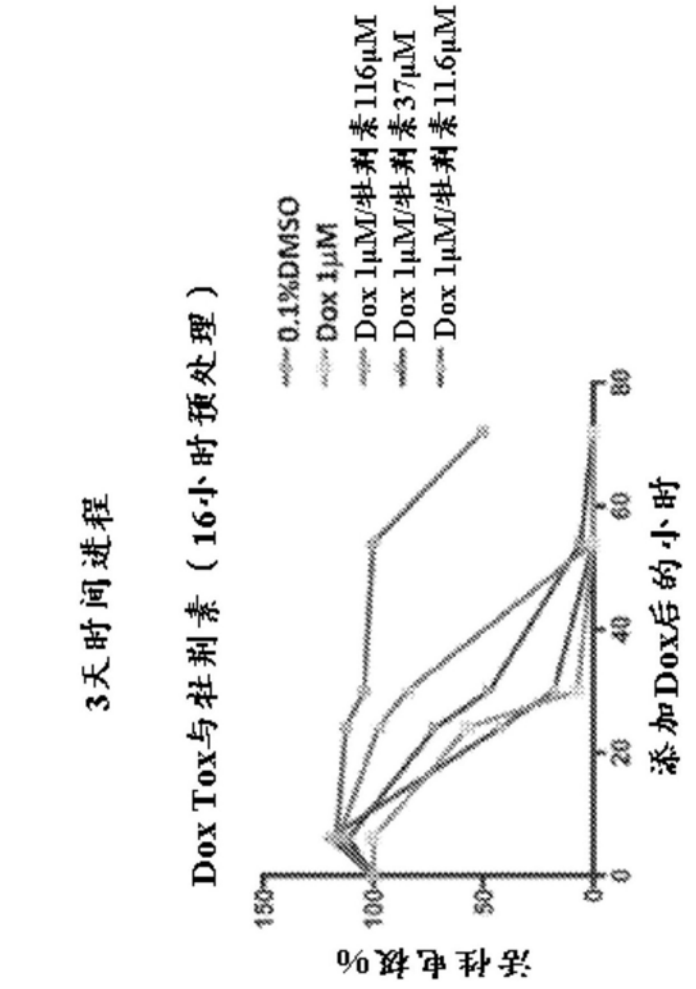

3天时间进程
Dox Tox与壮荆素（16小时预处理）
0.1%DMSO
Dox 1μM
Dox 1μM/壮荆素116μM
Dox 1μM/壮荆素37μM
Dox 1μM/壮荆素11.6μM
活性电极%
添加Dox后的小时
150
100
50
0
0
20
40
60
80

图28A

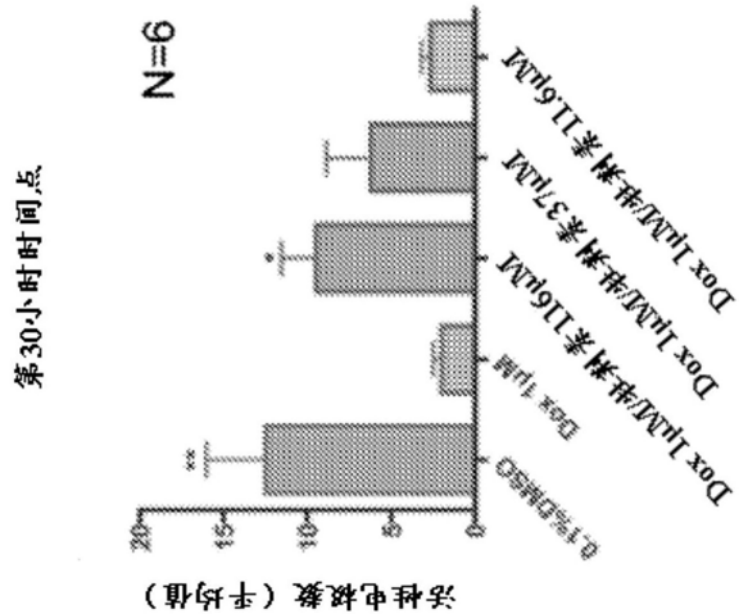

N=6
第30小时时间点
活性电极数（平均值）
0.1%DMSO
Dox 1μM
Dox 1μM/壮荆素116μM
Dox 1μM/壮荆素37μM
Dox 1μM/壮荆素11.6μM
**
*
0
5
10
15
20

图28B

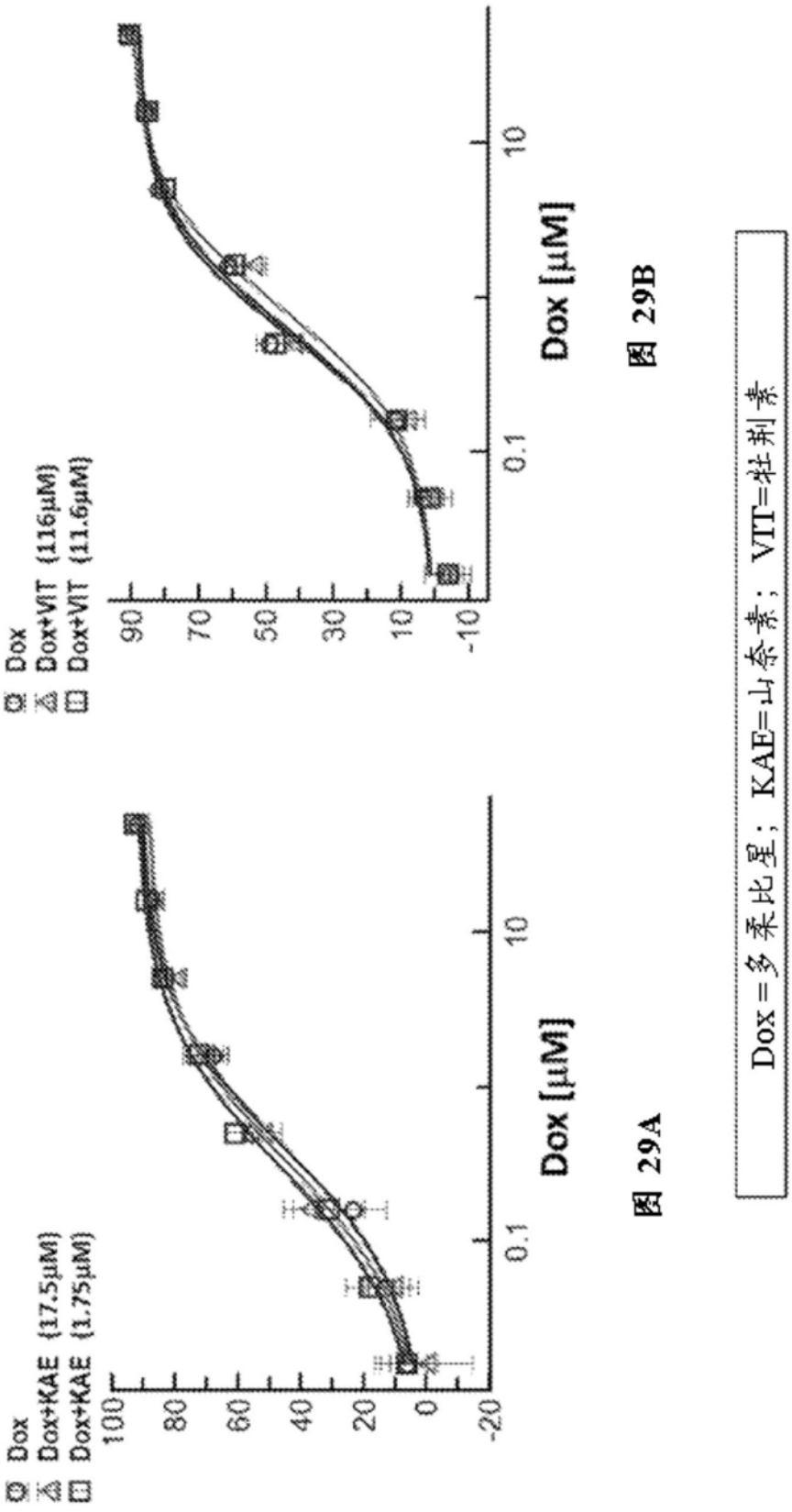


图 29A

图 29B

图29