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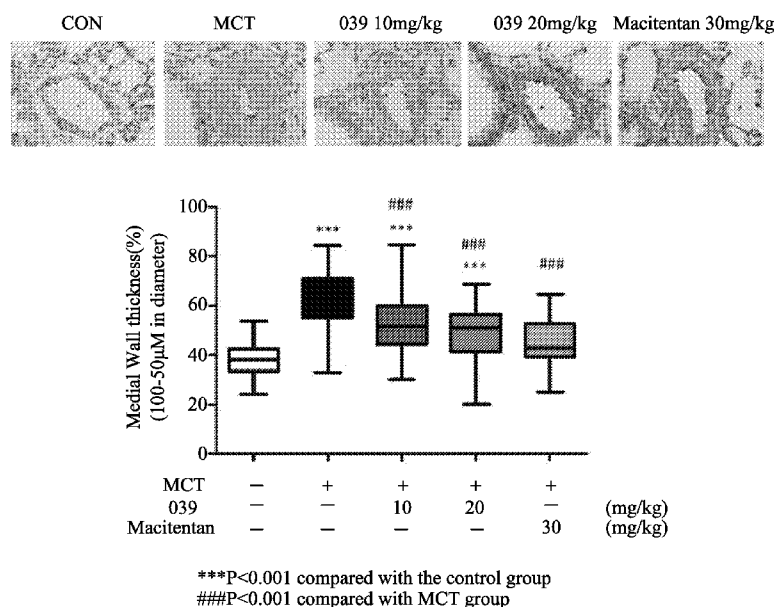
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FIG. 10



(57) Abstract: Provided is a method for preventing or treating pulmonary arterial hypertension in a subject in need thereof, including administering to the subject an effective amount of a 7- dehydrocholesterols or a salt, an oxide, a metabolite, or a derivative thereof.

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METHOD FOR PREVENTING OR TREATING PULMONARY ARTERIAL
HYPERTENSION BY USING 7-DEHYDROCHOLESTEROLS OR SALTS, OXIDES,
METABOLITES, OR DERIVATIVES THEREOF

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BACKGROUND

1. Technical Field

[0001] The present disclosure relates to a method for preventing or treating pulmonary arterial hypertension, particularly a method for preventing or treating pulmonary arterial hypertension by using 7-dehydrocholesterol or a salt, an oxide, a metabolite or a derivative thereof.

2. Description of Related Art

[0002] Pulmonary arterial hypertension (PAH) is a destructive pulmonary vascular disease characterized by persistently elevated mean arterial pressure in pulmonary vessels (≥ 25 mmHg at rest) and normal pulmonary capillary wedge pressure of no more than 15 mmHg accompanying by increase of vessels, thereby progressively leading to right heart failure and even death^{1,2}. Pulmonary arterial hypertension may be primary, hereditary, drug- or toxin-induced, or associated with other conditions including connective tissue diseases, HIV infection, portal hypertension, congenital heart disease, and schistosomiasis, etc.³ However, the pathogenesis of pulmonary arterial hypertension remains unclear until now.

[0003] Early pathological changes of tissues of pulmonary arterial hypertension include vascular endothelial fibrosis, smooth muscle cell proliferation and peripheral pulmonary artery obstruction⁴. Plexiform lesions are pathological changes in late-stage severe pulmonary arterial hypertension, which originate from the proliferation of pulmonary artery endothelial cells, smooth muscle cells, and circulating cells^{5,6}. Relevant genetic studies show that bone morphogenetic protein receptor type 2 (BMPR2) is an important gene affecting more than 70% of patients with familial pulmonary arterial hypertension (FPAH) and 10 to 20% of patients

with idiopathic pulmonary arterial hypertension (HPAH)⁷⁻¹⁰. New classification system reclassifies these patients as hereditary pulmonary arterial hypertension (HPAH).

[0004] BMPR2 is a member of the transforming growth factor- β (TGF- β) receptor superfamily. TGF- β is a multifunctional cytokine that is related to cell growth, differentiation, apoptosis, angiogenesis, wound healing, neuroprotection and immune regulation. Pathologies related to TGF- β include immunosuppression, inflammation, vascular sclerosis, neurodegeneration, tissue fibrosis, and cancer, etc., and promoting or inhibiting TGF- β has become the target for developing many new drugs.

[0005] 7-dehydrocholesterol (7-DHC) is a TGF- β receptor inhibitor, as disclosed in U.S. Patent No. 8,946,201, a certain amount of oxidized 7-DHC can effectively inhibit the activity of TGF- β in the subject, thereby treating and/or preventing skin diseases such as skin fibrosis, skin wounds, inflammation, and alopecia. PCT Patent Publication No. WO2009/138582 also indicates that compositions containing 7-DHC, its derivatives or natural extracts of plant or animal microorganisms containing 7-DHC can be used as cosmetics or food additives. U.S. Patent No. 10,683,324 mentions that 7-DHC can be used to treat or prevent cancer, as well as treat or prevent uncontrolled angiogenesis.

[0006] The drugs traditionally used to treat pulmonary arterial hypertension include calcium ion blockers, anticoagulants, diuretics or cardiotonic agents. In recent years, some new drugs have been developed for the treatment of pulmonary arterial hypertension, which can be divided into three categories according to the mechanism of action: (1) endothelin receptor antagonists; (2) phosphodiesterase type 5 inhibitors; and (3) prostacyclin analogs. However, both traditional drugs and novel drugs can only slow down the progression of the disease and temporarily improve the clinical symptoms of the patient.

[0007] Therefore, there is still an urgent need to develop a method or medicine effective for preventing or treating pulmonary arterial hypertension.

SUMMARY

[0008] The present disclosure provides a method for preventing or treating pulmonary arterial hypertension using a pharmaceutical composition comprising a TGF- β receptor inhibitor and a pharmaceutically acceptable excipient thereof.

[0009] In one aspect, in view of the foregoing, the present disclosure provides a pharmaceutical composition for preventing or treating pulmonary arterial hypertension in a subject in need thereof, comprising an effective amount of a TGF- β receptor inhibitor and a pharmaceutically acceptable excipient thereof.

[0010] In at least one embodiment of the present disclosure, the TGF- β receptor inhibitor is a 7-dehydrocholesterol or a salt, an oxide, a metabolite or a derivative thereof.

[0011] In at least one embodiment of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is administered to the subject in a dosage range of 1 mg/kg to 50 mg/kg.

[0012] In some embodiments of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is administered to the subject in a dosage range of 10 mg/kg to 20 mg/kg.

[0013] In at least one embodiment of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is a single active ingredient in the pharmaceutical composition. In other embodiments of the present disclosure, the pharmaceutical composition further comprises an additional active ingredient selected from the group consisting of a calcium blocker, an anticoagulant, a diuretic, a cardiotonic agent, an endothelin receptor antagonist, a phosphodiesterase type 5 inhibitor, a prostacyclin analog, and any combination thereof.

[0014] In another aspect, in view of the foregoing, the present disclosure provides a method for preventing or treating pulmonary arterial hypertension in a subject in need thereof, comprising administering to the subject a pharmaceutical

composition comprising an effective amount of a TGF- β receptor inhibitor and a pharmaceutically acceptable excipient thereof.

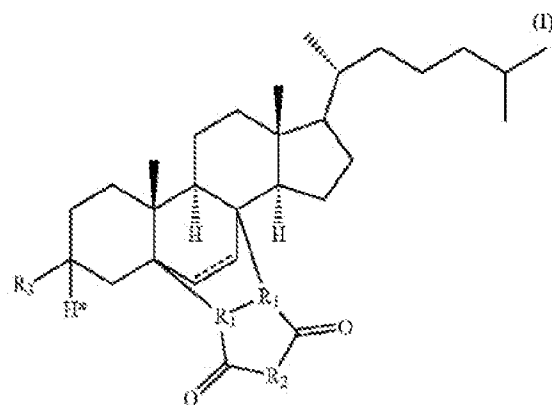
[0015] In at least one embodiment of the present disclosure, the TGF- β receptor inhibitor can reduce proliferation of pulmonary artery endothelial cells, smooth muscle cells, or a combination thereof in the subject.

[0016] In at least one embodiment of the present disclosure, the TGF- β receptor inhibitor is a 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof.

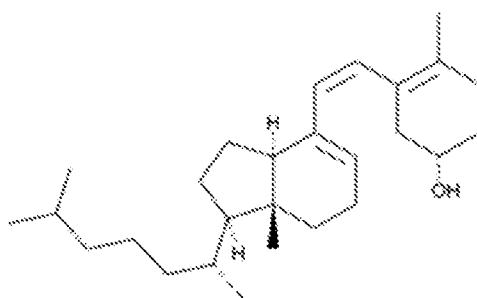
[0017] In at least one embodiment of the present disclosure, the salt of 7-dehydrocholesterol includes an acetate salt or a benzoate salt of a (3 β)-7-dehydrocholesterol or a derivative thereof.

[0018] In at least one embodiment of the present disclosure, the derivative of 7-dehydrocholesterol includes a cholecalciferol (i.e., vitamin D3) or a compound represented by formula (I), in which R_1 is CR_5 or N; R_3 is selected from the group consisting of $-O(CR_5)_nR_6$, $-OC(-O)(CR_5)_nR_6$, $-OC(=O)(CR_5)_nOR_5$ and $-OC(=O)C(R_5)=C(R_5)_2$; R_2 is selected from the group consisting of oxygen, sulfur, $C(R_4)_2$, and $N(R_4)$; R_4 is independently selected for each occurrence from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, OR_5 and $N(R_5)_2$; R_5 is independently selected for each occurrence from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl and substituted heteroarylalkyl; R_6 is selected from the group consisting of fluorine, chlorine, bromine, iodine, methanesulfonyl, toluenesulfonyl, $-OSi(R_5)_3$, $-C(=O)OR_5$ and $-C(=O)R_5$; dashed line represents a single bond or a double bond; and n is an integer from 1 to 10.

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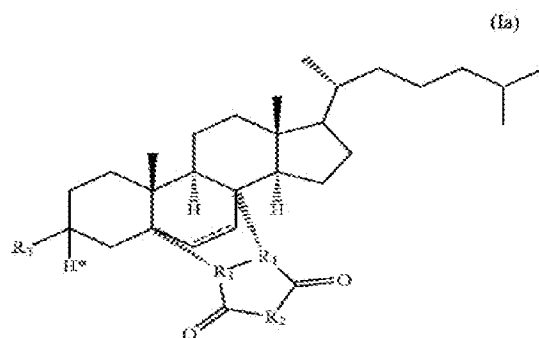


Compound represented by formula (I)

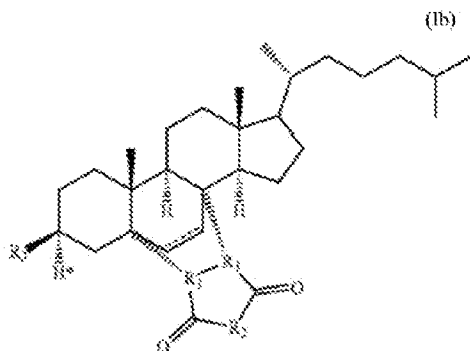


Cholecalciferol

5 [0019] In at least one embodiment of the present disclosure, the compound represented by formula (I) is the compound represented by formula (Ia) or a salt or a solvate thereof, in which R₁ to R₃ are as defined above:



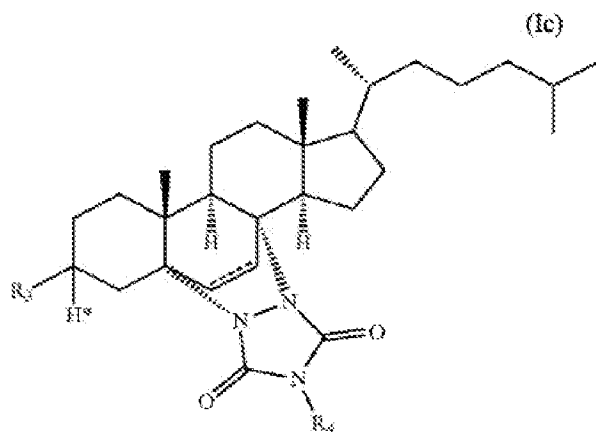
10 [0020] In at least one embodiment of the present disclosure, the compound represented by formula (I) is the compound represented by formula (Ib) or a salt or a solvate thereof, wherein R₁ to R₃ are as defined above:



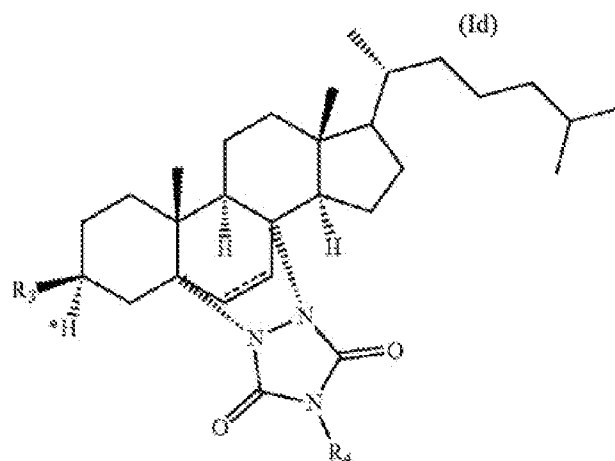
[0021] In at least one embodiment of the present disclosure, R_1 of the compound represented by formula (I) is N.

[0022] In at least one embodiment of the present disclosure, R_2 is $N(R_4)$, and R_4 is defined as above.

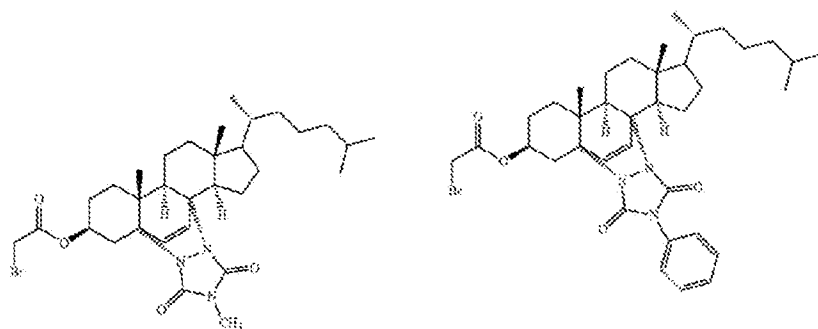
[0023] In at least one embodiment of the present disclosure, the compound represented by formula (I) is the compound represented by formula (Ic) or a salt or a solvate thereof, in which R_3 and R_4 are as defined above:



[0024] In at least one embodiment of the present disclosure, the compound represented by formula (I) is the compound represented by formula (Id) or a salt or a solvate thereof, in which R_3 and R_4 are as defined above:



[0025] In at least one embodiment of the present disclosure, the compound represented by formula (I) is

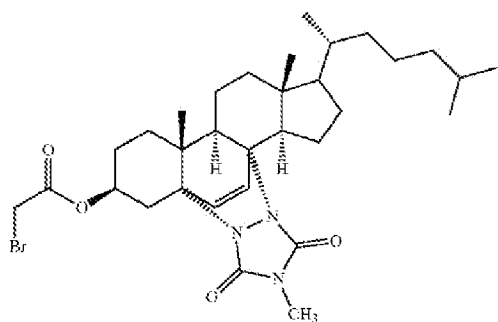


or

5 [0026] In at least one embodiment of the present disclosure, the subject is a mammal.

[0027] In at least one embodiment of the present disclosure, the subject is human.

[0028] In at least one embodiment of the present disclosure, the compound
10 represented by formula (I) is



[0029] In at least one embodiment of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is administered to the subject at a dose ranging from 1 mg/kg to 50 mg/kg.

5 [0030] In at least one embodiment of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is administered to the subject at a dose ranging from 10 mg/kg to 20 mg/kg.

[0031] In at least one embodiment of the present disclosure, the TGF- β receptor inhibitor can be administered to the subject in combination with an additional active ingredient to prevent or treat pulmonary arterial hypertension.

10 [0032] In at least one embodiment of the present disclosure, the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative is a single active ingredient in the pharmaceutical composition for preventing or treating the pulmonary arterial hypertension.

[0033] In other embodiments of the present disclosure, the additional active
15 ingredient is selected from the group consisting of a calcium blocker, an anticoagulant, a diuretic, a cardiotonic agent, an endothelin receptor antagonist, a phosphodiesterase type 5 inhibitor, a prostacyclin analog, and any combination thereof.

[0034] In other embodiments of the present disclosure, the TGF- β receptor
20 inhibitor reduces the proliferation of pulmonary artery endothelial cells, smooth muscle cells, or a combination thereof in the subject.

[0035] In other embodiments of the present disclosure, the pharmaceutically acceptable excipient includes a filler, a binder, a preservative, a disintegrant, a lubricant, a suspending agent, a wetting agent, a solvent, a surfactant, an acid, a
25 flavoring agent, polyethylene glycol, alkanes glycol, sebacic acid, dimethyl sulfoxide, alcohol or any combination thereof.

[0036] In other embodiments of the present disclosure, the pharmaceutical composition is a formulation selected from the group consisting of troches, tablets,

liquids, powders, granules, dispersants, pills, dripping pills, capsules, ointments, creams, emulsions, gels, patches, injections, inhalants, sprays and suppositories.

[0037] In other embodiments of the present disclosure, the pharmaceutical composition is subcutaneously, intravenously, intradermally, intraperitoneally, orally, intrabuccally, sublingually, or intramuscularly, or through respiratory tract, or lungs administered to the subject.

[0038] In other embodiments of the present disclosure, the pharmaceutical composition is administered to the subject at least once daily.

[0039] In other embodiments of the present disclosure, the pharmaceutical composition is administered to the subject for a period of at least 1 month.

[0040] In addition to the above, the present disclosure further provides a use of a pharmaceutical composition comprising an effective amount of 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof in the manufacture of a medicament for preventing or treating pulmonary arterial hypertension in a subject in need thereof. Moreover, the present disclosure further provides the use of an effective amount of 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof in the manufacture of a medicament for preventing or treating pulmonary arterial hypertension in a subject in need thereof.

[0041] Also, the present disclosure provides a pharmaceutical composition comprising an effective amount of 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof for use in preventing or treating pulmonary arterial hypertension in a subject in need thereof and an effective amount of 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof for use in preventing or treating pulmonary arterial hypertension in a subject in need thereof.

BRIEF DESCRIPTIONS OF THE DRAWINGS

[0042] The present disclosure can be more fully understood by reading the following description of the embodiments, with reference made to the accompanying drawings.

[0043] Figure 1 shows the cell survival assay (MTT assay) results of 7-DHC (039) in normal rat pulmonary artery smooth muscle cells (CON1), PAH rat pulmonary artery smooth muscle cells (rPASMCT4), human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASMCT4).

[0044] Figure 2 shows the conditions of the cell growth inhibitions of BrdU cell proliferation assay by 7-DHC (039) for normal rat pulmonary artery smooth muscle cells (rPASMCT4), PAH rat pulmonary artery smooth muscle cells (rPASMCT4), human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASMCT4) under the conditions of 10% FBS.

[0045] Figure 3 shows the conditions of the cell growth inhibitions of BrdU cell proliferation assay by 7-DHC (039) for normal rat pulmonary artery smooth muscle cells (rPASMCT4), PAH rat pulmonary artery smooth muscle cells (rPASMCT4), human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASMCT4) under the condition of 0.1% FBS, 100 pM TGF- β .

[0046] Figure 4 illustrates the administration schedule, animal survival status and body weight (BW) changes of the pulmonary hypertension animal model experiment of a control group (CON), MCT induced pulmonary hypertension rat group (MCT), 10 mg/kg 7-DHC treated pulmonary hypertension rat group (039 10 mg/kg), 20 mg/kg 7-DHC treated pulmonary hypertension rat group (039 20 mg/kg) and 50 mg/kg 042 compound treated pulmonary hypertension rat group (042 50 mg/kg). Graphs indicated by slashes represent dead rats in different groups.

[0047] Figure 5 shows the physiological parameters of the rats tested after administration in the pulmonary arterial hypertension animal model, including right ventricle pressure (RVP), systemic mean blood pressure (mbp), heart rate (HR) and right ventricle hypertrophy (RV/S+LV; right ventricle (RV) / septum (S) + left ventricle (LV)).

[0048] Figure 6 is immunohistological staining diagrams of the pulmonary artery vascular tissue section of the rats tested in the pulmonary arterial hypertension animal model, and a histogram of the pulmonary artery blood vessel thickening degree of rats in different administration groups, and the different administration groups include a control group (CON), pulmonary hypertension rat group (MCT), 10 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 10 mg”), 20 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 20 mg”), and 50 mg/kg 042 compound treated pulmonary hypertension rat group (expressed as “042 50 mg”).

[0049] Figure 7 shows the arterial gas analysis values of the rats tested after administration in the pulmonary hypertension animal model, including blood pH, oxygen pressure (PaO_2), carbon dioxide pressure (PaCO_2), total amount of carbon dioxide (TCO_2) in the blood of three forms (CO_2 , H_2CO_3 , and dissociated HCO_3^- ions), bicarbonate (HCO_3) in the form of dissociated HCO_3^- ions and base excess in the extracellular fluid compartment (BEecf).

[0050] Figure 8 illustrates the administration schedule, animal survival status and body weight (BW) changes of the pulmonary hypertension animal model experiments of a control group (CON), a pulmonary hypertension rat (MCT), 10 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 10 mg/kg”), 20 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 20 mg/kg”) and 30 mg/kg macitentan treated pulmonary hypertension rat group (expressed as “macitentan 30 mg/kg”). Graphs indicated by slashes represent dead rats in different groups.

[0051] Figure 9 shows the physiological parameters of the rats tested after administration in the pulmonary arterial hypertension animal model, including right ventricle pressure (RVP), systemic mean blood pressure (mbp), heart rate (HR) and right ventricle hypertrophy (RV/S+LV).

[0052] Figure 10 is immunohistological staining diagrams of the pulmonary artery vascular tissue section of the rats tested in the pulmonary arterial

hypertension animal model, and a histogram of the pulmonary artery blood vessel thickening degree of rats in different administration groups (statistical blood vessel wall thickness for blood vessels with a diameter between 50-100 μm), and the different administration groups include a control group (CON), pulmonary
5 hypertension rat group (MCT), 10 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 10 mg”), 20 mg/kg 7-DHC treated pulmonary hypertension rat group (expressed as “039 20 mg”), and 30 mg/kg macitentan treated pulmonary hypertension rat group (expressed as “macitentan 30 mg”).

[0053] Figure 11 shows the arterial gas analysis values of the rats tested after
10 administration in the pulmonary hypertension animal model, including carbon dioxide pressure (PaCO_2), oxygen pressure (PaO_2), total amount of carbon dioxide (TCO_2), oxygen saturation (sO_2), base excess in the extracellular fluid compartment (BEecf) and bicarbonate (HCO_3). The normal value of sO_2 is 93% to 100%. Under a certain PaO_2 , the percentage of oxygenated Hb in the blood sample to the total Hb
15 reflects the degree of combination of O_2 and Hb in the blood, but is less sensitive to hypoxia than PaO_2 .

[0054] Figure 12A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 039 compound in different concentrations (0, 0.5, 1, 10, 25, 50 and 75 μM).

20 [0055] Figure 12B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 039 compound in different concentration ranges (0 to 80 μM).

[0056] Figure 13A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 042 compound in different
25 concentrations (0, 0.01, 0.1, 1, 5, 10 and 25 μM).

[0057] Figure 13B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 042 compound in different concentration ranges (0 to 30 μM).

[0058] Figure 14A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 050 compound in different concentrations (0, 1, 5, 10, 25, 50 and 100 μ M).

5 [0059] Figure 14B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 050 compound in different concentration ranges (0 to 120 μ M).

[0060] Figure 15A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 053 compound in different concentrations (0, 50, 75, 100, 150 and 200 μ M).

10 [0061] Figure 15B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 053 compound in different concentration ranges (0 to 200 μ M).

[0062] Figure 16A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 056 compound in different
15 concentrations (0, 0.1, 1, 10, 50 and 100 μ M).

[0063] Figure 16B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 056 compound in different concentration ranges (0 to 120 μ M).

[0064] Figure 17A shows the conditions of inhibition of Pai-1 luciferase
20 activity with or without TGF- β stimulation by 057 compound in different concentrations (0, 0.01, 0.1, 1, 10 and 100 μ M).

[0065] Figure 17B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 057 compound in different concentration ranges (0 to 120 μ M).

25 [0066] Figure 18A shows the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by 203 compound in different concentrations (0, 5, 10, 25, 50 and 75 μ M).

[0067] Figure 18B shows the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by 203 compound in different concentration ranges (0 to 70 μ M).

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DETAILED DESCRIPTIONS

[0068] The following examples are used to illustrate the present disclosure. A person skilled in the art can easily conceive of the other advantages and effects of the present disclosure based on the invention of the specification. The present disclosure can also be implemented or applied as described in various examples. It is possible to modify or alter the following examples for carrying out the present disclosure without violating its spirit and scope, for different aspects and applications.

[0069] It should also be noted that the singular forms “a,” “an,” and “the” as used in this disclosure include plural referents unless expressly and unambiguously limited to one referent. In addition, the term “or” is used interchangeably with the term “and/or” unless the context clearly dictates otherwise.

[0070] As used herein, the term “comprising,” “having,” “including” or “containing” is used in reference to compositions, methods, and respective component(s) thereof, which are essential to the present disclosure, yet open to the inclusion of unspecified elements, whether essential or not. Additionally, the compositions of the present disclosure can be used to implement the methods of the present disclosure.

[0071] As used herein, the term “treating” or “treatment” refers to the administration of an effective amount of a 7-DHC or a salt or a derivative thereof to a subject in need thereof for curing, mitigating, relieving, remedying, ameliorating or preventing a disease its symptoms or its predisposition. This subject can be identified by a healthcare professional based on results from any suitable diagnostic method.

[0072] The present disclosure provides a method of treating pulmonary arterial hypertension in a subject in need thereof, comprising administering to the subject an effective amount of a 7-DHC or a salt or a derivative thereof.

[0073] As used herein, the term “an effective amount” refers to a therapeutic amount sufficient to result in the prevention or treatment of pulmonary arterial hypertension and the development, recurrence or onset of one or more symptoms thereof, or a therapeutic amount sufficient to enhance or improve the preventive effect of another therapy, reduce the severity and duration of a condition, improve one or more symptoms of a condition, prevent the progression of pulmonary arterial hypertension, and/or enhance or improve the therapeutic effect of another therapy.

[0074] The term “alkyl group” means a straight or branched saturated monovalent hydrocarbon chain having 1 to 12 carbon atoms. The straight chain or branched chain alkyl group having 1 to 6 carbon atoms is preferable. Examples thereof are methyl group, ethyl group, propyl group, isopropyl group, butyl group, t-butyl group, isobutyl group, pentyl group, hexyl group, isohexyl group, heptyl group, 4,4-dimethylpentyl group, octyl group, 2,2,4-trimethylpentyl group, nonyl group, decyl group, and various branched chain isomers thereof. Further, the alkyl group may optionally and independently be substituted by 1 to 4 substituents as listed below, if necessary.

[0075] The term “cycloalkyl group” means a monocyclic or bicyclic monovalent saturated hydrocarbon ring having 3 to 12 carbon atoms, and the monocyclic saturated hydrocarbon group having 3 to 7 carbon atoms is more preferable. Examples thereof are a monocyclic alkyl group and a bicyclic alkyl group such as cyclopropyl group, cyclobutyl group, cyclopentyl group, cyclohexyl group, cycloheptyl group, cyclooctyl group, cyclodecyl group, etc. These groups may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0076] The term “alkenyl group” means a straight or branched monovalent hydrocarbon chain having 2 to 12 carbon atoms and having at least one double bond.

Preferable alkenyl group is a straight chain or branched chain alkenyl group having 1 to 6 carbon atoms. Examples thereof are vinyl group, 2-propenyl group, 3-butenyl group, 2-butenyl group, 4-pentenyl group, 3-pentenyl group, 2-hexenyl group, 3-hexenyl group, 2-heptenyl group, 3-heptenyl group, 4-heptenyl group, 3-octenyl group, 3-nonenyl group, 4-decenyl group, 3-undecenyl group, 4-dodecenyl group, 4,8,12-tetradecatrienyl group, etc. The alkenyl group may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0077] The term “alkynyl group” means a straight or branched monovalent hydrocarbon chain having at least one triple bond. The preferable alkynyl group is a straight chain or branched chain alkynyl group having 1 to 6 carbon atoms. Examples thereof are 2-propynyl group, 3-butylnyl group, 2-butylnyl group, 4-pentylnyl group, 3-pentylnyl group, 2-hexynyl group, 3-hexynyl group, 2-heptynynl group, 3-heptynynl group, 4-heptynynl group, 3-octynynl group, 3-nonylnyl group, 4-decynynl group, 3-undecynynl group, 4-dodecynynl group, etc. The alkynyl group may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0078] The term “aryl group” means a monocyclic or bicyclic monovalent aromatic hydrocarbon group having 6 to 10 carbon atoms. Examples thereof are phenyl group, naphthyl group (including 1-naphthyl group and 2-naphthyl group). These groups may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0079] The term “aralkyl” as used alone or as part of another group refers to alkyl groups as described above having an aryl substituent. The aralkyl group may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0080] The term “heteroaryl” means a monocyclic or bicyclic monovalent aromatic hydrocarbon group having 6 to 10 carbon atoms, in which at least one carbon atom is substituted by at least one heteroatom, such as N, O or S. The heteroaryl group may optionally and independently be substituted by 1 to 4

substituents as mentioned below, if necessary.

[0081] The terms “heteroaralkyl” as used alone or as part of another group refers to alkyl groups as described above having an heteroaryl substituent. The heteroaralkyl group may optionally and independently be substituted by 1 to 4 substituents as mentioned below, if necessary.

[0082] The substituent for the above groups includes, for example, a halogen atom (e.g., fluorine, chlorine, bromine, iodine), a nitro group, a cyano group, an oxo group, a hydroxy group, a mercapto group, a carboxyl group, a sulfo group, an alkyl group, an alkenyl group, an alkynyl group, a cycloalkyl group, a cycloalkenyl group, a cycloalkynyl group, an aryl group, a heterocyclyl group, an alkoxy group, and a cycloalkyloxy group, but is not limited thereto.

[0083] In some embodiments of the present disclosure, the effective amount of 7-DHC or a salt and a derivative thereof may be 1 mg/kg to 50 mg/kg. In some embodiments, the lower limit of the dose may be 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg or 30 mg/kg, and the upper limit of the dose may be 50 mg/kg, 48 mg/kg, 45 mg/kg, 43 mg/kg, 40 mg/kg, 38 mg/kg, 35 mg/kg, 33 mg/kg, 30 mg/kg, 25 mg/kg, 24 mg/kg, 23 mg/kg, 22 mg/kg, 21 mg/kg or 20 mg/kg. For example, the dosage of the 7-DHC or a salt or a derivative thereof may be 1 mg/kg to 40 mg/kg, 5 mg/kg to 40 mg/kg, 5 mg/kg to 35 mg/kg, 10 mg/kg to 30 mg/kg, 10 mg/kg to 20 mg/kg, about 42 mg/kg, about 40 mg/kg, about 37 mg/kg, about 35 mg/kg, about 32 mg/kg, about 30 mg/kg, about 28 mg/kg, about 25 mg/kg, about 20 mg/kg, about 18 mg/kg, about 15 mg/kg, about 13 mg/kg or about 10 mg/kg.

[0084] As used herein, when referring to a number or range, a person having ordinary skill in the art can understand that it is intended to encompass an appropriate and reasonable range for the particular field to which the present disclosure relates.

[0085] In some embodiments of the present disclosure, the administration of 7-DHC or a salt or a derivative thereof may be, for example, once every two days,

once a day, twice a day, three times a day, or four times a day. In some embodiments of the present disclosure, the administration of 7-DHC may be administered three times per week.

[0086] In some embodiments of the present disclosure, the 7-DHC or a salt or a derivative thereof are administered subcutaneously, intravenously, intradermally, intraperitoneally, orally, intramuscularly or intracranially to the subject.

[0087] In some embodiments of the present disclosure, the 7-DHC or a salt or a derivative thereof may be administered to the subject for a period of time sufficient to prevent or treat pulmonary arterial hypertension. In some embodiments of the present disclosure, the sufficient period of time may depend on the species, gender, weight or age of the subject, the stage, symptoms or severity of the disease, as well as the route, time or frequency of administration, etc. In some embodiments of the present disclosure, the administration of 7-DHC or a salt or a derivative thereof is daily for at least 1 month. For example, the administration period of 7-DHC or a salt or a derivative thereof may last 1, 2, 3, 4 or 6 months, or 1, 2, 3 or 4 years, or even longer, as long as no side effects occur during the treatment, and there are no specific limitations in the present disclosure. In the embodiment illustrated in the present disclosure, the period may range from 1 month to 2 years. In other embodiments of the present disclosure, the period may range from 4 weeks to 12 months. In other embodiments of the present disclosure, the 7-DHC or a salt or a derivative thereof are administered daily for a period of at least 2 months.

[0088] In at least one embodiment of the present disclosure, the 7-DHC or a salt or a derivative thereof may be administered in an oral dosage form. In at least one embodiment of the present disclosure, the 7-DHC or a salt or a derivative thereof administered to the subject may be included in a pharmaceutical composition. In at least one embodiment of the present disclosure, the pharmaceutical composition of the present disclosure includes 7-DHC or a salt or a derivative thereof, and pharmaceutically acceptable excipients thereof. In at least one embodiment, the composition of the present disclosure may be formulated in a form suitable for oral

administration, such that the composition may be administered to the subject by oral delivery. In other embodiments of the present disclosure, the composition can be formulated into troches, tablets, liquids, powders, granules, dispersants, pills, dripping pills, capsules, ointments, creams, emulsions, gels, patches, injections, inhalants, sprays and suppositories. In some embodiments of the present disclosure, pharmaceutically acceptable excipients include, but are not limited to, fillers, binders, preservatives, disintegrants, lubricants, suspending agents, wetting agents, solvents, surfactant, acid, flavoring agent, polyethylene glycol (PEG), alkylene glycol, sebacic acid, dimethylsulfoxide, alcohol or any combination thereof.

[0089] The pharmaceutical composition of the present disclosure may include 7-DHC or a salt or a derivative thereof as a single active ingredient for preventing or treating pulmonary arterial hypertension. In other words, 7-DHC is served as the only active ingredient in the pharmaceutical composition of the present disclosure for preventing or treating pulmonary arterial hypertension. In this embodiment, the present disclosure provides a safe and effective therapy for preventing or treating pulmonary arterial hypertension by using only 7-DHC or a salt or a derivative thereof as the active ingredient.

[0090] In other embodiments of the present disclosure, unless the effects of the present disclosure would be inhibited, the compositions may be administered to the subject in combination with other active ingredients. In some embodiments of the present disclosure, the 7-DHC or a salt or a derivative thereof and other active ingredients can be made into a single composition or individual compositions and provided to a subject in need.

[0091] In at least one embodiment, the administration of 7-DHC or a salt or a derivative thereof in the method provided by the present disclosure can be combined with any suitable conventional therapy for pulmonary arterial hypertension. In at least one embodiment of the present disclosure, conventional therapies for pulmonary arterial hypertension include, but are not limited to, calcium blockers, anticoagulants, diuretics, cardiotonic agents, endothelin receptor antagonists,

phosphodiesterase type 5 inhibitors and prostacyclin analogs.

[0092] As used herein, the term “mammal” refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc., but is not limited thereto. Preferably, the mammal is human.

[0093] Multiple examples have been used to illustrate the present disclosure. The examples below should not be taken as a limit to the scope of the present disclosure.

EXAMPLES

[0094] Experimental methods and steps

[0095] The principle of cell viability assay (MTT assay) is to utilize MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide]), a yellow compound, which is a dye that accepts hydrogen ions and can act on cell mitochondria, to generate purple formazan crystals under the action of succinate dehydrogenase (SDH), and the amount of formazan crystals produced is directly proportional to the number of living cells. DMSO was used to dissolve the formazan crystals and O.D. 570 nm was measured, the O.D. value represents the activity of mitochondria, that is, the number of living cells, therefore, MTT assay can be used as an indicator of cell viability (toxicity). After the drug to be tested was added to the cell culture medium and co-cultured, MTT can be used to detect the toxicity of the drug to the cells and determine the drug's action concentration range. The MTT assay experimental procedure includes implanting 1.5×10^4 test cells into a 48-well plate, culturing them overnight at 37°C in a culture medium containing 10% FBS and different drug concentrations. The cell culture medium was aspirated, washed with phosphate buffered saline (PBS), then 10% MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added, placed in a 37°C incubator to react for 4 hours. Next, the MTT solution was aspirated and 100 μ l/well of DMSO was added to completely dissolve the MTT purple crystals. After mixing evenly, the absorbance values at wavelength 570 nm were measured.

[0096] BrdU (5-bromo-2-deoxyuridine) cell proliferation assay (BrdU assay)

[0097] A 48-well plate was used, and 1.5×10^4 cells were implanted in each well. After 24 hours, the medium was changed to serum-free culture medium and synchronized for 24 hours. A 2-fold concentration of the compound in serum-free culture medium is prepared and the cells were pretreated for 1 hour. Then, the same volume of culture medium containing 20% fetal bovine serum (FBS) was added to dilute the compound. The final concentration was 10% FBS culture medium and 1 times the concentration of the compound. In the experimental group that added TGF- β , the same volume of TGF- β culture medium containing 10 ng/mL was added, with a final concentration of 5 ng/mL TGF- β and 1 times the concentration of the compound, and in the experimental group added with TGF- β , the entire process was conducted in culture medium containing 0.1% serum. Then, BrdU reagent was added about 6 hours later, and the cells were collected the next day for enzyme-linked immunosorbent assay (ELISA) analysis, the compound treatment time was about 24 hours, and the BrdU treatment time was about 18 hours.

[0098] TGF- β stimulated Pai-1 luciferase assay

[0099] MLECs-clone32 cell line was used to measure the impact of different compounds on the downstream gene, plasminogenactivator inhibitor 1 (PAI-1) performance stimulated by TGF- β . MLECs-clone32 is an expression construct that is stably transfected for the Mv1Lu cell line, and the expression construct PAI-1 Luciferase contains a truncated (PAI-1) promoter fused to the firefly luciferase reporter gene^{11, 12}. The activity stimulated by the truncated PAI-I promoter was quantified by using a luciferase assay. The luciferase activity stimulated by TGF- β in cells without compound treatment was set as 100%, and the effects of each compound on TGF- β -stimulated PAI-I promoter activity were compared. The steps for measuring Pai-1 luciferase activity include using a 96-well plate (DMEM medium containing 10% FBS), seeding each well with 1.5×10^5 cells, and culturing in an incubator overnight. After discarding the medium the next day, cells in DMEM medium with or without the addition of test compounds were treated with

recombinant TGF- β (50 pM) and allowed to react in a 37°C incubator for 4 hours. The cell culture medium was aspirated, washed with PBS, and the activity of Pai-1 luciferase was evaluated by luciferase assay.

[0100] Pulmonary arterial hypertension animal experimental model

5 [0101] The present disclosure utilizes injection of monocrotaline to induce symptoms of pulmonary arterial hypertension in rats to establish a pulmonary arterial hypertension animal experimental model suitable for screening test drugs.

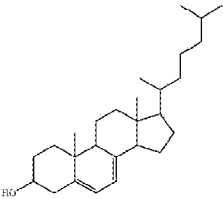
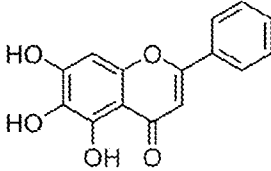
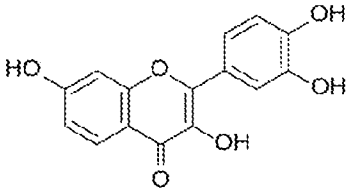
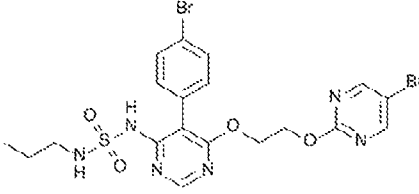
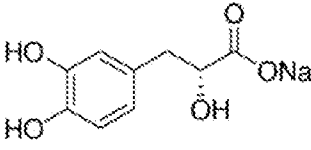
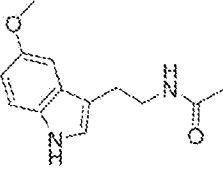
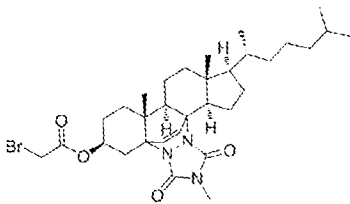
[0102] The diagnostic method of clinical pulmonary arterial hypertension standards is right heart catheterization, which is defined as mean pulmonary arterial pressure (mPAP) greater than or equal to 20 mmHg. The diagnostic criteria for pulmonary arterial hypertension, in addition to an increase in mean pulmonary arterial pressure, need to be combined with pulmonary vascular resistance (PVR) greater than 3 Wood unit. The right ventricle pressure (RVP) and systemic mean blood pressure (mbp) in rats with pulmonary arterial hypertension were measured
10 by Millar Model PCU200 pressure system, and the heart rate (HR) was also monitored at the same time. After measuring the hemodynamic data, the arterial blood was extracted from the carotid artery of the pulmonary arterial hypertension rat, and the Abbott blood oximeter (i-STAT) combined with Abbott's special chip cartridge (i-STAT Cartridge G3+) was used to measure arterial gas analysis data
15 such as pH, PaO₂, PaCO₂, HCO₃⁻ and base excess (BE) value in quantitative whole blood samples. The normal pH value is between 7.35 and 7.45, and the pH value indicates whether the experimental animal has the condition of acidemia, alkalemia, acidosis or alkalosis. The normal value of PaO₂ is between 80 and 100 mmHg, which reflects the effectiveness of gas exchange (ventilation/perfusion). The normal value
20 of PaCO₂ is between 35 and 45 (such as 38 and 42) mmHg, which can evaluate the effectiveness of alveolar ventilation. The acid produced by food, tissue decomposition, inflammation and hypoxia rely on the lungs to excrete volatile acid CO₂ (short-term) and the bicarbonate (HCO₃⁻) produced by the kidneys (long-term) to neutralize non-volatile acids, while the normal value of bicarbonate hydrogen

radical (HCO_3^-) is between 22 and 26 meq/L. BE is the base excess value, its normal value ranges from -2 to +2, the negative value represents the degree of alkali ion deficiency in the blood, a negative BE value (-) that is too high indicates metabolic acidosis, and a positive value (+) that is too high is metabolic alkalosis. At the end
5 of the experiment, the rats were sacrificed and their hearts were removed and the left and right ventricles were separated and weighed (RV)/(S)+(LV).

[0103] Example 1: Screening TGF- β receptor inhibitors by pulmonary arterial hypertension cell model

[0104] Five compounds to be tested were screened by cell survival assay (MTT
10 assay) and BrdU (5-bromo-2-deoxyuridine) cell proliferation assay, and the information of these compounds was listed in Table 1 below. Tables 2 and 3 respectively listed the growth inhibitions of on human pulmonary artery endothelial cells (hPAEC), human pulmonary artery smooth muscle cells (hPASMC), normal rat pulmonary artery smooth muscle cells (rPASMC, as the control group, represented
15 by "CON"), and rat pulmonary artery smooth muscle cells (represented by "MCT"), by the five compounds to be tested under the conditions of 10% FBS, 0.1% FBS and 100 pM TGF- β . The results showed that the growth inhibition treated with 7-DHC (No. 039) was the most significant.

[0105] Table 1: Codes, names and structures of the seven compounds

Code	Name	Structure
039	7-DHC	
042	Baicalein	
050	Fisetin	
053	Macitentan	
056	Danshensu	
057	Melatonin	
203	MeTC7	

[0106] Table 2: Cell growth inhibitions of the six compounds in 10% FBS

Code	Cell	1 μM	10 μM	100 μM	EC50
039	hPAEC	108%	76%	0.3%	47 μ M
	hPASC	108%	80%	12%	53 μ M
	CON	95%	46%	2.8%	38 μ M
	MCT	96%	40%	3.3%	37 μ M
Code	Cell	5 μM	25 μM	50 μM	EC50
042	hPAEC	89%	7%	4%	20 μ M
	hPASC	51%	36%	28%	5 μ M
	CON	16%	8%	9%	
	MCT	19%	11%	10%	
050	hPAEC	100%	14%	5%	22 μ M
	hPASC	90%	37%	24%	27 μ M
	CON	102%	71%	68%	59 μ M
	MCT	84%	64%	37%	38 μ M
053	hPAEC	86%	84%	77%	175 μ M
	hPASC	90%	82%	71%	100 μ M
	CON	101%	89%	88%	142 μ M
	MCT	90%	79%	82%	114 μ M
056	hPAEC	89%	88%	83%	272 μ M
	hPASC	81%	81%	71%	124 μ M
	CON	104%	88%	16%	35 μ M
	MCT	90%	40%	15%	25 μ M
Code	Cell	10 μM	100 μM	1000 μM	EC50
057	hPAEC	89%	91%	81%	
	hPASC	86%	89%	88%	
	CON	99%	101%	101%	
	MCT	93%	93%	88%	

*EC50 : concentration for 50% of maximal effect

[0107] Table 3: Cell growth inhibitions of the five compounds under the addition of 100 pM TGF- β

No.	Cell	5 μ M	25 μ M	50 μ M	EC50
039	hPAEC	111%	3.5%	3%	23 μ M
	hPASMC	95%	28%	12%	24 μ M
	CON	119%	33%	25%	30 μ M
	MCT	87%	9%	8.5%	20 μ M
042	hPAEC	70%	6%	5%	15 μ M
	hPASMC	38%	37%	33%	4 μ M
	CON	87%	65%	55%	52 μ M
	MCT	56%	10%	10%	9 μ M
050	hPAEC	148%	105%	18%	40 μ M
	hPASMC	119%	47%	40%	35 μ M
	CON	115%	102%	86%	106 μ M
	MCT	142%	126%	86%	79 μ M
053	hPAEC	113%	87%	29%	40 μ M
	hPASMC	102%	78%	61%	59 μ M
	CON	102%	86%	78%	96 μ M
	MCT	106%	94%	75%	87 μ M
056	hPAEC	121%	122%	104%	155 μ M
	hPASMC	114%	99%	84%	100 μ M
	CON	102%	94%	49%	50 μ M
	MCT	109%	94%	12%	36 μ M

[0108] Table 4: IC₅₀ values of the seven compounds inhibiting TGF- β stimulated Pai-1 luciferase activity (%)

Code	Name	IC ₅₀ value of compound inhibiting TGF- β -stimulated Pai-1 luciferase activity (%)
039	7-DHC	15.5 μ M
042	Baicalein	14.8 μ M
050	Fisetin	44.1 μ M
053	Macitentan	82.9 μ M
056	Danshensu	N/A
057	Melatonin	N/A
203	MeTC7	17.5 μ M

5 [0109] As shown in the results of the cell survival assay, no matter in the normal rat pulmonary artery smooth muscle cells (rPASM C CON1), rat pulmonary artery smooth muscle cells (rPASM C MCT4), human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASM C), when the concentration of 7-DHC (039) was increased to 100 μ M, cytotoxicity began to
10 occur (Figure 1).

[0110] In the BrdU cell proliferation assay, as shown in Figure 2, under the conditions of 10% FBS, when the concentration of 7-DHC (039) was 10 μ M, normal rat pulmonary artery smooth muscle cells (rPASM C CON1) and rat pulmonary artery smooth muscle cells (rPASM C MCT4) have significant inhibitory effects, and
15 inhibition of cell growth can also be observed for human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASM C).

[0111] As shown in Figure 3, under the conditions of 0.1% FBS, 100 pM TGF- β , when the concentration of 7-DHC (039) was 25 μ M, the normal rat pulmonary artery smooth muscle cells (rPASM C CON3) and rat pulmonary artery smooth muscle cells (rPASM C MCT2) all had significant inhibitory effects. In particular,
20

the inhibitory effect on rat pulmonary artery smooth muscle cells was more significant. Similarly, when the concentration of 7-DHC (039) was 25 μ M, inhibition of cell growth of human pulmonary artery endothelial cells (hPAEC) and human pulmonary artery smooth muscle cells (hPASMC) can also be observed.

5 [0112] Example 2: Physiological parameters, pulmonary artery thickening and arterial gas analysis after pulmonary arterial hypertension animals were administered with 7-DHC

[0113] Monocrotaline (MCT) was utilized to induce pulmonary hypertension rat animal model, the TGF- β receptor inhibitor 7-DHC (039) and compound No. 042 were tested via intraperitoneal (IP) administration, and the physiological parameters
10 of the animals were collected, wherein includes measuring right ventricle pressure, systemic arterial pressure, heartbeat and right ventricle hypertrophy, as well as observing and analyzing the pulmonary artery thickening and blood oxygenation levels through pathological sections of lung tissue. Specifically, 7-DHC (039) was divided into two groups with a dosage of 10 mg/kg or 20 mg/kg, the dosage of group
15 042 was 50 mg/kg, each dosage group has 3 rats ($n = 3$), while the control group (CON) and the pulmonary arterial hypertension rat group (MCT) had 6 rats ($n = 6$) respectively.

[0114] As shown in Figure 4, the weight and activity of the rats in the 042 administration group had significantly decreased in the second week of
20 administration, so they were sacrificed early on the 21st day after monocrotaline induction. On the 23rd day after monocrotaline induction, it was found that rats in the 20 mg/kg group of 7-DHC (039) had died, therefore, the pulmonary artery pressure could not be obtained, so the right ventricle pressure (RVP) measurement was started on the 24th day.

25 [0115] As shown in Figure 5, the average right ventricle pressure (RVP) of pulmonary arterial hypertension rats (MCT) was 28 mmHg, but right ventricle pressure can be reduced after administration of 7-DHC (039) (10 or 20 mg/kg). After the rats were sacrificed, the hearts were removed and the left and right ventricles were separated and weighed (RV/S+LV), the ratio of the left and right ventricles of

normal rats was approximately 25%, the average right ventricle hypertrophy ratio in pulmonary arterial hypertension rats (MCT) exceeds 50%. However, after administration of 7-DHC (039) (10 or 20 mg/kg), right ventricle hypertrophy was reduced, and 7-DHC (039) did not reduce systemic blood pressure and heartbeat, this shows that 10 mg/kg 7-DHC (039) has the effect of reducing right ventricle pressure and reducing right ventricle hypertrophy. In addition, the phenomenon of right ventricle hypertrophy in the 042-administered rats was slightly lower than that in the pulmonary hypertension rats (MCT).

[0116] In addition, observe the pulmonary artery tissue sections of the rats in the 042 administration group were observed, and immunohistological staining α -smooth muscle actin (referred to as α -SM actin or α -SMA) was used to confirm the position of the pulmonary artery, the results were shown in Figure 6, the average pulmonary artery blood vessel thickness (medial wall thickness) of pulmonary arterial hypertension rats (MCT) exceeded 55%. However, in the 10 mg/kg 7-DHC (039) group, the thickness of the pulmonary artery blood vessels was significantly reduced ($p < 0.001$). The above result showed that 10 mg/kg 7-DHC (039) has the effect of reducing the thickness of pulmonary artery blood vessels in rats with pulmonary arterial hypertension.

[0117] As shown in Figure 7, the pH value, PaO_2 and PaCO_2 of the control group (CON), pulmonary arterial hypertension rat group (MCT), 10 mg/kg 7-DHC (039) group (represented by "039-10") and 20 mg/kg 7-DHC (039) group (expressed as "039-20") did not show any significant differences, and the results may be caused by the longer operation time affecting the blood oxygen exchange function of the lungs. It was interesting that the normal value of base excess (BE) was generally between -2 and +2, however, the BE value in the MCT group was -5.5, indicating metabolic acidosis may be related to tissue hypoxia caused by tissue inflammation or tissue edema. The BE value of the 10 mg/kg 7-DHC (039) group was -2, and the BE value of the 20 mg/kg 7-DHC (039) group was 0. It thus can be seen that 7-DHC (039) can improve tissue inflammation or tissue edema, allowing the tissue to regain

oxygen, thereby terminating hypoxic, the lactic acid produced by hypoxia was therefore reduced, and metabolic acidosis was also improved.

[0118] Example 3: Comparison of physiological parameters, pulmonary artery thickening and arterial gas analysis values in rats with pulmonary arterial hypertension after administration of 7-DHC or macitentan

[0119] Macitentan was a clinical drug currently used for the treatment of pulmonary arterial hypertension, it was a new type of dual endothelin receptor antagonist (ERA) which has high affinity and can occupy the endothelin (ET) receptor on human pulmonary artery smooth muscle for a long time, thereby preventing endothelin-1 (ET-1) from binding to its receptors (ETA and ETB).

[0120] Monocrotaline was utilized to induce pulmonary arterial hypertension rat animal model, intraperitoneal injection (IP) TGF- β receptor inhibitor 7-DHC (039) and oral administration of macitentan (soluble in methylcellulose), 7-DHC (039) was divided into two groups according to the dosage: 10 mg/kg or 20 mg/kg, and the dosage of macitentan group was 30 mg/kg.

[0121] The body weight and activity of the rats in the macitentan group were not greatly affected during the second week of pulmonary arterial hypertension animal model administration, however, on the 23rd day after monocrotaline induction, the rats in the macitentan group died, so the pulmonary artery pressure of this group could not be obtained subsequently. By contrast, although the weight of rats in the 20 mg/kg 7-DHC (039) group decreased significantly, it did not affect their activity, and the survival rate was 100% (Figure 8).

[0122] On the 25th day after monocrotaline induction, the right ventricle pressure (RVP) of rats in each group was measured. As shown in Figure 9, the mean pulmonary artery pressure (RVP) of pulmonary arterial hypertension rats (MCT) was 28.97 mmHg, and the right ventricle pressure of the 10 mg/kg group and 20 mg/kg group administered with 7-DHC (039) was reduced respectively to 17.61 mmHg and 19.96 mmHg. After the rats were sacrificed, the hearts were removed, and the left and right ventricles were separated and weighed (right ventricle/septum + left

ventricle; RV/S+LV). The ratio of left and right ventricles in normal rats was approximately 26.7%, the average right ventricle hypertrophy ratio in pulmonary arterial hypertension rats (MCT) was approximately 71.64%, and the average right ventricle hypertrophy ratios in the 10 mg/kg group and 20 mg/kg group of administration of 7-DHC (039) were 53.96% and 62.97%, respectively, both of which significantly reduced the phenomenon of right ventricle hypertrophy. Moreover, 7-DHC (039) did not significantly reduce the systemic average blood pressure and heartbeat, and the administration of 7-DHC (039) having the effect of reducing right ventricle pressure was determined.

[0123] The pulmonary artery vascular tissue sections of rats in each administration group were observed, and the location of the pulmonary artery by immunohistological staining for α -smooth muscle actin were confirmed. As shown in Figure 10, the results showed that the mean arterial blood vessel thickness of pulmonary arterial hypertensive rats (MCT) exceeded 65%, but the 10 mg/kg group and 20 mg/kg group that were administered 7-DHC (039) significantly reduced the thickening of pulmonary arteries (### $p < 0.001$). Similarly, since macitentan was an endothelin-1 receptor antagonist, a reduction in blood vessel thickening was also observed in the macitentan group, but the survival rate was significantly worse than that of the 7-DHC (039) group. Therefore, the TGF- β receptor inhibitor 7-DHC can inhibit the cell proliferation and remodeling of pulmonary blood vessels by reducing TGF- β -related signaling.

[0124] Figure 11 indicated that there was no significant difference in the PaO_2 and PaCO_2 of the control group (CON), the pulmonary arterial hypertension rat group (MCT), the 10 mg/kg 7-DHC (039) group and the 20 mg/kg 7-DHC (039) group. This result may be caused by the long operation time affects the blood oxygen exchange function of the lungs. The normal value of base excess value (BE) was -2 to +2, the BE value in the MCT group was -6, indicating metabolic acidosis, which may be related to tissue hypoxia caused by tissue inflammation or tissue edema, while the BE value of the 10 mg/kg 7-DHC(039) group was -3, and the BE value of

the 20 mg/kg 7-DHC(039) group was -1.4. Therefore, it can be seen that 7-DHC (039) can improve tissue inflammation or tissue edema, allowing the tissue to regain oxygen, thereby terminating hypoxic, the lactic acid produced by hypoxia was reduced, and metabolic acidosis was also improved, the results were consistent with the results of Example 2.

[0125] Figures 12 to 18 showed the conditions of inhibition of different compounds in different concentration ranges including the conditions of inhibition of Pai-1 luciferase activity with or without TGF- β stimulation by compound 7-DHC (039), Baicalein (042), Fisetin (050), Macitentan (053), Danshensu (056), Melatonin (057) and MeTC7(203), and the conditions of inhibition of TGF- β -stimulated Pai-1 luciferase activity (%) by different compounds in different concentration ranges.

[0126] Based on the results of the inhibition of Pai-1 luciferase activity (%) stimulated by TGF- β , it can be found that among the tested compounds 7-DHC, Baicalein, Fisetin, Macitentan, Danshensu, Melatonin or MeTC7, only 7-DHC and 7-DHC derivative MeTC7 could significantly inhibit TGF- β -stimulated Pai-1 luciferase activity when the concentration was increased to 10 μ M, other compounds failed to have the same effect at similar concentrations. Specifically, for example, as shown in Figures 12A and 12B, 7-DHC had a significant inhibitory effect on the activity of Pai-1 luciferase stimulated by TGF- β when the concentration exceeds 10 μ M, and the reducing effect increased with concentration. For example, according to Figures 13A and 13B, when the concentration of compound Baicalein exceeded 10 μ M, a significant inhibitory effect on the activity of Pai-1 luciferase stimulated by TGF- β can be seen, and the reducing effect increased with concentration. For example, as can be seen in Figures 18A and 18B, compound MeTC7 had a significant inhibitory effect on the activity of Pai-1 luciferase stimulated by TGF- β when the concentration exceeds 10 μ M, and the reducing effect increases with concentration.

[0127] In addition, as shown in Table 4, compound 7-DHC, Baicalein, Fisetin, Macitentan, Danshensu, Melatonin, MeTC7 caused TGF- β -stimulated Pai-1 luciferase activity (%) to inhibit the concentration (IC_{50}) value of 50%, the IC_{50}

concentrations of the three compounds 7-DHC, Baicalein, and MeTC7 were lower than those of Macitentan, Danshensu, Fisetin, and Melatonin, and no inhibitory effect was detected. Obviously, compounds 7-DHC, Baicalein and MeTC7 had stronger inhibitory effects on TGF- β -stimulated Pai-1 luciferase activity than the other four compounds.

[0128] Even though numerous characteristics and advantages of the present disclosure have been set forth in the foregoing description, together with details of the structure and features of the invention, the invention is illustrative only. Changes may be made in the details, especially in matters of shape, size, and arrangement of parts within the principles of the invention to the full extent indicated by the broad general meaning of the terms in which the appended claims are expressed.

[0129] References:

1. McGoon M., Guttermann D., Steen V., Barst R., McCrory D.C., Fortin T.A., et al., "Screening, early detection, and diagnosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines." CHEST Journal. 2004; 126 (suppl.): 14S-34S.

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3. Simonneau G., Gatzoulis M.A., Adatia I., Celermajer D., Denton C., Ghofrani A., et al., "Updated clinical classification of pulmonary hypertension." Journal of the American College of Cardiology. 2013; 62 (25): D34-D41.

4. Humbert M., Morrell N.W., Archer S.L., Stenmark K.R., MacLean M.R., Lang I.M., et al. "Cellular and molecular pathobiology of pulmonary arterial

hypertension.” *Journal of the American College of Cardiology*. 2004; 43 (12s1): S13-S24.

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6. Jonigk D., Golpon H., Bockmeyer C.L., Maegel L., Hoeper M.M., Gottlieb J., et al. “Plexiform lesions in pulmonary arterial hypertension: composition, architecture, and microenvironment.” *The American Journal of Pathology*. 2011; 179 (1): 167-179.

7. Aldred M.A., Vijayakrishnan J., James V., Soubrier F., Gomez-Sanchez M.A., Martensson G., et al. “BMP2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension.” *Human Mutation*. 2006; 27 (2): 212-213.

8. Cogan J.D., Vnencak-Jones C.L., Phillips J.A., Lane K.B., Wheeler L.A., Robbins I.M., et al. “Gross BMP2 gene rearrangements constitute a new cause for primary pulmonary hypertension.” *Genetics in Medicine*. 2005; 7 (3): 169-174.

9. Cogan J.D., Pauciulo M.W., Batchman A.P., Prince M.A., Robbins I.M., Hedges L.K., et al. “High frequency of BMP2 exonic deletions/duplications in familial pulmonary arterial hypertension.” *American Journal of Respiratory and Critical Care Medicine*. 2006; 174 (5): 590-598.

10. Thomson J.R., Machado R.D., Pauciulo M.W., Morgan N.V., Humbert M., Elliott G.C., et al. “Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMP2-II, a receptor member of the TGF- β family.” *Journal of Medical Genetics*. 2000; 37 (10): 741-745.

11. An assay for transforming growth factor-beta using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. *Anal Biochem* 1994, 216:276–284.

12. Quantification of active and total transforming growth factor- β levels in
5 serum and solid organ tissues. *BMC Research Notes* 2012, 5:636

CLAIMS

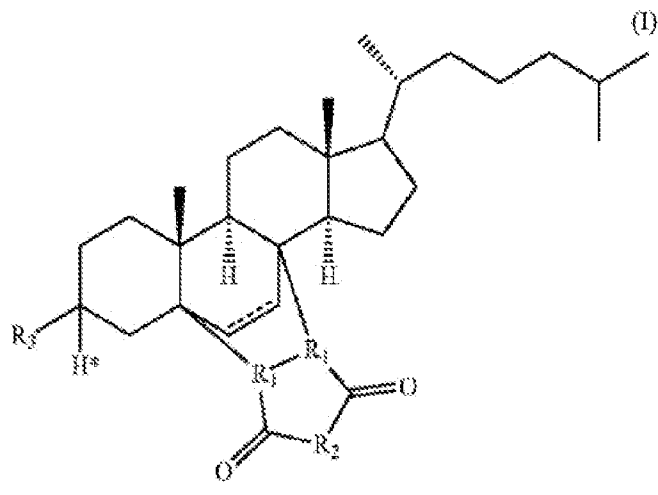
What is claimed is:

1. A method for preventing or treating pulmonary arterial hypertension in a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising an effective amount of a TGF- β receptor inhibitor and a pharmaceutically acceptable excipient thereof.

2. The method of claim 1, wherein the TGF- β receptor inhibitor is 7-dehydrocholesterol, a salt, an oxide, a metabolite or a derivative thereof.

3. The method of claim 2, wherein the 7-dehydrocholesterol salt comprises an acetate salt or a benzoate salt of (3 β)-7-dehydrocholesterol or a derivative thereof.

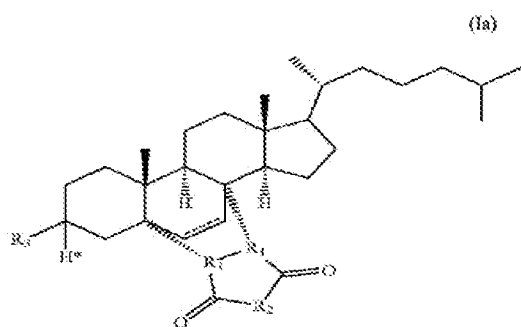
4. The method of claim 2, wherein the 7-dehydrocholesterol derivative comprises cholecalciferol or a compound represented by formula (I):



wherein R_1 is CR_5 or N ; R_3 is selected from the group consisting of $-O(CR_5)_nR_6$, $-OC(-O)(CR_5)_nR_6$, $-OC(=O)(CR_5)_nOR_5$ and $-OC(=O)C(R_5)=C(R_5)_2$; R_2 is selected from the group consisting of oxygen, sulfur, $C(R_4)_2$, and $N(R_4)$; R_4 is independently selected for each occurrence from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl, substituted heteroarylalkyl, OR_5 and $N(R_5)_2$; R_5 is independently selected for each occurrence from the group

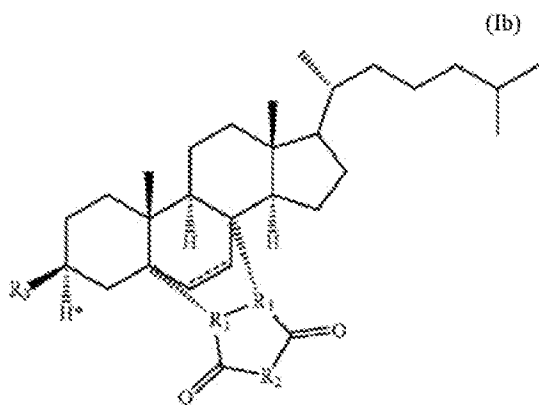
consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroaryl, substituted heteroaryl, heteroarylalkyl and substituted heteroarylalkyl; R_6 is selected from the group consisting of fluorine, chlorine, bromine, iodine, methanesulfonyl, toluenesulfonyl, $-\text{OSi}(R_5)_3$, $-\text{C}(=\text{O})\text{OR}_5$ and $-\text{C}(=\text{O})\text{R}_5$; the dashed lines are single or double bonds; and, n is an integer from 1 to 10.

5. The method of claim 4, wherein the compound represented by formula (I) is the compound represented by formula (Ia) or a salt or a solvate thereof,



, wherein R_1 to R_3 are as defined in claim 4.

6. The method of claim 5, wherein the compound represented by formula (I) is the compound represented by formula (Ib) or a salt or a solvate thereof,

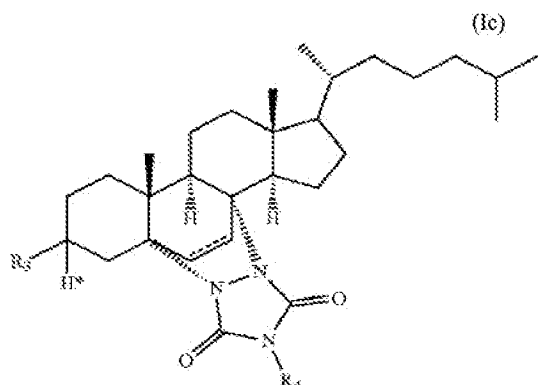


, wherein R_1 to R_3 are as defined in claim 4.

7. The method of claim 4, wherein R_1 is N.

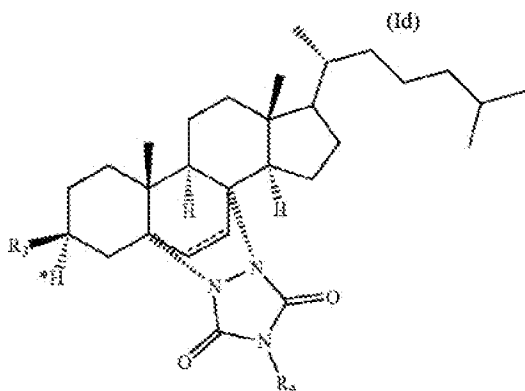
8. The method of claim 7, wherein R_2 is $\text{N}(\text{R}_4)$, and R_4 is as defined in claim 4.

9. The method of claim 8, wherein the compound represented by formula (I) is the compound represented by formula (Ic) or a salt or a solvate thereof,



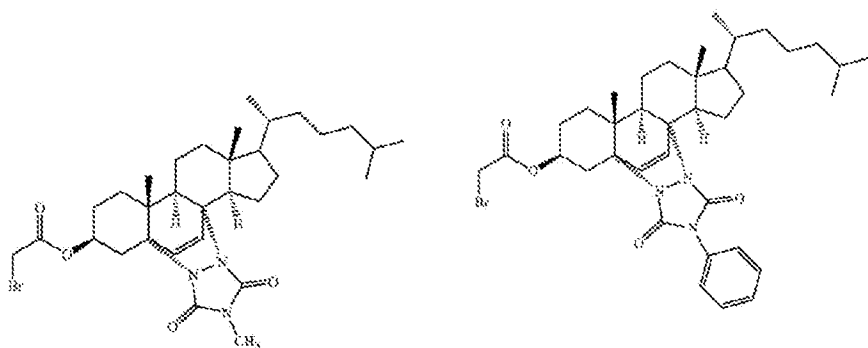
, wherein R₃ and R₄ are as defined in claim 4.

10. The method of claim 9, wherein the compound represented by formula (I) is the compound represented by formula (Id) or a salt or a solvate thereof,



, wherein R₃ and R₄ are as defined in claim 4.

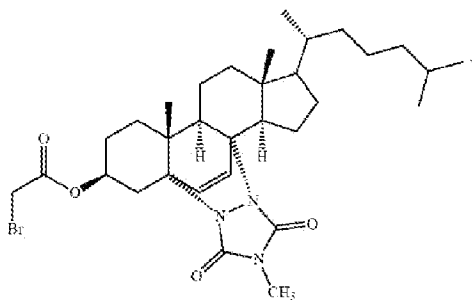
11. The method of claim 4, wherein the compound represented by formula (I) is



or

12. The method of claim 4, wherein the subject is a mammal.

13. The method of claim 4, wherein the compound represented by formula (I) is:



14. The method of claim 2, wherein the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative thereof is administered to the subject at a dose ranging from 1 mg/kg to 50 mg/kg.

5 15. The method of claim 1, wherein the 7-dehydrocholesterol, or a salt, an oxide, a metabolite or a derivative is the single active ingredient in the pharmaceutical composition for preventing or treating the pulmonary arterial hypertension.

16. The method of claim 1, wherein the pharmaceutical composition further comprises other active ingredients selected from the group consisting of calcium
10 blockers, anticoagulants, diuretics, cardiotonic agents, endothelin receptor antagonists, phosphodiesterase type 5 inhibitors, prostacyclin analogs, and any combination thereof.

17. The method of claim 1, wherein the TGF- β receptor inhibitor reduces the proliferation of pulmonary artery endothelial cells, smooth muscle cells, or a
15 combination thereof in the subject.

18. The method of claim 1, wherein the pharmaceutical composition is administered to the subject subcutaneously, intravenously, intradermally, intraperitoneally, orally, intrabuccally, sublingually, intramuscularly, into the respiratory tract, or into the lungs.

20 19. The method of claim 1, wherein the pharmaceutical composition is administered to the subject at least once daily.

20. The method of claim 1, wherein the pharmaceutical composition is administered to the subject for at least 1 month.

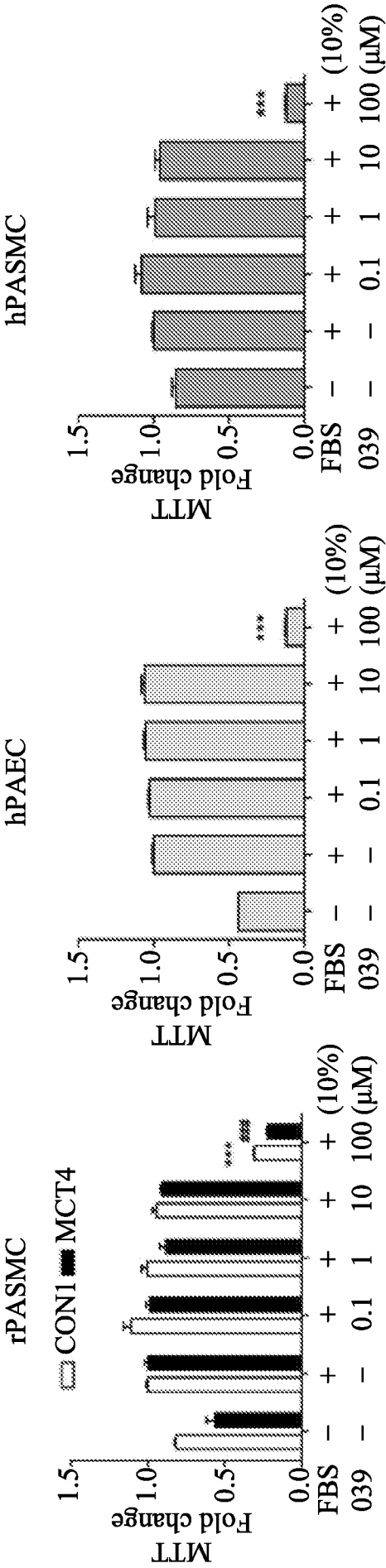


FIG. 1

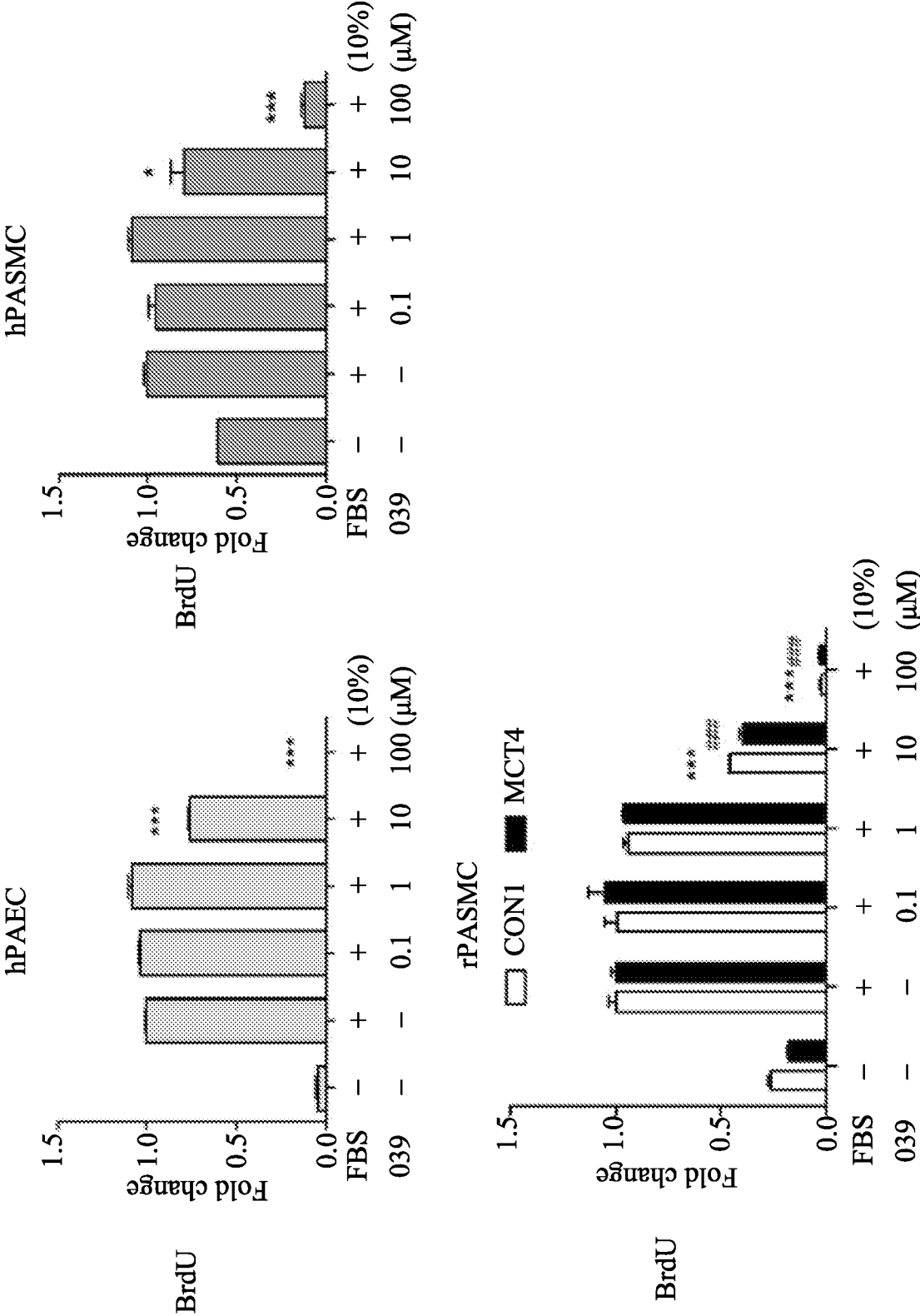


FIG. 2

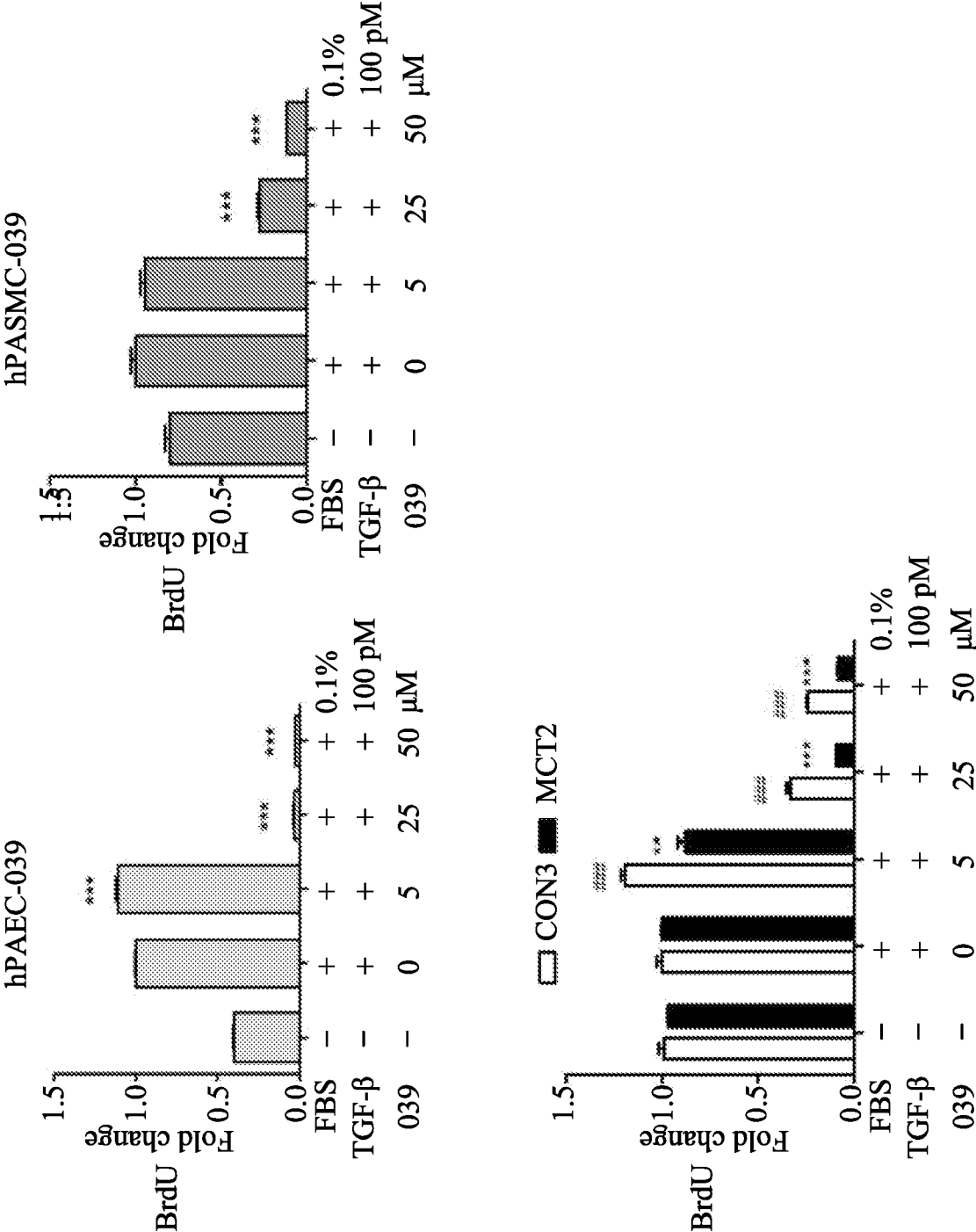


FIG. 3

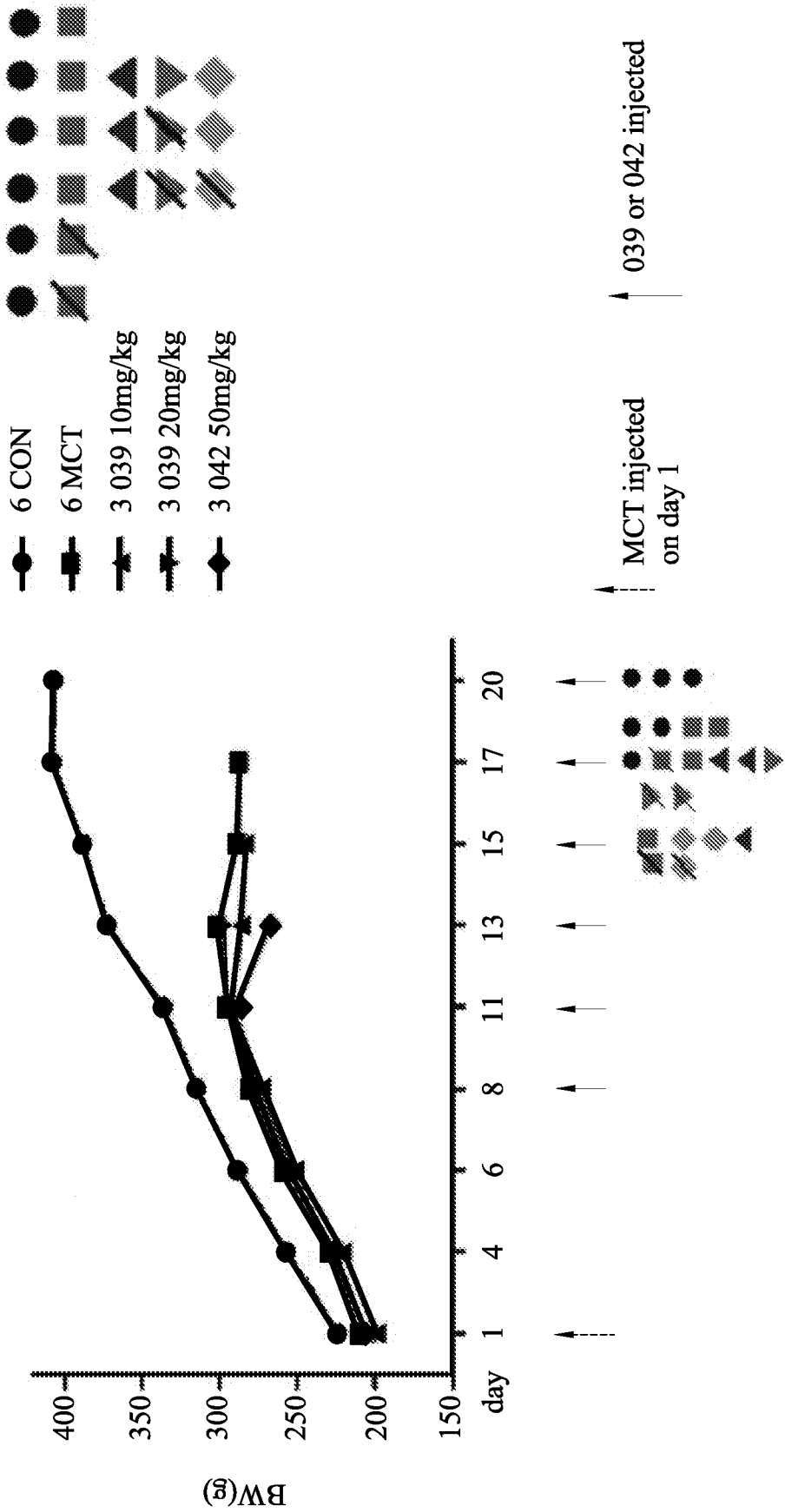


FIG. 4

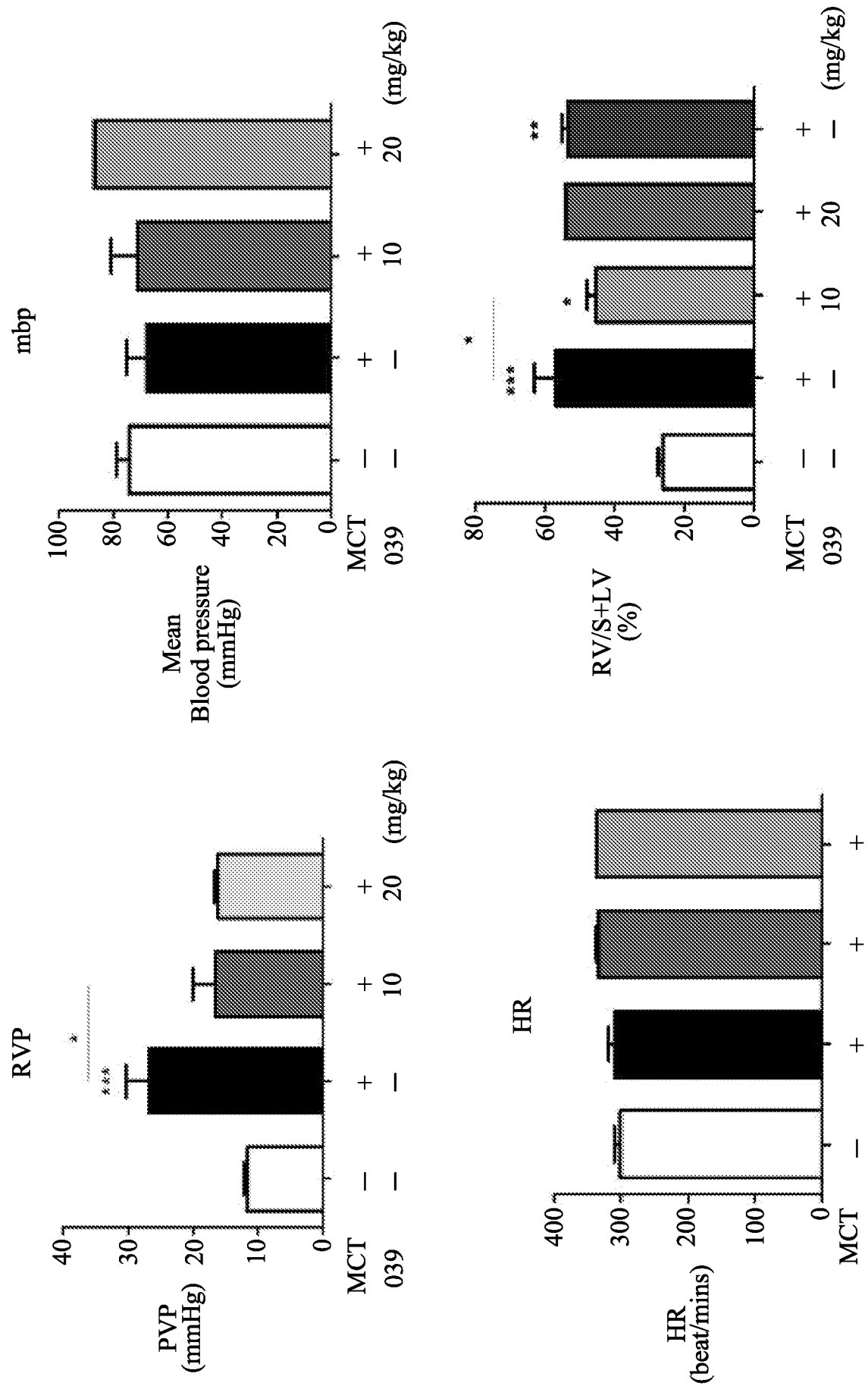


FIG. 5

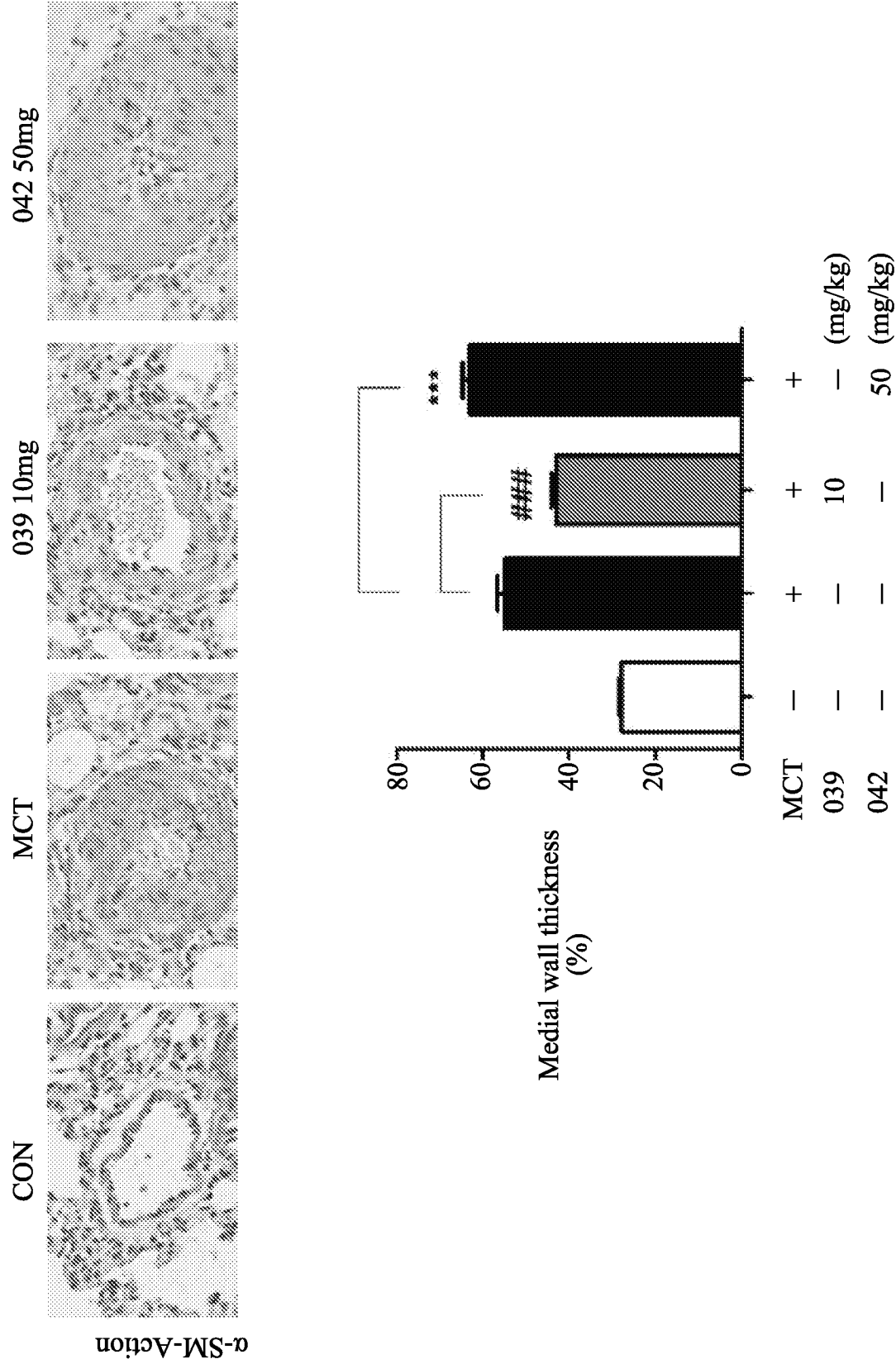


FIG. 6

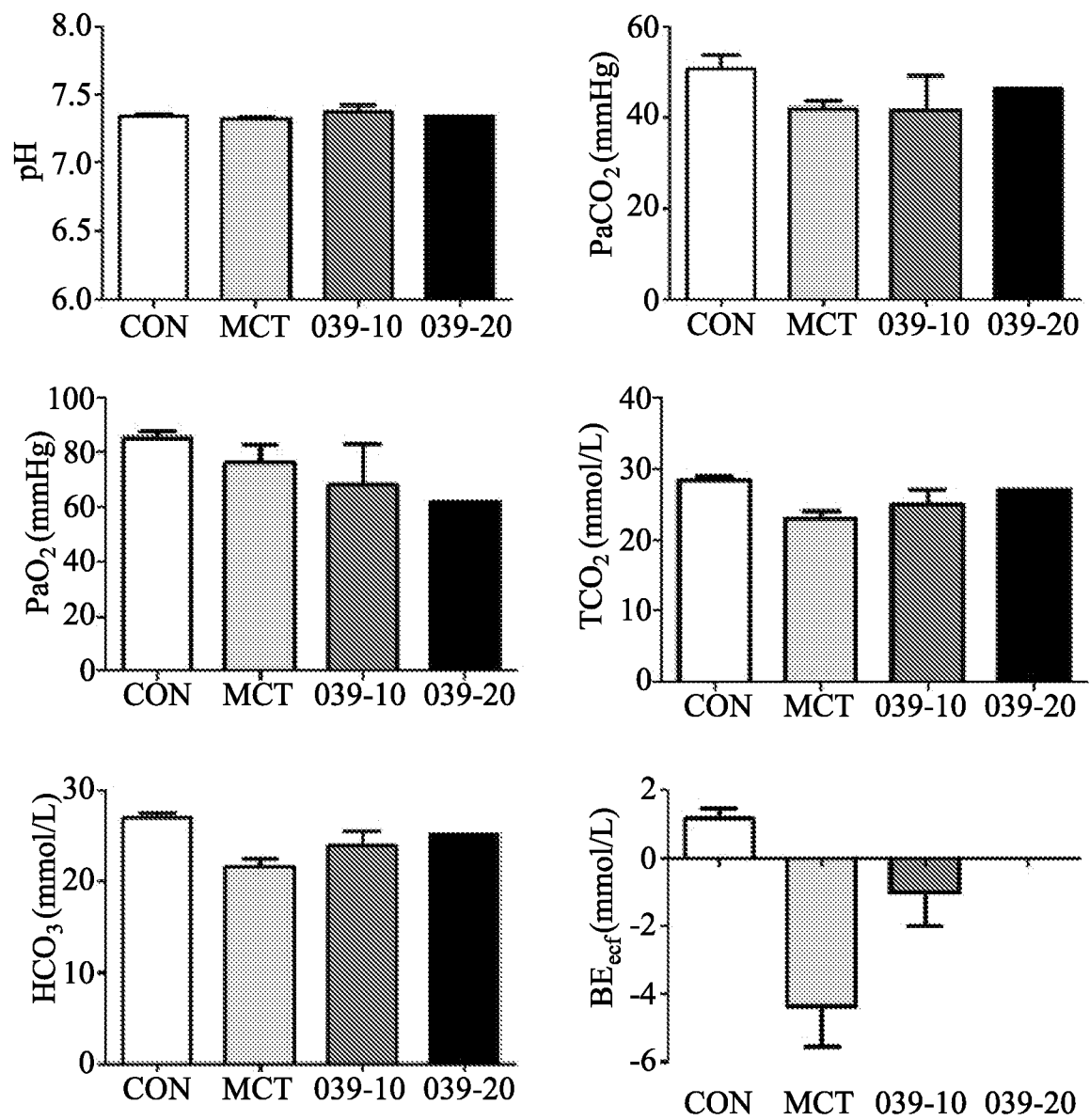


FIG. 7

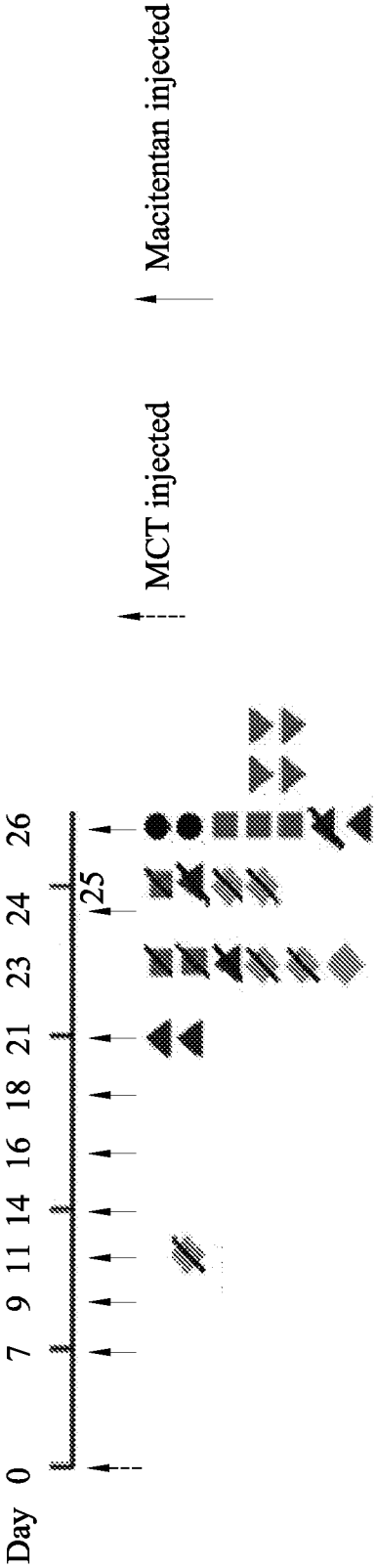
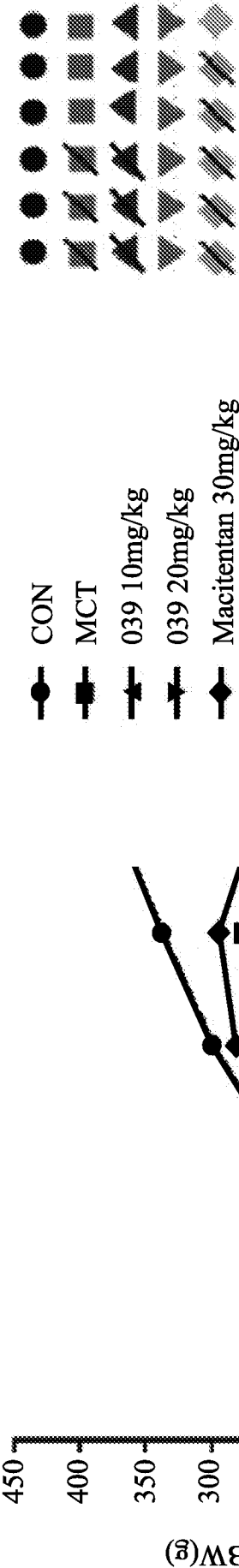


FIG. 8

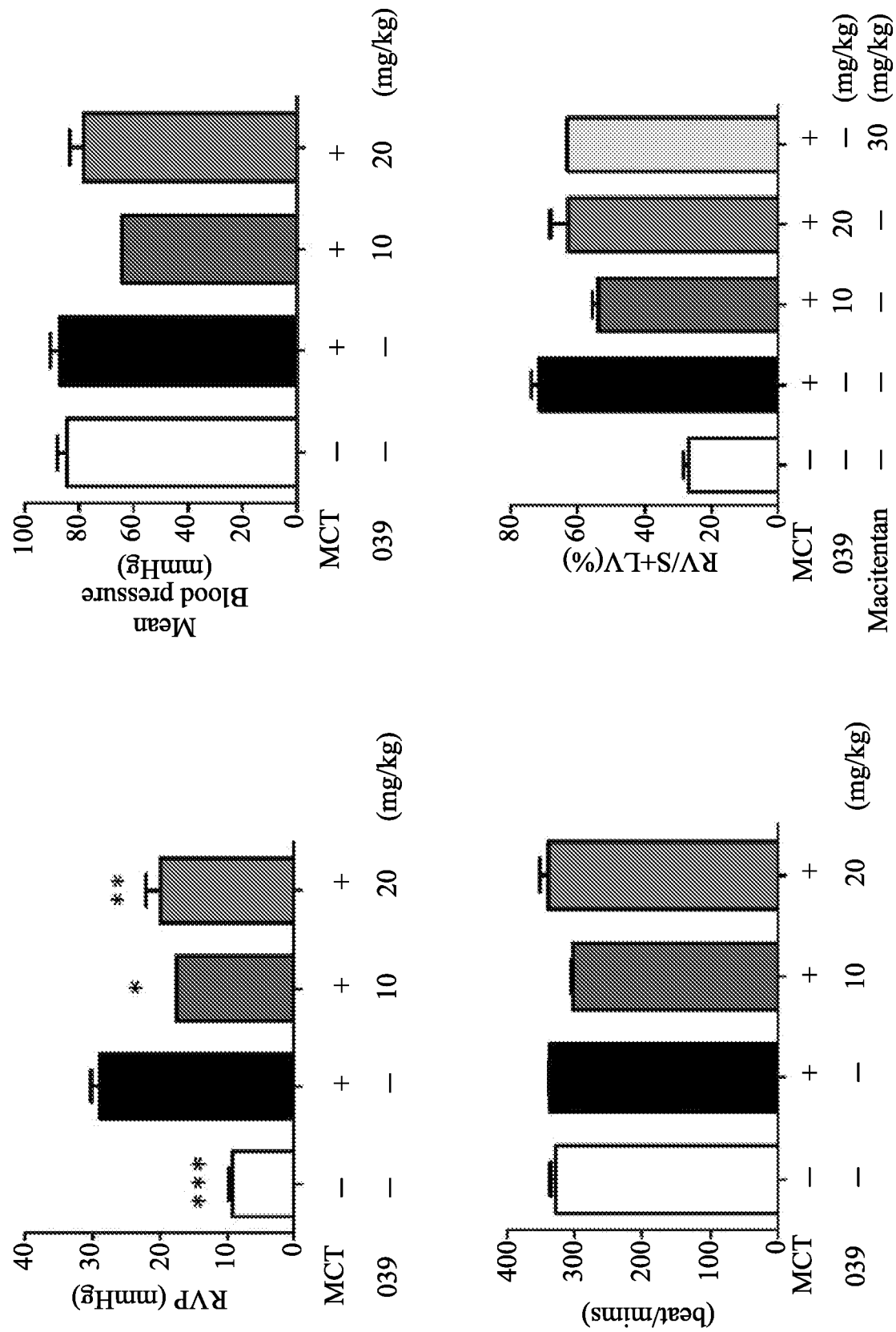


FIG. 9

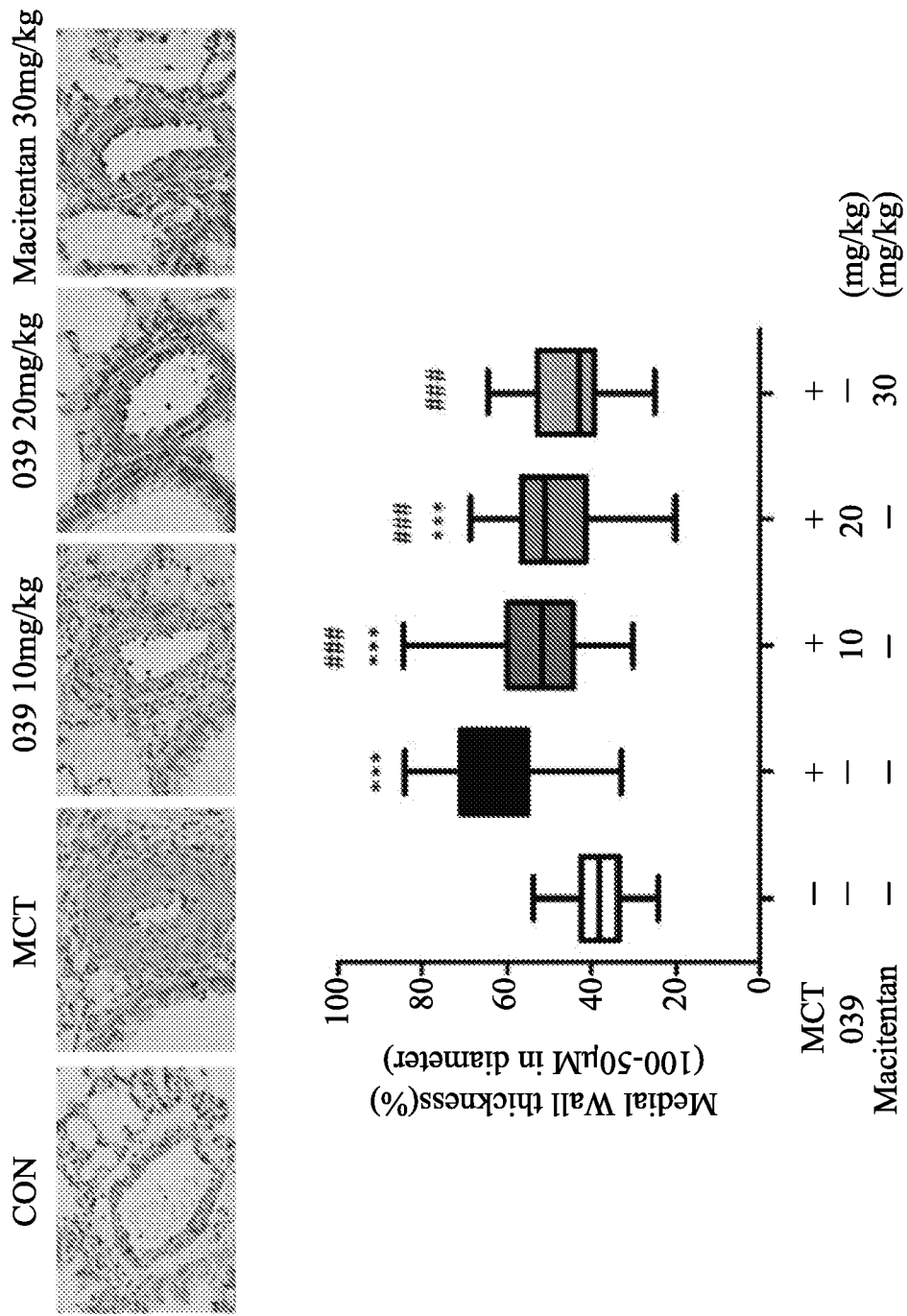


FIG. 10

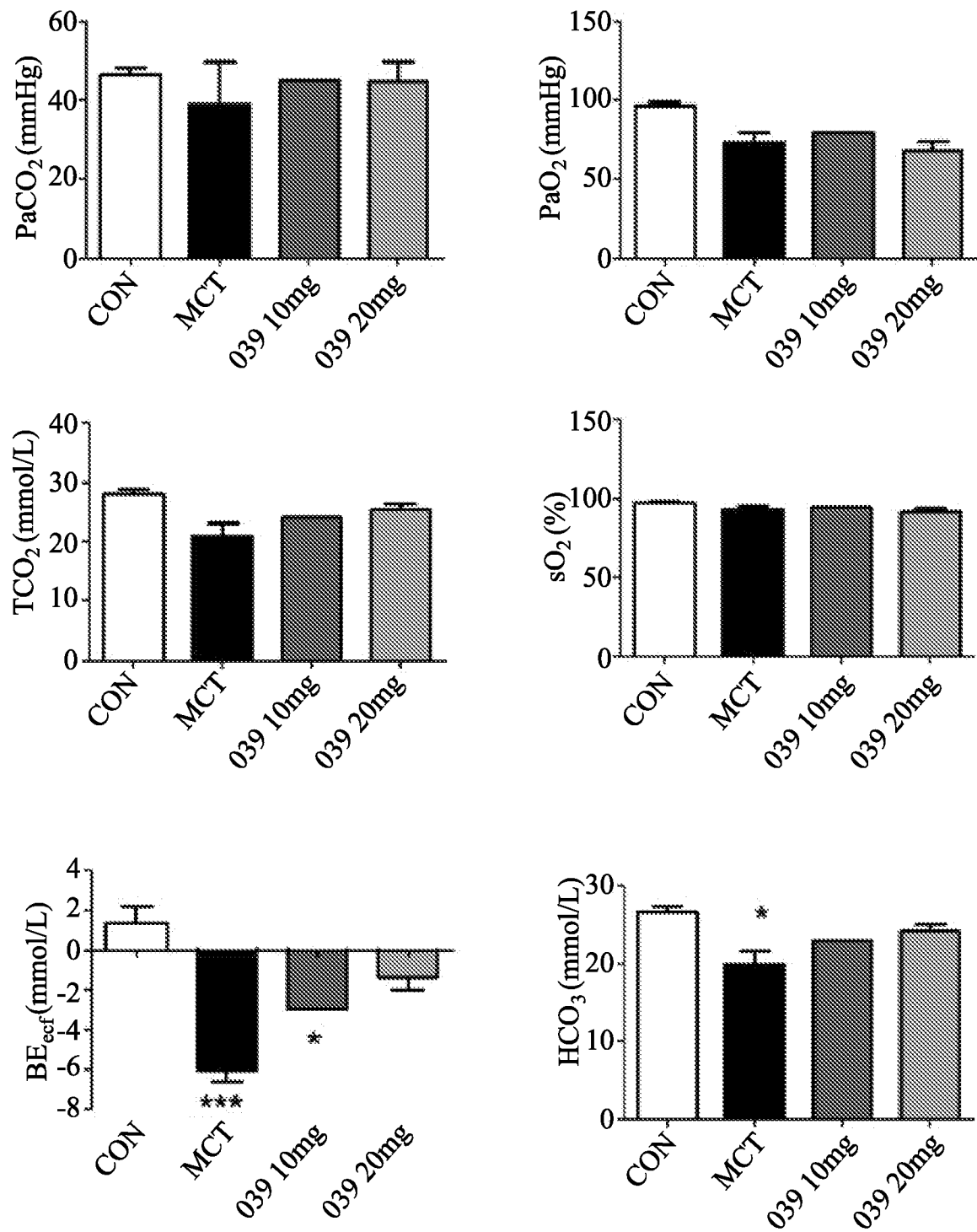


FIG. 11

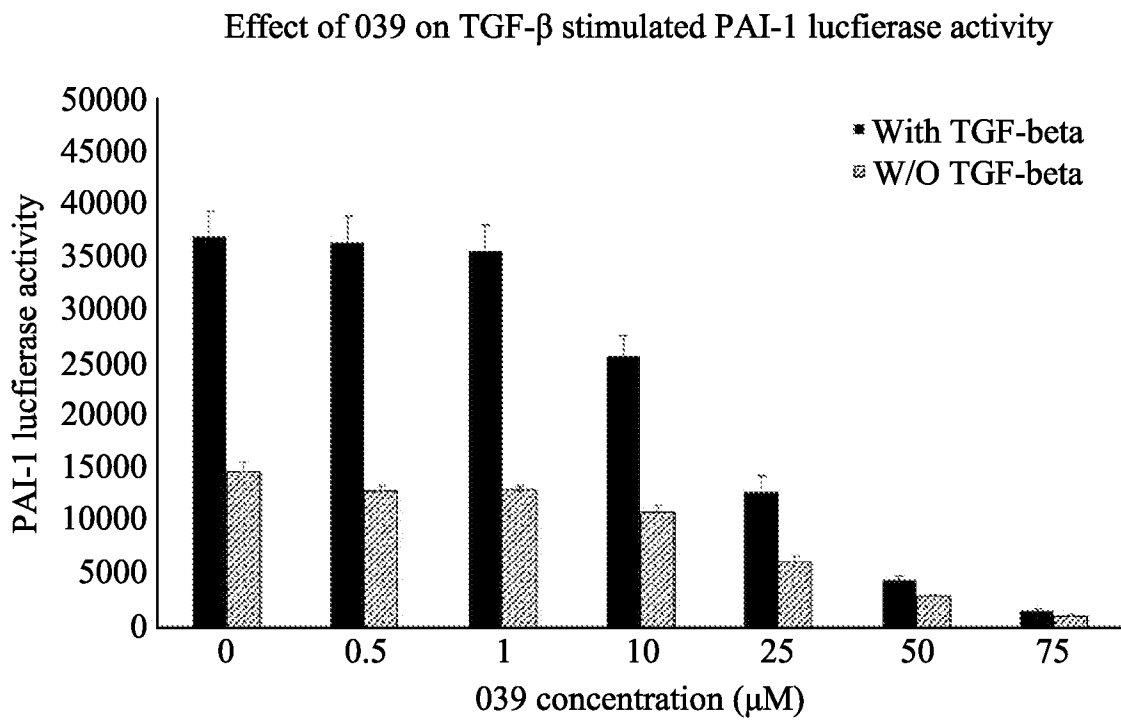


FIG. 12A

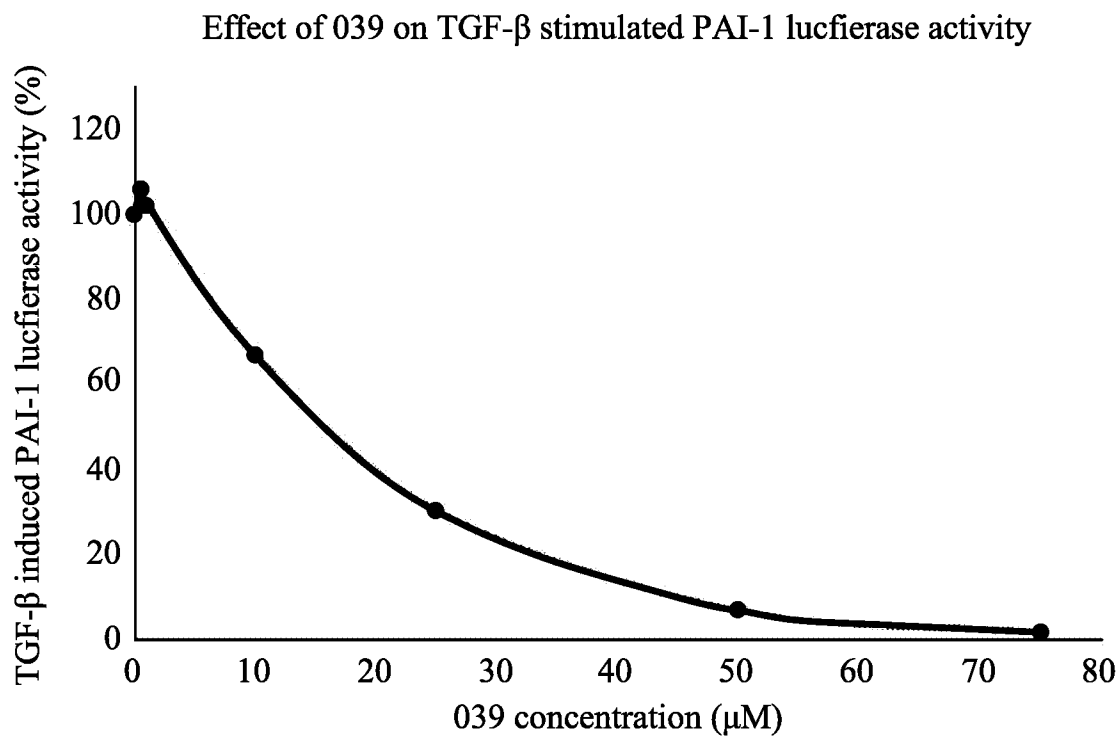


FIG. 12B

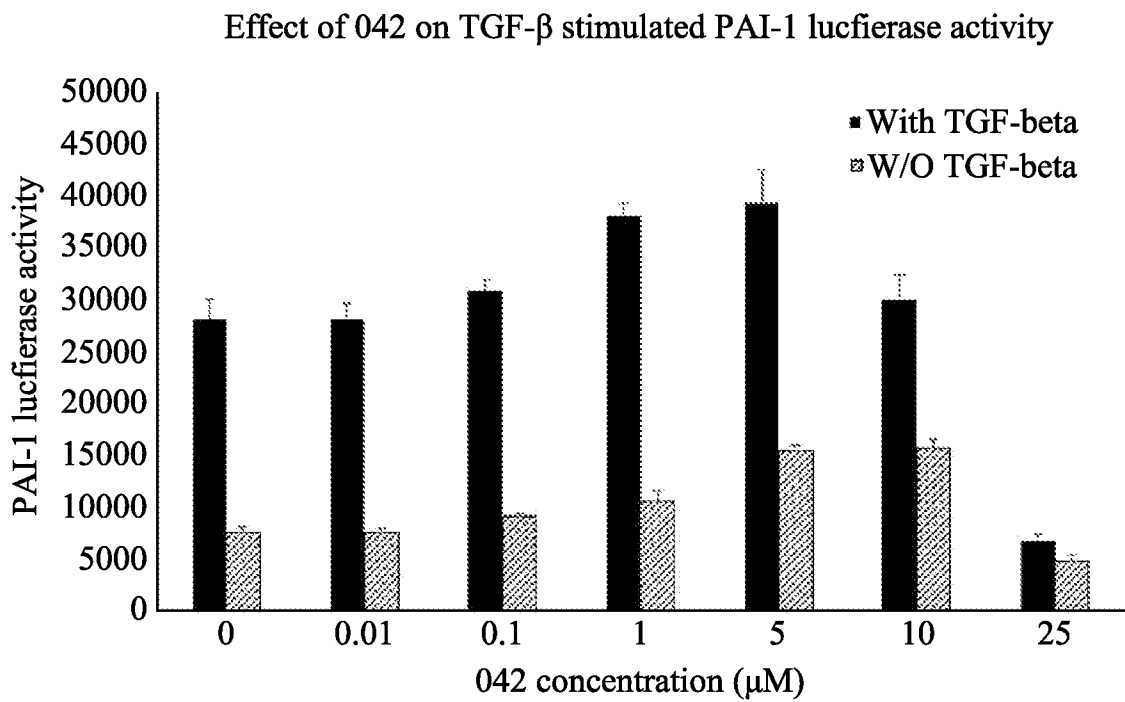


FIG. 13A

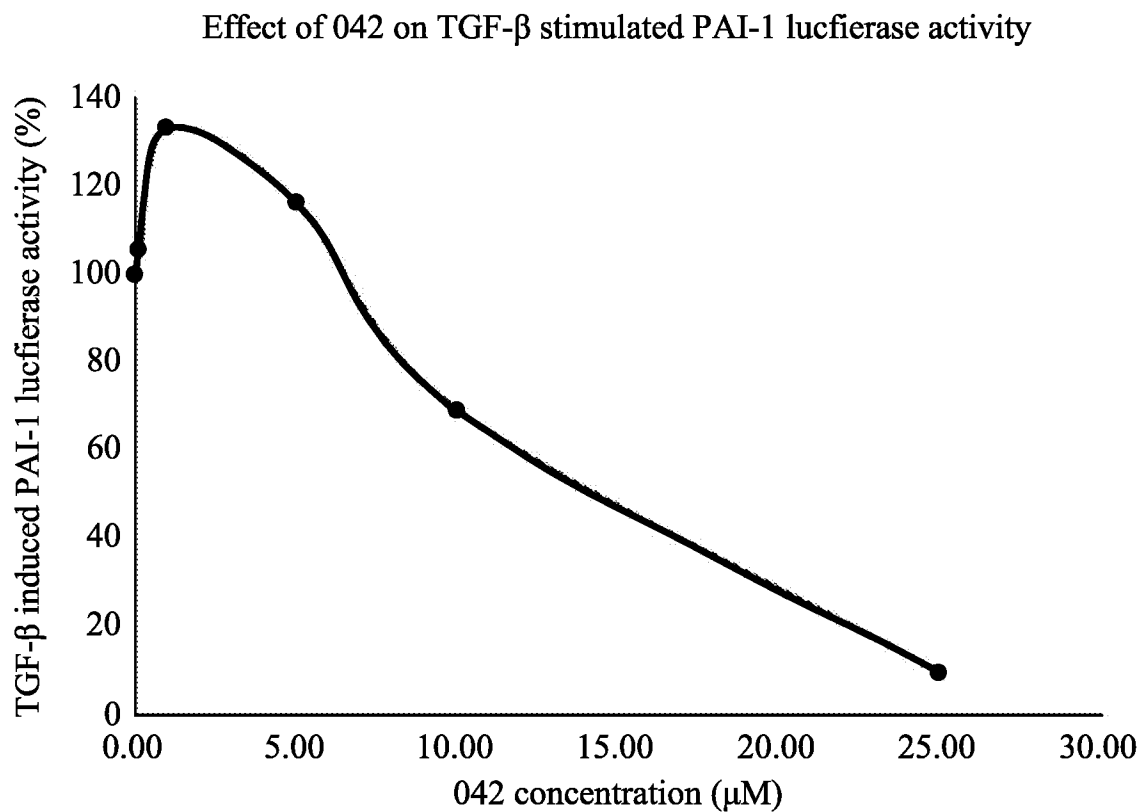


FIG. 13B

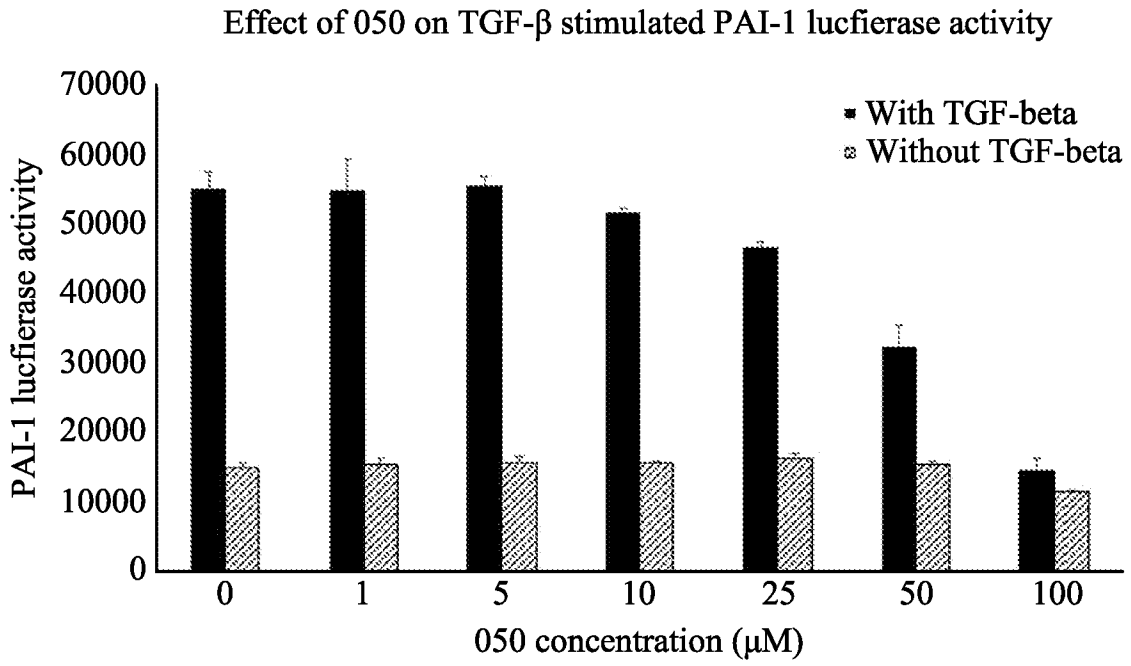


FIG. 14A

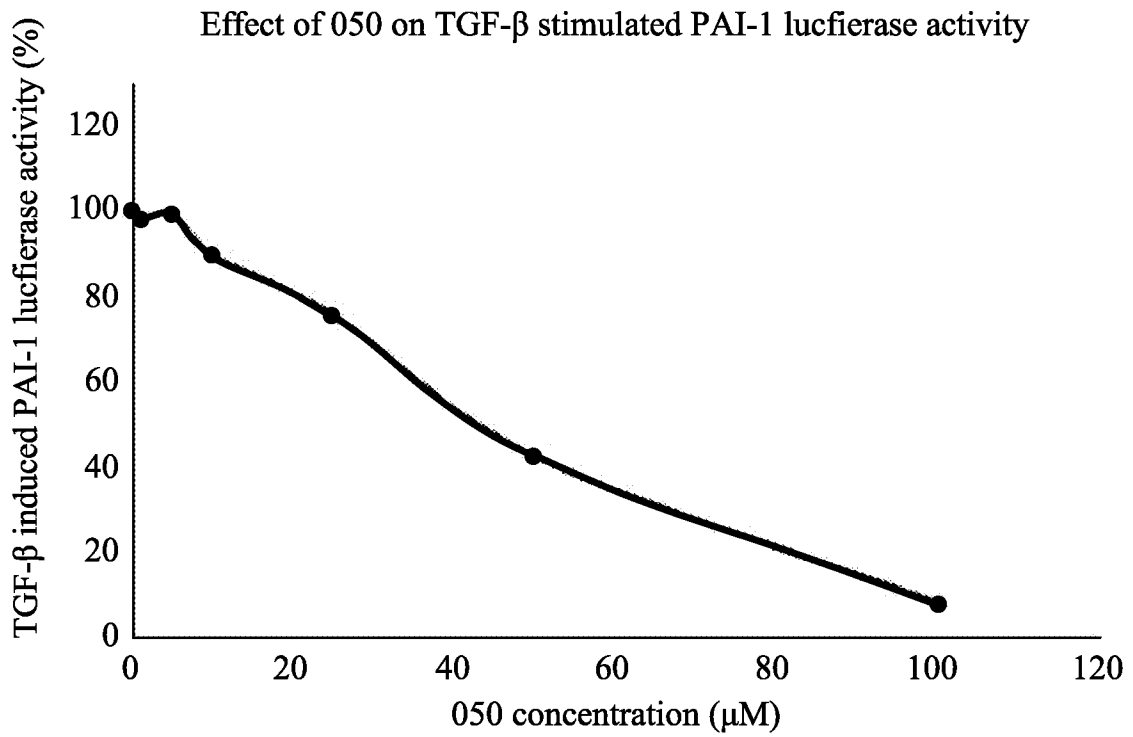


FIG. 14B

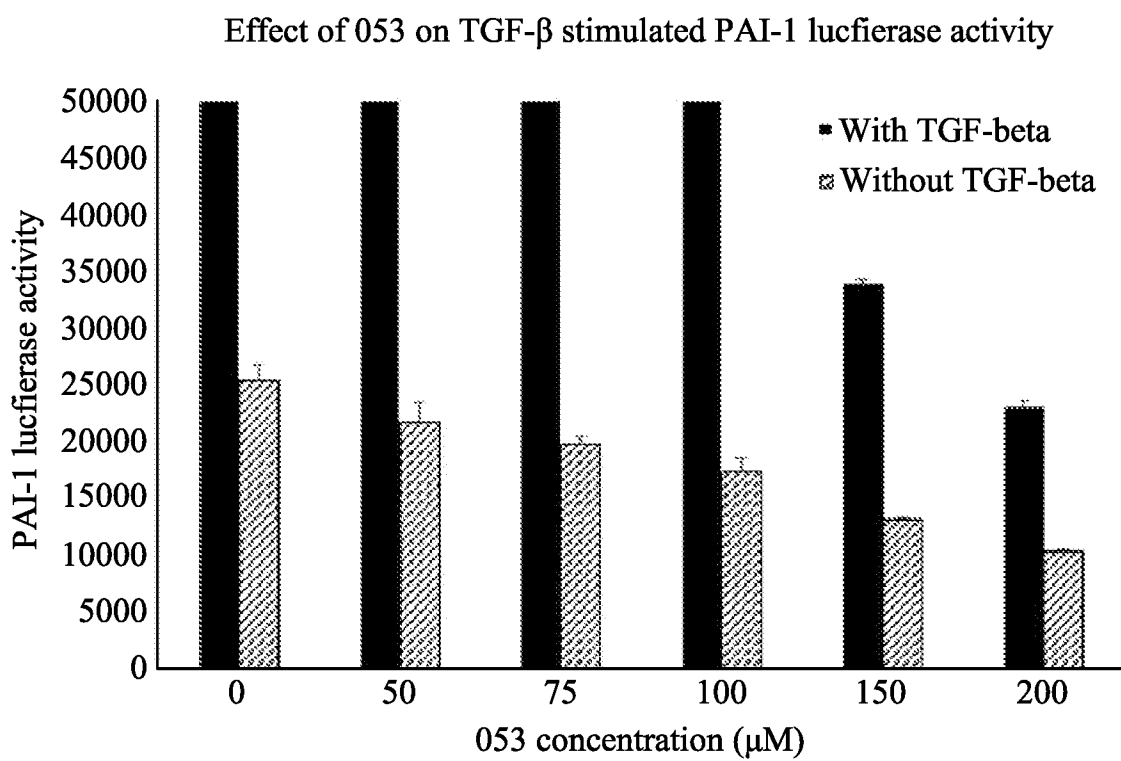


FIG. 15A

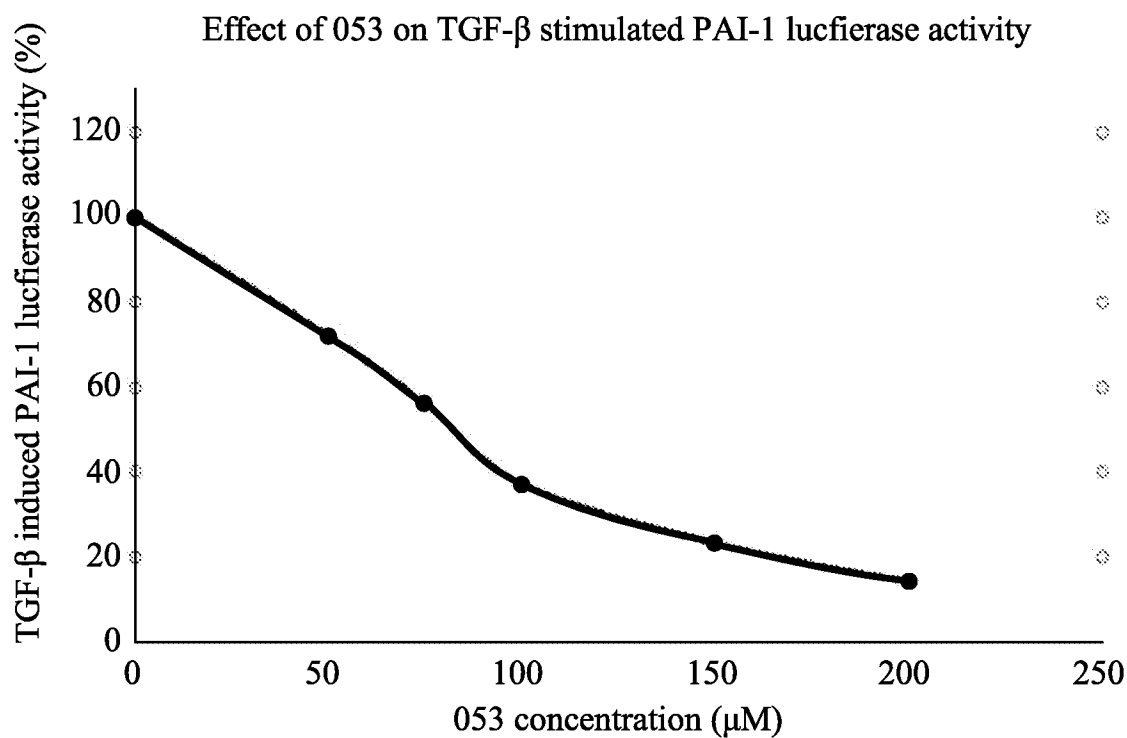


FIG. 15B

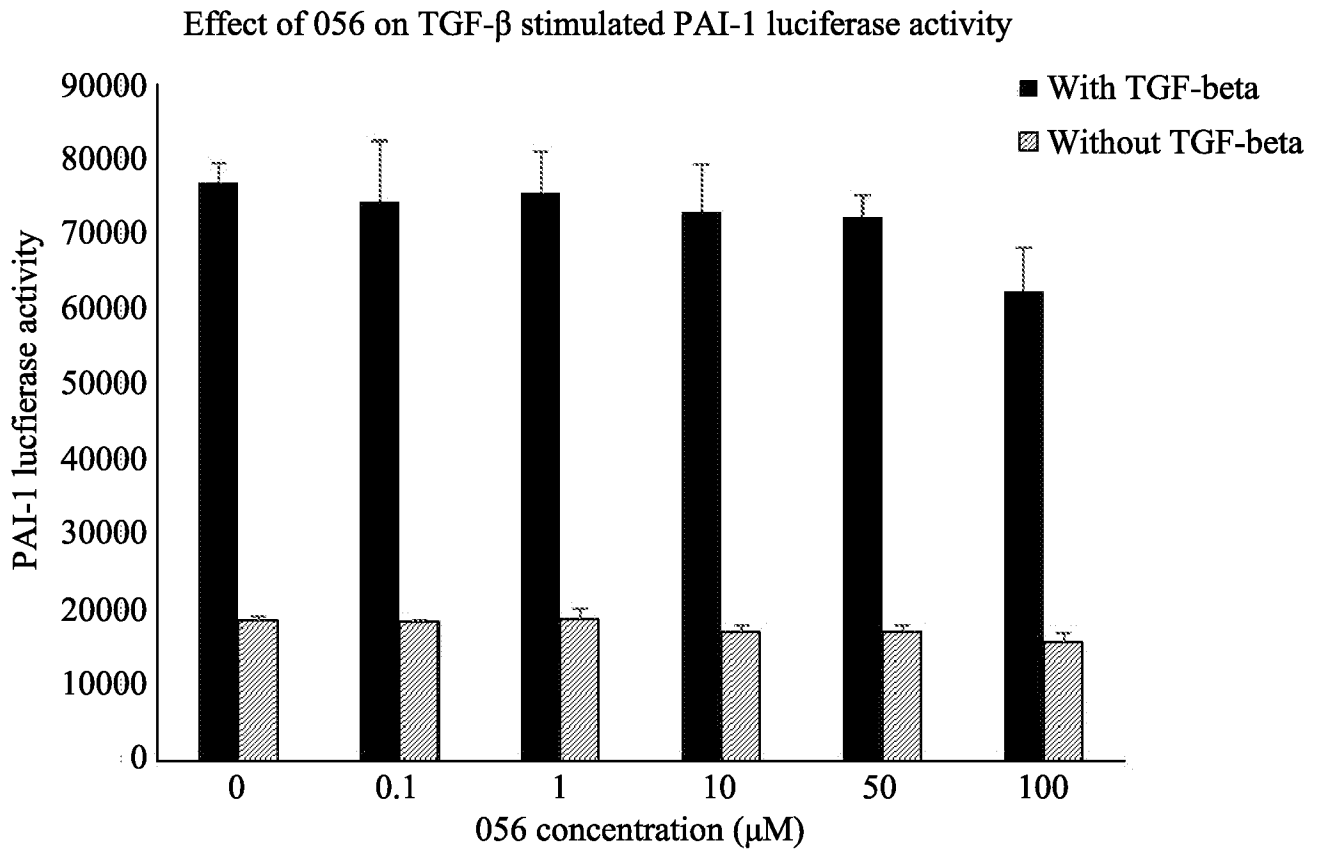


FIG. 16A

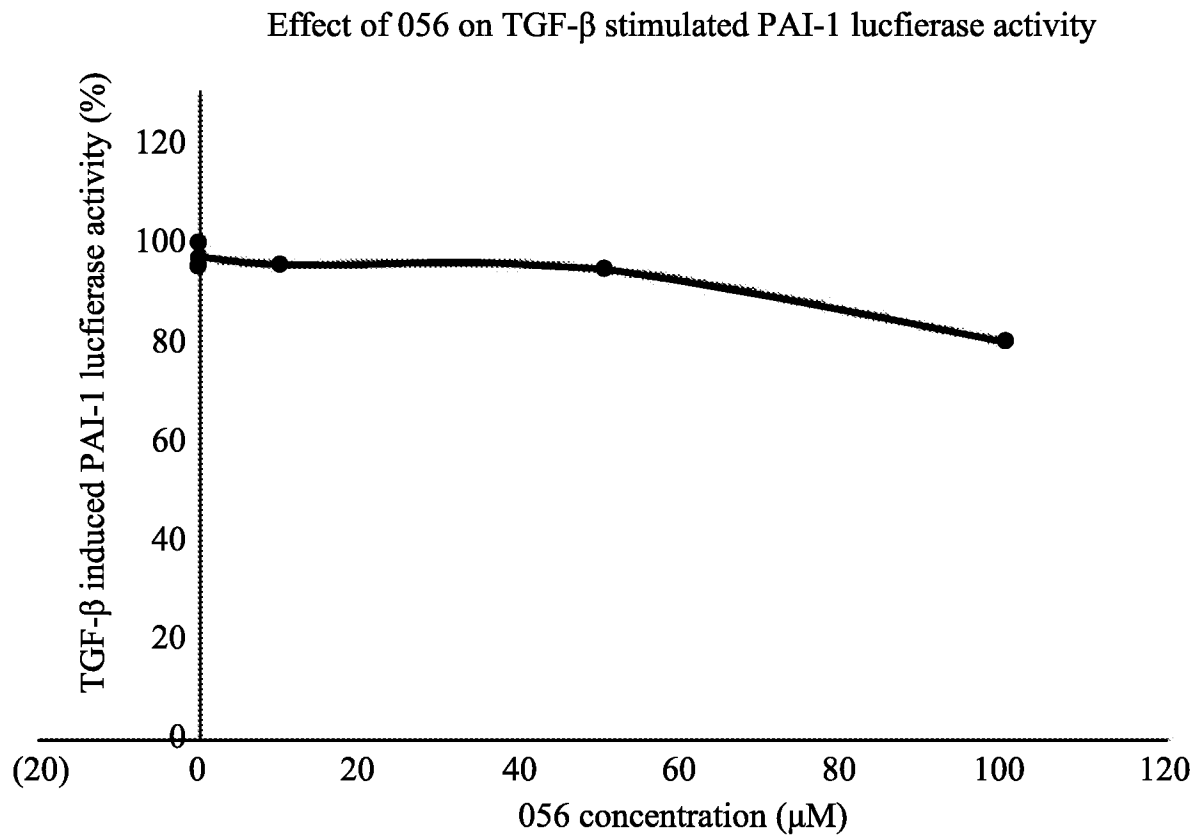


FIG. 16B

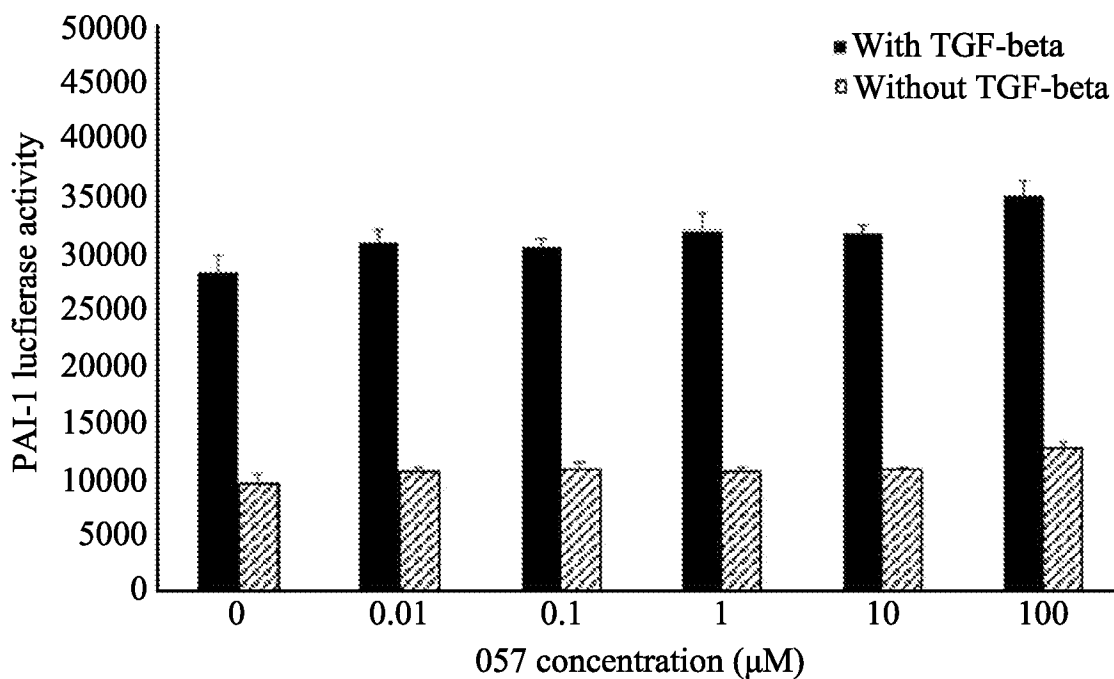
Effect of 057 on TGF- β stimulated PAI-1 luciferase activity

FIG. 17A

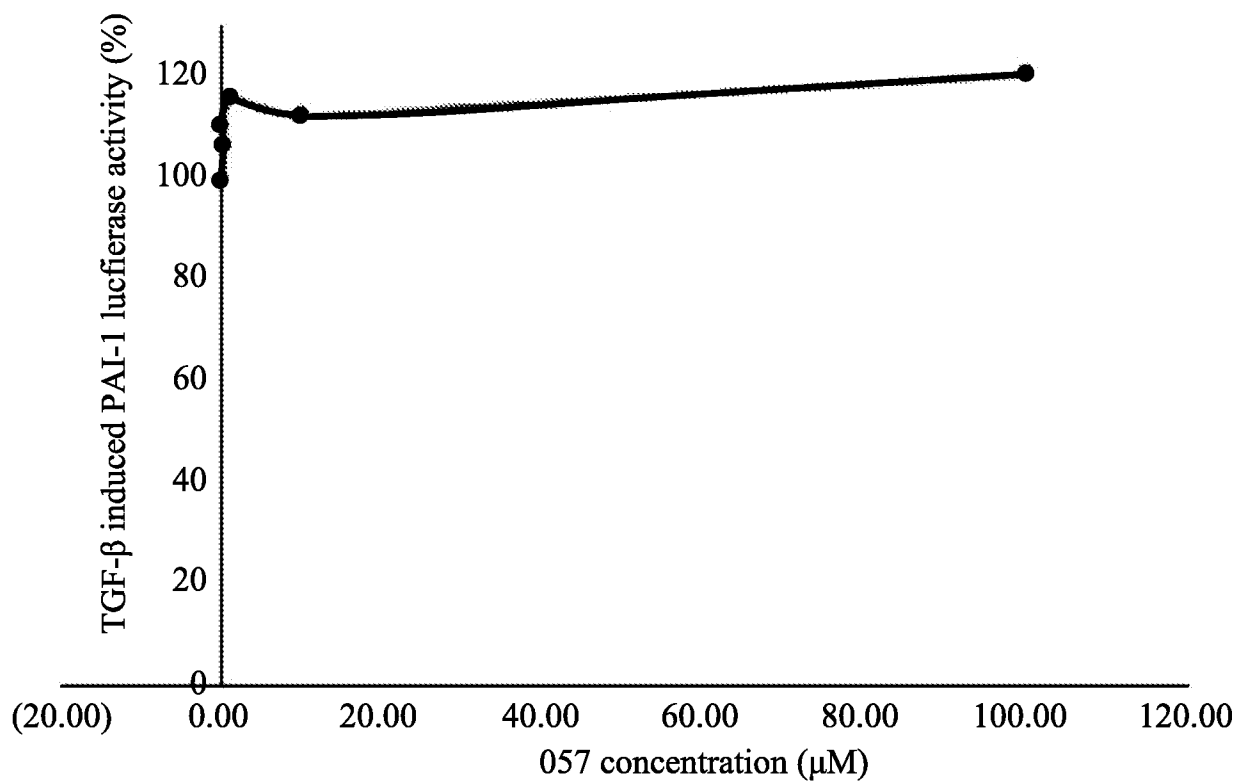
Effect of 057 on TGF- β stimulated PAI-1 luciferase activity

FIG. 17B

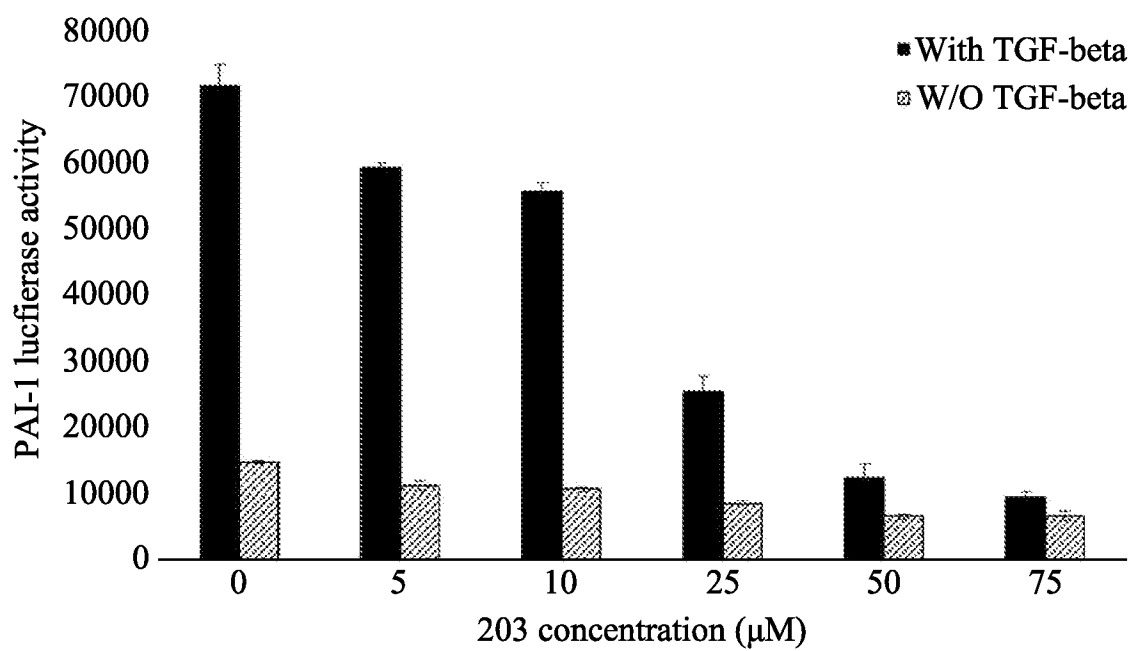
Effect of 203 on TGF- β stimulated PAI-1 luciferase activity

FIG. 18A

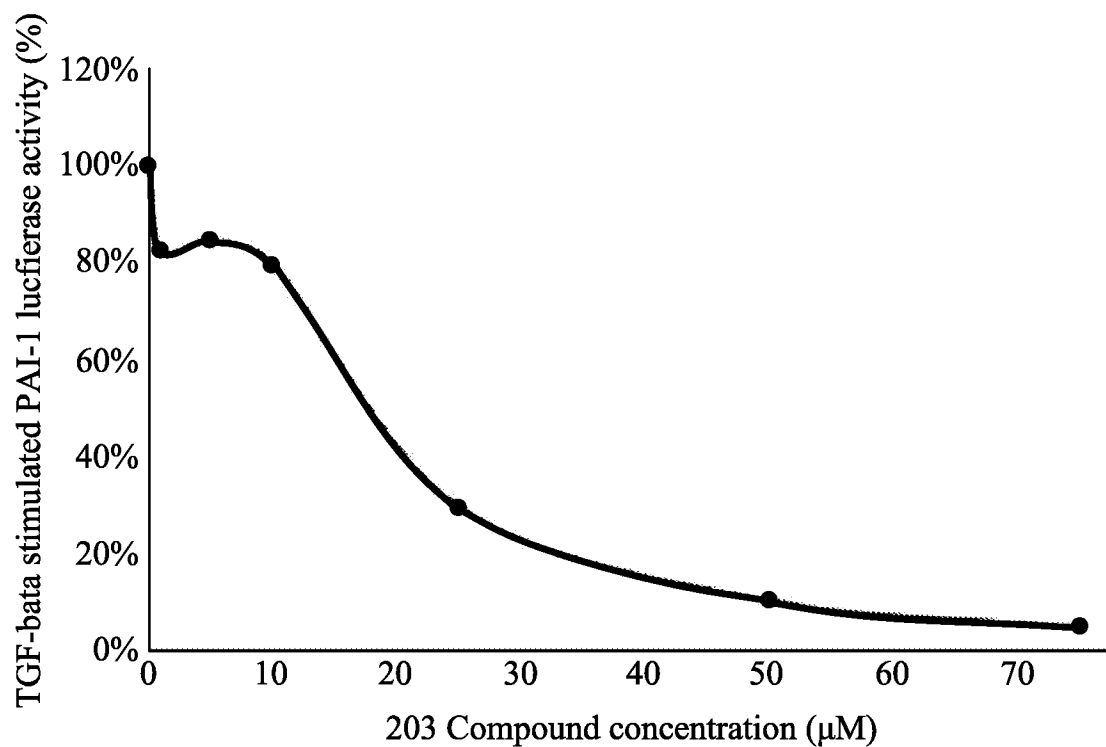
Effect of 203 on TGF- β stimulated PAI-1 luciferase activity

FIG. 18B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/MY2024/050047

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/593(2006.01)i; A61K 45/06(2006.01)i; A61P 9/12(2006.01)i; A61P 11/00(2006.01)i

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)

A61K 31/593(2006.01); A61K 31/56(2006.01); A61K 38/18(2006.01); A61K 45/06(2006.01); A61K 47/55(2017.01);
A61K 8/63(2006.01); C07J 9/00(2006.01); C07K 14/495(2006.01)

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal), STN(Registry, Caplus) & Keywords: pulmonary arterial hypertension, TGF- β receptor inhibitor,
7-dehydrocholesterol, cholecalciferol**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	US 2017-0202918 A1 (THE BRIGHAM AND WOMEN'S HOSPITAL, INC.) 20 July 2017 (2017-07-20) claims 1-3, 6-8; paragraphs [0013], [0047], [0058], [0061], [0085]	1,16-20 2-15
A	WO 2009-029656 A1 (AUXAGEN, INC. et al.) 05 March 2009 (2009-03-05) claims 1-15	1-20
A	YU, H. et al., "1,25(OH)2D3 attenuates pulmonary arterial hypertension via microRNA-204 mediated Tgfb β 2/Smad signaling", Experimental Cell Research, 2018, Vol. 362, pp. 311-323 abstract; page 311	1-20
A	CN 112370529 A (CHINA PHARMACEUTICAL UNIVERSITY) 19 February 2021 (2021-02-19) claims 1-8	1-20

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

* Special categories of cited documents:

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"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

16 October 2024

Date of mailing of the international search report

16 October 2024

Name and mailing address of the ISA/KR

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Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT

International application No.

PCT/MY2024/050047

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015-157262 A1 (WOMEN & INFANTS HOSPITAL OF RHODE ISLAND) 15 October 2015 (2015-10-15) claims 1-71	1-20

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/MY2024/050047

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
US	2017-0202918	A1	20 July 2017	CN	106794233	A	31 May 2017
				CN	106794233	B	12 November 2021
				CN	114470207	A	13 May 2022
				CN	114470207	B	19 September 2023
				EP	3174550	A1	07 June 2017
				EP	3174550	A4	20 June 2018
				EP	3174550	B1	29 January 2020
				EP	3693004	A1	12 August 2020
				JP	2017-523200	A	17 August 2017
				JP	2020-125353	A	20 August 2020
				JP	6955443	B2	27 October 2021
				US	11027014	B2	08 June 2021
				US	2019-0365857	A1	05 December 2019
				US	2021-0322548	A1	21 October 2021
				WO	2016-019368	A1	04 February 2016
WO	2009-029656	A1	05 March 2009	EP	2192904	A1	09 June 2010
				EP	2192904	A4	25 August 2010
				EP	2192904	B1	17 August 2016
				US	2009-0062247	A1	05 March 2009
				US	8946201	B2	03 February 2015
CN	112370529	A	19 February 2021	CN	112370529	B	11 November 2022
WO	2015-157262	A1	15 October 2015	EP	3128996	A1	15 February 2017
				EP	3128996	A4	29 November 2017
				EP	3128996	B1	02 June 2021
				US	10683324	B2	16 June 2020
				US	11034719	B2	15 June 2021
				US	2017-0029459	A1	02 February 2017
				US	2018-0273580	A1	27 September 2018
				US	2020-0291060	A1	17 September 2020
				US	9982010	B2	29 May 2018